Individual sPLS on ruminal bacterial community of 2 lambs responding differently to a high starch diet to discriminate potential clusters producing trans10-18:1 and trans11-18:1 fatty acids

Annabelle Meynadier, Laurent Cauquil, Francis Enjalbert, Monica Costa, Susana P.Alves, Rui J.B. Bessa

▶ To cite this version:

> Annabelle Meynadier, Laurent Cauquil, Francis Enjalbert, Monica Costa, Susana P.Alves, et al.. Individual sPLS on ruminal bacterial community of 2 lambs responding differently to a high starch diet to discriminate potential clusters producing trans10-18:1 and trans11-18:1 fatty acids. 10. International Symposium of the Nutrition of Herbivores, Sep 2018, Clermont-Ferrand, France. Cambridge Universtiy Press, Advances in Animal Biosciences, 9 (3), p.381, 2018, Proceedings of the 10 th International Symposium on the Nutrition of Herbivores (ISNH). hal-02872079

HAL Id: hal-02872079

https://hal.inrae.fr/hal-02872079v1

Submitted on 17 Jun 2020

ISSN 2040-4700

SEPTEMBER 2018

VOLUME 9 ISSUE 3

Advances in Animal Biosciences

Herbivore nutrition supporting sustainable intensification and agro-ecological approaches

Proceedings of the 10th International
Symposium on the Nutrition of Herbivores
ISNH 2018

10th
INTERNATIONAL
SYMPOSIUM
on the Nutrition
of Herbivores

Editors
René Baumont
Mathieu Silberberg
Isabelle Cassar-Malek

CAMBRIDGE
UNIVERSITY PRESS

Advances in Animal Biosciences

Aims and Scope

Advances in Animal Biosciences is an associated publication to the journal *animal*. It aims to publish high-quality conference, symposium and workshop proceedings about animal-related aspects of the life sciences with emphasis on farmed and other managed animals. These can be in the form of a book of abstracts, summaries or complete papers. The format will highlight the title of the meeting and organisations involved but the publications will have the added advantage of forming a series under *Advances in Animal Biosciences.*

Subject areas can include aspects of Breeding and Genetics, Nutrition, Physiology and Functional Biology of Systems, Behaviour, Health and Welfare, Livestock Farming Systems, Human Health and Product Quality.

However, due to the integrative nature of biological systems, monographs and conference proceedings dealing with the translation of basic and strategic science into the whole animal and farming system and the impact on Productivity, Product Quality, Food Security, the Environment, Climate Change and Humans will be particularly welcome.

Information for Conference Organisers

The Animal Consortium together with Cambridge University Press offers conference organisers a package that enables publication of high-quality conference, symposium and workshop proceedings about animal-related aspects of the life sciences with emphasis on farmed and other managed animals.

Summaries, abstracts or full papers may be published in *Advances in Animal Biosciences* and high-quality invited papers from these meetings may be submitted and published as a defined series in *animal.*

Conference organizers interested in publishing their proceedings should send an outline proposal for publication in *Advances in Animal Biosciences, animal,* or both journals to cko@cambridge.org. The publisher together with the Editors-in-Chief will then provide an estimate of costs and the procedures to be used.

Manuscripts submitted to *Advances in Animal Biosciences* will be reviewed by the Editor-in-Chief and papers submitted to *animal* will be peer reviewed. If accepted after review, proceedings will be published within 12 weeks of receipt by the Publisher.

Cover pictures provided by N. Bertrand, Ch. Slagmulder, F. Carreras and A. Ridel ©INRA.

Proceedings
of the 10th International Symposium
on the Nutrition of Herbivores
2018

Herbivore nutrition supporting sustainable intensification and agro-ecological approaches

Advances in Animal Biosciences

This book is part of a series, which is a companion to the journal ANIMAL

10th INTERNATIONAL SYMPOSIUM
on the Nutrition of Herbivores

2 to 6 September 2018, Clermont-Ferrand, France

These proceedings of the International Symposium on the Nutrition of Herbivores constitutes summaries of papers presented at the 10th conference in Clermont-Ferrand, France, 2nd – 6th September 2018.

Summaries have been reviewed and edited for English language; however, the Organising Committee and publisher accept no responsibility for their accuracy. Views expressed in all contributions are those of the authors and not those of the symposium's committee members.

This publication contains all summaries that were available at the time of going to press.

ISNH 2018 COMMITTEE MEMBERS

Local Organising Committee
Yannick Faulconnier
(General Secretary)
Pascal Champciaux
Christiane Espinasse
Pascale Francisco
Sabrina Gasser
Benoît Graulet
Pascal Payard
Bernard Sepchat
Mathieu Silberberg
Maryse Teyssedre

Local Scientific Committee
Isabelle Cassar-Malek *(Chair)*
René Baumont *(Chair)*
Fabienne Blanc
Maryline Boval
Bertrand Dumont
Philippe Faverdin
Géraldine Fleurance
Evelyne Forano
Nicolas Friggens
Eliel Gonzalez-Garcia
Diego Morgavi
Brigitte Picard
Mathieu Silberberg
Isabelle Veissier

International Advisory Committee
Ilias Kyriazakis, UK *(Chair)*
André Bannink, Netherlands
Isabelle Cassar-Malek, France
Trevor DeVries, Canada
Robert Forster, Canada
Pilar de Frutos, Spain
Pablo Gregorini, New Zealand
Sabrina Louise Greenwood, USA
Dianne Mayberry, Australia
David Pacheco, New Zealand
Tanda Panjaitan, India
Debra Patterson, UK
Nigel Scollan, UK
W. Brandon Smith, USA
Izabelle A. M. A. Teixeira, Brazil
Aila Vanhatalo, Finland
Guangyong Zhao, China

Editors
René Baumont
Mathieu Silberberg
Isabelle Cassar-Malek

ISNH 2018 Website : https://symposium.inra.fr/isnh2018

ISSN 2040-4700

FOREWORD

It is our pleasure to welcome you to the 10th Meeting of the International Symposium on the Nutrition of Herbivores (ISNH 2018). The objectives of the Symposium series are “to provide a forum for development, exchange and presentation of knowledge in the nutrition of terrestrial farmed and wild herbivores”.

The role of herbivores in food security and in the emergence of developing economies out of poverty is a key issue for the sustainable development of many countries. At the same time, however, herbivore production systems are major players in global environmental issues -due to their contribution to greenhouse gas emissions- and facing an increasing societal concern for farmed animal welfare and human health linked to meat and dairy consumption.

In this context, producing better with fewer inputs is the main challenge imposed to animal production by the increase of global food demand and environmental issues. Agro-ecology, mainly by stimulating natural processes to reduce inputs, proposing a framework to cope with this challenge is an emerging field in animal science. At the same time, precision techniques and the digital revolution in animal husbandry create opportunities for herbivore production systems that are more efficient and more respectful of animal welfare and health. ISNH 2018 will be a unique opportunity for scientists and other stakeholders to discuss herbivore nutrition in relation to these issues.

As previous Symposia in the series, ISNH 2018 covers a wide range of topics: from feed resources and basic herbivore nutrition to nutritional strategies to improve nutritional efficiency, health and animal welfare, reduce emissions and waste, and improve product quality. The sessions will address organisational levels from the genome to organ, animal, herd (and farm), and consider herbivores under temperate, Mediterranean and tropical climates. To address these topics, we have selected 22 invited conferences with the International Advisory Committee of the Symposium series. The programme also includes 422 selected communications, presented either orally (40) or as short presentations of posters (65), or as free-viewing posters. These communications come from 46 countries from all continents, showing the world-wide interest in herbivore nutrition.

We would like to address our warmest thanks to the members of the International Advisory Committee of the Symposium and Local Scientific Committee who built the Symposium programme; to all authors whose efforts allow us to propose this rich and diversified program; to the reviewers whose expertise was essential to the publication of the scientific contributions; to the editorial teams of Advances in Animal Biosciences and Animal journals; to all scientists and technicians involved in the organisation of the Symposium; to the management of INRA and to the numerous sponsors who supported us in setting up this Symposium.

Finally, we hope that the reading of this issue of Advances in Animal Biosciences will help you learn more about herbivore nutrition and find inspiration for future research and development in this area.

René Baumont, Mathieu Silberberg, Isabelle Cassar-Malek

Acknowledgements

The ISNH local scientific committee particularly thanks the reviewers who accepted to evaluate part of the submitted summaries.

- Harry Archimède
- Nathalie Bareille
- Denis Bastianelli
- René Baumont *
- Marc Benoit
- Laurence Bernard
- Fabienne Blanc
- François Bocquier
- Anne Boudon
- Maryline Boval *
- Gerardo Caja
- Gonzalo Cantalapiedra-Hijar
- Ramon Casals
- Isabelle Cassar-Malek *
- Mizeck Chagunda
- Frédérique Chaucheyras-Durand
- Anne de la Torre
- Virginie Decruyenaere
- Luc Delaby
- Rémy Delagarde
- Stuart Denman
- Richard Dewhurst
- Jan Dijsktra
- Bertrand Dumont *
- Nadège Edouard
- Marie-Pierre Ellies
- Maguy Eugène
- Philippe Faverdin *
- Anne Ferlay
- Géraldine Fleurance *
- William Foley
- Evelyne Forano *
- Dominique François
- Nicolas Friggens *
- Hervé Fritz
- Mohammed Gagaoua
- Sylvie Giger-Reverdin
- Eliel Gonzalez-Garcia *
- Iain Gordon
- Paul Greenwood
- Philippe Hassoun
- Lina Jaber
- Helene Jammes
- Magali Jouven
- Yann Serge Landau
- Yannick le Cozler
- Dorothée Ledoux
- Christine Leroux
- Didier Macheboeuf
- Christel Marie-Etancelin
- Cécile Martin
- Gaëlle Maxin
- Marie-Madeleine Mialon
- Peter Moate
- Aidan Moloney
- Valérie Monteils
- Carole Moreno
- Diego Morgavi *
- Pascale Mosoni
- Rafael Munoz-Tamayo
- Michel Naves
- Vincent Niderkorn
- Pierre Nozière
- Isabelle Ortigues-Marty
- David Pacheco
- Christelle Philippeau
- Brigitte Picard *
- José Pirès
- Milka Popova
- Gilles Renand
- Mathieu Silberberg *
- Kevin Sinclair
- Paulo Solgado
- Fabien Stark
- Flavie Tortereau
- Jaap van Milgen
- Isabelle Veissier
- Alexander Wezel

* Reviewer was session coordinator.

The ISNH 2018 Local Scientific Committee particularly thanks **Christiane Espinasse** for managing and assembling all summaries, **Bridget Hilton, Claire Reigate, Dr Daryl Borley, Clare Collett, Chelsea Davies, Claire Dodd, Richard Pizzey** and **Rebekah Weatherhead** for their skilled help when formatting and editing English language of the present book.

Table of contents

Programme

Session 1: Herbivore nutrition in a globalized world

Keynotes

759 Review: Domestic herbivores and food security: current contribution, trends and challenges for a sustainable development.
Mottet A., Teillard F., Boettcher P., De' Besi G., Besbes B.

760 Review: Role of herbivores in sustainable agriculture in sub-Saharan Africa.
Ayantunde A.A., Duncan A.J., Van Wijk M.T., Thorne P.

761 Review: Make ruminants green again - How can sustainable intensification and agroecology converge for a better future?
Dumont B., Groot J.C.J., Tichit M.

Oral communications

337 Ruminants can produce more human-edible protein than they eat
Laisse S., Baumont R., Veysset P., Benoit M., Madrange P., Rouillé B., Peyraud J.-L.

338 Farmers from the Central Tablelands of NSW (Australia) can promote both biodiversity and food security
Watt B.

339 Higher stocking rate spring lambing systems may not result in greater lamb production in southern Australia
Friend M., Robertson S.

Posters presented during workshops

340 How grassland management impacts legume species and their potential ecosystem services delivery?
Campion M., Hautier L., Dufrêne M., Stilmant D.

341 Silvopastoral systems with wild sunflower shrubs (*Tithonia diversifolia*) as alternative for competitive and sustainable milk production in high tropic: experience in Colombia
Angulo J., Mahecha L., Mejia E.

Short poster presentations

342 Milk and meat versus human-edible nutrient production from Costa Rica's livestock sector
Letelier P., Chacón M., Wattiaux M.A.

343 Towards protein self-sufficiency for both dairy and beef cattle
Rouillé B., Tranvoiz E., Cloët E., Le Pichon D., Férard A., Valance S., Bastien D., Freulon H.

344 Diversity management of ruminant farming systems in mountainous region of Tizi-Ouzou (Algeria)
Mouhous A., Kadi S.A., Djellal F., Guermah H., Berchiche M.

345 Herbage production and spring calving dairy cow performance from perennial ryegrass or mixed perennial ryegrass -white clover swards
Egan M., McAuliffe S., Hennessy D.

346 Legume hay is a valuable feed resource during dry season feed gaps in eastern Indonesia
Mayberry D., Dalgliesh N., Quigley S., Dida J., Praing J., Kana Hau D., Budisantoso E., Poppi D., Bell L.

Posters

Session 2: Microbial ecosystem and herbivore nutrition

Keynotes

Oral communications

Short poster presentations

Posters

373 **Evaluation of nutrient composition and *in vitro* digestibility of *Vigna radiata* straw using thin tailed sheep rumen fluid as microbial source**
Umami N., Suhartanto B., Suwignyo B.

374 **The degradation of lignocellulose was higher in the enriched co-culture of ruminal fungi and methanogens than the enriched co-culture of bacteria and methanogens**
Li Y., Jin W., Cheng Y., Zhu W.

375 **New fibrolytic enzymes as pre-treatment in dairy cow diet**
Refat B., Christensen D.A., McKinnon J.J., Yang W., Beattie A.D., McAllister T.A., Yu P.

376 **Eco-evolutionary association of rumen methanogens and acetogens with their hosts**
Li Z., Alberdi A., Wright A.-D.G., Mao S., Guan L., Li G.

377 **Effect of dietary supplementation of *Bacillus subtilis* and *Aspergillus oryzae* on rumen fermentation, growth traits and faecal microbial population of beef steers**
Lee J.-S., Zeleke A.W., Kim W.-S., Peng D.Q., Jo J.-H., Kim E.J., Lee H.-G.

378 **Ruminal protozoal numbers are not related to methane production in dairy cows**
Moate P., Deighton M., Jacobs J., Hannah M., Wales B., Williams S.R.

379 **Adaptation of goat rumen fluid to the fermentation of fungi treated wheat straw**
Ratni E., Sonnenberg A.S.M., Hendriks W.H., Cone J.W.

380 **Rumen bacteria in sheep: first evidence of host's genetic control**
Meynadier A., Gabinaud B., Tomas R., Menras J.-M., Pascal G., Enjalbert F., Allain C., Marie-Etancelin C.

381 **Individual sPLS on ruminal bacterial community of 2 lambs responding differently to a high starch diet to discriminate potential clusters producing trans10-18:1 and trans11-18:1 fatty acids**
Meynadier A., Cauquil L., Enjalbert F., Costa M., Alves S.P., Bessa R.J.B.

382 **Characterizing the pan and core rumen bacteria across individuals of a large cohort of dairy cows**
Xue M., Sun H., Wu X., Wang D., Wang J., Guan L., Liu J.

383 **The inhibitory action mode of nitrocompounds to rumen methanogenisis: A comparison of nitroethane, 2-nitroethanl and 2-nitro-1-propanol**
Zhang Z.-W., Yu L., Wang Y.-L., Wang W.-K., Si X.-M., Yang H.-J., Li S.-L.

384 **Effect of varying sources of protein in glucogenic dairy diets on *in vitro* rumen microbial nitrogen yield**
Eyni B., Danesh Mesgaran M., Vakili A., Valizadeh R.

385 **Effect of level and supplementation time of exogenous cellulase enzyme on *in vitro* rumen fermentation parameters of barley straws**
Ahmadi Bonakdar Y., Vakili A., Danesh Mesgaran M.

386 **Development of FibroChip, a functional DNA microarray as a targeted metatranscriptomic tool to monitor carbohydrate-active enzyme gene expression by rumen microbiota**
Comtet-Marre S., Chaucheyras-Durand F., Mosoni P., Bayat A., Peyret P., Forano E.

387 **Effect of using RumenBuff plus® on *in vitro* duodenal utilizable crude protein of two glucogenic diets**
Eyni B., Danesh Mesgaran M., Garajian F.

388 **Dynamic changes of main rumen microflora in sheep supplemented with molasses-urea**
Xue S., Li C., Alatengdalai X.

389 **Effect of dietary addition of EPA, DPA and DHA on rumen bacterial community in cows and ewes. An *in vitro* approach**
Carreño D., Belenguer Á., Pinloche E., Yáñez-Ruiz D.R., Toral P.G., Hervás G., McEwan N.R., Newbold C.J., Pilar F.

390 **Proteomic analysis provides insights into the molecular basis of rumen papillae growth during adaptation of sheep to high concentrate diets.**
Xu L., Zhu W., Dai C., Wang Y., Mao S.

Session 3: Precision herbivore nutrition

Keynotes

Oral communications

Short poster presentations

Posters

Session 4: Nutrition to improve milk and meat quality

Keynotes

766 **Review: Modulating ruminal lipid metabolism to improve the fatty acid composition of meat and milk from ruminants. Challenges and opportunities.**
Toral P.G., Monahan F.J., Hervás G., Frutos P., Moloney A.P.

767 **Review: Nutrigenomics of marbling and fatty acid profile in ruminant meat**
Ladeira M.M., Schoonmaker J.P., Swanson K.C., Ducket S.K., Gionbelli M.P., Rodrigues L.M., Teixeira P.D.

Oral communications

439 **Variations of global DNA methylation in peripheral blood mononuclear cells, in milk leukocytes and in milk epithelial cells in dairy cows: effects of micro-nutrient supplemented diet.**
Jammes H., Gasselin M., Boutinaud M., Prezelin A., Debournoux P., Mariani E., Zawadzki J.

440 **Feeding *Pistacia lentiscus* improves milk quality and curd properties in Damascus goats**
Argov-Argaman N., Hadaya O., Glasser T., Muklada H., Shemesh M., Landau Y.S.

441 **Effects of feeding a *Moringa oleifera* rachis and twig preparation to dairy cows on their milk production and composition, and plasma antioxidants**
Cui K., Zhang T., Tu Y., Diao Q.

442 **The farm-to-table continuum as an innovative trade-off approach for optimal management decisions in the beef sector: flavour clustering of young bull meat cuts.**
Gagaoua M., Picard B., Monteils V.

Posters presented during workshops

443 **Saliva samples as non-invasive proxies to determine the rumen 18-carbon fatty acid composition of dairy cows suffering or not from subacute ruminal acidosis**
Dewanckele L., Jing L., Stefanska B., Vlaeminck B., Van Straalen W., Koopmans A., Fievez V.

444 **Exploring the effects of dietary lipid content and digestibility on lipophilic contaminants transfer from feed to milk in dairy cows: insights from a physiologically-based toxicokinetic modelling approach**
Lerch S., Martin O., Fournier A., Henri J.

Short poster presentations

445 **Effects of starch-rich or lipid-supplemented diets that induce milk fat depression on lipid metabolism and methane emissions in Holstein lactating dairy cows**
Bougouin A., Martin C., Doreau M., Tixier E., Ferlay A.

446 **Effects of forage to concentrate ratio on the lactational performances of high- and mid-yielding dairy ewes in early-lactation**
Elhadi A., Caja G., Salama A.A.K., Mendivil M., Durán E., Albanell E.

447 **Comparison of milk polar lipid composition of cows and goats fed various lipid supplements**
Fougère H., Delavaud C., Emery S., Bertrand-Michel J., Bernard L.

448 **Alterations in ruminal biohydrogenation pathways and milk fatty acid composition during diet-induced milk fat depression in lactating cows**
Leskinen H., Ventto L., Kairenius P., Stefanski T., Shingfield K., Vilkki J.

449 **Adipose tissue transcriptome during transition period in dairy cows with low and high negative energy balance**
Mellouk N., Ramé C., Briant E., Humblot P., Ntallaris T., Naquin D., Dupont J.

Posters

Session 5: Feed resources and sustainable diets

Keynotes

Oral communications

Poster presented during workshops

Short poster presentations

Posters

578 **Meat production in grass based system with bulls and steers of double purpose Belgian Blue and Limousine breeds: ammonia emissions from their manure at barn and store**
Mathot M., Mertens A., Lambert R., Stilmant D., Decruyenaere V.

579 **Incorporation of multispecies swards into an intensive sheep grazing system**
Lynch M.B., Grace C., Sheridan H., Lott S., Brennan E., Fritch R., Boland T.M.

580 **Faba beans can replace soybean meal and rapeseed meal in diets for dairy cows**
Hansen N.P., Johansen M., Weisbjerg M.R.

581 **Performance and nutrient utilization of calves fed diets containing agro-industrial by-products and *Enterolobium cyclocarpum* leaves**
Onwuka C., Ayilara B., Oni A., Arigbede M.

582 **Understanding factors associated with grazing efficiency of perennial ryegrass**
Tubritt T., Byrne N., Gilliland T.J., Delaby L., Cummins D., O'Donovan M.

583 **Effect of dairy cow diet on milk and milk solids production in a spring calving dairy system**
Hennessy D., Hurley M.A., McAuliffe S.

584 **The wonder of willow tannin-rich tree (*Salix* spp. *Salicaceae*): a potentially valuable tree fodder for ruminants.**
Campbell M., Foskolos A., Stergiadis S., Richardson E., Humphrey C., Drake C., Mueller-Harvey I., Theodoridou K.

585 **Rumen parameter of West African dwarf goats offered concentrate supplement containing varying levels of *Leucaena leucocephala* leaves**
Adelusi O., Oni A., Ojo V., Aderinboye Y., Onwuka C.

586 **Grass-only and grass and clover seasonal sward structure effects on *in vivo* dry matter digestibility and intake on individually housed sheep**
Hurley M.A., Boland T.M., Hennessy D.

Session 6: Feed conversion efficiency towards productivity and reduction of excreta

Keynotes

770 **Review: Biological determinants of between-animal variation in feed efficiency of growing beef cattle**
Cantalapiedra-Hijar G., Abo-Ismail M., Carstens G.E., Guan L., Hegarty R., Kenny D.A., McGee M., Plastow G., Relling A., Ortigues-Marty I.

771 **Review: Selecting for improved feed efficiency and reduced methane emissions in dairy cattle**
Løvendahl P., Difford G.F., Li B., Chagunda M.G.G., Huhtanen P., Lidauer M.H., Lassen J., Lund P.

Oral communications

587 **Residual feed intake is positively related to beta hydroxybutyrate measured in blood plasma for adult Merino ewes**
Blumer S., Gardner G., Ferguson M., Thompson A.

588 **Comparing and combining proxies for methane emission of lactating Holstein Friesian cows**
Van Gastelen S., Hettinga K.A., Dijkstra J.

589 **Quebracho (*Schinopsis balansae*) extract in beef cattle fed high-roughage total mixed ration affects manure gas emissions**
Norris A., Tedeschi L.O., Casey K., Dubeux J., Foster J., Muir J., Pinchak W.

590 **Estimating the part of residual energy intake associated with real differences in feed efficiency and not with errors in dairy cows**
Fischer A., Friggens N.C., Berry D.P., Faverdin P.

Poster presented during workshops

591 **Effect of basal diet on the methane mitigation effect from dietary fat supplementation**
Alvarez P., Williams S.R., Jacobs J., Hannah M., Beauchemin K.A., Eckard R.J., Moate P.

Short poster presentations

592 **Application of partial least squares regression to predict feed efficiency based on feeding behaviour patterns in confined beef steers fed a concentrate diet**
Parsons I., Carstens G.E., Kayser W., Johnson J.

593 **Metabolic responses to an epinephrine challenge during mid-lactation and the dry period in dairy cows classed as high or low RFI measured during growth**
DiGiacomo K., Norris E., Dunshea F.R., Hayes B., Marett L., Wales W., Leury B.J.

594 **Genetic parameters and genome-wide association study for residual feed intake in pure breed Charolais young bulls**
Taussat S., Saintilan R., Renand G.

595 **Effect of increasing levels of dietary fibre and fibre digestibility on methane production in dairy cattle**
Benaouda M., Apodaca-Martínez G., González-Ronquillo M., Castelán-Ortega O.A.

596 **Multiplatform milk metabolomics highlights potential biomarkers related to methane emissions in dairy cows**
Yanibada B., Morgavi D.P., Canlet C., Pétéra M., Eugène M., Martin C., Boudra H.

Posters

597 **Impact of tannins on the net flux of ammonia, urea and glucose across the splanchnic tissues of sheep**
Kozloski G.V., Orlandi T., Stefanello S., Zeni D.S., Mezzomo M.P.

598 **Insights in dry matter intake prediction in growing goats**
Almeida A., Tedeschi L.O., Resende K., Biagioli B., Cannas A., Teixeira I.

599 **Influence of chestnut tannins on *in vitro* crude protein rumen degradability kinetics of red clover silage**
Herremans S., Decruyenaere V., Beckers Y., Froidmont E.

600 **Adding a native plant (*Allium mongolicum*) in the feed diet of cattle decreases methane emissions**
Xie K., Zhang Che., Wang Z., Hou F.

601 ***In vitro* investigation of the ruminal digestion kinetics of different N fractions of 15N-labelled red clover silages and dried forage**
Vaga M., Huhtanen P.

602 ***In silico* identification of low invasive biomarkers for feed efficiency in cattle**
Cassar-Malek I., Bonnet M.

603 **Effect of inclusion of capulin leaf (*Prunus salicifolia*) in goats as a strategy for methane mitigation *in vitro***
Robles Jimenez L., Ruiz Perez J.A., DiLorenzo N., Castelán-Ortega O.A., Osorio Avalos J., González-Ronquillo M.

604 **Effects of substrate on *in vitro* methane inhibition by 3-nitrooxypropanol**
Alvarez P., Moate P., Williams S.R., Jacobs J., Beauchemin K.A., Durmic Z., Vadhanabhuti J., Eckard R.J.

605 **A partial life cycle assessment of the greenhouse gas mitigation potential of feeding 3-nitrooxypropanol on two Australian dairy farms**
Alvarez P., Little S., Moate P., Jacobs J., Beauchemin K.A., Eckard R.J.

606 **Effects of dietary neutral detergent fibre and non-fibre carbohydrates ratio on methanogenesis, rumen fermentation and rumen microbial flora in sheep**
Liu Y., Ding J., Cheng S., Deng K., Diao Q., Cui K., Tu Y.

607 ***In vitro* effect of heat physically processed flaxseed on ruminal fermentation and methane emission response**
Mohammadi F., Danesh Mesgaran M., Vakili A., Dehghani M.

608 **An antimethanogenic index for meadow plants consumed by ruminants**
Macheboeuf D., Cornu A., Kerros S., Recoquillay F.

609 **Kinetics of nitrogen secretion in milk and excretion in urine and faeces of four common dairy feeds intrinsically enriched with 15N**
Reed K.F., Barros T., Powell J.M., Wattiaux M.A.

610 **Association rule mining to help detect plant phenolic compounds putatively involved in decreased ruminal methane production *in vitro***
Macheboeuf D., Guillaume S., Leguay C., Kerros S., Cornu A.

611 **Daily methane emissions and yields in dairy cows are predicted from methane to carbon dioxide ratio in breath**
Moate P., Deighton M., Hannah M., Jacobs J., Wales B., Williams S.R.

612 **Effects of dietary protein level and lysine/methionine ratio on performance, nutrient digestibility for weaned calves**
Kong F., Yun Q., Tu Y., Cui K., Tao H., Diao Q.

613 **Are dietary strategies to mitigate enteric methane emission effective across ruminant species?**
Van Gastelen S., Dijkstra J., Bannink A.

614 **Responses to incomplete essential amino acid profiles at the same metabolizable protein supply in lactating dairy cows**
Nichols K., Bannink A., Dijkstra J.

615 **The combined effects of supplementing monensin and 3-nitrooxypropanol on methane emissions, growth rate, and feed conversion efficiency in beef cattle fed high forage and high grain diets**
Vyas D., Alemu A., McGinn S., Duval S., Kindermann M., Beauchemin K.A.

616 **The effect of γ-aminobutyric acid addition on *in vitro* ruminal fermentation characteristics and methane production of a high concentration ration**
Wang Y.-L., Zhang Z.-H., Wen Y., Yang H.-J., Li S.-L.

617 **The consistency of feed efficiency ranking and the mechanism explaining efficiency variation among growing calves**
Aviv A., Shabtay A., Cohen-Zinder M., Aharoni Y., Miron J., Halachmi I., Haim A., Tedeschi L.O., Carstens G.E., Johnson K., Brosh A.

618 **A candidate proteomic signature from the plasma of Charolais bulls to phenotype feed efficiency**
Cassar-Malek I., Cantalapiedra-Hijar G., Delavaud A., Bonnet M.

619 **Effect of brewer's grains and rapeseed meal on methane emissions and milk production of dairy cows**
Van Wesemael D., Vandaele L., De Campeneere S., Fievez V., Peiren N.

620 **The combined effects of supplementing 3-nitrooxypropanol and lipids on emissions of methane and hydrogen, digestibility, and rumen fermentation in beef cattle fed a forage based diet**
Smith M., Vyas D., Kung L., Duval S., Kindermann M., Beauchemin K.A.

Session 7: Interactions between nutrition, genetics and environment

Keynotes

772 **Review: The influence of genotype x environment interactions on the robustness of dairy cows for grazing systems**
Roche J.R., Berry D.P., Delaby L., Dillon P.G., Horan B., Macdonald K.A., Neal M.

773 **Review: Epigenetics, developmental programming and nutrition in herbivores**
Chavatte-Palmer P., Velazquez M.A., Jammes H., Duranthon V.

Oral communications

652 **How do animals differing in experience and genetic merit behave and perform when grazing biodiverse mountainous pastures?**
Koczura M., Bouchon M., Turille G., Berard J., Kreuzer M., Farruggia A., Martin B.

653 **Genetic markers are associated with the ruminal microbiome and metabolome in grain and sugar challenged dairy heifers**
Golder H., Thomson J., Denman S.E., McSweeney C.S., Lean I.

654 **Serotonin regulates maternal calcium homeostasis during the transition period of cashmere goat**
Jin L., Sun H., Namei R., Sang D., Li S.-L., Zhang Cho., Zhang Chu., Ru T.

Short poster presentations

655 **Relationship between the expressions of volatile fatty acid absorption genes and circadian clock factor genes in ruminal epithelium**
Wang M., Gao J., Ouyang J., Jiang B., Loor J.-J.

656 **Dietary nitrogen influences the hepatic signalling pathway of the somatotropic axis in young goats**
Firmenich C., Schnepel N., Muscher-Banse A.

657 **Effects of dietary protein level of lactating dairy cows on epigenetic changes in their offspring**
Bach A., Crompton L., Fàbregas F., Arís A., Reynolds C.

658 **Feed restriction modifies liver transcriptomic profile of suckling Assaf lambs**
Santos A., Giráldez F.J., Kenny D.A., Frutos J., Andrés S.

659 **Effects of birth weight on meat fatty acid profile of fattening lambs**
Valdés C., Santos A., Frutos J., Giráldez F.J., Andrés S.

Posters

660 **Regression analysis of body measurements to estimate body weight in Ghezel sheep breed**
Ebrahimi M., Moghaddam G., Rafat S.A., Alijani S.

661 **Effect of α-lipoic acid on oxidative status, lipid metabolic parameters and liver enzyme activities in transition dairy cows**
Wang H., Li J.

662 **Body weight parameters and progesterone concentration of Ghezel ewe lambs taken over two years**
Ebrahimi M., Fardoost B., Moghaddam G.

663 **The effect of pregnancy toxemia on gluconeogenesis in both maternal and fetal livers**
Xue Y., Guo C., Hu Fa., Xie F., Liu J., Mao S.

664 **Enteric methane emission of lactating Holstein and Jersey cows fed two levels and two sources of forage neutral detergent fibre**
Uddin M.E., Santana O.I., Wickert T., D'huyvetter D., Wattiaux M.A.

665 **Comparison of blood metabolites and progesterone of Ghezel ewe lambs at one and two years old**
Ebrahimi M., Fardoost B., Moghaddam G., Rafat S.A.

666 **Variability in growth of weaned beef cattle during the dry season in northern Australia**
Quigley S., Streeter S., Schatz T., Anderson S., Poppi D.

667 **Forage intake and foraging strategy of gestation beef cows with changes in herbage allowance and cow genetic group grazing Campos grassland**
Soca P., Claramunt M., Scarlato S., Do Carmo M.

Session 8: Wild herbivores

Keynotes

774 **Review: Livestock production increasingly influences wildlife across the globe**
Gordon I.J.

775 **Review: Using physiologically-based models to predict population responses to phytochemicals by wild vertebrate herbivores**
Forbey J.S., Liu R., Caughlin T.T., Matocq M.D., Vucetich J.A., Kohl K.D., Dearing M.D., Felton A.M.

Oral communication

668 **Retention marker excretion suggests incomplete digesta mixing across primates**
Matsuda I., Espinosa-Gómez F.C., Sha J., Marlena D., Nijboer J., Ortmann S., Schwarm A., Clauss M.

Short poster presentation

669 **The role of salivary proteins in the consumption of tannin-rich foods in a neotropical monkey (*Alouatta pigra*)**
Espinosa-Gómez F.C., Serio-Silva J.C., Ruiz-May E., Sandoval-Castro C., Santiago-García J.D., Aristizábal J., Chapman C.A.

Posters

670 **Diet overlap between water buffaloes and Lesser White-fronted Goose**
Karmiris I., Papachristou T., Platis P., Kazantzidis S.

671 **Feeding behaviour of alpaca and llamas co-grazing on Andean highlands in Peru and the interactions with spatial distribution of available vegetation**
Castro-Montoya J., Hoehn D., Gómez C., Dickhöfer U.

672 **Nutrients composition of feedstuffs fed to animals in the National Gardens of South Africa**
Mwimbi K.U., Mbatha K.

Session 9: Nutritional management of health and welfare

Keynotes

Oral communications

Short poster presentations

Posters

Session 10: Adaptation of herbivores to climate changes and diverse environments

Keynotes

Oral communications

Short poster presentations

Posters

Session 11: Converting scientific knowledge into innovation in herbivore nutrition

Keynote

Oral communication

Short poster presentations

Posters

Acidosis & rumen health satellite conference

Ruminants can produce more human-edible protein than they eat

Sarah Laisse[1,2], René Baumont[3], Patrick Veysset[3], Marc Benoit[3], Pauline Madrange[4], Benoît Rouillé[2], Jean-Louis Peyraud[5]

[1]IFIP, Le Rheu, France, [2]Idele, Le Rheu, France, [3]INRA, UMR Herbivores, Saint-Genès-Champanelle, France, [4]Idele, Aubière, France, [5]INRA, UMR PEGASE, Saint-Gilles, France

E-mail: rene.baumont@inra.fr

Take home message The use of grass and non-edible by-products are the key factors to improve the net protein efficiency of ruminant productions.

Introduction Ruminant production are perceived as less efficient and in competition with crops for the human food supply. It takes more than 3 kg of plant protein to produce one kg of milk protein and between 5 and 10 kg to produce one kg of bovine meat proteins (Peyraud and Peeters 2016). But, the feed/food competition only concerns those proteins of plant origin that are consumed by animals and that are consumable by human. The amount of edible protein of animal origin produced by kg of human-edible protein of plant origin used in animal feeding is an unbiased view of the contribution of livestock to protein production (Wilkinson, 2011; Ertl *et al.,* 2015). The aim of this work was to evaluate the potential of ruminant livestock for net production of protein for human consumption in the French context.

Material & methods The total protein efficiency was calculated as *total protein output / total protein input* and the net protein efficiency as *human-edible protein output / human-edible protein input*; with output = all animal products; and input = all feeds consumed. The human-edible protein output includes all edible protein of animal origin (milk, meat, edible offal and by-products), and the human-edible protein input includes those proteins of plants which are edible for human but used as feed. A table of human-edible protein fraction (hepF) of the main feedstuffs used in France was built from literature data and interviews with the food industry experts (Laisse *et al.,* 2016). Two scenarios for hepF values were considered: in the "current situation", hepF of wheat, maize grain, soybean meal and rapeseed or sunflower meal were estimated respectively at 66, 15, 60 and 0%; the hepF values of forages were estimated at 0%, except for maize silage (hepF set at 10% to consider the grain fraction). In the "potential scenario" hepF were increased to take into account the progress in protein extraction from food industry (e.g. protein extraction from rapeseed meals) and the potential changes in eating habits (e.g. more consumption of whole meal flours). The total and the net protein efficiency were then calculated using data from real farms collected in the farm network surveys from INRA, IDELE and Chambres d'Agriculture. For each type of production, we present results (Figure 1) from systems with contrasted hepF values: Maize silage and soybean meal based system (DC1) vs. grass based system (DC2) in dairy cattle; high maize silage and soybean meal (BC1) vs. low maize silage and soybean meal (BC2) in beef cattle; high concentrate (Sh1) vs. low concentrate (Sh2) in sheep.

Results & discussion As expected, total protein efficiency of ruminant production systems is low and varied between 0.05 (BC1) and 0.24 (DC1) (Figure 1). The net protein efficiency is much higher than the total protein efficiency and reached or exceeded a value of 1 for DC1 and DC2, BC2 and Sh2. This means that all ruminant production systems have the potential to produce more human-edible protein than they consume and, contribute positively to the production of protein for human nutrition. Figure 1 reveals large variation in net protein efficiency within each type of ruminant production system. Grass-based dairy cow systems with low consumption of concentrates (DC2) can produce 2.5 times more human-edible protein than they consume.

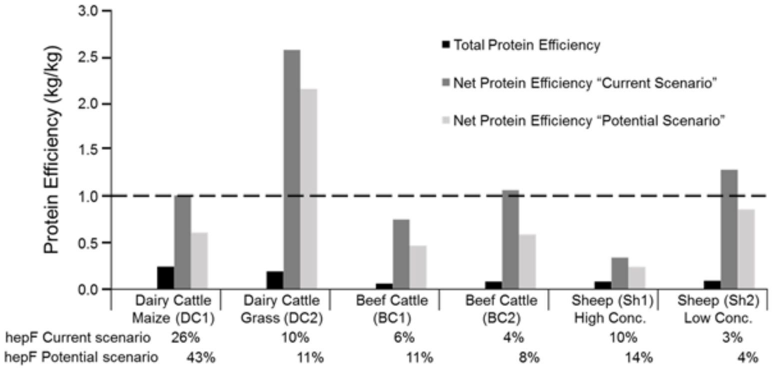


Figure 1 Total and net protein efficiency of ruminant livestock systems, according to the human-edible fraction of the diet (hepF) for current and potential scenarios.

Conclusion The results clearly show that grassland based dairy systems have a high contribution to the protein supply for food, and that beef and sheep production can also make a net positive contribution to the protein supply for humans.

Acknowledgements This work was supported by GIS Elevages Demain (www.gis-elevages-demain.org/).

References

Ertl P, Klocker H, Hörtenhuber S, Knaus W and Zollitsch W 2015. Agricultural systems 137, 119-125.
Laisse S, Rouillé B, Baumont R and Peyraud JL 2016. Rencontres Recherches Ruminants, 23, 263-266.
Peyraud JL and Peeters A 2016. Grassland Science in Europe 21, 29-43.
Wilkinson JMR 2011 Animal 5, 1014-1022.

Farmers from the Central Tablelands of NSW (Australia) can promote both biodiversity and food security

Bruce Watt

Central Tablelands Local Land Services, Bathurst, NSW, Australia

E-mail: bruce.watt@lls.nsw.gov.au

Take home message Farmers can promote both food security and biodiversity by carefully utilising native ecosystems.

Introduction The livestock sector both in Australia and elsewhere has been blamed for a substantial contribution to air pollution, global warming, land, soil and water degradation and for reducing biodiversity (FAO 2006). Critics of grazing livestock also observe that it is more efficient to produce food and fibre from cropping systems than from livestock and that it is particularly inefficient to grow crops to feed livestock. Following these criticisms, livestock farming would thus appear as a threat to food security. While these criticisms are acknowledged, this argument overlooks the complementarity of many cropping and livestock systems and the fact that 89% of the world and 94% of Australia is non arable (World Bank 2018). We thus rather argue that livestock systems (both in the Central Tablelands of NSW and elsewhere) can produce food from areas unsuited to cropping, can complement cropping systems and can contribute to preserving biodiversity because they can operate in native grasslands and woodlands.

Case study In the Central Tablelands of NSW, many farmers adopt practices to enhance biodiversity and incentive programs are available to assist this. An example related to grassland management comes from endangered grassy box gum woodlands that are preserved on private land through a Federal Government Program that pays the farmer to manage the grazing of these grassy woodlands, including restricting grazing to autumn, winter and early spring to enable the flowering of native grasses and forbs and to facilitate the regeneration of trees. Another example at landscape scale comes from a recent initiative using community support and government funds to encourage farmers to preserve and enhance wooded corridors that might be used by the iconic native koala (*Phascolatus cinereus*). At this early stage, the benefits of this program have not been assessed.

Results & discussion Following the initial clearing of the land west of the mountains in NSW, Victoria and Queensland, soil organic matter levels declined markedly and crop yields declined as eons of soil fertility were utilised. The wheat fallow rotations with superphosphate did not reverse these declines. Sustainable production was only achieved when mixed legume grass pastures were incorporated into farming rotations. Now in the arable areas of the Central Tablelands, most farmers run mixed cropping (primarily cereals and canola) and livestock (primarily beef cattle and sheep). These enterprises are complementary with a lower risk than all-cropping systems since pasture-based rotations can replenish soil nitrogen, organic matter and structure and reduce the build-up of herbicide resistant weed species. Livestock can also utilise crop residues, spread labour requirement and diversify income sources, thereby lowering risk (Reeve 1987). This complementarity enhances production per unit area and this contributes to Australian food and fibre production and exports.

Only 11.4% of land on the Central Tablelands is suitable for regular cultivation while a further 31% is suited to occasional cultivation. Grazing livestock are almost the only enterprise that can be run on this land (with the possible exception of silviculture and tourism). Livestock can utilise native pastures and woodlands producing income for farmers and food and fibre from land with limited usage options. As might be expected on an isolated island continent, a high proportion of Australia's plant and animal species are endemic. With only 10.08% of the landmass formally protected for conservation and the greatest number of threatened ecosystems in the highly cleared land of SE Australia, many of these species exist on land also utilised for grazing livestock (Figgis 2004). While it is recognised that grazing livestock can degrade land and threatened biodiversity they can also be managed in a manner that helps maintain native pastures and woodlands and therefore preserve biodiversity.

Conclusion Farmers from the Central Tablelands of NSW can promote food security by producing meat and wool from land that is unsuited to cropping or by using practices that complement cropping. They can also promote biodiversity by preserving native ecosystems using appropriate grazing strategies.

References

Behrendt K and Eppleston J 2011. The threats, realities and opportunities of grassland farming in the Central Tablelands. Proceedings of the 26th Annual Conference of the Grassland Society of NSW. Bathurst, Australia.
Figgis P 2004. Conservation on Private Land: The Australian Experience. IUCN Gland. pp i -31.
Reeve TG 1987. Pastures in cropping systems. In Temperate Pastures, their production, use and management. Edited by Wheeler JL, Pearson CJ and Robards GE.
Steinfeld H 2006. Livestock's Long Shadow. Food and Agriculture Organisation of the United Nations.
World Arable Land, World Bank accessed 15 February 2018 https://data.worldbank.org/indicator/AG.LND.ARBL.ZS

Higher stocking rate spring lambing systems may not result in greater lamb production in southern Australia

Michael Friend[2,1], Susan Robertson[1,2]

[1]*Charles Sturt University, Wagga Wagga, NSW, Australia,* [2]*Graham Centre for Agricultural Innovation, Wagga Wagga, NSW, Australia*

E-mail: mfriend@csu.edu.au

Take home message Flexible lower stocking rate sheep systems can produce similar or more product/ha at lower risk than higher stocking rates.

Introduction Spring lambing systems have been recommended in southern Australia for increasing farm profits by allowing higher stocking rates (matching feed supply with demand) to produce more product/ha (Warn *et al.,* 2006).

Material & methods An experiment was conducted near Tarcutta, NSW (147º31'E 35º12'S, long term average rainfall 665mm) between 2006 and 2010, using a randomised block design with 3 replicates of 3 treatments. By area, each 5.2 ha replicate comprised 20% lucerne, 20% tall fescue and 60% phalaris. The three treatments were: Winter lambing Merino (WLM) – merino ewes joined to merino rams to lamb in July; Later Lambing (LL) – Merino ewes with 50% joined to Merino and 50% joined to terminal rams lambing from the first week in September; Split-Lambing (SL) – 50% of ewes lambing to terminal rams in July and the other 50% lamb to Merino rams in September. The same mid-winter stocking rate (10.5 dry sheep equivalents/ha) was used in all treatments in each year; with the difference in lambing time meaning the LL enterprise carried nearly twice as many ewes/ha as the WLM enterprise, because in mid-winter WLM ewes were late-pregnant/lactating and so had higher energy demands than LL ewes that were only in early pregnancy. Lambs were sold either at weaning, or later when pasture conditions allowed. Ewes were supplementary fed when required (and removed from plots and fully hand fed when pasture thresholds were reached to ensure pasture persistence) and managed to achieve a body condition score of 3 at joining and lambing. Rainfall experienced during the experiment was characterised by three years of significantly below average rainfall (<540 mm) and one very wet year (1186 mm).

Results & discussion The lower stocking rate, earlier lambing WLM system produced less wool, lambs and lambs weaned/ha than the other systems, but a similar lamb sold/ha to the highest stocking rate LL system (Table 1). While the LL system was most productive in terms of wool and number of lambs weaned, spring conditions during most years of the experiment precluded retaining lambs to finish to higher weights, and resulted in less lamb sold/ha than the lower stocking rate SL system, and required much greater levels of supplementary feed. The inherent flexibility of the SL system enabled lambs to be sold at marketable weights in poor years, but retained to generate more lamb/ha in better years.

Table 1 Mean annual production per hectare and supplement fed during 2006-2010.

	Ewes/ha	Wool (kg/ha)	No. lambs weaned/ha	Lamb weaned (kg live/ha)	Lamb sold (kg live/ha)	Supplement (kg/ha)
WLM	4.6	15.4a	4.5a	137a	173a	355
SL	6.0	19.0b	5.6b	162b	195b	585
LL	8.4	27.6c	7.2c	171b	175a	995
SEM	-	0.41	0.23	6.7	6.5	-

Ewe mortality was similar (P=0.14) between systems. Clean wool produced per ewe was similar between the lowest and highest stocking rate systems (3.4 kg), and there were only small differences in mean fibre diameter (19.1 – 20.0 µm) and staple strength (30 – 35 N/ktex), indicating management prevented the negative effects of higher stocking rates on individual animal performance reported in other studies (Lloyd Davies and Southey 2001; Fitzgerald 1976).

Conclusion Higher stocking rate spring lambing systems can be managed to ensure individual animal production is not compromised and increase production/ha, but entail significant risk. Intermediate stocking rate systems with more flexibility enable increased production in favourable years and reduced supplementation in poor years.

Acknowledgements Funding was received from Future Farm Industries CRC, Meat and Livestock Australia, and Australian Wool Innovation.

References

Fitzgerald R 1976. Australian Journal of Agricultural Research 27, 261-275.
Lloyd Davies H and Southey I N 2001. Australian Journal of Experimental Agriculture 41, 161-168.
Warn L, Webb Ware J, Salmon L, Donnelly J and Alcock D 2006. Final Report for Project 1.2.6. Sheep Co-op Research Centre.

How grassland management impacts legume species and their potential ecosystem services delivery?

Morgane Campion[1], Louis Hautier[1], Marc Dufrêne[2], Didier Stilmant[1]

[1]*Walloon Agricultural Research Centre, Gembloux, Belgium,* [2]*Gembloux Agro-Bio Tech, Gembloux, Belgium*

E-mail: m.campion@cra.wallonie.be

Take home message Moderate and organic mown management allows legumes and their potential ecosystem services to be maintained.

Introduction In grasslands, legumes are known to play key roles in the supply of both production and regulation services such as soil structure improvement, high nutritive value and digestibility, carbon storage, livestock health (tannins), and nitrogen supply. Here, we assessed on the field, the link between mown grassland management and plant diversity with a special attention for main plant group diversity in order to sustain ecosystem services delivery.

Material & methods Mown permanent grasslands were selected (n=33) on agricultural holdings, in three pedoclimatic regions (Ardenne, Famenne, Pays de Herve), to cover a gradient of five management intensity: (Int-I) intensive mowing [three to four cuts in the season; mineral and organic inputs], (Int-II) organic intensive mowing [three to four cuts in the season; organic inputs] (Int-III) mowing on 15 June [two cuts in the season, mineral and organic inputs], (Int-IV) natural meadow [agri-environment scheme allowing two cuts in the season from mid-June and only organic inputs], and (Int-V) grassland with high biological value [agri-environment scheme allowing one to two cuts in the season from July and no fertilization]. These contrasted managements were applied for at least 5 years. Floristic diversity of each grassland was characterized by the Braun-Blanquet method. Within 6 quadrats (1*1m) each plant species were recorded and its cover – abundance (%) was estimated. Floristic surveys were carried out in such a way that the flora is at the same stage of development, according to the pedoclimatic regions, at the time of sampling and were analysed with Tukey (HSD) tests to compare them to each other.

Results & discussion The proportion of legumes in the survey is influenced by management intensity (Fig 1). Indeed, as expected, there is a greater proportion of legumes when management is moderate with organic inputs only [Ard Int-IV] compared to intensive ones [Ard Int-I; Fam Int-I ($p < 0.01$, Fig 1); Her Int-I ($p<0.05$, Fig 1)], and if mineral inputs are used [Ard Int-III ($p<0.01$, Fig 1)].

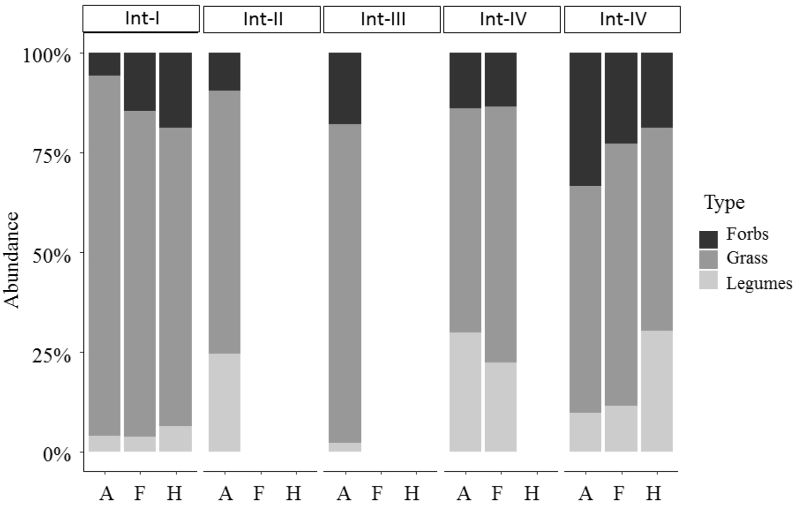


Figure 1 Relative abundance of main plant groups (grass, legumes and forbs) according to mown grassland management intensity in three pedoclimatic regions, A: Ardenne, F: Famenne, H: Herve.

For example, legumes such as Lotus corniculatus, Vicia sativa, Medicago lupulina or Lathyrus pratensis are only observed in sites where mineral inputs are absent. But, we also observed that grasslands with a more extensive management scheme have a lower proportion of legumes than grasslands under moderate organic management scheme [Ard Int-V; Fam Int-V ($p<0.01$, Figure 1)].

Conclusion Our observations seem to be consistent with the literature (Marriott *et al.*, 2004; Klimek *et al.*, 2007): organic moderate grassland management enhances floristic diversity, but in particular the proportion of legumes which provide a large range of useful ecosystem services, in the sward. Our future objectives are to link the floristic diversity with biological parameters of soil fertility such as bacterial and fungi diversity and to study the impact of grassland management on their potential ecosystem services delivery.

Acknowledgements The authors thank the Walloon Agricultural Research Centre (CRA-W), which finances this research.

References

Klimek S, Richter A, Hofmann M and Isselstein J. 2007. Biological Conservation 134, 559-570.
Marriott C A, Fothergill M, Jeangros B, Scotton, M, and Louault F 2004. Agronomie 24, 447-462.

Silvopastoral systems with wild sunflower shrubs (*Tithonia diversifolia*) as alternative for competitive and sustainable milk production in high tropic: experience in Colombia

Joaquin Angulo, Liliana Mahecha, Estefania Mejia

University of Antioquia, Medellin, Colombia

E-mail: liliana.mahecha@udea.edu.co

Take home message Producing milk at lower cost in silvopastoral systems with lower use of fertilization.

Introduction Most livestock production systems in Colombia are developed in the traditional way of felling and burning of forests, which has resulted in low tree cover, unprotected soils and biodiversity losses. In turn, susceptibility to erosion and high dependence on chemical inputs increases both production costs and environmental impacts. Hence, the need to generate new forms of production, in which livestock production is based on natural resources and allows farmers to be more efficient and competitive on local, national and international markets. Our objective is to compare forage availability, milk yield and composition, and milk production cost in a non-fertilised silvopastoral system and in a traditional fertilized monoculture of kikuyo grass.

Material & methods The experiment was developed on La Montaña farm belonging to the University of Antioquia, in Antioquia-Colombia (2400 ma.s.l, 15°C average temperature, 75% humidity). Intensive non-fertilised silvopastoral system (ISS) was composed of kikuyo grass (*Cenchrus clandestinus*), 1110 wild sunflower shrubs (*Tithonia diversifolia*) distributed into 40 furrows at an approximate distance of 8 meters between shrubs, and alder trees (*Alnus acuminata*) spread out in the pastures. *Tithonia diversifolia*, is not a legume, is instead a herbaceous flowering plant in the Asteraceae family that has the ability to establish association with mycorrhizae and mobilize N and P making them available to the associated pasture. Alder is a tree that is not a legume but has the property of fixing atmospheric N; the tree's foliage was not available to cows. ISS was established in March 2014 using strategic fertilization in accordance with soil analyses. In addition, commercial mycorrhizal soil was used to plant the wild sunflower shrubs. Animals were grazing ISS from September 2014. The evaluation was carried out in March 2015. Since planting until the end of this evaluation, ISS was not fertilized. MONO was managed with Fertilization (550kg N/ha/year + organic manure after each rotation as commonly handled on farms). Rotational stripgrazing was used in both systems, so 1.4 ha was set apart for each system distributed into 36 grazing strips with an average of 389 square meters each. In each production system, a 1-day occupation period was used per grazing strip as well as a 35-day rest period for kikuyu and 70 days for wild sunflower. Twelve pure Holstein cows, at second or more lactation were balanced in the two treatments based on liveweight (on average 537 and 548 kg, for SSP and MONO, respectively) and in milk (on average 110 DIM at the start of measurements). All cows were supplemented with the same concentrate (8 kg/cow/day). The experiment was developed during one grazing rotation (36 days). Each sampling period had 7 days of habituation and 5 days of measurement. There were 12 days between sampling periods. Evaluated variables were forage availability (double sampling), daily milk yield, milk protein and fatty acid composition, and milk production cost. For milk, experimental design consisted of a Latin square design 2 x 2 x 12 with two treatments (ISS vs MONO), two sampling periods and 12 cows. A crossover was performed between sampling period 1 (P1) and sampling period 2 (P2) of each trial. Forage availability was analyzed by using the T student test and data was calculated per hectare year according to the number of grazing.

Results & discussion Total forage availability was higher in ISS than MONO (43.7 vs. 40.8 t DM/ha/year, respectively, $p< 0.05$). Likewise, milk per hectare per year was higher in ISS (32400 vs. 29900 l/ha.year, respectively; $p<0.05$). Protein and fat yield were similar in both systems (0.9 vs. 0.9 kg fat/cow/day and 0.8 vs 0.8 kg protein/cow/day ($p>0.05$) but ISS produced 8% more protein and fat per ha and per year due to higher milk yield. Milk produced in ISS had a 14% lower cost per l than in MONO due to the non-use of fertilization in the SSP and the higher milk yield per ha and per year.

Conclusion Non-fertilised silvopastoral system based on non-legume sunflower shrubs and legume trees increased available biomass and consequently milk yield per ha and year, without affecting milk composition. Milk production costs were lower due to the higher productivity of the system and lower input costs. In the future, it will be necessary to evaluate if the silvopastoral system sustains its productivity without fertilization, and to analyse the effect of shrub and tree relative abundance in the paddocks. Initial modelling simulations suggest that forage availability and milk production could be sustained by five fertilisations per year (instead of 10 in grass monocultures), which would reduce production cost by 9%.

Acknowledgements Project 2012 AS180031 (Ministry of Agriculture and Rural Development of Antioquia, National University of Colombia and the University of Antioquia) and CODI project University of Antioquia 910-2014.

References

Mahecha L, Escobar JP, Suárez JF and Restrepo LF 2007. Livestock Research for Rural Development 19

Gallego-Castro L, Mahecha L and Angulo Arizala J 2015. Crecimiento y desarrollo de Tithonia diversifolia Hemsl. A Gray en condiciones de trópico alto.

Gallego LA, Mahecha L and Angulo J 2017. Agronomía Mesoamericana 281, 213-222.

Milk and meat versus human-edible nutrient production from Costa Rica's livestock sector

Paulina Letelier[1], Mauricio Chacón[2], Michel Wattiaux[1]

[1]*Department of Dairy Science, University of Wisconsin-Madison, Madison, WI, USA,* [2]*Ministry of Agriculture and Livestock of Costa Rica, San José, Central, Costa Rica*

E-mail: letelier@wisc.edu

Take home message Milk drives the production of human-edible nutrient production in non-specialised (dual purpose) livestock systems of Costa Rica.

Introduction The definition of the functional unit and the issue of milk and meat allocation in LCA is particularly critical in developing countries where farms remain less specialised than in developed countries. The aim of this study was to quantify the relative contribution of milk and meat to human-edible protein, fat, and energy (from fat and protein), referred to herein as human-edible nutrients (HEN) from livestock systems of Costa Rica.

Material & methods A survey of 683 farms was conducted on six regions of Costa Rica from April to May 2016. Income from sold milk was used to identify five systems based on self-reported percentage of farm income from milk: Specialised Beef (SB; <1%), Non-Specialised Toward Beef (NSTB; 1-39%), Non-Specialised (NS; 40-59%), Non-Specialised Toward Dairy (NSTD; 60-89%), and Specialised Dairy (SD; 90-100%). In order to compare systems, calculations were based on the products generated yearly from one representative animal of the herd (weighted average based on breed and performance). Eight variables were studied, including: milk production, bone-free meat (BFM) production, and the estimation of human-edible protein, fat, and energy from milk and BFM. Production of milk was calculated using the breed proportion. Production of BFM was calculated using animal performance values from Costa Rica's national records and animals sold in a year (% of herd size, which was assumed to be constant) from survey data (Gómez, 2016). Protein and fat content of milk were estimated from milk yield and breed proportion. Human-edible protein and fat content of BFM were calculated using referential values of nutrient composition of food (Menchú *et al.,* 2012). Human-edible energy was calculated assuming 4.25 and 9.00 kcal per gram of protein and fat, respectively (Merrill *et al.,* 1955). Data were analysed with R-Studio. Kruskal-Wallis test was used to evaluate differences across systems and Wilcoxon tests was used to separate the medians.

Results & discussion In Costa Rica, dual-purpose is the main breed in non-specialised systems. Simpson *et al*, (1993) stated that dual-purpose cattle are relatively inefficient species, less suitable for intensive systems. However, data in Table 1 suggest that the presence of dual-purpose breed in the system contributes to greater outputs of HEN compared with SB system. The production of HEN is driven mainly by milk, increasing with milk specialisation across systems. Hence, BFM is a substantially less efficient way of deliver HEN than milk. Environmentally, the incorporation of milk production to a livestock system would most likely dilute the greenhouse gases emission per unit of HEN.

Table 1 Annual production of Milk, BFM, and HEN for a representative animal in five livestock systems of Costa Rica.

Item	SB (n=412)	NSTB (n=54)	NS (n=30)	NSTD (n=32)	SD (n=155)	*P*-value
Dual-purpose breed (%)	39^{a}	68.6^{b}	68.3^{b}	50^{a}	12.1^{c}	***
Milk (kg)	94 ± 360.3^{a}	$1{,}843\pm1{,}232.5^{b}$	$2{,}054\pm2{,}185.1^{b}$	$3{,}744\pm2{,}518.4^{c}$	$5{,}193\pm3{,}343.2^{d}$	***
BFM (kg)	47 ± 16.4^{a}	38 ± 12.4^{b}	28 ± 8.0^{c}	37 ± 19.6^{b}	12 ± 10.1^{d}	***
HE protein [1] (kg)	12 ± 12.9^{a}	69 ± 40.8^{b}	72 ± 71.2^{b}	134 ± 90.4^{c}	180 ± 121.0^{c}	***
HE fat[1] (kg)	5 ± 15.1^{a}	75 ± 48.3^{b}	75 ± 69.9^{b}	121 ± 79.4^{c}	136 ± 102^{c}	***
Energy from HE protein[1] (Mcal)	52 ± 54.8^{a}	294 ± 173.0^{b}	307 ± 303.0^{b}	567 ± 384.0^{c}	762 ± 514.0^{d}	***
Energy from HE fat[1] (Mcal)	43 ± 144.1^{a}	673 ± 435.0^{b}	678 ± 630.2^{b}	$1{,}099\pm715.0^{c}$	$1{,}223\pm919.1^{c}$	***

[1] Outputs of human-edible (HE) protein, fat and energy from milk and BFM

Conclusion Non-specialised farms contribute substantially to milk and BFM outputs of the livestock systems of Costa Rica. In these systems milk drives the production of HEN as the relative contribution of BFM is very low. Thus we anticipate that incorporation of milk production in the livestock system might be a strategy to decrease emissions per unit of HEN. Future efforts to assess the greenhouse gases emissions from these systems may consider relying on HEN as a functional unit.

Acknowledgements This research was funded by USDA, Foreign Agricultural Services grant #TA-CR-17-001.

References

Gómez AI 2016. San José CR Boletín Estadístico Agropecuario nº26. SEPSA
Menchú MT and Méndez H. 2012. Guatemala: Tabla de Composición de Alimentos de Centroamerica. 2ed. INCAP/OPS.
Merrill A and Watt B. 1973. USDA ARS Handbook #74.
Simpson JR and Conrad JH 1993. Journal of Dairy Science 76, 1744-1752.

Towards protein self-sufficiency for both dairy and beef cattle

Benoît Rouillé[3], Elodie Tranvoiz[1], Estelle Cloët[1], Daniel Le Pichon[1], Alexis Férard[2], Sophie Valance[4], Didier Bastien[3], Henry Freulon[5]

[1]Chambre d'agriculture de Bretagne, Rennes, France, [2]Arvalis Institut du végétal, La Chapelle Saint-Sauveur, France, [3]Institut de l'Elevage, Paris, France, [4]Chambre d'agriculture des Pays de la Loire, Angers, France, [5]Pôle Agronomique Ouest, Rennes, France

E-mail: benoit.rouille@idele.fr

Take home message Increasing protein farm self-sufficiency is practically feasible with good performances for both animal and economy.

Introduction Ruminant's diets require rations rich in energy and protein. Maize silage is a basis of many rations in Brittany and Pays de la Loire (France). Nevertheless maize has a low content in digestible protein and must be combined with feedstuffs rich in protein, very often rapeseed meal or imported soybean meal. Thus, given the fact that profitability of cattle production is proportionally linked to the concentrates costs, farmers seek to produce as much as possible their feed resources on their farms. Therefore, through the program called "SOS Protein", we tested the interest to increase protein self-sufficiency, using roughages production, at the farm scale for both dairy and beef cattle.

Material & methods This program leads to the involvement of five experimental farms in Brittany and in Pays de la Loire (France): two for dairy cows and three for beef production. All the work done on the experimental stations has only one objective: to search protein autonomy by decreasing soybean meal importation. The complementarity of the overall program implemented makes it possible to study different types of forage (hay, wrapped bales, silage, dehydrated), different plant species (alfalfa, perennial ryegrass, Hybride Ryegrass, white clover, red clover, crimson clover) and different types of animals (dairy cows, young bulls, heifers, cull cows) and some breeds (Prim Holstein, Limousin, Charolais). This makes it possible to cover a majority of situations in the West part of France.

Results & discussion In dairy production, it is possible to use alfalfa silage in the cow's ration, while maintaining an acceptable level of milk production. In order not to degrade the animal performance too much, the alfalfa:maize silage ratio should be around 20:80. The soybean meal requirements can be reduced by 50%. At the same time, the use of legume silage required a supplementation with on-farm produced high energy feedstuffs such as cereals. From an economic point of view, the use of alfalfa silage decreases by a maximum of 5 €/1000 liters of milk the feeding cost. It has impacts on the final income over feed costs that represents € 2,500 per year for a farm producing 500,000 liters per year. It has been shown that increasing farm self-sufficiency usually needs to extend more or less the farm main forage area depending on the different crop production yields.

For grass silage in conventional agriculture, the results are inconsistent, showing the difficulty of harvesting a high quality forage at an early stage, positive for the protein content. Despite this constraint, farm incomes over feed costs were slightly affected (-2 € / 1000 liters) in bad year but increased +11 € / 1000 liters in a good year. However, this technique does not involve a change of system and can therefore be implemented quickly and simply. In organic production, in this trial, the search for better fodder has significantly improved milk performance (raw milk +3.3 kg/cow/day). This has repercussions on the income over feed cost (+14 € / 1000 liters).

In beef production, it is possible to use high-protein forage produced on the farm. The positive inputs in farm forage, energy and protein autonomies are significant. Growth performance is sometimes negatively impacted especially when the energy density of the ration is reduced due to the introduction of legumes silages. Further works are necessary to get new and more conclusive insights regarding the economic aspects to consider in this investigation.

Conclusion The search for improving farm protein autonomy, in dairy and beef cattle production farms, by including alternative forages is a real opportunity for the farms in Brittany and in Pays de la Loire regions of France. Whether from grass silage or alfalfa in different forms, the gain of autonomy is possible. In most of the cases, a consideration of the economy must be realized for at least keeping farm incomes over feed costs.

Acknowledgements We thank Europe, Regional council of Brittany who funded the project 4AGEPROD, carried out by the association "Pole Agronomique de l'Ouest". We also thank other partners involved in this study: Chambre d'agriculture des Pays de la Loire, Chambre d'agriculture de Bretagne, Arvalis-Institut du Végétal, Institut de l'élevage-Idele.

Diversity management of ruminant farming systems in mountainous region of Tizi-Ouzou (Algeria)

Mouhous Azeddine[1], Kadi Si Ammar[2], Djellal Farid[3], Guermah Hocine[4], Berchiche Mokrane[5]

[1]Université Mouloud Mammeri, Tizi-Ouzou, Algeria, [2]Université Mouloud Mammeri, Tizi-Ouzou, Algeria, [3]Université Farhat Abbas, Setif, Algeria, [4]Université de M'sila, M'sila, Algeria, [5]Université Mouloud Mammeri, Tizi-Ouzou, Algeria

E-mail: mouhousazeddine@yahoo.fr

Take home message Characterization of livestock systems and their viability.

Introduction In mountainous areas, farming systems have undergone major changes in recent decades. Consequently, livestock which was previously conducted by an extensive model has passed to systems which incorporate new modes of organization and where production is now mainly intended for the market to obtain sources of income (Mouhous, 2015). These changes are driven by development policies, strong population growth and decreased forage sole. This study aims to characterize the livestock systems and identify the most viable in the long term.

Material & methods The study area was the District of Tizi-Ouzou located in the center of the coastal area of Algeria. Its area is 2976 km² with a population density of 400 inhabitants / km². From January to May 2012, a survey was conducted in the region and involved 165 farms with different livestock species (cattle, sheep and goats). The questionnaire was structured in rubrics relating to the social situation, the structural and functional characterization of livestock farming. Data collected have been the subject of multi-varied analysis, Multiple factor analysis (MFA) and hierarchical ascending clustering (HAC) using the R software version 2.15.3-win. In order to conduct a typology of farms and discriminate factors that affect management strategies and decision-making of farmers, five Explanatory variables were used.

Results & discussion The total of the eigenvalues is 11.3% with Dim1 (6.66%) and Dim2 (4.66%). Variables; livestock numbers, supplementary feeding and dairy production are correlated and highly discriminatory, but number of workers and pasture surface are correlated but weakly discriminating. The type 1 strategy is to breed goats for their milk and meat, and type 2 develops sheep and goat farms mainly for the production of meat and secondarily goat's milk. Species diversity is part of an anti-risk strategy adopted by breeders (Alary *et al.,* 2011). The strategy of the type 3 is based on the diversification of products (meat and milk). Whereas type 4 is mainly based on milk. The viability of dairy farms depends in part on the support of public policies (production subsidies and the guarantee of milk sales). All systems face several constraints; low forage sole, dependence on subsidies, imported concentrates and the weak succession. However, goat farms can develop through the structuring of the goat sector and the labeling of its products. Dairy cattle farms can be maintained by diversifying their products and better cope with market disturbances.

Table 1 Main characteristics of different types of livestock systems in the mountainous region of Tizi-Ouzou.

Types	1	2	3	4
Characteristics	Goat dominance	Sheep dominance	Dairy specialized cattle	Dairy dominance
Number of workers on the farm	1.68 (0.15)	2.44 (0.23)	2.29 (0.20)	2.48 (0.18)
Livestock numbers	goat: 19 (3.70) sheep: 8 (2.10)	goat: 10 (3.10) sheep: 37 (11.71) dairy cattle: 2 (0.66)	dairy cattle: 15 (1.32)	dairy cattle: 22 (2.22) sheep: 2 (1.47) goat: 7 (3.22)
supplementary feeding (kg / head / day)	0.5 (0.05)	0.65 (0.08) (sheep and goat)	8.22 (0.27)	dairy cattle:8.45 (0.37) goat, sheep:0.45 (0.11)
Dairy production	1.8 (0.34) l/goat/day	1.7 (0.20) l/goat/day 9.6 (1.63) l/dairy cow/day	11.8 (1.21) l/ dairy cow /day	10.7 (0.32) l/ dairy cow /day
Pasture surface (ha)	9.29 (1.54)	21.76 (2.05)	19 (2.7)	6.88 (1)

( ): standard error

Conclusion In extensively dominated small ruminant systems, the low recourse on inputs or external financing combined with the existence of a solvent market enhances the viability of these systems. The structuring of the goat spinneret and the valorization of its products will allow the development and viability of this spinneret. In contrast, the viability of cattle systems is highly dependent on the existence of a guaranteed market supported by public policies, subsidies for milk production, and off-farm income. The diversification of products (milk and meat) makes it possible to reduce the vulnerability of these farms.

References

Alary V, Duteurtre G and Faye B, 2011. INRA Productions Animales 24, 145-156.
Mouhous A 2015. These Doctorat. Ecole Nationale Supérieure d’Agronomie, El Harrach, Alger, 275 p.

Herbage production and spring calving dairy cow performance from perennial ryegrass or mixed perennial ryegrass -white clover swards

Michael Egan, Stephen McAuliffe, Deirdre Hennessy

Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland

E-mail: michael.egan@teagasc.ie

Take home message White clover can play an integral role in intensive pasture based systems of milk production at reduced levels of N fertiliser application.

Introduction Nitrogen (N) fertiliser is used in pasture-based milk production systems to ensure that an adequate supply of high quality herbage is available to feed dairy cows. Nitrogen fertiliser use is limited under the EU Nitrates Directive (Council Directive 91/676/EEC). However, farms with high stocking rates (>2.5 cows/ha) have a high feed demand, and N fixed from the atmosphere by white clover (clover) can supply additional N for herbage growth. Applying N fertiliser to perennial ryegrass (PRG)-clover swards can compensate for low clover growth rates in spring. Research has shown the benefits of clover over PRG for milk production, particularly in the second half of the year (July onwards) (Egan *et al.*, 2017). The objective of this experiment was to examine the effect of including clover in PRG swards fertilised with 150 kg N/ha compared to a PRG only sward fertilised with 250 kg N/ha on herbage and milk production in an intensive pasture-based spring calving dairy production system.

Material & methods A full lactation farm systems experiment (February to November) was undertaken at Teagasc, Moorepark, Fermoy, Co. Cork Ireland from 2013 to 2016. There were two experimental treatments: PRG -only fertilised with 250 kg N/ha (Gr250) and PRG -clover fertilised with 150 kg N/ha (Cl150), at a common stocking rate of 2.74 cows/ha. Swards were rotationally grazed 9 times from February to November. There were 14, 17, 18 and 18 cows per treatment in 2013, 2014, 2015 and 2016, respectively. Swards were rotationally grazed to a target post grazing sward height of 4 cm. Pre-grazing herbage mass was measured with an Etesia mower (Etesia UK Ltd., Warwick, UK) twice weekly. Sward clover content was measured in the Cl150 paddocks prior to grazing. Milk yield was measured daily and milk solids (fat, protein, lactose) were measured weekly. Data were analysed using PROC MIXED in SAS with terms for treatment, time and associated interactions. Fixed terms were treatment and week or rotation and random terms were cow and paddock.

Results & discussion Mean annual herbage production was similar (P>0.05) on all treatments (14.4 t DM/ha) across the four years of the experiment (Table 1). Average annual sward clover content was 27% (range 25.5% - 28%) on the Cl150 treatment. Sward clover content did not decline (P>0.05) in the four years of the study. Milk fat, protein and lactose (data not shown) concentrations were not significantly different (P>0.05) across sward type. Daily and cumulative milk yield was greater (P<0.001) on the Cl150 treatment compared to the Gr250. Cows grazing the Cl150 treatment had a 7% greater (P<0.01) milk solids/cow and /ha compared to the Gr250 treatment (Table 1).

Table 1 Average daily milk and milk solids yield, annual milk solids yield and annual herbage production on PRG only swards fertilised with 250 kg N/ha (Gr250) and PRG -clover swards fertilised with 150 kg N/ha (Cl150) and average annual sward clover content during the experimental period (2013 to 2016), a P>0.05 was considered not significant (NS).

	Gr250	Cl150	SE	Level of significance
Milk yield (kg/cow/d)	21.47	22.57	0.28	<0.001
Milk solids (kg/cow/d)	1.73	1.81	0.021	<0.01
Cumulative milk solids (kg/cow/year)	460	493	5.6	<0.001
Cumulative milk solids (kg/ha/year)	1,261	1,353	15.3	<0.01
Annual herbage production (kg DM/ha)	14,544	14,350	900	NS
Sward clover content (%)	-	27	1.95	-

Conclusions Based on the results of this experiment there is potential to reduce N fertiliser application to PRG clover swards from May onwards, while maintaining herbage production and increases animal performance compared to PRG -only swards. This offers a considerable potential saving to the farmer in terms of reduced N fertiliser application. The study shows that clover can have a role in intensive pasture-based milk production systems and offers the potential to reduce fertiliser N application and increase animal performance, while maintaining adequate levels of sward clover content.

References

Egan M, Lynch MB and Hennessy D 2017. Journal of Agricultural Science 155, 657–668.

Legume hay is a valuable feed resource during dry season feed gaps in eastern Indonesia

Dianne Mayberry[1], Neal Dalgliesh[2,3], Simon Quigley[6], John Dida[5], Jefri Praing[5], Debora Kana Hau[4], Esnawan Budisantoso[4,7], Dennis Poppi[6], Lindsay Bell[2]

[1]CSIRO Agriculture and Food, Brisbane, Queensland, Australia, [2]CSIRO Agriculture and Food, Toowoomba, Queensland, Australia, [3]Dalgliesh Ag, Toowoomba, Queensland, Australia, [4]BPTP, Naibonat, Nusa Tenggara Timor, Indonesia, [5]BPTP, Waingapu, Nusa Tenggara Timor, Indonesia, [6]University of Queensland, Gatton, Queensland, Australia, [7]Indonesia-Australia Commercial Cattle Breeding Program, Jakarta, Java, Indonesia

E-mail: dianne.mayberry@csiro.au

Take home message Legume hay can be used to maintain liveweight gain of cattle during the mid-late dry season.

Introduction Increasing beef supply in Indonesia will require an increase in the number of breeding cows, improvement in the reproductive performance of these cows, and increased productivity of smallholder fattening operations. Growth and reproduction rates of cattle raised by smallholder farmers are often limited by inadequate nutrition (Mayberry *et al.*, 2016). Integration of herbaceous legumes into existing rice and maize cropping systems provides an opportunity to increase the amount and quality of feed available to livestock, without compromising crop yields or requiring an increase in the area of land planted to forages. Herbaceous legumes can be dried, baled and stored for use during feed gaps. The aim of this project was to demonstrate the feeding value of legume hay for different classes of cattle during the dry season in eastern Indonesia.

Material & methods On-farm feeding trials were used to demonstrate the feeding value of dried legume hay when included in a range of existing livestock feeding systems. The amount of hay offered was based on recommendations for supplementation in Mayberry *et al.,* (2016). The results of two trials from differing production systems with different classes and breeds of cattle are presented here to illustrate the broad application of legume hays as a dry season feed resource. In the first trial, 10 weaned Bali calves (*Bos javanicus,* 8 months, 82 kg) were either tether-grazed on native pastures, or penned and fed native grass *ad libitium* plus legume hay (*Clitoria* and *Centrosema* sp.) at 15 g DM/kg liveweight. The trial ran from the beginning of the dry season (April) to the middle of the wet season (February), and liveweight was measured every 2 weeks. In the second trial, 30 Ongole (*Bos indicus*) cows (2.3 years, 195 kg) grazed communal pastures during the day from the early dry season (July) to early wet season (December). Half the cows were provided with dried legume hay (*Clitoria*) at 5 g DM/kg liveweight overnight. Liveweight and body condition score were measured monthly. T-tests were used to compare average liveweight gain of cattle within each trial.

Results & discussion In both trials, cattle gained weight in the early to mid-dry season regardless of if they were provided with legume hay (Figure 1 & 2). In the mid-late dry season, unsupplemented calves and cows stopped gaining weight and lost body condition (data not shown). In comparison, cattle provided with legume hay were able to continue to gain weight during the late-dry and early-wet season. Average liveweight gain of cattle fed legume hay (calves 0.22 kg/head/day, cows 0.15 kg/head/day) was higher than unsupplemented cattle (calves 0.05 kg/head/day, cows 0.04 kg/head/day) in both experiments ($P<0.001$). Increased liveweight gain of calves means they would reach slaughter weight or sexual maturity sooner. Maintenance of cow liveweight and body condition score means they are more likely to conceive a calf.

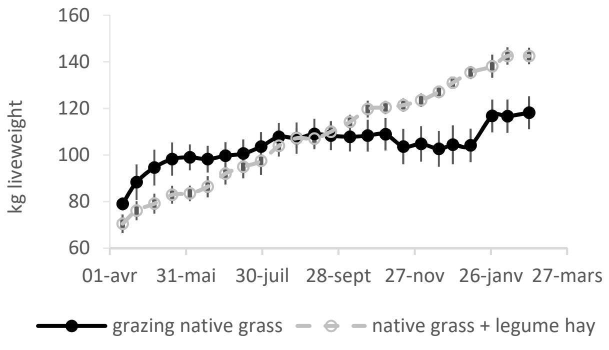


Figure 1 Liveweight (mean ± SE) of weaned Bali calves.

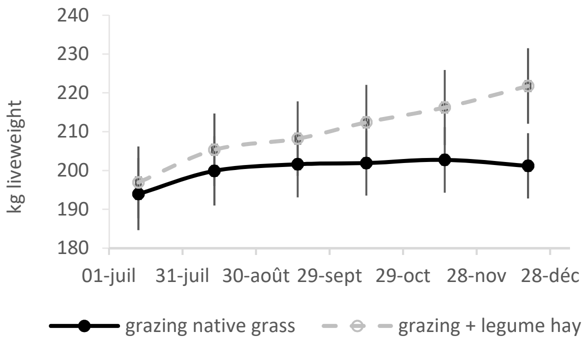


Figure 2 Liveweight (mean ± SE) of Ongole cows.

Conclusion Herbaceous legumes can be used to increase cattle production in eastern Indonesia. The most valuable time to supplement cattle with legumes is from the mid-dry season until the early wet season.

Acknowledgements This research was funded by the Australian Centre for International Agricultural Research.

References

Mayberry D, Cowley F, Cramb R, Poppi D, Quigley S, McCosker K and Priyanti A 2016. Final Report 2016-16. Australian Centre for International Agricultural Research.

Effect of herbage allowance on metabolic hormones, reproductive response and calf weight of primiparous beef cows with temporary suckling restriction and flushing grazing Campos

Martín Claramunt[1], Ana Meikle[2], Pablo Soca[3]

[1]*Universidad de la República, Treinta y Tres, Uruguay,* [2]*Universidad de la República, Montevideo, Uruguay,* [3]*Universidad de la República, Paysandú, Uruguay*

E-mail: martinclaramunt@gmail.com

Take home message Increasing the annual herbage allowance from 2.5 to 4 kgDM/kgLW improved the reproductive and productive response in primiparous beef cows primarily associated to an increase in mid gestation/autumn nutrition and IGF-1 concentrations during winter/last third of gestation.

Introduction Cow-calf systems in the Campos region have low pregnancy rates and calf weaning weight that affect the profitability and threatened his sustainability. Increasing herbage allowance (HA; Sollenberger *et al.,* 2005) from 2.5 to 4 kgDM/kgLW improves calf weaning weight and reproductive response of primiparous beef cows (Claramunt *et al.,* 2017). Changes in insulin, IGF and leptin concentrations are associated with postpartum anoestrus, probability of conception (Hess *et al.,* 2005) and milk production; however, the effect of HA on metabolic hormones have not been studied in primiparous beef cows. The objective of this study was to evaluate the effect of HA during an annual cycle on cow BCS and LW, anoestrus postpartum, calving conception interval, milk yield and calf weight and insulin, IGF-1, leptin concentrations in primiparous beef cows with suckling restriction and flushing.

Material & methods Thirty-one primiparous spring calving beef cows (LW 394 ± 4.4 kg; BCS: 5.7 ± 0.1 (1-8 scale)) were allocated from -150 (autumn) to 195 (summer) days relative to calving to a completely randomized block design of two HA of natives pastures of Campos region (primarily C4 grasses). The HA treatments (the ratio between herbage mass and stocking rate, kg DM/kg LW; Sollenberger *et al.,* 2005) fluctuated throughout the seasons as follows: autumn 5 and 3, winter 3 and 3, spring and summer 4 and 2 kg DM/kg LW for High and Low HA, respectively (4 vs 2.5 kg DM/kg LW annual mean). Autumn, winter, spring and summer correspond to -150 to -90, -90 to 0, 0 to 90 and 90 to 180 days relative to calving periods, respectively. The grazing system was continuous, and the put-and-take method (Mott and Lucas 1952) was employed to adjust HA. At 86 ± 12 days postpartum, all cows were submitted to calf suckling restrictions with nose plates (12 days) and flushing (2 kg/cow.day whole-rice bran for 22 days), six days prior to bull introduction. We evaluate the herbage mass, insulin, IGF-1, leptin, cow BCS, cow and calf LW, milk yield, anoestrus postpartum (samples from 70 to 125 days postpartum) and calving conception interval. Data were analyzed using general linear models and mixed models with repeated measures using SAS (SAS Institute Inc., Cary, NC, USA).

Results & discussion Herbage mass tended to be greater in High HA compared to Low HA (1379 vs 1112 ± 65 kg/ha; $P = 0.09$). Cows´ BCS during -90 to 16 days relative to calving tended to be greater (4.6 vs 4.1 ± 0.17; HA*days: $P = 0.07$) and cow LW during -50 to 16 days relative to calving was greater (379 vs 350 ± 7 kg; HA*days: $P < 0.01$) in High compared to Low HA. LW and BCS did not differ between 16 days postpartum to the end of the experiment. Milk yield was not affected by HA (5.1 vs 4.7 ± 0.21 kg; $P = 0.2$) and calf weight did not differ at 0 (34 vs 31.5 ± 4 kg), 60 (88.5 vs 80 ± 4 kg) and 120 (143 vs 133 ± 4 kg) days from parturition; however, calf weight was greater at weaning in High compared to Low HA (194 vs 179 ± 3.3 kg; $P < 0.05$). Insulin and leptin were not affected by HA but IGF-1 was greater at -90 (128 vs 87 ± 9.2 ng/mL), -50 (98 vs 75 ± 9.7 ng/mL) and tended to be greater from temporary weaning (86 days postpartum) up to 65 days after in High HA compared to Low HA (123 vs 102 ± 8.8 ng/mL; $P = 0.1$). The similar insulin, leptin, cow LW and BCS after 16 days postpartum, milk yield and calf weight until 120 days suggest similar energy balance between treatments. Therefore, the tendency to greater IGF-1 in High compared to Low can be partially explained by the better prepartum nutrition as is reported during the implementation of temporary weaning and flushing in primiparous beef cows (Soca *et al.,* 2013). Duration of postpartum anoestrus was not affected by HA (121 ± 1.9 days; $P = 0.8$) while calving conception interval tended to be shorter in High HA cows compared to Low HA (121 vs 133 ± 5 days; $P = 0.08$). IGF-1 concentrations likely increase the probability of ovulation after 125 days postpartum (end of ovulation evaluation) and the fertility of those ovulations (Hess *et al.,* 2005).

Conclusion Increasing the HA improved the reproductive and productive response in primiparous beef cows primarily associated to an increase in mid gestation/autumn nutrition and IGF-1 concentrations.

References

Claramunt M, Fernández-Foren A, Soca P 2017 Animal Production Science. https://doi.org/10.1071/AN16601
Hess B, Lake SL, Scholljegerdes EJ, Weston TR, Nayigihugu V, Molle JDC and Moss GE 2005. Journal of Animal Science 83 (E. Suppl.), E90-E106.
Mott G and Lucas H 1952. In `Proceedings of the international Grassland Congress.´ pp. 1380-1385. State College Press, PA.
Soca P, Carriquiry M, Claramunt M, Ruprechter G and Meikle A 2013. Animal Production Science 54, 862-868.
Sollenberger LE, Moore JE, Allen VG and Pedreira CGS 2005. Crop Science 45, 896–900

Re-design of livestock farming systems with an agroecological approach in campos grassland. First step: understand the functioning and results of the systems

Ignacio Paparamborda, Pablo Soca

Faculty of Agronomy. UdelaR, Montevideo, Uruguay

E-mail: ipaparamborda@fagro.edu.uy

Take home message Agroecological principles are necessary to redesign LFS in Campos region.

Introduction Livestock farming systems (LFS), in particular cow-calf system, are the main activity in the Pampa Biome grasslands. In Uruguay, LFS have low production and economic income, and are vulnerable to climate variability. Inputs technologies strategy (e.g. improved pasture, feed supplementation) increase LFS vulnerability to prices and climate variability. The redesign of the LFS with an agroecological approach, based on low inputs use, natural grassland management and processes techniques implementation, is necessary (Dumont *et al.,* 2014). With this, natural grassland production and forage intake can be increased, and at the same time improve soil quality and diversity (Nabinger *et al.,* 2011). Co-innovation (Dogliotti *et al.,* 2014) is an appropriate framework for this, but requires knowing the heterogeneity of farms. The objective of this work is to describe the LFS of Uruguay in terms of its structure, operation and results.

Material & methods Based on census data a stratified sample of LFS according to their stocking rate was defined. Sample size was defined by Neyman method. A survey to the 244 LFS of the sample was done during 2016. The first farm typology was based on variables related to farm structure (total area, own/leased area ratio, natural grassland area, stocking rate, sheep/cow ratio) and management practices index (MPI, Paparamborda, 2017). MPI summarizes practices on differential feeding management according to body condition and physiological state, mating period, suckling control, month of weaning and pregnancy diagnosis. Cluster analysis was used. For 69 farms, a second classification in three groups (managers, non-managers and partial managers) was made based on spatio-temporal grazing management (STGM), considering the use of paddocks throughout the year by different animal categories, MPI and stocking rate. Productive differences between groups were estimated using ANOVA and compared using Tukey´s test.

Results & discussion Six different farm groups in terms of system structure and MPI were identified, but they did not differ in their production levels (Table 1). Production levels remained generally low in all types. The high intensity of grazing relative to grassland stocking rate capacity and the limited implementation of management practice on herd (low MPI) in all groups, would explain these poor productive performances. However, when analyzing production levels and MPI between STGM groups, differences where identified. While managers group achieved 91 kg beef meat ha^{-1} year^{-1} with 53 MPI, Non-Managers achieved 70 kg ha^{-1} year^{-1} with 19 MPI ($p < 0.05$). Partial managers group has an intermediate result and did not differ from the other groups. MPI and production levels of managers group is higher but still improvable

Table 1 Characteristics and management traits in the six groups of livestock farms (mean ± standard deviation).

Variable	1 (n=58)	2 (n=28)	3 (n=33)	4 (n=25)	5 (n=53)	6 (n=40)
Total grazing area (ha)	147 ± 120	416 ± 183	229 ± 122	255 ± 193	172 ± 130	113 ± 85
Land property/Total area	0.04 ± 0.01	0.22 ± 0.24	0.90 ± 0.16	0.93 ± 0.12	0.13 ± 0.23	0.82 ± 0.22
Improved pastures/Total grazing area	0.09 ± 0.19	0.05 ± 0.07	0.05 ± 0.08	0.12 ± 0.13	0.18 ± 0.22	0.20 ± 0.30
Total Stocking rate (LU ha^{-1})	1.11 ± 0.43	0.69 ± 0.27	0.66 ± 0.24	1.10 ± 0.24	1.33 ± 0.46	1.20 ± 0.36
Sheep Stocking rate (LU ha^{-1})	0.37 ± 0.37	0.15 ± 0.13	0.29 ± 0.25	0.21 ± 0.14	0.2 ± 0.21	0.28 ± 0.29
Beef Stocking rate (LU ha^{-1})	0.74 ± 0.39	0.54 ± 0.22	0.36 ± 0.19	0.90 ± 0.30	1.10 ± 0.48	0.91 ± 0.32
Sheep/Cow Ratio	4.77 ± 5.96	1.88 ± 1.76	7.62 ± 9.40	1.69 ± 1.41	1.69 ± 2.00	2.32 ± 2.90
Beef Meat yield kg ha^{-1}	65 ± 38	62 ± 31	47 ± 33	84 ±45	87 ± 34	85 ± 34
Sheep Meat yield kg ha^{-1}	15 ± 15	11 ± 9	12 ± 12	11 ± 10	12 ± 13	21 ± 26
Wool yield kg ha^{-1}	8 ± 7	4 ± 4	6 ± 4	5 ± 4	6 ± 5	6 ± 6
Management Practice Index (0 to 100)	5 ± 11	39 ± 21	15 ± 16	45 ± 14	49 ± 15	7 ± 14

Conclusion Production levels were defined by the STGM, and not by the resources' availability or improved area. Therefore, the opportunity for improvement in LFS is based on a better management of the natural grassland and the implementation of process techniques in herd, which means put into practice the agroecological principles.

References

Dogliotti S, García MC, Peluffo S, Dieste JP, Pedemonte AJ, Bacigalupe GF and RossingWAH 2014. Agricultural Systems 126, 76-86

Dumont B, González-García E, Thomas M, Fortun-Lamothe L, Ducrot C, Dourmad J, and Tichit M 2014. Animal 8, 1382-1393

Nabinger C, Carvalho P, Pinto E, Mezzalira J, Brambilla D and Boggiano P 2011. Asociación Latinoamericana de Produccion Animal. 19, 27-34

Paparamborda I 2017. MSc thesis. Universidad de la República, Uruguay.

349

Sheep apparently prefer provenances of the Australian native shrub *Rhagodia preissii* that are smaller and have lower nutritional value

Hayley C. Norman, Matthew G. Wilmot, Elizabeth Hulm, Paul Young, Joshua Hendry

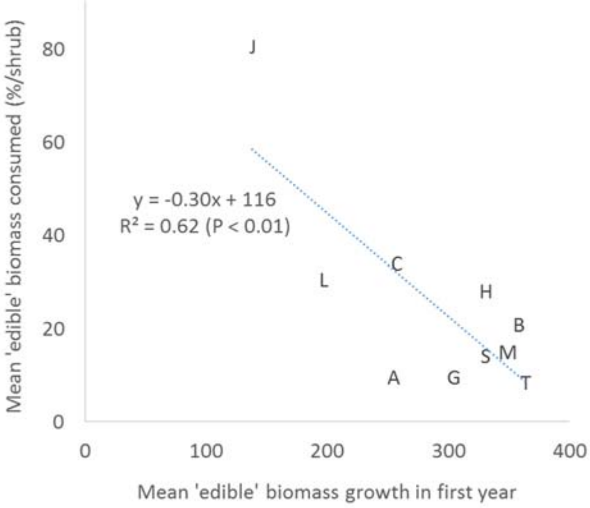
CSIRO Agriculture and Food, Perth, Western Australia, Australia

E-mail: hayley.norman@csiro.au

Take home message Although often rewarding, using sheep preferences to inform plant domestication can lead to challenges!

Introduction Rhagodia *(Rhagodia preissii* Moq.) is a shrub from the Chenopodiaceae family that is found on dry, sandy and infertile soils in Western Australia. In the past 15 years, there has been some interest in this species as a forage to complement the diets of sheep grazing poor-quality crop residues in autumn. While biomass production and nutritional value appear promising, it has been noted that sheep and cattle are often reluctant to consume the biomass. The aim of our project is to identify genotypes of rhagodia that are preferred by livestock, have higher energy values, lower toxins and good agronomic potential. In this study, we tested the hypothesis that sheep would preferentially graze provenances with higher energy and crude protein in addition to lower oxalate and saponin concentrations.

Material & methods Seeds from 15 female plants within ten spatially diverse native populations were collected in 2014 and grown in a nursery. Seedlings (n=12000) were planted on three experimental sites in winter 2015. Each site consisted of 24 seedlings from each of the 150 females, planted into six replicated blocks. This paper presents data from the first year of growth and subsequent grazing at the Tammin experimental site (deep yellow sand with 325 mm annual rainfall). A year after planting, 'edible' biomass (leaves and stems <3mm diameter) was visually assessed and plants were subsampled for laboratory analysis (n=45 samples/provenance). Traits measured include digestible organic matter in the dry matter (DOMD), neutral and acid detergent fibres (NDF, ADF), crude protein (CP), ash, nitrate, oxalate and saponin. The day after sampling, 1.5 year old Merino wethers, with recent experience of grazing rhagodia, were introduced to the site. During the four week grazing, oaten hay of moderate quality and fresh water were available in unlimited quantities. Relative preferences were assessed with a visual defoliation score on each shrub on a weekly basis during grazing. Data were analysed by ANOVA and provenance means were compared in a correlation matrix.



ıass

Results & discussion Our hypothesis was not supported; the sheep preferentially grazed rhagodia provenances that were characterised by lower DOMD (correlation -0.84, $P < 0.01$), lower CP (correlation -0.61, $P < 0.1$), higher ash (correlation 0.90, $P < 0.001$) and higher oxalate (correlation 0.78, $P < 0.01$). This finding was in contrast to our previous work with old man saltbush (*Atriplex nummularia*), where sheep consistently preferred provenances with higher energy values. While there were significant differences between provenances in DOMD ($P < 0.001$), the differences were not large (64 - 72%). Assuming that the sheep could detect a difference in energy values, the small differences may not have been enough to motivate selective grazing. Similarly, the range in CP values was well above the nutritional requirement of wethers (17 to 23% DM). Apparent selection for high ash is not expected as it was in excess of levels that sheep would normally consume (15 to 24% DM), however the oaten hay would have diluted the salt content of the diet. We would have anticipated that the high oxalate and saponin concentrations in the biomass (13-28 mg/g DM oxalate and 8.5 to 13.7 % DM saponin) would have had a negative impact on selection. It is possible that sheep could not detect these compounds or other factors were confounding. Figure 1 shows that preferred provenances tended to produce less biomass.

Conclusion There were significant differences between provenances in nutritional traits, biomass production and relative palatability to sheep. While early data suggests that relative palatability may not be linked to improved production traits at a provenance level, within provenance variation is promising.

Silvopastoral system with wild sunflower shrubs (*Tithonia diversifolia*) and polyunsaturated fatty acid supplementation: productive and environmental effects

Lilliana Mahecha, Joaquin Angulo, Leonardo Cardona

University of Antioquia, Medellin, Colombia

E-mail: liliana.mahecha@udea.edu.co

Take home message Producing milk efficiently while being environmentally friendly.

Introduction In Colombia, the majority of dairy farming systems in high tropic are developed under monoculture of *Cenchrus clandestinus* grass (kikuyo) with high nitrogen fertilization; the excess of nitrogen and the deficit of energy offered in the diet predominate as well as the lack of trees in the pastures. The design and implementation of innovative animal production systems that incorporate nutritional strategies in order to contribute to a competitive and sustainable livestock are thus necessary. Intensive silvopastoral systems (ISS) appear a promising alternative. *Tithonia diversifolia,* is not a legume, instead is an herbaceous flowering plant in the Asteraceae family, considered a traditional forage with a great potential for using in ISS in different agro-ecological conditions. Likewise, it has been found that lipid supplementation is a way to increase the energy density of the diet and reduce greenhouse gas emissions. The objective of this work was to evaluate the joint effect of ISS and supplementation with polyunsaturated fatty acids on Holstein cow dry matter intake (DMI), milk yield and composition, animal N balance and animal methane emissions.

Material & methods The experiment was developed in La Montaña farm belonging to the University of Antioquia, in Antioquia-Colombia (2400 ma.s.l, 15°C average temperature, 75% humidity). Treatments were intensive silvopastoral system (ISS) vs. traditional fertilized monoculture of *C. clandestinus* here called MONO crossed with three levels of supplementation. ISS was composed by kikuyo grass, 1110 wild sunflower shrubs (*T.diversifolia*) distributed into 40 furrows at an approximate distance of 8 meters between shrubs, 40 Alder trees (*Alnus acuminate*) spread out in the pastures; ISS was established in March 2014 using strategic fertilization in accordance with soil analyses. In addition, commercial mycorrhizal soil was used to plant the wild sunflower shrubs. The evaluation was carried out in 2016 during 70 days. Since planting until the end of this evaluation, ISS was not fertilized. MONO was managed with Fertilization (550kg N/ha/year + organic manure after each rotation). Rotational stripgrazing was used in both systems, so 1.4 ha was set apart for each system distributed into 36 grazing strips with an average of 389 square meters each. In each production system, a 1-day occupation period was used per grazing strip as well as a 35-day rest period for kikuyu and 70 days for wild sunflower. The control concentrate (D1) included 3% of commercial saturated fat, D2 included 1% soybean oil, 0.5% fish oil, and 1.5% bypass fat rich in n-3 fatty acids, and D3 included 2.5% soybean oil, and 0.5% fish oil. Twelve pure Holstein cows at second or more lactation were used while being between 60-150 days in milk at the start of measurements. Cows were distributed in 6 treatments (2 cows/treatment). A randomized complete block design with a matrix arrangement 2 x 3 (two systems x three diets) and six treatments was used. The evaluated variables were milk yield and composition, DM intake (using chrome as an internal marker and the agronomic method), nitrogen balance in cows (measuring creatinine in urine, N in urine, N feaces, N diet, N milk) and methane emissions estimated by mathematic models (Ellis *et al.,* 2007 (model 1); Mills *et al.,* 2003 (model 2)).

Results Total DM intake was higher in cows grazing ISS than MONO (19.3±0.38 vs 17.3±0.62 kg/cow/d; p=0.0002). Forage intake of cows in ISS was composed by 82.5% of grass and 17.5% of *T. diversifolia.* There were significant differences between systems for fat corrected milk (24.6±0.8 vs 21.9±0.68 l/cow/d; p=0.0001), fat yield (0.89±0.02 vs 0.79±0.02 kg/cow/d; p=0.0001), protein yield (0.83±0.02 vs 0.72±0.02 kg/cow/d, p=0.0001), apparent N digestibility (75.7±0.56 % vs. 74.0±0.56 %, p=0.0419) and efficiency in the use of nitrogen (23.2±2.58% vs. 19.4±2.58 %, p=0.0191) being higher in ISS than in MONO. ISS was lower than MONO for milk somatic cells (67.3±6.7 vs. 125±20.8 cellx 1000/ml, p=0.0130), N excretion to the environment (183±6.4 vs. 215±6.4 g/cow/d from urine, p= 0.0030; 139.9±4.7 vs. 150.7±4.7 g/cow/d from faeces, p=0.11) and methane emissions (model 1: 2413±122 vs. 2470±132 CO_2 eq/cow, p=0.0001; model 2: 3018±39 vs. 3185±37 CO_2 eq/cow, p=0.0067). There was significant effect of diets on milk yield (D1: 26.2, D2: 26.5, D3: 27.0 l/cow/d; p<0.05) and fat yield (D1: 0.80, D2: 0.90, D3: 0.82 kg/cow/day; p <0.05). Nitrogen digestibility was different according to the diet (D1: 73.0, D2: 75.0, D3: 76.2 %; P= 0.02). Interactions between system and diet was not significant for any of the variables.

Conclusion Results indicate that it is possible to produce milk in high tropic being efficient and friendly with the environment in an unfertilised intensive silvopastoral systems. Conditions for success of ISS are the presence of 1110 shrubs of *T.diversifolia* that complement nutritionally the diet of the animals and are associated with mycorrhizae mobilizing N and P and making it available for pasture by reducing their demand for external fertilizers; additionally, the Alder trees are N-fixators and serve as a shade for the animals. Trees and shrubs create a microclimate on the ground that favours its activity. Unsaturated supplementation with polyunsaturated fatty acids is a good complementary alternative to these systems as it increases N digestibility, while remaining at a relatively low cost.

Acknowledgements Project 2012 AS180031 (Ministry of Agriculture and Rural Development of Antioquia, National University of Colombia and the University of Antioquia) and CODI project University of Antioquia 910-2014.

References

Ellis, J.L., Kebreab, E., Odongo, N.E., McBride, B.W., Okine, E.K., France, J. 2007. Journal of Dairy Science, 90:3456–3467.

Mills JAN, Kebreab E, Yates CM, Crompton LA, Cammell SB, Dhanoa MS, Agnew RE and Francia J 2003. Journal of Animal Science 81, 3141–3150.

Determination of an enteric methane emission factor for Holstein cows under tropical conditions

Sandra Posada-Ochoa, Ricardo Rosero-Noguera, Sebastian Bedoya-Mazo, Gustavo Villegas-Henao

Universidad de Antioquia, Research Group in Agrarian Science - GRICA, Medellín, Antioquia, Colombia

E-mail: jaime.rosero@udea.edu.co

Take home message Differences in livestock systems and nutritional management around the world make it necessary to establish methane emission factors adjusted to geographic regions.

Introduction Livestock generates greenhouse gases (GHG). Steinfeld *et al* (2006) estimated that this sector contributes about 18% of the total anthropogenic GHG emissions. To design methane (CH_4) mitigation strategies it is necessary to establish an initial inventory of emissions using accurate emission factors. Several factors determine CH_4 production, mainly food availability, dry matter intake (DMI) and its nutritional quality. The objective of this work was to determine a CH_4 enteric emission factor for dairy cows in the Colombian tropics.

Material & methods The experiment was conducted in "La Montaña" farm, property of Universidad de Antioquia (Colombia) in San Pedro de los Milagros (Antioquia, Colombia) at 2600 meters above sea level, with 15°C average temperature and 68% relative humidity. Forty-five Holstein-Friesian cows with the following characteristics were used: 552±30 kg body weight, 160±41 days in milk, and 21.74±4.3 kg milk/day. The cows were confined in individual pens throughout the experimental period. The animals consumed Kikuyu grass (*Cenchrus clandestinus*, at 40-d regrowth) *ad libitum* and a concentrate supplement at milking times (at 6 and 14 hours) in a proportion of 1 kg concentrate per 3.5 kg milk. The animals were kept in open circuit respirometric chambers (RC) for 24-hour periods to determine CH_4 production. The total volume of each chamber was 25 m^3. Air was continuously suctioned from RC at 1000 L/min by a mass-flow system (Flowkit-500, Sable Systems International, Las Vegas, NV, USA) that automatically corrected air volume (flow) for humidity (RH-300 Analyzer, Sable Systems International). Samples of chamber and atmospheric air were analyzed for methane (MA-10 Methane Analyzer, Sable Systems International). The software automatically recorded the results each second (ExpeData-UI2, Sable Systems International). Within the RC, dry matter intake was determined as the difference between feed offered and orts. The CH_4 emission factor (EF) was calculated according to the expression (IPCC 2006): EF, kg CH_4/animal/year= ((GEI*4.184) * (Ym/100)*365)/55.65, where: GEI=gross energy intake (Mcal/animal/day); Ym= gross energy that is converted to CH_4 (%); 55.65= energy density of CH_4 (MJ/kg).

Results & discussion The proportion in which energy consumed is converted to CH_4 depends on the interaction of factors associated with animal and feed. In this study, 6.1% of the energy consumed by the animals was converted to CH_4. This value is close to 6.5% suggested by the IPCC (2006) for dairy cows in developing countries. The EF was 115.5±21.3 kg CH_4/animal/year. The IPCC (2006) recommends EF of 72 and 128 kg CH_4/animal/year in Latin America and North America, respectively. Clearly, EF of 72 kg CH_4/animal /year is inappropriate for milk farms in Colombia. Purebred animals predominate in these farms, which are managed under rotational grazing and supplemented with grain according to yield (> 6000 liters/lactation). Considering DMI, feed quality and nutritional management, an EF closer to the one recommended for North America and Western Europe would be more appropriate.

Table 1 Emission factor and methane production based on dry matter intake and performance of Holstein cows.

Item	Mean	Standard deviation
DMI, kg/animal/day	16.5	2.7
GEI, Mcal/animal/day	71.6	11.7
4.0% fat-corrected milk, kg/animal/day	21.7	4.3
Gross energy loss as methane, Mcal/day	4.2	0.8
Y_m, %	6.1	1.5
Emission factor, kg methane/animal/year	115.5	21.3
Methane, g/kg of dry matter intake	22.4	5.7
Methane, g/kg of 4.0% fat-corrected milk	15.3	4.8

Conclusion The EF suggested by the IPCC (2006) for dairy cows in Latin America (72 kg CH_4/animal/year) underestimates CH_4 emissions. Ym is an important parameter for estimating national and global emission inventories; therefore, it is necessary to know DMI, especially of grazing animals. The inappropriate use of EF negatively affects GHG inventories.

References

Steinfeld H, Gerber P, Wassenaar T, Castel V, Rosales, M and de Haan C 2006. Livestock's long shadow. FAO.
IPCC 2006 Guidelines for National Greenhouse Gas Inventories. IGES, Japan.

The overall structure of rumen bacterial communities significantly differentiates animals by diet but not by their methane emission phenotype

Ilma Tapio, Alireza Bayat, Seppo Ahvenjärvi, Tytti Luukkonen, Johanna Vilkki

Natural Resources Institute Finland, Jokioinen, Finland

E-mail: ilma.tapio@luke.fi

Take home message Low- and high-methane emitting cows hold their ranking over different diets. The overall structure of rumen bacterial communities significantly differentiates animals by diet but not by their methane emission phenotype.

Introduction In this experiment, we tested if ranking of cows based on methane (CH_4) yield (CH_4/dry matter intake, DMI) is defined by the animal or rumen microbiome, and whether the ranking is stable irrespective of the diet fed to the animal.

Material & methods One hundred Nordic Red cows in mid-lactation were ranked according to CH_4/DMI using respiratory chambers. Five low- and five high-ChH_4 emitters were fitted with rumen cannulas and subjected to three diets in three 35-d periods. Cows received high grass (HG, 70:30), low grass (LG, 30:70) or red clover (RC, 50:50) based-diets differing in forage to concentrate ratio. Rumen digesta samples were collected on d 24 of each experimental period, freeze-dried and grinded for DNA extraction following Yu and Morrison (2004) method. Rumen bacterial and archaeal community composition was analysed by sequencing V4 region of 16S rRNA gene on Illumina MiSeq platform. Sequencing data was quality checked and processed using Qiime v. 1.9 (Caporaso *et al.*, 2010). Greengenes 13_8 (McDonald *et al.*, 2012) and RIM-DB (Seedorf *et al.*, 2014) databases were used for bacterial and archaeal taxonomical classification, respectively. Clustering of samples based on the diet and group (high- or low-CH_4 emitters) was evaluated by using Bray-Curtis as dissimilarity measure and nonmetric multidimensional scaling (NMDS) as ordination method. Distance based permutation multivariate analysis of variance (adonis) was used to assess significant differences with respect to the diet and CH_4 phenotype group. Significance was tested after 999 permutations as implemented in *vegan* (R package).

Results & discussion Comparison between diets and CH_4 phenotype groups in terms of DM intake, milk yield, digestibility, CH_4 emissions, rumen fill and rumen molar acetate to propionate ratio was documented by Bayat *et al.*, (2017). Results suggested that CH_4/DMI ranking of the cows is animal dependent and is not defined by the diet.

The overall structure of rumen bacterial-archaeal communities, evaluated by two-dimensional NMDS plot based on Bray-Curtis dissimilarities, showed significant clustering of samples by diet (adonis test p = 0.001) (Figure 1). However, within-diet sample sub-division based on high- or low-CH_4 emitter grouping was not significant (p = 0.54).

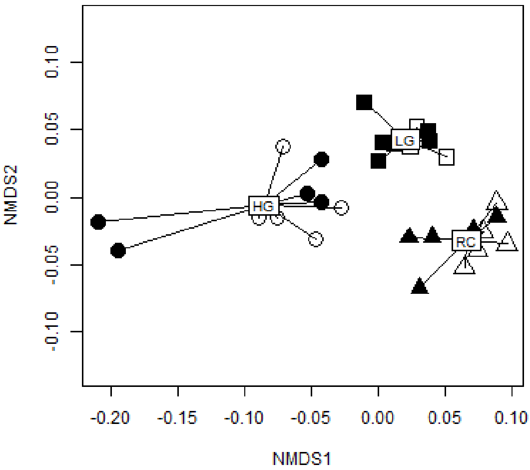


Figure 1 NMDS plot of bacteria and archaea combined data. Individual animals are identified based on the high/low-CH4 emitters group: HG diet (●, low- vs ○, high-emitters), LG diet (■, low- vs □, high-emitters), RC diet (▲, low- vs Δ, high-emitters).

Conclusion CH_4/DMI ranking of cows is animal specific and is not affected by the diets offered. Overall, rumen bacterial community structure is not significantly differentiating high- and low-CH_4 emitter groups. To better understand rumen microbiome association with CH_4 phenotype, further analysis will be performed on individual microbial taxa.

Acknowledgements This research was conducted as part of the Global Network project funded by the Academy of Finland (No.281337) through FACCE-JPI initiative.

References

Bayat AR, Luukkonen T, Kairenius P, Leskinen H, Hurme T, Ahvenjärvi S and Vilkki J 2017. Journal of Dairy Science 100, 220.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J and Knight R 2010. Nature Methods 7, 335-336.

McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, Andersen GL, Knight R, Hugenholtz P 2012. The ISME Journal. 6, 610.

Seedorf H, Kittelmann S, Henderson G and Janssen PH 2014. PeerJ. 2, p.e494.

Yu Z and Morrison M 2004. BioTechniques 36, 808-812.

Dynamic changes in ruminal fermentation and bacterial communities following rumen liquid transfer for sheep suffering from acute rumen acidosis

Junhua Liu, Hongwei Li, Weiyun Zhu, Shengyong Mao

Nanjing Agricultural University, Nanjing, Jiangsu Province, China

E-mail: liujunhua0011@163.com

Take home message These results revealed that rumen liquid transfer has a beneficial effect on re-establishing normal rumen fermentation and bacterial homeostasis for sheep suffering from rumen acidosis.

Introduction Previous studies showed that ruminal acidosis, a common ruminal digestive disorder in dairy or beef cattle, who have been fed high amounts of easily fermentable carbohydrates, can disturb the composition of ruminal microbial community and ruminal metabolism and lead to marked reductions in microbial fermentation activity and animal performance (Owens *et al.,* 1998). There is an acceptance among ruminant scientists that the rapid restoration of the ruminal bacterial homeostasis with rumen acidosis will facilitate the normal rumen function (Cole and Hutcheson, 1985, Fluharty *et al.,* 1996).Over the years, rumen fluid transfer (RT) has been applied to treat indigestion diseases in ruminants (DePeters and George, 2014), but little information is available regarding the underlying mechanisms involved in the beneficial effects of rumen transfaunation on rumen fermentation and function. Thus, the objectives of this study were to explore the dynamic changes in ruminal fermentation and bacterial communities following RT for sheep suffering from acute rumen acidosis.

Material & methods A group of 20 male Hu-sheep (30.55 ± 0.90kg of body weight), fitted with permanently ruminal fistula, were used in this experiment. Fifteen healthy sheep were assigned randomly to either the normal group (NRG, n = 5) or the oligofructose treatment group (OTG, n = 10) and five sheep were used as the donor animal. Acute rumen acidosis was induced by intraruminal administration of oligofructose (21 g/kg body weight) successfully for all 10 sheep, which were then assigned randomly to two subgroups after 2-day of acidosis, including control group (received 1 liter of saline, CON, n = 5) and rumen fluid transfer group (received 1 liter of rumen fluid of donor sheep, RT, n = 5). Ruminal content was collected on day 0 (before RT) and up to 14 days after RT to determine the dynamic changes in rumen fermentation and bacterial communities using Illumina MiSeq Sequencing.

Results & discussion The results showed that, compared with the CON, RT increased ($P < 0.05$) the concentration of acetate on day 0.25, 2, 3, and 14, and total volatile fatty acid on 0.25 day, 1 day and 14 days in the rumen content. Meanwhile, RT decreased ($P < 0.05$) the level of lactate from 0.5 day to 12 days in the rumen content. When compared with CON, the LPS concentration in RT was significantly decreased in the plasma of jejunal vein ($P = 0.028$) on day 5 after RT, and the concentration of LPS in RT was both lower than in CON on day 5 ($P = 0.033$) and day 14 ($P = 0.014$) after RT in the rumen content. Principal coordinate analysis showed that RT facilitates rapid rebuilt ruminal bacterial homeostasis from a markedly dysbiotic acidosis state to one representative of normal ruminal microbiota. Furthermore, RT also significantly increased ($P < 0.05$) the number of OTUs, Ace and Chao value in rumen microbial community at 1 day and 2 days post-RT. At the genus level, our data showed that, on day 1 after transplantation, as compared with CON, the relative abundance of Unclassified Christensenellaceae and unclassified Bacteroidales increased ($P < 0.05$) in RT. On day 2 after transplantation, the relative abundance of unclassified Prevotellaceae ($P = 0.028$), unclassified Ruminococcaceae ($P = 0.047$) and *Acetitomaculum* ($P = 0.016$) were higher in RT than in CON. On day 4 after transplantation, the percentage of unclassified Ruminococcaceae ($P = 0.016$) increased more in RT compared to that in CON.

Conclusion In summary, our data revealed that RT increased the concentrations of acetate and total VFA, and decreased the concentration of lactate and LPS translocation, and RT also increased the diversity of ruminal microbiota in sheep with acute rumen acidosis within two days after RT. In addition, our findings also revealed that the ruminal microbial community can be recovered in sheep with acute rumen acidosis within 14 days, and RT may accelerate the recovery of ruminal microbial community diversity. These findings may further provide new insights into restoring the composition and functions of dyspeptic ruminal microbial communities in ruminants.

Acknowledgements This work was supported by the Natural Science Foundation of China (NSFC) (31372339).

References

Cole NA and Hutcheson DP 1985. Journal of Animal Science 61, 692-701.
DePeters EJ and George LW 2014. Immunology Letters 162, 69-76.
Fluharty FL, Loerch SC, and Dehority BA 1996. Journal of Animal Science 74, 465-474.
Owens FN, Secrist DS, Hill WJ and Gill DR 1998. Journal of Animal Science 76, 275-286.

Colostrum feeding and management is critical in shaping colon microbiota during the first 12h of life in dairy calves

Yang Song[1], Nilusha Malmuthuge[2,1], Fuyong Li[1], Le Luo Guan[1]

[1]*University of Alberta, Edmonton, Canada,* [2]*University of Saskatchewan, Saskatoon, Canada*

E-mail: lguan@ualberta.ca

Take home message Colostrum feeding can enhance the colonization of beneficial bacteria and inhibit the colonization of opportunistic pathogenic bacteria in the colon during the first 12h of life in dairy calves. Feeding heat-treated colostrum (60°C, 60min) fortifies these effects.

Introduction Colostrum feeding is crucial to facilitate the passive transfer of immunity in neonatal calves. It has been reported that feeding heat-treated colostrum influences microbial colonization process in the small intestine of dairy calves (Malmuthuge *et al.*, 2015). However, effects of colostrum feeding on the colon microbial colonization during early life is unknown. Our recent study has revealed the importance of microbiota in the hindgut of neonatal calves (Song *et al.*, 2018), therefore, this study aimed to identify how different colostrum feeding strategies affect the colon microbiota in calves within the first 12h of life.

Material & methods Thirty-two Holstein bull calves were randomly assigned to three treatment groups: fresh colostrum (FC, n=12), heat-treated (60°C, 60min) colostrum (HC, n=12) and no colostrum (NC, n=8). Calves in FC and HC group were fed 2 L of colostrum within one hour after birth, while calves in NC groups received no colostrum or water during the whole experimental period. Calves were humanely euthanized at 6 h (NC, n=4; FC, n=6; HC, n=6) and 12 h (NC, n=4; FC, n=6; HC, n=6) after birth. Quantitative real time-PCR and amplicon sequencing of 16S rRNA genes were used to estimate the abundance of bacterial groups of interest (total bacteria, *Bifidobacterium*, *Escherichia coli* and *Clostridium* cluster XIVa) and profile microbial community in the colon tissue.

Results & discussion A higher proportion of *Clostridium cluster XIVa* and *Bifidobacterium,* and a lower abundance of *E.coli* were detected in the colon with colostrum feeding (NC vs. FC, Table 1). Additionally, the abundance of *Enterobacteriaceae* (NC, 90.44±5.88%; FC, 63.80±8.07%, *P*=0.07) and *Escherichia-Shigella* (NC, 89.10±5.66%; FC, 58.46±8.39, *P*=0.02) decreased at 12h in FC calves (Figure 1). Comparing to FC, HC feeding reduced *Enterobacteriaceae* (FC, 63.80±8.07%, HC, 40.25±7.59%, *P*=0.05) and *E.coli* (FC, 58.46±8.39%, HC, 39.12±7.35%, *P*=0.08), and increased *Bifidobacterium* at 12h. Considering the importance of *Clostridium* cluster XIVa and *Bifidobacterium* in immune system development (Dong *et al.*, 2010; Ivanov and Honda 2012), and the potential pathogenic effect of *Enterobacteriaceae* and *Escherichia-Shigella* in calf diarrhea, colostrum feeding, especially HC can benefit calves during early life.

Table 1 Effect of fresh colostrum and heat-treated colostrum on bacterial densities during the first 12h.

Time	Bacterial groups	Treatment			*P*-value		
		NC	FC	HC	NC vs. FC	FC vs. HC	NC vs. HC
6h	Total bacteria	$2.43\times10^{9} \pm 2.31\times10^{9}$	$9.83\times10^{10} \pm 7.60\times10^{10}$	$1.56\times10^{11} \pm 1.83\times10^{11}$	0.28	0.47	0.14
	Bifidobacterium	16.50±1.75	33.01±4.69	40.25±4.13	<0.01	0.01	0.01
	Clostridium cluster XIVa	0.45±0.38	3.53±2.91	8.39±3.58	0.02	0.01	0.01
	E.coli	0.06±0.02	0.02±0.01	0.01±0.00	<0.01	0.10	0.01
12h	Total bacteria	$1.02\times10^{10} \pm 0.55\times10^{10}$	$4.69\times10^{12} \pm 4.15\times10^{12}$	$1.61\times10^{12} \pm 1.09\times10^{12}$	0.02	0.09	0.02
	Bifidobacterium	20.71±1.28	46.88±6.59	57.40±4.16	<0.01	<0.01	<0.01
	Clostridium cluster XIVa	0.60±0.59	15.08±5.75	19.47±8.88	<0.01	0.31	<0.01
	E.coli	0.28±0.05	0.02±0.01	0.01±0.00	<0.01	<0.01	<0.01

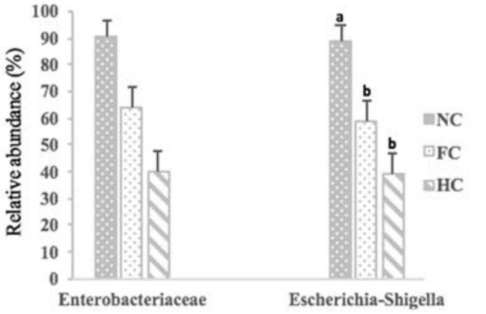


Figure 1 Effect of fresh and heat-treated colostrum on the relative abundance of *Enterobacteriaceae* and *Escherichia–Shigella*.

Conclusion This study suggests colostrum feeding can alter the the colonization of microbiota in the colon of dairy calves during first 12h of life. Furthermore, feeding heat-treated colostrum fortifies the effects of fresh colostrum by enhancing the colonization of beneficial bacteria and reducing that of potential pathogens.

Acknowledgements The authors thank the funding support provided by ALMA (2011F129R) and NSERC discovery grant.

References

Dong P, Yang Y and Wang W P 2010. Early Human Development 86, 51–8.
Ivanov II and Honda K 2012. Cell Host Microbe 12, 496–508.
Malmuthuge N, Chen Y, Liang G, Goonewardene LA, and Guan LL 2015. Journal of Dairy Science 98, 8044–8053.
Song Y, Malmuthuge N, Steele MA, and Guan LL 2018. FEMS Microbiology Ecology 94, DOI: 10.1093/femsec/fix179.

Early life inoculation of calves does not affect the rumen but the faecal microbiota

Stefan Muetzel, Sarah Lewis, German Molano, David Pacheco

AgResearch Ltd., Palmerston North, New Zealand

E-mail: stefan.muetzel@agresearch.co.nz

Take home message The rumen microbial community appears to be driven by the substrate provided, while the hindgut community could be more malleable to interventions.

Introduction The rumen microbial ecosystem is characterised by its high diversity and high redundancy, to enable it to break down complex and varied plant carbohydrates. There have been many attempts to increase fibre degradation and/or reduce the amount of protein degradation in order to improve the efficiency of the rumen fermentation. In principle this can be done by manipulating the substrates or the microbial community. It has been long established that the rumen community in an adult animal is very stable and basically can only be changed by maintaining a constant selection pressure, while the rumen of young animals appears to be more malleable (Yáñez-Ruiz *et al.,* 2015). In this experiment we attempted to imprint a microbial community in the rumen of calves by means of inoculation with rumen fluid from an adult cow.

Material & methods A group of 16 calves (Holstein Friesian x Hereford) at 4 d of age were divided into two groups. One group received 5ml of filtered rumen fluid orally at days 4, 7 and 10, while the other group served as a control with no inoculation. The calves were reared indoors receiving 4 L/d of milk replacer and calf starter pellets *ad libitum*. The animals were weaned from milk at the end of week 8, and subsequently transferred outside onto pasture. Rumen and faecal samples for short chain fatty acid and microbial analysis were collected at week 2.5, 4.5 and 10. Blood samples for immune parameters were collected at 0.5 and 6 weeks of age.

Results & discussion Over the entire treatment period the weight and the average daily gain of two treatment groups was similar. The rumen contents of the treatment group showed a lower proportion of butyrate at 2.5 weeks. At 4.5 weeks the butyrate concentration were similar but the proportion of acetate in the treatment group was higher and the proportion of propionate was lower. At 10 weeks of age the SCFA proportions were similar between the two treatment groups. The proportion of acetate at 10 weeks was higher compared to weeks 2.5 and 4.5. This was most probably driven by the change in diet from calf starter pellets to pasture. Compared to the rumen, the faecal profile of SCFA is higher in acetate and, in similar fashion to the rumen, the proportion of acetate increases over time. The rumen bacterial community showed a slight separation between the treatments shortly after the end of the inoculation at week 2.5 but no differences between the control and the treatment groups was observed at weeks 4.5 and 10. The variability of the rumen microbial community was very high at week 2.5 compare to later periods. At any time the rumen community was clearly different from the faecal community. The rumen was dominated to a higher degree by the phylum of Bacteriodetes and had a lower proportion of Firmicutes independent of the sampling time. Proteobacteria were only present at a higher proportion at week 2.5 and 4.5 but not at week 10. Presently, we cannot determine whether this is due to age of the calves or the change in diet, since both have been reported to decrease the proportion of Proteobacteria(Dias *et al.,* 2017; Fernando *et al.,* 2010). The bacterial community in the faecal samples were similar at week 2.5 but diverged at weeks 4.5 and 10 between the two groups indicating a response of the hindgut community to the inoculation. This result raises the question whether early life inoculation can be used to alter the hindgut community with possible beneficial effects for animal health.

Conclusion Early life inoculation has little effect on the rumen microbial ecosystem but may be an option to manipulate the hindgut community of calves.

Acknowledgements Funding for this study was provided by the AgResearch 'Curiosity Fund'.

References

Dias J, Marcondes MI, Noronha MF, Resende RT, Machado FS, Mantovani HC, Dill-McFarland KA and Suen G 2017. Frontiers in Microbiology 8.

Fernando SC, Purvis HT, Najar FZ, Sukharnikov LO, Krehbiel CR, Nagaraja TG, Roe BA and Desilva U 2010. Applied and Environmental Microbiology 76, 7482–7490.

Yáñez-Ruiz DR, Abecia L and Newbold CJ 2015. Frontiers in Microbiology 6.

Early life supplementation of extruded linseed or an essential oil blend increases daily weight gain of Holstein youngstock resulting in earlier insemination, but does not change methane emissions

Sieglinde Debruyne[1,2], Nico Peiren[2], Hannes Cattrysse[2], Sam De Campeneere[2], Ellen De Keyser[3], Karen Goossens[2], Veerle Fievez[1], Leen Vandaele[2]

[1]*Laboratory for Animal Nutrition and Animal Product Quality, Department of Animal Sciences and Aquatic Ecology, Faculty of Bioscience Engineering, Ghent University, Gent, Belgium,* [2]*Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Animal Sciences Unit, Melle, Belgium,* [3]*Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Plant Sciences Unit, Melle, Belgium.*

E-mail: sieglinde.debruyne@ugent.be

Take home message Supplementing extruded linseed or an essential oil blend to calves in early life can increase daily gain leading to higher live weight and earlier insemination, but does not reduce methane emissions.

Introduction Several studies have shown that linseed (poly-unsaturated fatty acids) and essential oils have the potential to alter the rumen microbiota and reduce methane (CH_4) emissions (Martin *et al.,* 2008, Castro-Montoya *et al.,* 2015). Next to this, recent studies show the possibility of permanently altering the rumen microbiota using feed supplements in early life (Abecia *et al.,* 2013, De Barbieri *et al.,* 2015). In this way, the disadvantages of feeding anti-methanogenic supplements to the adult ruminant (e.g. resilience of the rumen microbiota, adaptation, negative effects on dry matter intake and cost) could be by-passed. The objective of this study was to reduce CH_4 emissions of dairy heifers on the long-term through a nutritional intervention from birth until ca. 4 months of age. We further hypothesized that feeding linseed or essential oils in early life could improve daily weight gain compared to calves that do not receive this, potentially resulting in positive effects on age at first calving and first lactation performance (Soberon *et al.,* 2012).

Material & methods Two different feed supplements were investigated: extruded linseed crumble (68% linseed, Group Aveve/ Dumoulin, Leuven, Belgium) and an essential oil blend (Agolin® Ruminant, Agolin SA, Bière, Switzerland). Thirty-four newborn female Holstein calves were randomly assigned to a control group (CON, n=12), a group receiving extruded linseed crumble (22 g/d with step-wise increase until 578 g/d; LIN, n=11) or a group receiving the essential oil blend (0.5 g/d; ESS, n=11). From birth until ca. 4 months old (=treatment period), calves were housed individually, and supplements were fed twice daily. After this period, calves went into group housing and all animals received the same ration. Rumen fluid was sampled at ca. 1, 4 and 6 months by oesophageal tubing, and used for extraction of total DNA and RNA. From this, abundance of total eubacteria (16S rRNA), methanogens (16S rRNA and *mcrA*) and protozoa (18S rRNA) were determined using specific primers and qPCR. Also activity of methanogens was measured as the abundance of *mcrA* transcripts using RT-qPCR. *In vivo* CH_4 emissions were measured at ca. 4, 6, 12 and 18 months in open circuit chambers. From 3 weeks before until 3 weeks after calving, a Greenfeed system was used to measure CH_4 emissions of the heifers in the stable. Milk production, dry matter intake and body weight was further measured until 5 wks in lactation.

Results & discussion No difference in CH_4 emissions during the treatment period nor a programmed reduction later in life was found. This was also reflected in the equal abundances of rumen eubacteria and methanogens, and similar methanogenic activities. Irrespective of treatment, calves were lacking rumen protozoa at 1 and 4 months old, probably caused by the isolated rearing conditions. Compared to CON calves, LIN and ESS calves tended to have a better feed and energy conversion ratio until weaning ($P = 0.10$ and $P = 0.06$, respectively). The LIN and ESS calves had a higher daily gain during the treatment period ($P = 0.02$), which led to a persistently higher bodyweight until 2 wk before calving and a 1 month earlier first insemination ($P = 0.03$). However, age at first calving was not different because a number of heifers in each treatment group needed multiple inseminations. First lactation milk production and composition until 35 days in milk did not differ, nor did the production of colostral IgG concentrations.

Conclusion No (programmed) CH_4 reduction was established through the nutritional interventions. However, daily gain in early life was improved, leading to one month earlier insemination. Possibly the dose or the availability of the supplements was too low to (permanently) alter the rumen microbiota during the treatment period.

Acknowledgements Financial support has been provided within the frame of the FACCE JPI Multi-partner Call on Agricultural Greenhouse Gas Research (RumenStability). Sieglinde Debruyne received a personal PhD grant from the government agency Flanders Innovation & Entrepreneurship (VLAIO). The trial was co-financed by Group Aveve/ Dumoulin and Agolin SA and further supported by a STSM Grant from the COST Action FA1302 (www.methagene.eu).

References

Abecia L, Martín-García AI, Martínez G, Newbold CJ and Yáñez-Ruiz DR 2013. Journal of Animal Science 91, 4832–4840.
Castro-Montoya J, Peiren N, Cone JW, Zweifel B, Fievez, V and De Campeneere S 2015. Livestock Science 180, 134-142.
De Barbieri I, Hegarty RS, Silveira C, Gulino LM, Oddy VH, Gilbert RA, Klieve AV and Ouwerkerk D 2015. Small Ruminant Research 129, 48–59.
Martin C, Rouel J, Jouany JP, Doreau M and Chilliard Y 2008. Journal of Animal Science 86, 2642–2650.
Soberon F, Raffrenato E, Everett RW and Van Amburgh ME 2012. Journal of Dairy Science 95, 783–793.

Characterisation of the rumen archaeal and bacterial populations in Charolais and Holstein Friesian steers offered a pasture based diet phenotypically divergent for residual feed intake

Emily McGovern[1,2], Mark McGee[1], Claire Fitzsimons[1], Paul Smith[1], Sean Coyle[1], David Kenny[1,2], Alan Kelly[2], Sinead Waters[1]

[1]Teagasc, Meath, Ireland, [2]University College Dublin, Dublin, Ireland

E-mail: emily.mcgovern@teagasc.ie

Take home message The effect of RFI phenotype on rumen archaeal and bacterial populations of beef cattle is not consistent across breed type.

Introduction The escalating global population and increasing demand for animal protein supply are creating environmental challenges. The profitability of beef production systems requires achieving maximum outputs using minimum inputs (Hill 2012). Given that feed accounts for the single largest variable cost in ruminant production systems (Finneran, Crosson *et al.,* 2010); global interest has focused on the identification of cattle that are more feed efficient (Hill 2012). Residual feed intake (RFI) is a widely used and accepted measure of feed efficiency in cattle. It is defined as the difference between an animal's actual versus its predicted feed intake (Herd and Arthur 2009). Irish beef is produced predominantly using a diet of grazed and ensiled grass as these have been deemed the cheapest forms of feed available (Finneran, Crosson *et al.,* 2010). The rumen bacterial populations dictate the host's feed degradation capacity and subsequent nutrient supply to the host ruminant. Archaeal methanogen populations in the rumen are responsible for methane production; an energetically wasteful process for the host animal and a major contributor to global anthropogenic greenhouse gas emission (Hill 2012). The objective in this study was to explore the effect of RFI phenotype on the archaeal and bacterial populations present in the rumen of steers divergent for RFI fed a pasture based diet. It has been established that different breeds vary in feed efficiency capacity (Hill 2012), therefore, both Charolais and Holstein Friesian steers were utilised in this study.

Material & methods Residual feed intake was calculated for each animal as the difference between actual dry matter intake (DMI) and expected DMI for Charolais (CH) steers (n=90) and Holstein Friesian (HF) steers (n=77). The steers were fed zero-grazed grass (DM 196 g/kg) *ad libitum*. During the experimental phase rumen fluid samples were collected for each animal (mean bodyweight ± SD of CH = 561.6± 39.8 kg, HF = 482.5± 39.5 kg) via stomach intubation for volatile fatty acid (VFA) and microbial analysis. Rumen fluid samples were immediately frozen in liquid nitrogen and then stored at -80ºC. Rumen fluid samples from steers deemed the most (LRFI) (mean CH RFI= -0.56 n=9, mean HF RFI = -0.82 n=10) and least efficient (HRFI) (mean CH RFI =0.58 n=10, mean HF RFI= 0.72, n=9) were subject to VFA and microbiomic analysis. The VFA concentration of rumen fluid was measured using gas chromatography. DNA was extracted using the repeated bead beating and column purification method (Yu and Morrison 2004). *16S* DNA libraries were constructed and sequenced on the Illumina MiSeq platform. A combination of *de novo* and reference based OTU identification was carried out, clustering sequences at 97% similarity and aligning a representative sequence to the Greengenes database (version gg_13_8). A non-parametric statistical analysis between treatment groups was conducted with R (v 3.4.2).

Results & discussion Overall archaeal and bacterial population diversity differed between CH HRFI and LRFI steers ($P<0.05$). This was accompanied by increased diversity coverage and dominance in LRFI steers. VFA results showed that the LRFI CH steers had reduced butyrate concentration ($P<0.1$) and an increased acetate:propionate ratio ($P<0.05$). No difference in diversity was observed between HF HRFI and LRFI steers. There was an increase in acetate, propionate, isobutyrate, valerate and total VFA concentrations in LRFI in comparison to HRFI HF steers ($P<0.05$). The phyla; SR1 and TM7 were negatively correlated with RFI ($P<0.05$) in CH steers. In HF steers; *Euryarchaeota* was negatively correlated with RFI ($P<0.05$) and *Cyanobacteria* and *Proteobacteria* were positively correlated with RFI ($P<0.05$). Only one genus *Moryella,* exhibited a relationship in both CH and HF steers, however, it was positively correlated with RFI in HF steers and negatively correlated with RFI in CH steers. Nine OTUs were correlated with both HF and CH steers ($P<0.05$). Only two of these OTUs belonging to the *Lachnospiraceae* and *Ruminococcaceae* families had a positive relationship with RFI in both breeds ($P<0.05$).

Conclusion The results indicate that host ruminant feed efficiency phenotype influences the rumen microbial ecosystem however; this effect appears to be different dependent on the genetics of the host animal.

References

Hill, R. A. 2012. Feed efficiency in the beef industry, John Wiley & Sons.
Finneran, E, Crosson P, O'kiely P, Shalloo L, Forristal D and Wallace M 2010. Journal of Farm Management 14, 95-116.
Herd R and Arthur P 2009. Journal of Animal Ccience. 87, E64-E71.
Yu Z and Morrison M 2004. BioTechniques 36, 808-812.

Changes in rumen metabolites from lambs fed a feed additive containing linseed oil and garlic essential oil

Ulli M. Hohenester[1], Cristina Saro[1], Marine Gauthier[1], Stephanie Durand[2], Milka Popova[1], Diego P. Morgavi[1], Hamid Boudra[1]

[1]*INRA, Unité Mixte de Recherches sur les Herbivores, Saint Genès Champanelle, France,* [2]*INRA, PFEM, Saint Genès Champanelle, France*

E-mail: ulli.hohenester@inra.fr

Take home message Combining metabolomics and microbiota composition data aid the understanding of microbial functions.

Introduction Rumen metabolites are useful markers to monitor rumen function and follow up changes associated to feeding practices. Volatile fatty acids and ammonia are widely reported in the literature. However, the use of other metabolites is less common. The aim of this study was to assess the suitability of a non-targeted metabolomic approach for the discovery of metabolites associated to the rumen microbiota. To this end, we compared rumen fluid from lambs receiving or not a feed additive that modulated the microbiota (Saro-Higuera *et al.,* 2016).

Material & methods Sixty lambs of 16 weeks of age were allotted into two equal groups (weight, sex ratio) receiving or not a feed additive composed of garlic essential oil (allyl polysulfides) and linseed oil (rich in unsaturated C18 fatty acids) for four weeks. Rumen contents were sampled before feeding at the end of the treatment period and analysed by two complementary analytical platforms: liquid chromatography coupled to mass spectrometry (LC-MS-QTOF) and nuclear magnetic resonance spectroscopy (NMR). Both MS and NMR data were analysed by chemometrics tools (Simca-P13, Umetrics, Sweden). Previously obtained microbial data were incorporated in the analysis.

Results & discussion Results indicate eight metabolites in NMR (five volatile fatty acids, two aromatic acids and one amino acid) and 10 metabolites in MS (choline, 3-deoxycarnitine, pipecolic acid and seven not yet described metabolites) that discriminate the two groups. These seven metabolites possess a common m-amino-pyridine skeleton with different mono-, di- or tri-hydroxy-alkyl groups in ortho and para positions, suggesting a shared metabolic pathway. None of these 18 metabolites could be associated to the composition of the feed additive. We previously observed that the treatment modulated rumen bacterial and archaeal communities, up to 12 bacterial and 1 archaeal (Methanomassilicoccaceae) families that were significantly influenced by the additive (Saro-Higuera *et al.,* 2016). Pearson correlation analyses (univariate) and Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA; multivariate) were performed between the signals in LC-MS and the relative abundance of different microbial groups. Taxons belonging to Methanomasiliicoccaceae (A6) and Fibrobacteres B31 were positively or negatively related with 8 out of the 10 metabolites suggesting a relationship between these two microbes. The result of this Pearson correlation is also visible in the OPLS-DA loading plot shown in Figure 1. Pipecolic acid correlated with protozoal genera in the OPLS-DA in agreement with the known production of this metabolite by rumen protozoa (Onodera and Kandatsu, 1972). 3-Deoxycarnitine, also known as gama-butyrobetaine, is an end-product of the gama-butyrobetaine-crotonobetaine-carnitine cycle, found in some anaerobic bacteria (Meadows *et. al.*, 2015).

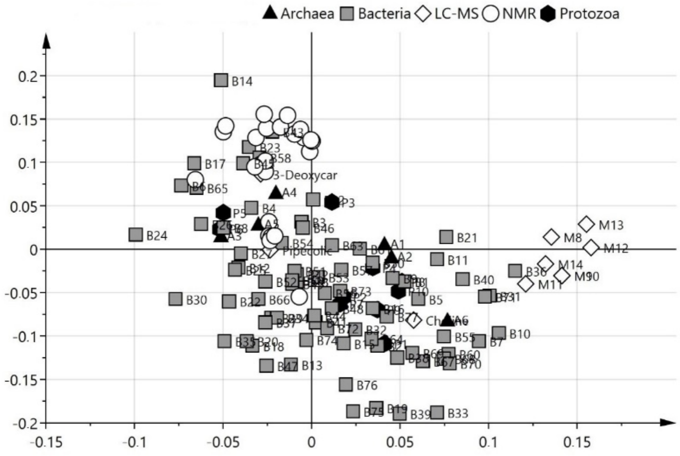


Figure 1 OPLS-DA loading plot combining rumen metabolomics and microbiota data. Each symbols represent all samples of one rumen metabolite detected by NMR (circle), LC-MS (diamond), archaeal clade (triangle), bacterial familie (square) or protozoal genera (Hexagon).

Conclusion This study shows that changes occurring in the ruminal ecosystem can be better interpreted by combining metabolomics and microbiota data.

Acknowledgements This research was supported by the French National Research Agency (ANR) through the FACCE-JPI project RumenStability.

References

Meadows JA and Wargo MJ 2015. Microbiology 161, 1161-1174

Onodera R and Kandatsu M 1972. Agricultural and Biological Chemistry 36, 1989-1995.

Saro-Higuera C, Bernard M, Graviou D, Rochette Y, Martin C, Doreau M, Boudra A, Popova M and Morgavi D. 2016. 67th. Annual Meeting of the European Federation of Animal Science (EAAP). Wageningen (NLD): Academic Publishers.

Effects of N-acyl homoserine lactone (AHL) lactonase or *Artemisia* seed polysaccharide on rumen bacterial diversity

Shengli Li, Haizhou Sun, Lu Jin, Chunhua Zhang, Dan Sang, Chongzhi Zhang

Institute of Animal Nutrition and Feed Research, Inner Mongolia Academy of Agricultural & Animal Husbandry Sciences, Hohhot, Inner Mongolia, China

E-mail: lshl2000@163.com

Take home message The objective of this experiment was to investigate changes in rumen bacterial diversity in sheep fed N-acyl Homoserine Lactone (AHL) Lactonase or *Artemisia* Seed Polysaccharide; the results indicated the two supplements had indirect or direct antimicrobial effects.

Introduction The AHL-lactonases, a quorum quenching enzyme, inactivates AHLs by hydrolysing the lactone bond to produce corresponding N-acyl homoserines involved in bacterial quorum sensing (Greenberg *et al.,* 2017) and therefore represent a novel approach to regulate rumen micro-ecology. *Artemisia sphaerocephala* Krasch (Asteraceae) is widely distributed in northwest of China. The surface of its seeds is covered by a layer of gum, *Artemisia* seed polysaccharide (ASP). Studies have confirmed that ASP exhibits medicinal effects, including anticancer properties, antioxidant properties, and immunity enhancement in rats (Zhang *et al.,* 2006). The host immune system regulates the gut microbiota indirectly. Polysaccharides play extensive roles in host–gut microbiota symbiosis (Nathan and Eric, 2017). So, ASP may influence of the diversity of rumen bacteria. The NFC/NDF Level is also a key factor influencing ruminal bacterial community diversity and composition.

Material & methods This experiment was conducted to study the effects of AHL Lactonase (AHLL) or ASP on rumen bacterial diversity at different dietary NFC (Non-Fiber Carbohydrate)/NDF (Neutral Detergent Fiber) ratios *in vivo*. Nine ruminal cannulated Ordos Fine Wool Sheep were randomly assigned to three dietary treatments with different NFC/NDF ratios (0.48, 0.76, and 1.53) named diet A, B and C respectively. Then we had three period trials, the first period was the control period, the sheep only took 3 basal diets; then the AHLL period, the basal diet + 0.3% AHLL; and last, the ASP period, the basal diet + 1.0% ASP for diet A and C,0.5% ASP for diet B, respectively. Each experimental period was 16 d, and the animal has a period of adaptation among trials. The ruminal content samples were collected at 2h following the morning feeding on d 15 and d 16 of each experimental period. Samples were collected from four locations in the rumen and filtered through four layers of cheesecloth stored frozen at -20°C for bacterial profiling. The analysis of amplified and sequenced 16S rRNA genes was used for bacterial diversity studies.

Results & discussion The bacteria shannon index and simpson index decreased when supplemented AHLL and ASP in diet B. A total of 27,720 operational taxonomic units (OTUs) were obtained, the numbers of typical OTUs was ASP> control> AHLL. The relative abundance of bacterial phila showed that *Firmicutes* and *Bacteroidetes* were the dominant populations within the rumen flora. Data analysis was carried out by using Metastats analysis, at genus level, with the adding of AHLL, *Synergistetes* was significantly higher in diet A. In contrast, proportions of *Tenericutes* was reduced for diet B ($P<0.05$). The percentage of *Fibrobacteres* increased for diet B were increased with the adding of ASP ($P<0.05$). By comparing family, genus and species richness, we found that *Campylobacter*, *Porphyromonadaceae* decreased by adding AHLL, and *Desulfovibrionaceae, Vibrio*, *Campylobacter jejuni* disappeared by adding ASP ($P<0.05$).

Conclusion Collectively, the results indicated that N-acyl Homoserine Lactone (AHL) Lactonase or *Artemisia* Seed Polysaccharide altered bacterial populations in the rumen of sheep, possibly through indirect or direct antimicrobial effects.

Acknowledgements Thanks to Beijing Challenge Group who supported N-acyl Homoserine Lactone (AHL) Lactonase for this work.

References

Greenberg EP, Whiteley M, and Diggle SP 2017. Nature 551, 313–320
Zhang J, Huang YL, Hou TD, and Wang YP. 2006. Swiss Medical Weekly 136, 529-532
Nathan TP and Eric CM. 2017. Annual Review of Microbiology 71, 349-369

Identification of potential docosahexaenoic acid (22:6*n*-3) biohydrogenating rumen bacteria by inoculum dilution-to-enrichment technique

Jeyamalar Jeyanathan[1], Bruno Vlaeminck[1], Marlene Escobar[1], Dung Viet Nguyen[2], Peter Bossier[2], Veerle Fievez[1]

[1]Laboratory for Animal Nutrition and Animal Product Quality (Lanupro), Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium, [2]Laboratory of Aquaculture and Artemia Reference Center, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

E-mail: jeyamalar.jeyanathan@ugent.be

Take home message Inoculum dilution-to-enrichment technique and bacterial 16S rRNA gene amplicon sequencing helped to identify several potential bacterial genera involve in 22:6*n*-3 biohydrogenation in the rumen.

Introduction Rumen bacteria are mainly responsible for the biohydrogenation of polyunsaturated fatty acids in the rumen. However, knowledge on biohydrogenation of docosahexaenoic acid (22:6*n*-3) is limited in the rumen. *Butyrivibrio proteoclasticus* is the only rumen bacteria known to date to be involved in 22:6*n*-3 hydrogenation. Studies showed that 22:6*n*-3 is extensively hydrogenated in the rumen. As such, there can be more than one bacterial species involved in 22:6*n*-3 hydrogenation. Identifying these bacterial species will help to understand the 22:6*n*-3 metabolism and more importantly the intermediate products formed in the rumen. In this study, we attempted to identify potential bacterial species involved in biohydrogenation of 22:6*n*-3 in the rumen by an inoculum dilution-to-enrichment technique in combination with bacterial 16S rRNA gene amplicon sequencing.

Materials & methods Rumen inoculum was obtained from three adult sheep fed grass hay and a grain-based concentrate diet according to their maintenance requirement. This inoculum was serially diluted to produce cultures with different amounts of rumen inocula (dilution ranges from 10^2 to 10^{14}) and incubated in Hungate tubes containing rumen bacterial growth media supplemented with 22:6*n*-3 (10 µg/mL). After 48-h incubation at 39 °C, pH was measured, and samples were collected for volatile fatty acids (VFA), residual 22:6*n*-3 and bacterial composition analysis. The bacterial 16S rRNA gene amplicon sequencing was performed to all the samples (dilutions 10^2 to 10^{14}) using illumina Miseq V3-technology (Macrogen, Korea).

Results & discussion The amounts of residual 22:6*n*-3 after a 48-h incubation progressively accumulated and the total VFA production progressively reduced as the inoculum was more diluted (Figure 1). Relative abundance analysis of 16S rRNA gene amplicon sequencing and correlation analysis identified several bacterial genera negatively correlated with residual 22:6*n*-3 (Table 1).

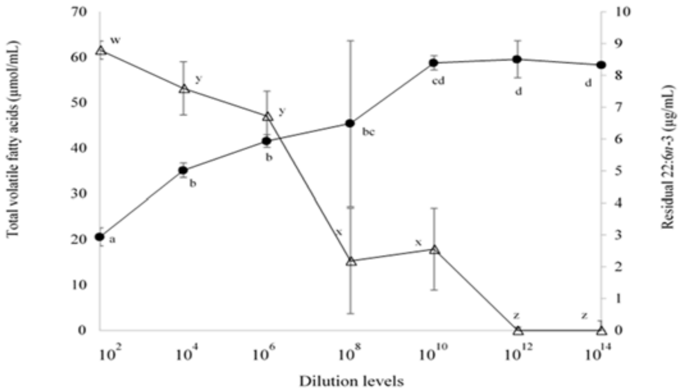


Figure 1 Residual 22:6*n*-3 (●) and VFA production (Δ) after 48-h of incubation. Within the parameter 'residual 22:6*n*-3' or 'total volatile fatty acids', data points with different letters are significantly different ($P < 0.05$).

Table 1 List of bacterial genera showing negative correlations with residual 22:6n-3

	Spearman	
Genera	R	P
Treponema	-0.79	<0.01
Catenibacterium	-0.78	<0.01
RC9_gut_group	-0.72	<0.01
Pseudobutyrivibrio	-0.64	0.01
Succinivibrio	-0.47	0.07
Selenomonas	-0.45	0.08

Conclusion Inoculum dilution-to-enrichment technique and bacterial 16S rRNA gene amplicon sequencing helped to identify several potential bacterial genera that may be involved in 22:6*n*-3 biohydrogenation in the rumen. Further pure culture studies are needed to confirm their involvement in 22:6*n*-3 hydrogenation.

Acknowledgements The study was funded by the Fund for Scientific Research-Flanders (Belgium) and the Special Research Fund of the Ghent University.

Intestinal digestibility of amino acids in canola and soybean meals using cecectomized roosters and comparison of methods to determine intestinal N digestibility

D. R. Ouellet[1], A. Rouissi[1,2], K. Békri[1,2], D. Pellerin[2], H. Lapierre[1]

[1]Agriculture et Agroalimentaire Canada, QC, Canada, [2]Dépt. des Sci. Animales Univerité Laval, QC, Canada

E-mail: daniel.ouellet@agr.gc.ca

Take home message The average intestinal digestibility of amino acids (AA) of rumen undegraded protein (RUP) of two sources of canola meal (CM) varied between 0.66 and 0.79 and was lower than the AA digestibility of RUP of soybean meal (SBM). These results support that the reason for the underestimation of metabolizable protein (MP) supply using NRC (2001) with inclusion of CM in dairy rations is not related to an underestimation of the digestibility of the RUP of CM.

Introduction Adequate evaluation of AA supply from RUP is necessary to properly balance dairy rations for AA. The low intestinal digestibility of RUP from CM (0.75) used by NRC (2001) reduces the estimated MP supply when CM substitutes other protein sources in dairy rations. Despite this decrease in the estimated MP supply, many studies have reported a positive milk protein response when CM substituted another protein source (Martineau *et al.,* 2013). The objective of this research was to estimate intestinal AA digestibility of RUP from CM and SBM using cecectomized roosters and to compare these results to N digestibility of the RUP of CM and SBM obtained using *in vitro*, *in sacco*, and *in vivo* methodologies.

Materials & methods The intestinal digestibility of 12-hour residues of rumen incubation of three protein sources [commercial CM (CCM), ^{15}N-CM (LCM; experimental CM produced using ^{15}N fertilization; Rouissi *et al.,* 2017) and SBM] was estimated using cecectomized roosters (Chen *et al.,* 2015). Briefly, seven birds fasted 48 hours were used to estimate the endogenous AA excretion while four birds were fed 20 g of one of the three protein sources and their excreta collected for 48 hours to estimate AA digestibility. Protein sources and pooled excreta were analyzed for AA composition. Based on the N content of each AA, N digestibility was also calculated. The three-step *in vitro* procedure (Calsamiglia and Stern, 1995) was performed in quadruplicate on three different days to estimate N digestibility of the three protein sources. The estimations of N digestibility of CCM, LCM, and SBM using mobile bags and that of LCM using ^{15}N labelling have been described and reported previously (Rouissi *et al.,* 2017).

Results & discussion Using the cecectomized roosters, the AA digestibility of CCM varied from 0.12 for Gly to 0.86 for Trp (Figure 1). For LCM, the AA digestibility was intermediate among the sources evaluated (0.48 for Gly to 0.93 for Trp). The digestibility of all AA of SBM was close to or greater than 0.90 except for Gly (0.69). The N digestibility calculated based on individual AA digestibility averaged 0.65, 0.80 and 0.93 for CCM, LCM, and SBM, respectively. Three-step *in vitro* N digestibility averaged 0.56 (SD 0.026), 0.67 (SD 0.023), and 0.81 (SD 0.046) for CCM, LCM and SBM, respectively. Using the mobile bags (10 µm pore size), the N intestinal digestibility averaged 0.65, 0.76, and 0.97 for CCM, LCM, and SBM, respectively; N digestibility of LCM introduced directly into the duodenal cannula and recovered in faeces averaged 0.73 (Rouissi *et al.,* 2017). The latter 2 methods gave values in the same range than those measured with the cecectomized roosters but higher than determined with the three-step method.

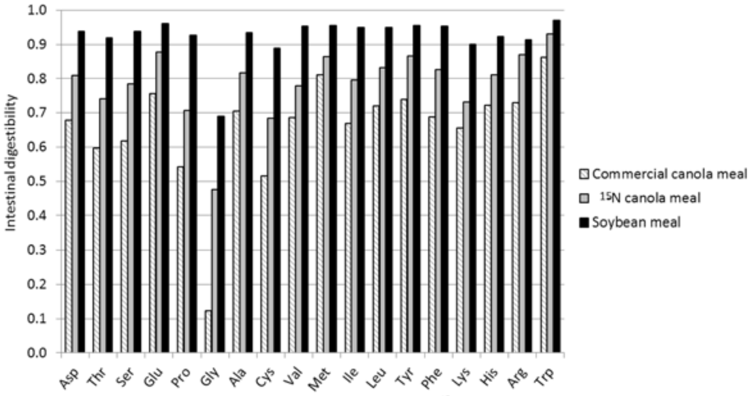


Figure 1 Amino acid digestibility of commercial canola meal, ^{15}N-canola meal and soybean meal using cecectomized roosters.

Conclusion Four methods were used to estimate the intestinal digestibility of the RUP of CM and three methods were used for SBM. All methods showed a lower intestinal digestibility of the RUP of CM than SBM; *in sacco* and *in vivo* methods are yielding higher values than *in vitro* procedure. It seems that the current low estimation of intestinal digestibility of the RUP of CM is not an underestimation: other reasons must justify the observed lower prediction of MP supply with inclusion of CM in dairy rations, despite the observed positive milk protein response.

Acknowledgements This research was funded by the Canadian Canola Council and the Agri-Science Cluster initiative of Agriculture and Agri-Food Canada.

References

Calsamiglia S and Stern MD 1995. Journal of Animal Science. 73, 1459-1465
Chen X, Parr C, Utterback P and Parsons C M 2015. Poultry Science 94, 984-991
Martineau R, Ouellet DR and Lapierre H 2013. Journal of Dairy Science 96, 1701-1714
NRC 2001, Natl. Acad. Press;
Rouissi A, Lapierre H, Pellerin D, Békri K and Ouellet DR 2017. Journal of Dairy Science 100 (suppl. 2) T195.

In vitro fermentation of barley or sugarbeet pulp in response to dry or pre-activated *Saccharomyces cerevisiae* yeasts additive under an incubation pH dropping to 6.0

Pilar Tejeda, Zahia Amanzougarene, Hector Calvo, Manuel Fondevila

Faculty of veterinary, Department of food science and Animal Nutrition, Zaragoza, Spain

E-mail: zahiaagro@yahoo.fr

Take home message Pre-activation of yeasts improves its effect on rumen fermentation, mainly on fibrous substrates.

Introduction Adding ruminant diets with yeasts stabilises rumen pH and increases fibre degradation. However, effects are often low and variable, probably because yeasts are mostly added to the feed in dry form. Optimum yeast growth conditions (30-35ºC, pH 4.4-5.6, aerobiosis, Walsh and Martin, 1977) differ from rumen environment (39-40ºC, pH 5.8-6.8, strict anaerobiosis), and should not ensure their active metabolic stage for affecting fermentation. Most studies report effects on fibre digestion in forage or mixed diets; thus, we hypothesized the inclusion of dry or pre-activated yeasts on starch or fibre fermentation under conditions simulating high-concentrate feeding is studied.

Material & methods Barley (B) and sugarbeet pulp (P) were chosen as models of starch and digestible fibre sources used in high concentrate ruminant feeds. *S. cerevisiae* live yeast (MUCL39885; 9 x 10^9 cfu/g, AMBIOTEC Balance, Spain) was added in dry form (DY) or pre-activated (AY) by aerobic culturing at 30ºC for 24 h in water with a nutritional enhancer (80 mg/mL), and dispensed in bottles to give 1.4 mg yeast/g substrate. Three 24 h incubations series were carried out, at fixed pH of 6.2-6.0, with triplicated bottles with 500 mg substrate and 80 mL incubation medium with 10 mL/100 mL rumen inoculum from ewes given daily 0.9 kg (0.45 kg concentrate/ kg dry matter). A third treatment with rumen liquid but no yeasts (RL) was included. The average of treatment bottles in each series was considered the experimental unit. Results per incubation time were analysed by ANOVA as a Split-plot design, with substrate (B, P) as main plot, inoculum (RL, DY, AY) as subplot and the incubation series as block. Means were compared by the Tukey test.

Results In a previous incubation study, concentration (log cfu/mL, n=4) of *S. cerevisiae* in DY and AY at 0 h was 8.89 and 8.99, whereas after 6 h was higher with AY than DY (6.83 *vs.* 5.76; P=0.007) and differences disappeared after 12 h (6.26 *vs.* 6.29), showing that AY grows earlier. In this work, accumulated gas production (Figure 1) with B was higher than P from 4 h onwards (P<0.05). Among inocula, from 2 to 18 h yeast treatments (AY, DY) promoted higher gas volume than RL, but response was always higher with AY than DY (P<0.05). The interaction substrate x inoculum (4 to 12 h, P<0.05) indicates that with P as substrate AY was highest (P<0.05), while no effect of DY over RL was detected. In contrast, with B as substrate differences persisted between AY and RL but not between AY and DY, and gas volume with DY was higher than RL only at 8 and 12 h. Accordingly, rate of gas production (Figure 2) was highest with AY up to 6 h and differences between DY and RL were also shown at 6 and 12 h. These inoculum differences were only manifested with P as substrate.

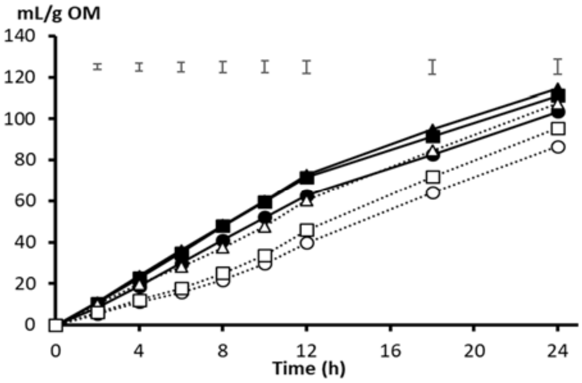


Figure 1 Total gas volume from B (solid lines) or P (dotted lines) incubated with RL (●,○), DY (■,□) or AY (▲,△). Upper bars show standard error of means (n=3)

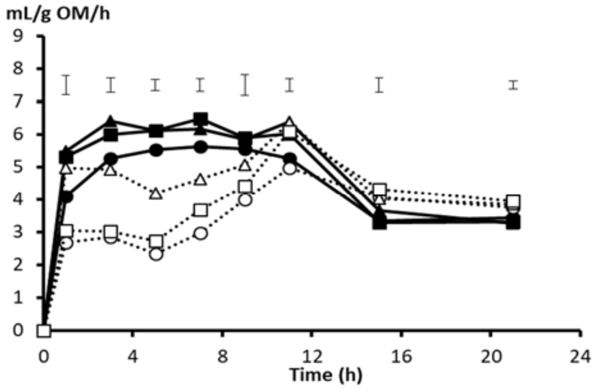


Figure 2 Rate of gas production (mL/h) from B (solid lines) or P (dotted lines) incubated with RL (●,○), DY (■,□) or AY(▲,△).Upper bars show standard error of means (n=3).

Conclusion The increase in yeast concentration promoted by pre-activation of *S. cerevisiae* allows for a positive response in rumen microbial fermentative activity at pH 6.2-6.0, in both rate and extent, compared to yeasts added in dry form. The magnitude of response in fermentation was higher on the fibrous (sugarbeet pulp) than the starchy (barley) substrate.

Acknowledgements Spanish Government (project AGL2013-46820-P), Government of Aragon and European Social Funds

References

Walsh RM and Martin PA 1977. Journal of the Institute of Brewing 83, 169-172.

The effects of different forages on diversity of rumen bacteria of Tibetan sheep

Xiongxiong Cui, Zhaofeng Wang, Fujiang Hou, Fenghua Chang

State Key Laboratory of Grassland Agro-ecosystems,College of Pastoral Agriculture Science and Technology, Lanzhou University,, lanzhou, China

E-mail: cuixx15@lzu.edu.cn

Take home message An in-depth understanding of the genetic diversity of rumen bacteria is helpful for feeding ruminants and producing higher quality livestock products.

Introduction Rumen microbes play an important role in degenerating cellulose of ruminants. Former studies have implied that host, feed and environment all affect the genetic diversity of rumen microbes (Douglas *et al.,* 2016). Tibetan sheep is an important kind of livestock in the Qinghai-Tibetan Plateau, which provides a variety of products for local people. Oats is a main species in artificial grassland, with significant forage value, and plays an important role in feeding livestock of the alpine pastoral areas in winter. Thus, how oat affect the rumen bacterial community composition of Tibetan sheep and how the bacteria adapt to these diets is worthy of investigation. The objectives of the present study using next-generation sequencing targeting the V4 region of bacterial 16S rRNA gene, to investigate differences of bacterial community composition in the rumen of Tibetan sheep fed native and domesticated diets.

Material & methods The study was conducted at the Maqu County, China. With nine1-year-old Tibetan sheep with a uniform healthy weight. They were divided into two groups, fed with oat (NDF = 54.20% and CP = 7.86% DM) and natural grass (NDF = 50.09% and CP = 9.79% DM). There were 4 sheep in the natural grass group and 4 in the oat group.

Results & discussion Taxonomic distribution analysed by NGS revealed the prevalence of Firmicutes and Bacteroidetes phyla in all Tibetan sheep, a significantly higher proportion of Bacteroidetes and Proteobacteria in oat-fed sheep and a significantly lower proportion of the Bacteroidetes in the grass-fed sheep(Fig1).The UPGMA clustering tree showed that all samples tended to cluster together in accordance with their own ration treatment (Fig1).The result is based on UniFrac's weighted principal coordinate analysis (PCoA) (Fig 2). The contribution rate of the first principal component is 57.47%, and the second principal component is 13.16%, The samples from oat-fed sheep is distributed on the left side of the graph, and the samples from grass-fed sheep is on the right side, indicating that there are significant differences in the structure of the rumen bacterial community of oat-fed and grass-fed sheep ($P < 0.05$). Data of the present study demonstrated that diets affected the clustering of the bacterial communities, and the difference between the two groups is significant.

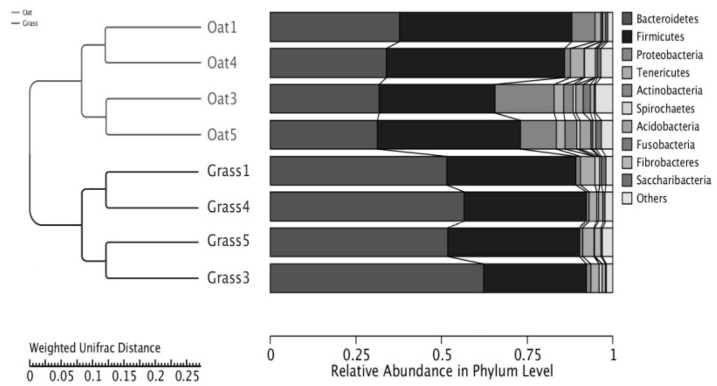


Figure 1 Effects of different forage on ruminal bacteria composition at phylum level and UPGMA clustering tree based on weighted unifrac distance of Tibetan sheep.

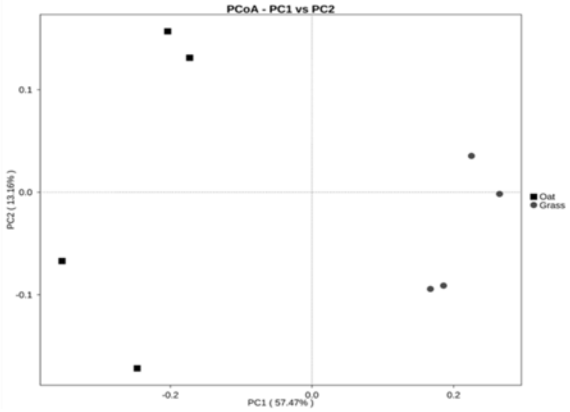


Figure 2 Principal coordinate analysis (PCoA) Generated by a weighted UniFrac analysis on the Tibetan sheep rumen fluid samples

Conclusion In conclusion, the current study suggested that rumen bacterial community composition were altered in the oat and natural grass diets. These conclusions are of great importance for the targeted improvement of energy use efficiency in Tibetan sheep.

Acknowledgements This research was supported by the National Natural Science Fund (31672472).

References

Godoy-Vitorino F, Goldfarb KC, Karaoz U, Leal S, Garcia-Amado MA, Hugenholtz P, Tringe SG, Brodie EL and Dominguez-Bello MG 2012. The ISME journal 6, 531–541.

Douglas J-L, Worgan HJ, Easton GL, Poret L, Wolf BT, Edwards A, Davies E, Ross D and McEwan NR 2016. Journal of Applied Microbiology 120, 1382-1389.

The bacterial and archaeal community structures and methanogenic potential of the cecal microbiota of goats fed high-grain diet or hay diet

Wei Jin, Yin Li, Yanfen Cheng, Shengyong Mao, Weiyun Zhu

Nanjing Agricultural University, Nanjing, China

E-mail: jinwei@njau.edu.cn

Take home message High-grain diet caused the goat cecal excessive fermentation and methane production.

Introduction The caecum play an important role in the feed fermentation of the ruminants, however, limited information is available for the cecal microbiota and the methane production potential. We hypothesized that the high-grain diet would increase organic matter arrival and fermentation in the cecum of ruminants, and would induce changes in the cecal microbiota toward to producing more methane. Thus, we described the cecal size, digesta components, microbiota of the local Chinese goats fed hay diet or high-grain diet. The methanogenesis potential and the metabolic profiles of the cecal microbiota were also evaluated.

Material & methods The experiment was conducted at Nanjing Agricultural University. Twelve fistulated male goats (local chinese species, eight-month-old) were used in this study. All animals were fed a hay diet *ad libitum* for 2 weeks before the start of the experiment to ensure adaptation to the low energy diet. Then, goats were randomly assigned to complete hay diet treatment (Hay; 0% concentrate; $n=6$) or high grain diet treatment (HG; 75% concentrate; $n=6$), and placed in individual pens (1.2×1.2 m) with free access to water. On d 50, goats were slaughted and cecal digesta was collected. The community structures of bacteria and archaea in the cecal digesta were determined using high throughput sequencing of 16S rRNA genes (Dennis *et al.,* 2013; Su *et al.,* 2014) and real-time PCR (Jin *et al.,* 2017). The methanogenesis potential and metabolic characteristics of the cecal microbiota were determined using the *in vitro* fermentation with ground corn or hay as substrates (Popova *et al.,* 2013). The statistical calculations were carried out with the tests in SPSS. The normality of the distribution of variables was tested by the Shapiro–Wilk test. The independent samples T-test procedure (one-way ANOVA for the data of the *in vitro* fermentation) was used to analyze the variables that have a normal distribution.The variables that have a non-normal distribution were analyzed using the Kruskal–Wallis test procedure. Signifcance was declared at $P< 0.05$.

Results & discussion The results showed that HG significantly increased not only the length and weight of the cecum, but also the proportion of starch and CP in the cecal content ($P<0.05$). The pH of the cecal content declined in the HG group ($P<0.05$). The VFAs and NH_3-N concentration increased ($P<0.05$). The abundances of bacteria and archaea increased ($P<0.05$), while the abundance of anaerobic fungi decreased ($P<0.05$). HG altered the community structures of bacteria and archaea. Of the classified genera, *Bifidobacterium*, *Prevotella*, and *Treponema* were significantly higher in the HG group ($P<0.05$), while *Akkermansia*, *Oscillospira*, and *Coprococcus* were significantly higher in the Hay group ($P<0.05$). *Methanosphaerea stadtmanae* was higher in the HG group ($P<0.05$), while *Methanospharea* sp. ISO3-F5 was higher in Hay group ($P<0.05$). The cecal microbiota of HG group, *in vitro* fermentation on corn, produced significantly higher methane and VFAs ($P<0.05$), while the cecal microbiota of Hay group produced significantly higher lactate and lower methane ($P<0.05$).

Conclusion HG diet caused a large amount of fermentable substrates flowing into the hindgut of goats, resulting in an excessive fermentation and a dramatically increase of the abundance of bacteria and archaea in the caecum. The community structures of bacteria and archaea were changed by HG diet. The cecal microbiota of goats fed hay diet performed lactate-type fermenation with corn as substrate, in contrast, the cecal microbiota of goats fed HG diet performed methane-VFA-type fermentation. Moreover, the majority of the bacterial sequences were unclassified at the genus level. Further research should aim to isolate, speciate, and identify the function of these unclassified bacteria to better understand their roles in the caecum. Furthermore, more systematic studies are needed to investigate the effect of cecal excessive fermentation on the health of ruminants.

Acknowledgements This research was funded by the National Key Research and Development Program of China (2017YFD0500500), and the National Natural Science Foundation of China (31301999).

References

Dennis KL, Wang Y, Blatner NR, Wang S, Saadalla A, Trudeau E, Roers A, Weaver CT, Lee JJ, Gilbert JA, Chang EB and Khazaie K. 2013. Cancer Reseach 73, 5905-5913.
Jin W, Meng Z, Wang J, Cheng Y, Zhu, W 2017. Current Microbiology 74, 891-898.
Popova M, Morgavi DP and Martin, C. 2013. Applied and Environmental Microbiology 79, 1777-1786.
Su Y, Bian G, Zhu Z, Smidt H, Zhu W 2014. Archaea 2014, 547908.

The relationships between the predicted metabolic parameters of protein and biomolecular spectroscopic features in different batches of corn by-products from the same processing plant

Hangshu Xin, Kaijing Sun, Yonggen Zhang

Northeast Agricultural University, Harbin, China

E-mail: xinhangshu@163.com

Take home message Protein metabolic characteristic and spectroscopic structural features in corn by-products from the same processing plant were affected by batch with the exception of corn germ and corn gluten meal.

Introduction Corn by-products, such as corn gluten meal, corn gluten feed and corn germ cake, are regarded as good protein/fibre sources to ruminant animals. However, a critical concern is whether the quality of by-products from different batches is consistent (Nuez-Ortin and Yu, 2009). Therefore, this study was conducted to investigate 1) the National Research Council (NRC) model predicted metabolic parameters of proteins; 2) the bio-molecular spectral profile associated with nutrient supplies and 3) the relationship between modelled nutrient parameters and spectral profiles in the three different batches of corn by-products from the same processing plant.

Material & methods Four kinds of corn by-product were used in this study: corn gluten feed, corn gluten meal, corn germ and corn germ cake. The whole corn kernel was included as a reference. All the samples were obtained from Cargill (Cargill Animal Nutrition (Harbin), Co., Ltd., Harbin, China). According to the processing rules, corn gluten feed was mainly from the corn fibre, corn germ cake was the remaining from the corn oil extraction, and the corn gluten meal was obtained from the starch extraction. Forty-five samples were collected from three batches (1, 2, 3) of corn kernel (three samples per batch) for processing and three batches (I, II, III) of corresponding by-products (three samples per batch). The collection was carried out over 10 months. Three dry dairy cows each fitted with a rumen cannula were used in our *in situ* study to obtain the nutrients ruminal degradation parameters which were key factors to estimate the metabolic parameters of protein calculated by the methods described in the NRC. The spectrum data of by-product were collected using a Fourier transform infrared spectroscopy (FT/IR) (BRUKER ALPHA-T, Germany) at the Chemistry Molecular Structure Analysis Lab at the College of Science, Northeast Agricultural University (Harbin, China). Each sample was scanned ten times. All the data were statically analysed using the Mixed procedure of SAS 9.2 (SAS Institute, 2009).

Results & discussion Different kinds of corn by-product showed significant ($P < 0.0001$) characteristics of metabolic protein supply. The predicted rumen degradable protein (RDP) and the absorbed rumen undegraded feed protein in the small intestine (ARUP) were highest in the corn gluten meal (RDP, 121 g/kg DM; MP, 519 g/kg DM) and lowest in the corn kernel (RDP, 28 g/kg DM; MP, 41 g/kg DM). As a result, the corn gluten meal had the highest content of the total truly absorbed protein in the small intestine (MP), followed by corn germ cake, corn germ, corn gluten feed and corn kernel. The same tendency was observed in feed milk value (FMV) among these corn by-product samples. In addition, batch effects on MP and FMV values were found in all the samples with the exception of corn germ and corn gluten meal, indicating those two by-products had relatively stable metabolic protein supply. Consistently, the spectral area ratios (protein amide to carbohydrate) were not changed in corn germ for area ratios of protein amide I to total carbohydrate (TCHO), protein amide I to non-structural carbohydrate (NSCHO), protein amide II to structural carbohydrate (SCHO) and protein amide II to NSCHO and corn gluten meal for protein amide I to SCHO, protein amide I to NSCHO, protein amide II to SCHO and protein amide II to NSCHO from three batches in the same processing plant. The MP and FMV contents in all the corn samples had strong relations with spectral ratios of protein to carbohydrate with the exception of area ratio of protein amide I to cellulosic compounds. Our data are in full agreement with the report published by Gamage *et al.,* (2012).

Conclusion Protein metabolic characteristic and spectroscopic structural features in corn by-product from the same processing plant were affected by batch with the exception of corn germ and corn gluten meal. Strong relationships were also found between metabolic parameters and area ratios of protein amide peak to carbohydrate peaks.

Acknowledgements This study was financially supported by Heilongjiang Provincial Science Foundation Project (LC2017011).

References

Gamage IH, Jonker A, Christensen DA and Yu P 2012. Journal of Dairy Science 95, 6695–6715.

Nuez-Ortín WG and Yu P 2009. Journal of the Science of Food and Agriculture 89, 1754-1761.

Methane production and fermentative parameters of *Tithonia diversifolia/Pennisetum purpureum* silage enriched or not with lactic acid bacteria strains

Vilma Holguín[1], Mario Cuchillo[2], Siriwan Martens[2], Jairo Mora-Delgado[1]

[1]*Universidad del Tolima, Ibagué, Colombia,* [2]*Centro Internacional de Agricultura Tropical, Cali, Colombia*

E-mail: jrmora@ut.edu.co

Take home message: *Tithonia diversifolia* in silage reduce methane emission.

Introduction *Tithonia diversifolia* (TD) has great potential for feeding ruminant and monogastric because it has a high content of protein and minerals and a good acceptance by the animals for consumption. To select the best fermentation parameters of the materials favoring the availability of nutrients for rumen microorganisms we evaluated the association of TD and *Pennisetum purpureum* (PP) for silage making and the dynamics of gas production.

Material & methods TD and PP were harvested, wilted, chopped and ensiling in micro-silages (1kg). It was arranged in four different proportions as treatments: T1: 100/0; T2: 67/33; T3: 33/67; and T4: 0/100 (FM weight). Each micro-silage was inoculated as follows: control, lactic acid bacteria *(LAB)* strain T735 (identified as *Lactobacillus paracasei*), SIL-ALL$^{®4x4}$, resulting in 12 treatments in total. *In vitro* gas production was measured at 3, 6, 12, 24, 33, 48, 60, 72, 96, 120 and 144 h in triplicate. A factorial experimental design was used with two factors. The first factor, was the inclusion level of the TD/PP and the second factor, was the inoculant used: $Yij = \mu + PPi + Ij + I \times PPij + \varepsilon$; where Y = is the target variable, μ is the overall mean; I = inoculant (control; T735; SIL-ALL$^{®4x4}$), PP = proportion of grass in the silage (0/100, 33/67, 67/33, and 100/0) and ε = random experimental error. Analysis of variance was performed and statistical differences were detected by Duncan mean comparisons ($P<0.05$). A Gompertz equation was used to modeling gas accumulation from different treatments. Analyses of the net production of CH4 were made in the gas generated at 72 h incubation of 1 g of silage.

Results & discussion Gas accumulated at 144 hours in T1 (161.16 ml) had statistical differences ($P<0.0001$) compared to the other treatments. An increased gas production was found in T4 (0/100; TD/PP) at 144 h (204.12 mL). The results of mixture (TD/PP) indicate that silages prepared with higher inclusion of TD, independent of LAB inoculation, produced fewer gases, ranging between 161.2 and 174.45 mL/g. meaning that larger amount of inclusion of TD in ensiling process decrease the gas production. Gompertz equation results indicate that the highest rate of gas production were: T4 and T3 with 3.34, 3.20 mL/h, respectively. Lower values (3.03 and 3.09 mL/h) were reported for the treatments with higher proportion of TD, respectively). It can be concluded that the treatments with a high percentage of TD presented the lowest values of gas production ($P<0.0001$), while treatments with higher grass inclusion produced more gas. Less methane was produced in T1 compared to T2, T3 and T4, but no significant differences were found among the latter three. Rumen microorganisms and enzymes, first attack fermentable carbohydrates and then, with the colonization of the fiber and its degradation, gas production increases (Dhanoa *et al.,* 2000; Fondevila & Barrios, 2001).

Table 1 Methane production per gram of dry matter silage incubated at different levels of inclusion of TD /PP

Treatments	CH_4 (mg/mL)	CH_4 (mg/g of DDM)
T1: 100/0	49.36 ± 9.2b	77.51±14.49a
T2: 67/33	55.93±5.2ab	85.38±7.87ab
T3: 33/67	62.09±11.05a	92.57±16.47ab
T4: 0/100	64.06±9.7a	99.44±15.03b

DDM: Degraded Dry Matter. Common letters are not statistically different ($p > 0.05$)

Conclusion The treatments with a high proportion *T. diversifolia* presented the lowest values of gas production, while treatments with higher grass inclusion produced more gas.

Acknowledgements To Universidad del Tolima; to Universidad Nacional de Colombia and to the CIAT.

References:

Dhanoa MS, Lopez S, Dijkstra J, Davies DR, Sanderson R, Williams BA, Sileshi Z and France J 2000. The British Journal of Nutrition 83, 131–142.

Fondevila A and Barrios M 2001. Revista Cubana de Ciencia Agrícola 35, 3.

367

Rumen methanogens exhibit specific energetic and growth dynamics: a modelling approach

Rafael Muñoz-Tamayo[1], Milka Popova[2], Diego P. Morgavi[2], Dominique Graviou[2], Jean-Pierre Morel[3], Gérard Fonty[3], Nicole Morel-Desrosiers[3]

[1]UMR Modélisation Systémique Appliquée aux Ruminants, INRA, AgroParisTech, Université Paris-Saclay, Paris, France, [2]UMR1213 Herbivores, Institut National de la Recherche Agronomique, VetAgro Sup, Clermont Université, Université de Lyon, Saint Genès-Champanelle, France, [3]Laboratoire Microorganismes: Génome et Environnement, Université Clermont Auvergne, UMR CNRS/UCA 6023, Clermont-Ferrand, France

E-mail: rafael.munoz-tamayo@inra.fr

Take home message: Three rumen methanogens display differences in growth and energetic dynamics.

Introduction Rumen methanogenic archaea are specialized microbes that play a central role in rumen metabolism. By lowering the hydrogen concentration in the rumen, methanogens favour fermentation thermodynamics and enhance substrate utilization by fermenting microbes. It is commmonly assumed that energetic differences among methanogenic archaea are due to the presence/absence of cytochromes (Thauer *et al.,* 2008). This hypothesis implies that rumen methanogens (cytochrome-lacking archaea) exhibit similar energetics. To enhance the understanding of rumen methanogenesis, we investigated the energetics and extent of the methanogenesis of three representative rumen methanogens. Our purpose was to quantitatively characterize the dynamics of hydrogen utilization, methane production, growth and heat flux by integrating thermodynamics, microbiology and mathematical modelling.

Material & methods We performed *in vitro* growth experiments with *Methanobrevibacter ruminantium*, *Methanobrevibacter smithii* and *Methanobacterium formicium* with a gas mix of H_2 (80%) and CO_2 (20%) as substrates. Microbial growth was followed by measuring the OD_{660} and quantifying the functional *mcrA* gene using qPCR (Popova *et al.,* 2013). Gas composition was measured by gas chromatography. In complement, thermodynamic experiments were performed in isothermal microcalorimeters (Bricheux *et al.,* 2013) to quantify the methanogenesis heat flux. On the basis of a rumen fermentation model (Muñoz-Tamayo *et al.,* 2016), we built a dynamic model with an energetic-based kinetic function (Desmond-Le Quemener and Bouchez, 2014) to describe the experimental data from growth experiments.

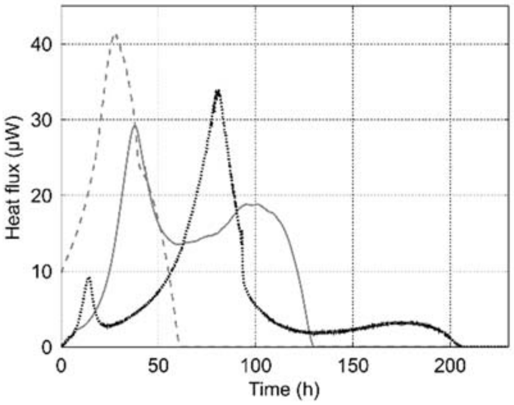


Figure 1 A. Isothermal calorimetric curves for *M. ruminantium* (dashed line), *M. smithii* (solid line) and *M. formicium* (dotted line).

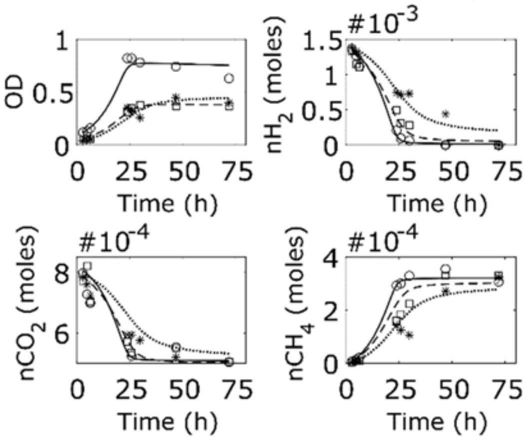


Figure 2 Experimental dynamic data of methanogenesis by *M. ruminantium* (*), *M. smithii* (o) and *M. formicium* (□) are compared against model predicted responses: dotted, solid and dashed lines respectively.

Results & discussion Figure 1 shows the specific energetic dynamics among rumen methanogens. Methanogenesis is characterized by heat flow peaks that account for catabolic and anabolic reactions whose intensities vary between species. Figure 2 illlustrates the capability of the dynamic model for representing growth, substrate utilisation and methane production. Kinetic differences among the three methanogens may be explained by the specific energetics of the microbes.

Conclusion Our study revealed species-specific metabolism kinetics and energetic dynamics among three rumen methanogens. Our dynamic model captured efficiently the dynamics of methanogens metabolism. The results provide new information on the dynamics and ecology of the three archaeal species. Additional experiments with other dominant rumen methanogens will be needed to enlarge the scope of our study and enhance understanding of rumen methanogens ecology. This understanding can be useful to develop novel rumen models with high prediction capabilities.

References

Bricheux G, Bonnet JL, Bohatier J, Morel JP and Morel-Desrosiers N 2013. Ecotoxicology and Environmental Safety 98, 88-94.
Desmond-Le Quemener E and Bouchez T 2014. The Isme Journal 8, 1747-1751.
Muñoz-Tamayo R, Giger-Reverdin S and Sauvant D 2016. Animal Feed Science and Technology 220, 1-21.
Popova M, Morgavi D and Martin C 2013. Applied and Environmental Microbiology 79, 1777-1786.
Thauer RK, Kaster AK, Seedorf H, Buckel W and Hedderich R 2008. Nature Reviews Microbiology 6, 579-591.

Effect of various silage additives applied in corn, alfalfa and whole barley on *in vitro* duodenum utilizable crude protein in dairy cows

Mohsen Danesh Mesgaran, Seyyed Mohsen Hosseni, Alireza Vakili, Abbasali Nasserian

Dept. Animal Science, Ferdowsi University of Mashhad, Mashhad, Iran

E-mail: danesh@um.ac.ir

Take home message Inclusion of urea and formic acid to silages increase utilizable crude protein at duodenum (uCP)

Introduction Silages have become the necessary forage component in the ration of dairy cows over the last few decades (Khan *et al.,* 2015). Silages are often a wise choice when selecting forages for providing fiber and energy needed to optimize rumen function (Kolver *et al.,* 2001). Although protein concentration in silages is much lower than concentrates, dry matter intake of silages is high and therefore protein entering into the digestive tract is considerable. Hence, the importance of inclusion of additives in silages to prevent protein proteolysis is indisputable. Protein utilization in silages is poor and is related to proteolytic activity by crop enzymes and further microbial breakdown of protein during ensilage. Thus, we tested urea (nutrient additive for enrich crude protein) , Biomin® inoculants (fermentation stimulant) and formic acid (fermentation inhibitor) on *in vitro* duodenum utilizable crude protein in dairy cows and its effectiveness.

Material & methods The whole crop corn (hybrid 700) in September 17, 2016 at the 2/3 milk-line stage of kernel maturity, whole barley forage (Hordeum vulgare L.) in April 20, 2016 at dough stage, and alfalfa forage (Medicago sativa L.) in May 10, 2016 in third cutting at the 40% bloom stage were harvested and chopped, then ensiled in laboratory mini-silos and opened after 90 days. Silage additives were urea, formic acid, and Biomin® inoculant (Biomin GmbH, Industriestrasse 213130 Herzogenburg, Austria). The Biomin® inoculant (BioStabil strains), which containing a blend of Enterococcus faecium (DSM 3530), Lactobacillus brevis (DSM 19456) and Lactobacillus plantarum (DSM 19457) was applied in liquid at least 2 x 10^5 colony forming units per gram for both corn and whole barley silages. Formic acid was used at about four litres per ton and 10% higher for alfalfa forage. Urea was added to corn and whole barley at 10.8 and 21.6 g/kg based on dry matter. *In vitro* duodenum utilizable crude protein was determined using the modified Hohenheim gas test (modHGT) based on the method described by Edmunds *et al.,* (2012) to mimic *in vivo* duodenal condition. The modHGT follows procedures of the gas test with a chemical alteration of 2 g/l increase in NH_4HCO_3 and 2 g/l reduction in $NaHCO_3$ in the buffer solution. Gas production (GP) was also recorded at 24 h for use in the calculation of metabolizable energy (ME). NH3-N concentration was determined by using a modified phenol-hypochlorite reaction, and used in the following calculation (Edmunds *et al.,* 2012): uCP (g/kg DM)=((NH3-N_{blank}+N_{sample}-NH3-N_{sample})/weight(mg DM)) × 6.25 × 1000), ME (MJ/kg DM) = 7.81 + 0.07559 GP - 0.00384 Ash + 0.00565 CP + 0.01898 fat - 0.00831 ADF. The Dunnett test was used between a control (untreated) and all other means (treated with additives) to compare differences within each crop. Differences among means were tested using the LSMEANS test.

Results & discussion *In vitro* duodenum utilizable crude protein value was higher in alfalfa silage than those of corn and whole barley silages. Inclusion of urea, regardless of crops and levels, increased uCP at 8 h. and effective uCP at different assumed passage rate. Nevertheless, the ratio of uCP to CP for urea-treated corn and whole barley silages were lower than those of their controls ($P< 0.05$). The ratio of uCP to CP at 48 h. unlike 24 h. was higher in silages treated with 21.6 g/kg urea than respective controls ($P< 0.05$). These various results may be explained by variety of microbial activity to protein degradation during incubation. *In vitro* duodenum utilizable crude protein was not affected by formic acid in whole barley silage, although treated alfalfa silages had higher uCP than control. These results confirmed reports from researchers who noted that an increase in microbial synthesis in rumen and microbial-N flow at the duodenum with addition of formic acid to alfalfa has occurred (Jaakkola *et al.,* 2006). On the other hand, silage treatments with acid might decrease the apparent digestibility of CP in the rumen and elevate the proportion of by-pass protein flowing to the duodenum. Applying inoculant did not result in a consistent change in uCP at 8 and 48 h after incubation in corn and whole barley silages ($P>0.05$), but effective uCP was influenced in treated whole barley silage. Metabolizable energy was lower in formic acid treated-alfalfa silage, but inclusion of urea and inoculant had no significant effect on metabolizable energy.

Conclusion In conclusion, urea and formic acid additives may improve animal performance by increasing utilizable CP entering at intestine.

Acknowledgements Researchers gratefully acknowledge funding from department of animal science, Ferdowsi University of Mashhad, Iran.

References

Edmunds B, Sudekum KH, Spiekers H, Schuster M and Schwarz FJ 2012. Animal Feed Science and Technology 175, 106-113.
Jaakkola S, Kaunisto V and Huhtanen P 2006. Grass Forage Science 61, 282-292.
Khan NA, Yu P, Ali M, Cone JW and Hendriks WH 2015. Food Science and Agriculture 95, 238-252.
Kolver ES, Roche JR, Miller D and Densley R 2001. Proceedings of the New Zealand Grassland Association. New Zealand. 63, 289–293

The relationship between efficiency of microbial crude protein production and rumen microbial community structure in steers fed tropical pastures

Karen Harper[1], Tanda Panjaitan[2], Lisa Gulino[3], Simon Quigley[1], Athol Klieve[1], Diane Ouwerkerk[3], Dennis Poppi[1]

[1]*School of Agriculture and Food Science, The University of Queensland, Gatton, Queensland, Australia,* [2]*Balai Pengkajian Teknologi Pertanian Nusa Tenggara Barat, Lombok, Indonesia,* [3]*Queensland Department of Agriculture and Fisheries, Queensland, Australia*

E-mail: karen.harper@uq.edu.au

Take home message The supplementation of spirulina algae to steers fed tropical pastures, significantly increased the efficiency of microbial crude protein production, however there was no simple, direct relationship between this increase in efficiency and the increase in diversity of the rumen bacterial community.

Introduction Microbial crude protein (MCP) production is the major protein supply to the host, and can provide up to 100% of the protein supply to the host under low CP diets. MCP is a function of the metabolisable energy intake and efficiency of MCP production (EMCP), therefore an important strategy to optimize MCP is to maximize EMCP. In practice EMCP of tropical forages rarely exceeds 130 g MCP/kg digestible organic matter (DOM). This value is the average value in the feeding standards when rumen degradable nitrogen (RDN) is adequate (SCA 2007). Various forms of supplementation to tropical forages have provided only limited improvements in EMCP values when RDN is adequate. An exception to this is the supplementation of Spirulina algae (*Spirulina platensis*) to Mitchell Grass (*Astrebla* spp.) in steers (Panjaitan *et al.,* 2015). Supplementation of Spirulina at 5.7 g DM/kg weight (W).day (equivalent to 290 g RDP/kg DOM) resulted in an EMCP value of 166 g MCP/kg DOM compared to control steers. The supplementation with Spirulina provides a model to compare the rumen microbiome in steers supplemented with different N sources and with resultant different EMCP.

Material & Methods Details of the animal trial are found in Panjaitan *et al.,* (2015). Briefly the dietary treatments were; a control of Mitchell Grass fed *ad libitum* (3.8% CP forage with 56 g RDP/kg DOM) and supplementation with increasing levels of Spirulina (0, 0.5, 1.4, 2.5 and 6.1 g DM/kg W.day). At the end of each experimental period, rumen fluid was collected, DNA extracted (Yu and Forster 2005) and PCR was performed using fusion primers (Integrated DNA technologies) based on 16S rRNA gene primers 341f and 787r. Pyrosequencing was undertaken on a Roche 454 Genome Sequencer FLX (Roche Diagnostics Corporation, Mannheim, Germany) at the Australian Genomic Research Facility at the University of Queensland. Data was analysed using the QIIMEs pipeline of software (Caporaso *et al.,* 2010). Statistical analyses of alpha diversity indices were performed using ANOVA with orthogonal terms in GenStat.

Results & discussion The supplementation of Spirulina was accompanied with large changes in rumen metabolic parameters, such as increases in EMCP, DOM intake, digestibility, digestion rate and passage rate, and a decrease in digesta retention time in the rumen (Panjaitan *et al.,* 2015). The addition of Spirulina significantly increased the richness and diversity of bacteria in the rumen and reflected the more complex nutrient input associated with Spirulina supplementation. There was, however, no simple direct relationship between diversity indices and EMCP, for diversity peaked at 130 g RDP/kg DOM while EMCP peaked at 290 g RDP/kg DOM. The lower retention times associated with the 170 and 290 g RDP/kg DOM spirulina treatments may have caused the reduction in the richness of the microbial community. Providing stability to the rumen bacterial community was a large diverse core group of 63 shared bacterial species which were identified in liquid phase of all sampled steers. This core group of bacteria was unchanged even though rumen dilution rates and true protein intake were significantly increased in the high spirulina treatments.

Conclusion The improvements in EMCP in this experiment, were related more to a mass action effect of the rumen bacterial community, due to more nutrients and better growth conditions in the rumen, rather than to major changes of the microbial community structure.

Acknowledgements This work was supported by the Meat and Livestock Australia and the University of Queensland.

References

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. Nature Methods 7, 335-336.
Panjaitan T, Quigley SP, McLennan SR Swain T and Poppi DP 2015. Animal Production Science 55, 535-543.
SCA (2007) Feeding standards for Australian livestock. Standing committee on Agriculture, CSIRO. Australia.
Yu Z and Forster RJ 2005. Nucleic acid extraction, oligonucleotide probes and PCR methods. In Methods in Gut Microbial Ecology for Ruminants (eds. H.P.S. Makkar and C.S. McSweeney), pp. 81–104. Springer Netherlands, Dordrecht.

Ruminal methanogens diversity in finishing grazing beef cattle supplemented with encapsulated nitrate

Yury Tatiana Granja Salcedo[1], Rodolfo Maciel Fernandes[1,2], Rafael Cononenco de Araujo[3], Gustavo Rezende Siqueira[1,2], Flávio Dutra de Resende[1,2], Luciano Takeshi Kishi[1], Telma Teresinha Berchielli[1]

[1]Faculdade de Ciências Agrárias e Veterinárias (FCAV), Univ Estadual Paulista–UNESP, Jaboticabal, São Paulo, Brazil, [2]Agência Paulista de Tecnologia dos Agronegócios (APTA), Colina, São Paulo, Brazil, [3]GRASP Ind. & Com. LTDA, Curitiba, Paraná, Brazil

E-mail: yurygranjasalcedo@gmail.com

Take home message Encapsulated nitrate reduces *Methanobrevibacter* abundance in finish grazing beef cattle.

Introduction Nitrate is an effective methane inhibitor and potential nitrogen source of non-protein nitrogen for cattle (Zhao *et al.*, 2015). However, the knowledge about how the archaea community respond to nitrate and products of its reduction have been limited. Thus, our study aimed understand the effects of encapsulated nitrate on rumen methanogens diversity in grazing beef cattle.

Material & methods Experiment were conducted during 432 days at ATPA in Colina, SP, Brazil. We used twelve castrated Nellore steers fitted with ruminal silicone cannula and allocated in twelve *Brachiaria brizantha cv. Marandu* paddocks distributed in a complete randomized block (two experimental areas) design, with two supplements: without nitrate with nitrogen equivalent substituted by urea (Control) and with 70 g of encapsulated nitrate/100 kg BW, corresponding to 47 g NO3- / 100 kg BW (EN). Nitrate source was the double salt of calcium nitrate and ammonium decahydrate (GRASP Ind. & Com. LTDA). Samples of 60 g per animal (a mix of liquid and solid) from the dorsal, central, and ventral regions of the rumen were collected through the ruminal cannula, immediately a Fast DNA SPIN Kit for Soil extraction kit was used to extract metagenomic DNA from 200 mg of sample. DNA concentrations were measured spectrophotometrically and fluorometrically. Archaeal 16S rRNA gene amplicons were generated using primers by Kittelmann *et al.,* (2013) to amplify the V6 to V8 regions. Sequencing was performed using the Ion Torrent Personal Genome Machine using the Ion 314™ Chip Kit v2 at the LMseq at FCAV in Jaboticabal, Brazil. Quantitative Insights Into Microbial Ecology (QIIME) software package version 1.9.1 were used to filter reads and determine Operational Taxonomic Units (OTUs) and the Usearch algorithm was used to cluster the reads OTUs with a 97% cutoff, and to assign the taxonomy using the Ribosomal Database Project (RDPII) version 10. Statistical analyses were performed with the assistance of R software, differences were considered significant at $p < 0.05$ using the Wilcoxon test.

Results A mean of 3131 ± 589 sequences per sample were produced. Total OTUs (467±51), Chao (518.47±34) and Simpson (0.96±0.01) indices were not affected by EN supplementation ($P > 0.05$). However, comparison of the archaeal communities by principal coordinate analysis (PCoA) using the weighted Unifrac distance (Figure 2), explains 56.14% of the variation in the data and confirmed a separation between Control and EN groups. *Euryarchaeota* was the only phyla identified and 11% of the sequences could not be classified at the phylum level. *Methanobrevibacter* was the most abundant genus in the archaeal community (95.30 ± 5.07%), followed by *Methanomassiliicoccus* (3.14 ± 1.31%) and *Methanobacterium* (1.79 ± 0.78%). The relative abundance of *Euryarchaeota* phylum ($P = 0.028$) and *Methanobrevibacter* genus ($P = 0.007$) were lesser in the rumen of steers supplemented with EN (Figure 2).

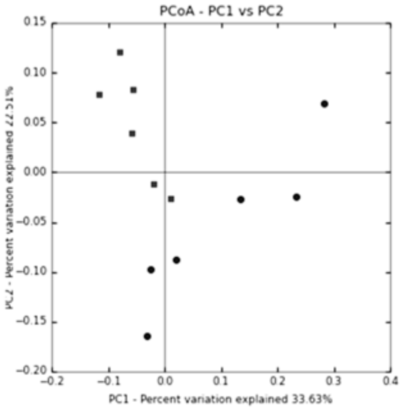


Figure 1 Weighted Unifrac diversity PCoA to explore dissimilarities in microbial composition among Control (square) and encapsulate nitrate (circle) groups.

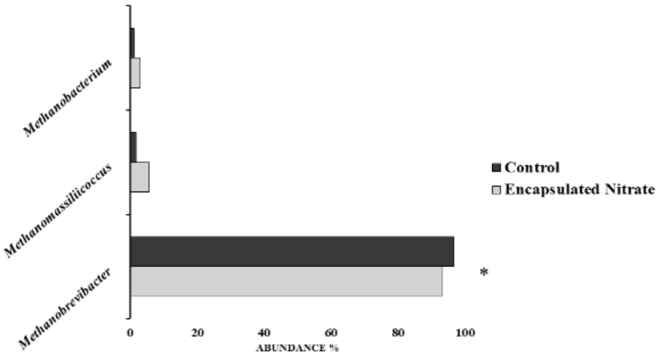


Figure 2: Ruminal methanogens abundance at genus level in finish grazing beef cattle supplemented with encapsulated nitrate. * $p < 0.05$ using Wilcoxon test.

Conclusion Encapsulated nitrate supplementation does not affect richness and alpha diversity indices in the rumen of grazing beef cattle. However, it does have a negative effect on the *Euryarchaeota* phylum and *Methanobrevibacter* genus.

Acknowledgements This research was supported by grants from São Paulo Research Foundation (FAPESP).

References

Kittelmann S, Seedorf H, Walters WA, Clemente JC, Knight R, Gordon JI and Janssen PH 2013. PloS One 8: e47879.
Zhao L, Meng Q, Ren L, Liu W, Zhang X, Huo Y and Zhou Z. 2015 Asian-Australasian Journal of Animal Sciences 28, 1433-1441.

Effect of lipid availabilities and crude glycerine association in the diet of feedlot beef cattle on rumen biohydrogenation

Yury Tatiana Granja Salcedo, Arturo Samuel Gomez Insuasti, Kenia Larissa Gomes Carvalho Alves, Bruna Gabriela Bosquini, Raquel de Oliveira Andrade, Yasmin Andrade Pinheiro, Juliana Duarte Messana, Yeison Fabian Murillo Alfonso, Telma Teresinha Berchielli

Faculdade de Ciências Agrárias e Veterinárias (FCAV), Univ Estadual Paulista–UNESP, Jaboticabal, São Paulo, Brazil

E-mail: yurygranjasalcedo@gmail.com

Take home message Calcium salts in cattle may be a strategy to increase the deposition of healthy unsaturated fatty acids in meat.

Introduction Dietary strategy to reduce the biohydrogenation (BH) extent includes the use of vegetables oils associated with glycerine (CG) which can restrict the rumen BH in high concentrate diets (Granja-Salcedo *et al.,* 2017). Thus, we evaluated the effect of different lipid rumen-availabilities associated with CG in the diet on rumen BH in feedlot beef cattle.

Material & methods We used eight castrated Nellore steers (BW 554.13 ± 18 kg) fitted with a duodenal silicone cannula, distributed in a double, simultaneous, Latin square 4 × 4 design with four treatments (diets) and four experimental periods (20 days). Diets were formulated for a gain of 1.25 kg/day and had 70% of concentrate using corn silage as source of roughage. Concentrates contained ground corn, urea (10 g/kg), mineral salts (30 g/kg), and different lipid availabilities, as follows: no additional fat (CO), whole soybeans (WS), soybean oil (SO), and calcium salts (CS) (Megalac-E®), all diets contained 100 g/kg dry matter (DM) of CG (Table 1). Duodenal content (100 mL) samples were obtained by cannula and were collected at 6-h intervals on the 19th and 20th day of each experimental period. The sample collection during the 20th day was delayed ensuring that every 3 h slot in a 24 h period was properly represented. Indigestible neutral detergent fibre (NDFi), an indicator of the daily flow of DM in the duodenum, was assessed by in situ incubation of feeds offered, leftovers, faeces, and duodenum samples for 288 hours. Total fatty acids (FA) were extracted from the feed ingredients, leftovers and duodenum samples using the methylation method (Palmquist and Jenkins, 2003). 1 mL of methyl nonadecanoate (C19:0) at 2.0% was added to each sample, which was used as the internal standard for FA quantification. FA profile was quantified by gas chromatography (GC Shimatzu model 20-10, automatic injection) using a SP-2560 capillary column (100 m × 0.25 mm diameter, 0.02 mm thick), and hexane as carrier gas. Data were analysed as a double 4 × 4 Latin square design balanced for residual effects using the PROC MIXED procedure of SAS. Diet effects were studied by three orthogonal contrasts.

Results Additional fat in the diet increased the ruminal BH rates of linoleic acid (C18:2 cis 9 cis 12), MUFA, PUFA, and UFA (Table 2; $P < 0.05$). In addition, SO diet exhibited a higher rate of ruminal BH of oleic acid (C18:1 cis 9), linoleic acid (C18:2 cis 9 cis 12), and PUFA ($P < 0.05$). CS diet resulted in lower ruminal BH rates of octadecenoic acid (C18:1 trans 6), oleic acid (C18:1 cis 9), linolenic acid (C18:3 cis 9 cis 12 cis 15), MUFA, and total UFA than WS ($P < 0.05$).

Table 1 Experimental diets: proportion of ingredients, bromatologic composition, and fatty acid (FA) profiles.

	CO	WS	SO	CS
Corn silage, % DM	30.00	30.00	30.00	30.00
Ground corn, % DM	47.89	46.48	45.71	45.40
Soybean meal, % DM	8.11	-	8.56	8.63
Whole Soybean, % DM	-	9.52	-	-
Soybean oil, % DM	-	-	1.73	-
Rumen - protected fat, % DM	-	-	-	1.97
Crude Glycerine, % DM	10.0	10.0	10.0	10.0
Dry matter, % DM	69.03	68.27	68.03	67.65
Crude protein, % DM	16.44	17.09	15.52	15.91
Ether extract, % DM	3.74	5.26	4.98	4.79
Metabolizable energy, MJ/kg DM	12.17	12.55	12.47	12.43
C18:1 n-9, g/kg DM	4.51	7.67	7.47	6.68
C18:2 n-6, g/kg DM	7.49	16.63	16.86	17.54
C18:3 n-3, g/kg DM	1.12	1.96	1.56	1.51
Total UFA, g/kg DM	17.06	34.74	33.68	33.26

Table 2 Rate of ruminal biohydrogenation (BH) of fatty acids (% of total ingested) in beef cattle fed different lipid availabilities associated to crude glycerine.

	Diet*				SEM	P-value[1]		
	CO	WS	SO	CS		FS	PF	PT
C18:1 *t-6*	66.45	71.33	62.50	60.71	66.45	0.183	0.144	<0.001
C18:1 n-9	42.97	51.98	53.98	42.84	42.97	0.362	0.041	0.007
C18:2 n-6	74.56	85.70	93.69	86.14	74.56	<0.001	<0.001	0.264
C18:3 n-3	52.29	55.46	30.02	17.68	52.29	0.216	0.124	<0.001
MUFA	3.10	25.03	14.15	5.24	5.25	0.011	0.174	<0.001
PUFA	71.64	82.50	88.30	80.73	71.64	<0.001	0.027	0.641
Total UFA	37.54	56.65	56.77	50.79	51.12	<0.001	0.137	0.015

[1] Orthogonal contrasts: FS= fat supplementation effect (CO vs. WS + SO + CS); PF= rumen-protected effect (SO vs. WS + CS); PT= protection type effect (WS vs. CS). * all diets contained crude glycerine.

Conclusions In feedlot diets, CG and rumen-protected forms of lipids association do not always prevent the ruminal BH. However, the association of CG and calcium salts in beef cattle may be a nutritional strategy to increase the deposition of healthy UFA in meat and limit the BH of unsaturated FA.

Acknowledgements: This research was supported by grants from São Paulo Research Foundation (FAPESP).

References

Granja-Salcedo YT, Souza VC, Dias LAV, Gomez IAS, Messana JD and Berchielli TT. 2017 Animal Feed Science and Technology 225, 195-204

Palmquist DL, Jenkins TC. 2003 Journal of Animal Science 81, 3250–3264.

The interaction of rumen degradable protein and rumen fluid dilution rate on efficiency of microbial protein production in the rumen of sheep and cattle

Leandro Martins, Simon Quigley, Stuart McLennan, Dennis Poppi

University of Queensland, Gatton, Queensland, Australia

E-mail: d.poppi@uq.edu.au

Take home message There is large variation in efficiency of microbial protein production in the rumen which can be exploited but is not explained simply by rumen degradable protein (RDP) or rumen fluid dilution rate

Introduction Microbial protein (MCP) provides most of the metabolisable protein to the host but the efficiency of microbial protein production (eMCP) varies widely, in part due to limitations in RDN but, even when that is adequate, there is still large variation. In the various feeding standards this is explained by the need for a proportion of degradable protein in the diet and also because of effects of intake on rumen fluid dilution rate. The feeding standards calculate that eMCP varies from 130 to 170 g MCP/kg digestible organic matter (DOM) (SCA 2007) under adequate RDP but tropical forages promote low values often <130 g MCP/kg DOM (Bowen et al 2016). The interaction between RDP and rumen fluid dilution rate was investigated to explain and develop better predictive equations.

Material & methods Databases were searched and papers that yielded data about rumen water retention time (RWRT, or dilution rate), levels of RDP intake and digestible organic matter (DOM) intake and eMCP were used in the subsequent analyses. Information was also collected on crude protein, organic matter, neutral detergent fibre, estimated RDP/CP, organic matter digestibility, NDF digestibility, dry matter intake and live weight for both sheep and cattle. In total, 34 journal articles and 4 theses from UQ library met the criteria of having values for eMCP, RDP, RWRT and NDF content in the same experiment which resulted in 173 individual data (treatments) and 1274 experimental units. Linear models of polynomial regression were used, in order to describe the influence of NDF, RWRT and protein on the eMCP in the rumen. The analyses were made in the PROC REG procedure of the SAS (Statistical Analysis System, version 9.2) software. The function WEIGTH was used to number the experimental units, in the different treatments from different studies.

Results & discussion The average of eMCP from all collected data was 123 g MCP/kg of DOM varying from 26 to 239 g MCP/kg DOM. A linear model explained some of the variation where eMCP = 46.191 + 0.170 RDP + 12.716 RWRT – 0.465 RWRT^2 – 0.619 NDF

r^2= 0.47. Whilst the mean value for eMCP agrees with various feeding standards the variability is large and not explained very well by using RDP, RWRT and NDF suggesting that other unknown factors (eg. other nutrients or rumen microbiome) are equally important. RDP showed the greatest correlation with the eMCP. In the presented model, the maximum eMCP was reached with RDP and CP values of 480.8 g RDP/kg DOM and 443.8 g CP/kg DOM, respectively, which represent diets with 288 g RDP and 266 g CP/kg DM. Using high levels of RDP to increase eMCP is a very inefficient and undesirable approach. An important modulator of eMCP is the rumen dilution rate or RWRT, and in the present study this variable presented a quadratic effect when correlated with the eMCP, showing small variation in the maximum eMCP between 6 and 8 hours of retention time, but a big decrease in the eMCP when RWRT increased from 8 to 24 hours. The interest here is in the quantitative values where temperate forages tend to have low RWRT whilst tropical forages have much higher values.

Conclusion RDP is the most important modulator of eMCP with maximal values reached at very high excess RDP which unfortunately is not a practical form of manipulation. Similarly RWRT affects eMCP in a quadratic fashion increasing as RWRT decreases. NDF appears to have a linear negative effect on EMCP as NDF increases.

Acknowledgements Leandro Martin was supported by Universidade Federal de Viçosa and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the award of a scholarship and hosted by The University of Queensland.

References

Bowen MK, Poppi DP and McLennan SR 2017. Animal Production Science 57, 1702.
SCA (2007) Feeding standards for Australian livestock. Standing committee on Agriculture, CSIRO. Australia.

Evaluation of nutrient composition and *in vitro* digestibility of *Vigna radiata* straw using thin tailed sheep rumen fluid as microbial source

Nafiatul Umami, Bambang Suhartanto, Bambang Suwignyo

Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia

E-mail: nafiatul.umami@ugm.ac.id

Take home message Indonesia has several local varieties of Mung Bean (*Vigna radiata*). This plant can be planted with the primary product for food and straw as feed. Thin tailed sheep as local sheep from Indonesia can use this straw as feed.

Introduction One species of legumes as crop and forage were known as Mung bean (*Vigna radiata*), mainly grown in the tropical area of Asia, is a crucial summer-growing, annual plant. *Vigna radiata* has a short growing period and is the relatively drought-tolerant crop. Mung bean originated in South and Southeast Asian regions. It is widely grown in Indonesia, Thailand, Myanmar, India, Pakistan, Bangladesh, Philippines, and China (Ullah *et al.,*) 2004). Indonesia has several *Vigna radiata* varieties namely Vima, Kenari, and Yogya. The information about specific nutrient characteristic was important because affected by production and quality. The chemical composition of forage is crucial, particularly in combination with *in vitro* digestibility, to evaluate the nutritive value of legume species, which are palatable for thin-tailed sheep in Indonesia. *In vitro* digestion techniques using rumen liquor as a microbial inoculum (Tilley and Terry, 1963) have proved useful in examination examining the digestibility of many feeds. This research was aimed to investigate the nutrient composition and *in vitro* digestibility of *Vigna radiata* straw using rumen fluid of thin tail sheep (local Indonesian sheep).

Material & methods Green Bean (*Vigna radiata*) straw samples (Vima, Kenari and Yogya) produced at Yogyakarta Indonesia were tested. Samples were ground in a hammer mill and analysed for dry matter (DM), crude protein (CP, Kjeldahl N×6.25), ash, ether extract (EE) and crude fibre (CF) according to the methods outlined by the AOAC (2000). Samples of *Vigna radiata* straw (0.5 g) were added to vessels with thin tailed sheep rumen fluid (10 ml) and buffer solution (40 ml) in the first batch fermentation. At the end of the first batch fermentation, supernatant were discarded and added pepsin solution (50 ml) then incubated for 48 h and dry matter (DM) and organic matter (OM) digestibility was determined.

Results & discussion Three varieties of *Vigna radiata* were used to test straw production in Yogyakarta Indonesia. The results showed that Vima produced 440.38 g/m^2 of dry matter yield. It was the highest dry matter yield production ($P<0.01$). All the samples had similar organic matter content. The crude protein content of *Vigna radiata* straw ranged from 124.1 g/kg for Kenari and 147.7 g/kg for Yogya. *In vitro* dry and organic matter digestibility was the lowest for Kenari compared Vima and Yogya ($P<0.01$). It was due to the fiber content. The component of fiber include cellulose, hemicellulose and lignin. Increased fiber content can decreased the digestibility. Feed containing high fiber could interfere feed digestibility because it need a lot of energy to be digested.

Table 1 Chemical composition (g/kg dry matter) and *in vitro* digestibility (g//kg) of several varieties of *Vigna radiata* straw on 60 days after planting.

Variety	DM	OM	CP	CF	EE	Ash	IVDMD	IVOMD
Vima	470.2	864.4	142.6^b	84.9^b	42.7^a	135.6	683.0^a	665.3^a
Kenari	504.0	880.0	124.1^a	48.0^a	63.4^b	120.0	775.6^b	767.7^b
Yogya	584.8	893.8	147.7^b	47.8^a	63.6^b	110.3	781.2^b	769.6^b
SEM	40.1	50.7	10.1	2.6	2.3	18.0	56.5	49.0

$^{a, b}$: the different superscript in the same column indicated significant different ($P<0.01$).

DM= dry matter, OM= organic matter, CP= crude protein, CF= crude fiber, EE= extract ether, IVDMD= *invitro* dry matter digestibility, IVOMD= *in vitro* organic matter digestibility. SEM= Standard error of the means.

Conclusion Based on the research, it could be concluded that of the *Vigna radiata* straw varieties the Yogya variety had the highest CP and the same *in vitro* dry matter and organic matter digestibility as the Kenari variety.

Acknowledgements This research was supported in part by Fundamental research program Faculty of Animal Science.Universitas Gadjah Mada Indonesia.

References

AOAC. 2000. Official methods of analysis. Association of Official Analytical Chemists. 17th ed. Arlington, VA, USA.
Tilley JMA and Terry RA 1963. Grass and Forage Science. 18, 104-111
Ullah R, Zain U, Muhamad A and Akash T 2014. The Scientific World. 2014, ID 871753 1-5.

The degradation of lignocellulose was higher in the enriched co-culture of ruminal fungi and methanogens than the enriched co-culture of bacteria and methanogens

Yuanfei Li, Wei Jin, Yanfen Cheng, Weiyun Zhu

National Center for International Research on Animal Gut Nutrition, Jiangsu Key Laboratory of Gastrointestinal Nutrition and Animal Health, Laboratory of Gastrointestinal Microbiology, Nanjing Agricul, Nanjing, China

E-mail: 373366867@qq.com

Take home message Lignocellulose degradation by enriched co-culture of ruminal fungi and methanogens was significantly higher than the enriched co-culture of bacteria and methanogens.

Introduction The rumen is a complicated microbial ecosystem, which includes anaerobic fungi, bacteria, protozoa and methanogens. Ruminants themselves cannot utilize the lignocellulosic plant biomass as they have no ability to produce biomass-degrading enzymes. On the other hand, the symbiotic microorganisms can hydrolyze the biomass to generate energy for themselves, as well as for the host ruminants. Reports have indicated that anaerobic fungi contributed to greater degradation of plant cell walls compared to bacteria as revealed by selecting the microbial groups in rumen fluids (Akin *et al.,* 1989; Lee *et al.,* 2000). However, the metabolic profiles and extent of lignocellulosic degradation by anaerobic fungi and bacteria in the presence of aboriginal methanogens are unclarified. Thus, the objective of the present study was to evaluate *In vitro* degradation of crude lignocellulose by ruminal fungi and bacteria in the presence of ruminal methanogens and their metabolic profiles.

Material & methods Rumen contents were collected from four crossbred boer-goat (body weight 26.0±1.8 Kg), fistulated a permanent cannula into rumen, and mixed up at 2 h before the morning feeding (forage to concentrate mixtures 7:3). Medium was prepared according to Davies *et al.,* (1993) with 1% (w/v) rice straw or wheat straw as carbon source. Antibiotics were used to select the microbial groups by adding into the autoclaved fresh media prior to inoculation as follows: (a) whole ruminal content (WRC) cultures, without adding antibiotics; (b) bacterial-methanogen (B+M) enriched cultures, adding cycloheximide to the final concentration of 0.25 mg/ml; (c) fungal-methanogen (F+M) enriched cultures, adding penicillin plus streptomycin to the final concentrations of 1915 U ml^{-1} and 2031 U ml^{-1}, respectively. The bottles were incubated at 39 °C for 70 h. At intervals, the gas production and methane were monitored. Aliquots of 10 ml supernatant were collected for the analysis of metabolites (short-chain fatty acids, NH_3-N and lactate). Aliquots of 7 ml cultures were collected for DNA extraction and subsequently for the analysis of microbial abundance. The residual substrates were collected for the analysis of DM, NDF and ADF. For comparison among groups, the statistically significant differences between mean values of digestibility of subtrates, gas and methane production, concentrations of volatile fatty acids, lactate and NH_3-N, and quantitative real-time PCR data were analyzed using one-way analysis of variance (ANOVA) (SPSS v.20, SPSS, Chicago, IL, USA). Multiple comparisons among means were carried out by Duncan-test when significance was detected.

Results Gas production from F+M was significantly higher than that from B+M ($P<0.05$) irrespective of the substrate. In both rice and wheat straw, methane production in F+M group was significantly higher than that from B+M and WRC ($P<0.05$). For rice and wheat straw, DM digestibility (DMD), NDF digestibility (NDFD), acid detergent solution digestibility (ADSD) and ADF digestibility (ADFD) in F+M group were comparable with WRC group, and were significantly higher than that from B+M ($P<0.05$). Additionally, in wheat straw, ADSD of F+M group was significantly higher than that of WRC group ($P<0.05$). For short-chain fatty acids (C_2-C_5), in both rice and wheat straw, only acetate and butyrate were accumulated in F+M group, whereas C_2-C_5 volatile fatty acids were substantially accumulated in B+M and WRC group. Acetate and formate accumulated in F+M group were dramatically higher than that in B+M and WRC group ($P<0.05$). However, concentration of NH_3-N in F+M group was significantly lower than that in B+M and WRC group ($P<0.05$). In F+M group, the respective population mean sizes were 2.51×10^6, 1.26×10^9 and 2.34×10^9 copy number / ml for anaerobic fungi, methanogens and bacteria in rice straw and 2.04×10^6, 9.12×10^8 and 1.66×10^9 in wheat straw. In B+M group, the mean number of anaerobic fungi, methanogens and bacteria was 8.51×10^3, 6.92×10^8 and 4.07×10^9 in rice straw and 1.10×10^4, 9.12×10^8 and 6.46×10^9 in wheat straw. In WRC group, the population of anaerobic fungi, methanogens and bacteria was 2.34×10^4, 4.27×10^8 and 3.24×10^9 copy number / ml in rice straw and 2.88×10^4, 3.72×10^8 and 3.16×10^9 in wheat straw.

Conclusions In the presence of ruminal methanogens, enriched anaerobic fungi had significantly higher degradation of rice and wheat straw compared with ruminal bacteria. The metabolic profile of F+M group was distinctly different from that of B+M and WRC group. The enhanced growth of methanogens in F+M group resulted in the promoted methane production, which revealed a synergistic interaction between anaerobic fungi and methanogens. Overall, anaerobic fungi played very important roles in cell wall degradation in the presence of ruminal methanogens. Further studies on the interaction between anaerobic fungi and methanogens are needed to investigate the promotion of lignocellulose degradation and the reduction of methane emissions.

Acknowledgements This research was funded by National Key Research and Development Program of China (2017YFD0500505) and the Natural Science Foundation of China (31772627).

References

Akin DE, Lyon CE, Windham WR and Rigsby LL. 1989. Applied and Environmental Microbiology 55, 611-616.
Davies DR, Theodorou MK, Lawrence MI and Trinci AP 1993. Microbiology 6, 1395-400.
Lee SS, Ha JK and Cheng KJ 2000. Applied and Environmental Microbiology 66, 3807-3813.

New fibrolytic enzymes as pre-treatment in dairy cow diet

B. Refat[1], D.A. Christensen[1], J.J. McKinnon[1], W. Yang[3], A.D. Beattie[2], T.A. McAllister[3], Peiqiang Yu[1]

[1]*Department of Animal and Poultry Science, College of Agricultural an Bioresources, University of Saskatchewan, Saskatoon, Canada,* [2]*Crop Development Center, Department of Plant Sciences, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, Canada,* [3]*Lethbridge Research and Development Center, Agriculture and Agri-Food Canada, Lethbridge, Canada*

E-mail: peiqiang.yu@usask.ca

Take home message Based on the current study, the optimum dosage of the new fibrolytic enzymes was 0.75 mL enzyme per kg of total mixed ration on dry matter basis. Applying this dosage improved fiber digestibility, and fat-corrected milk yield. The feed efficiency increased by up to 7% without any increase in dry matter intake compared to the control diet.

Introduction Recently, several new barley forage varieties have been developed in Western Canada. In previous studies, three newly developed barley forage varieties were selected based on their varying rate of *in vitro* NDF digestibility (NDFD) to study the effects of barley silage on lactational performance of dairy cows (Refat *et al.,* 2017) and growth performance of beef cattle (Nair *et al.,* 2017). To our knowledge, there is no available report documenting lactational performance response by cows fed with new fibrolytic enzymes applied to specific barley variety. The objectives of this study were to evaluate the effects of pre-treating dairy cow rations with a fibrolytic enzyme (FE) obtained from Trichoderma reesei-derived fungal extract on lactational performance, digestibility, and feeding behavior in response to feeding barley silage-based diet.

Material & methods Before starting the dairy trial, *in vitro* incubations were conducted to select the appropriate doses (0, 0.25, 0.5, 0.75, 1, and 1.25 ml of FE / kg dry matter (DM) of silage) with the newly developed barley silage for the dairy performance and metabolic studies. FE was obtained from Trichoderma reesei-derived fungal extract (VistaPre-T, ABVista, Wiltshire, UK). The dairy trial was performed using eight Holstein dairy cows. The cows were blocked by their parity and assigned randomly to one of 4 treatments: 0, 0.5, 0.75, and 1 mL of FE / kg DM of diet in a replicated Latin square design. The pre-treatment was applied to the diet during the mixing process of diets and then TMR diet stayed for one hour before feeding. The data were analyzed using Proc Mixed SAS 9.4. Polynomial contrasts were used to determine the curvilinear effect of increasing the amount of FE application.

Results & discussion The addition of FE linearly increased *in vitro* DM digestibility and tended to improve the *in vitro* neutral detergent fiber (NDF) digestibility in barley silage. Applying FE quadratically increased yields of fat-corrected milk and energy-corrected milk, and tended to increase feed efficiency. The milk fat yield was also quadratically increased by the addition of FE. The milk protein concentration linearly improved by applying the FE.

Conclusion In conclusion, dairy cows fed pre-treated barley silage-based diet with FE extracted from Trichoderma reesei-derived fungal extract increased feed efficiency without affecting DM intake. This positive effect of adding FE could benefit the dairy industry in Western Canada where barley silage-based diets are common. Further studies are warranted to evaluate the effect of FE on lactational performance of dairy cows during early lactation, as dairy cows typically face many metabolic challenges.

Acknowledgements The authors would like to thank Niu and Morgen Horbin for their helpful assistance in the project. Financial support for SRP Feed Research Chair (Dr. P. Yu) from the Saskatchewan Ministry of Agriculture Strategic Research Chair Programs in Feeds R&D, the Natural Sciences and Engineering Research Council of Canada, the Saskatchewan Agriculture Development Fund, SaskMilk, the Saskatchewan Forage Network, and Western Grain Research Foundation is acknowledged. The authors acknowledge AB Vista (Wiltshire, UK) for providing samples of their commercial products for use in this study.

References

Nair J, Beattie AD, Christensen D, Yu P, McAllister T, Damiran D and McKinnon JJ 2018. Canadian Journal of Animal Science 98, 299–310.

Refat B, Christensen DA, Mckinnon JJ, Prates LL, Nair J, Beattie AD, Yang W, McAllister TA and Yu P 2017. Canadian Journal of Animal Science 98, 177–186.

Eco-evolutionary association of rumen methanogens and acetogens with their hosts

Zhipeng Li[1], Antton Alberdi[2], André-Denis G Wright[3], Shengyong Mao[4], Leluo Guan[5], Guangyu Li[1]

[1]Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences, Changchun, China, [2]University of Copenhagen, Copenhagen, Denmark, [3]University of Arizona, Tucson, USA, [4]Nanjing Agricultural University, Nanjing, China, [5]University of Alberta, Edmonton, Canada

E-mail: zhplicaas@163.com

Take home message In this study, we firstly report the presence of core methanogens (methane producing archaea) and acetogens (acetate producing bacteria) inhabiting the rumen of 14 ruminants distributed across three families. Secondly, we show that there is no compositional interdependence between both microbial communities, and that methanogenic and acetogenic profiles are host species-dependent. Lastly, the co-occurrence analysis reveal that interactions between microorganisms and metabolites is changed with the methane yield of the host species.

Introduction Climate change resulting from greenhouse gases (CHG), such as carbon dioxide (CO_2) and methane (CH_4), is one of the major challenges facing humans. Although most climate change policies target CO_2, CH_4 accounts for about 10% of all greenhouse gas emissions, and about one third of CH_4 emissions result from digestion of plant compounds in the rumen of livestock ruminants. Methanogens in rumen are the main contributors of enteric methane by reduction of hydrogen, while reductive acetogenesis by acetogens is a possibly alternative sink to dispose hydrogen. Thus, a thorough understanding of the association between methanogens and acetogens is crucial to reduce methane emissions.

Material & methods We investigated and compared the rumen methanogen and acetogen communities in 97 individuals from 14 ruminant species, including 9 deer species (Cervidae), 2 bovid species (cattle and sheep), and 1 moschid species (forest musk deer), based on the methanogens 16S rRNA and *acsB* genes using next generation seqeuncing. We examined if rumen methanogen and acetogen populations display parallel arrangements and aligned with their host phylogenies, and elucidating if the interactive relationships between the methanogens and acetogens change according to their host's methane emissions.

Results & discussion *Methanobrevibacter* spp. and acetogens associated with Eubacteriaceae were identified as core methanogens and acetogens, respectively, while some other methanogens and acetogens exhibited host specificity. Acetogen and methanogen communities exhibited no compositional correlation across the studied ruminants, and host phylogenetic signal on the composition of both communities was also absent. However, the acetogen community composition and the *Methanobrevibacter gottschalkii* abundance showed positive and negative correlation with the papillation degree of the rumen wall, respectively. Finally, co-occurrence showed the variation of the estimated methane yields was characterized by the interactive patterns between methanogens, acetogens, and metabolites.

Conclusion Our results show that rumen methanogens and acetogen communities have low compositional interdependence and do not parallel host evolution, suggesting the strategies for mitigating methane production should be based on a species-specific community and interaction in rumen.

Acknowledgements This work was supported by the Natural Science Foundation of China (NSFC) (31501984), and Natural Science Foundation of Jilin Province (20170101158JC) to ZPL. AA was supported by The Danish Council for Independent Research grant (DFF 5051-00033). Special thanks to Prof. Jiakun Wang at Zhejiang University for the assistance.

References

Bergman EN 1990. Physiological Reviews 70, 567–590.
Brooks AW, Kohl KD, Brucker RM, van Opstal EJ and Bordenstein SR 2016. PLoS biology 14, e2000225.
Cantalapiedra JL, Fitzjohn RG, Kuhn TS, Fernández MH, DeMiguel D, Azanza B, Morales J and Mooers AØ 2014. Dietary innovations spurred the diversification of ruminants during the Caenozoic. Proceedings. Biological Sciences 281, 20132746.
Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J and Knight R 2010. Nature Methods 7, 335–336.
Carberry CA, Waters SM, Waters SM, Kenny DA and Creevey CJ 2014. Applied and Environmental Microbiology 80, 586–594.
Clauss M, Hofmann RR, Fickel J, Streich WJ and Hummel J 2009. Journal of Morphology 270, 929–942.

Effect of dietary supplementation of *Bacillus subtilis* and *Aspergillus oryzae* on rumen fermentation, growth traits and faecal microbial population of beef steers

Jae-Sung Lee[1,2], Agalu Wondimagegnehu Zeleke[1], Won-Seob Kim[1,2], Dong Qiao Peng[1,2], Jang-Hoon Jo[1,2], Eun Joong Kim[3], Hong-Gu Lee[1,2]

[1]*Department of Animal Science and Technology, College of Animal Bioscience and Technology, Konkuk University, Seoul, Republic of Korea,* [2]*Team of An Educational Program for Specialists in Global Animal Science, Brain Korea 21 Plus Project, Seoul, Republic of Korea,* [3]*Department of Animal Science, Kyungpook National University, Sangju, Republic of Korea*

E-mail: foodleeking@gmail.com

Take home message Combining different strains of probiotic contributes to an improvement of growth traits via rumen fermentation promotion.

Introduction Few studies reported that addition of *Bacillus subtilis* (BS) and *Aspergillus oryzae* (AO) to ruminal diets results in various responses in ruminants (Wiedmeier *et al.,* 1987; Beharka *et al.,* 1991; Sun *et al.,* 2011). In all of these studies, BS or AO was added individually to the diet. Very few results are reported from diet supplementation with combined BS and AO in ruminants. So, we investigated the effects of dietary supplementation of BS and AO on *in vitro* rumen fermentation traits as well as the growth performance and faecal microbial population of beef cattle.

Material & methods For an *in vitro* rumen fermentation test, 30 serum bottles were divided into two treatments with three replications, which were designed as follows: Control (fermentation of 0.3 g of TMR + 30 ml ruminal fluid without probiotic) and Treatment (control + 0.2% BS and AO) at 0, 3, 6, 12 and 24-hours fermentation time to investigate the effect of probiotics contained live species of BO (2.0×10^7 cfu/g) and AO (1.0×10^6 cfu/g) on *in vitro* rumen fermentation traits. For an *in vivo* trial, subsequently, 20 Korean native steers (average age 10.1 months, 303 ± 7.0 kg of BW) were randomly assigned to two treatments: Control (TMR without any probiotics, n = 10) and Treatment (TMR with 0.2% BS and AO/kg DM basis, n = 10), and were given this once daily by top dressing on the TMR for 140 days. Steers were weighed before feeding and watering at the onset and the end of the experiment. Feed intake was calculated daily by deducting the residual feed from the total supplied feed. Faecal samples collected from the rectum of steers at 140 days were used for counting the beneficial bacteria (*Lactobacillus spp* and *Bacillus spp*) and pathogenic microbes (*Salmonella* and *E. coli*) using agar cultivation. Statistical analyses were conducted to compare between control and treatment by using Student's *t*-test of the JMP 5.0 software package (SAS Institute Inc., Cary, NC, USA). A probability of less than 0.05 was considered statistically significant.

Results & discussion *In vitro* study showed that probiotic combined with BS and AO increases ruminal levels of dry matter disappearance and VFA ($p < 0.05$), but does not affect rumen pH compared to the control. *In vivo* trial revealed that incorporation of BS and AO to TMR diet markedly ameliorates growth performance by increasing average daily gain (ADG; $p < 0.01$) and feed efficiency ($p < 0.001$) and reducing the elimination of pathogenic microbes ($p < 0.05$) (Figure 1).

Table 1 Effect of probiotic supplementation on the growth traits of beef steers (140 days).

	Control	Treatment	SEM	P-value
Initial BW, kg	306	302	6.996	0.961
Final BW, kg	437	459	7.241	0.472
ADG[1], kg/day	0.9	1.1	0.022	0.001
Feed efficiency	0.09	0.11	0.002	<0.001

[1]ADG = average daily gain

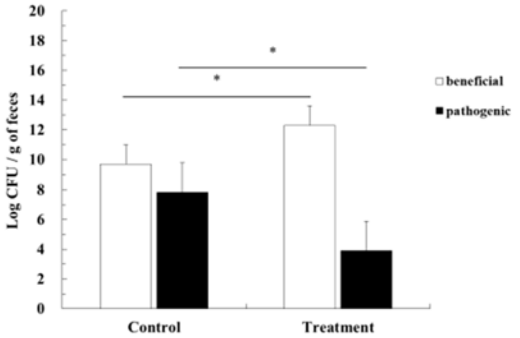


Figure 1 Effect of probiotic supplementation on the faecal microbes of beef steers (140 days).

Conclusion Dietary supplementation of probiotic (BS and AO) resulted in positive responses for the ADG, feed efficiency, faecal microbial population and *in vitro* rumen fermentation parameters, demonstrating that the probiotic supplementation combined BS and AO can be widely used in the livestock industry as a functional feed additive to improve the growth performance as well as the gut health of beef cattle.

References

Sun P, Wang JQ, and Zhang HT 2011. Animal Feed Science and Technology 164, 154–160.
Beharka AA, Nagaraja TG, and Morrill JL 1991. Journal of Dairy Science 74, 4326–4336.
Wiedmeier RD, Arambel MJ, and Walters JL 1987. Journal of Dairy Science 70, 2063–2068.

Ruminal protozoal numbers are not related to methane production in dairy cows

Peter Moate[1], Matthew Deighton[2], Joe Jacobs[1], Murray Hannah[1], Bill Wales[1], Sean Richard Williams[1]

[1]*DEDJTR, Ellinbank, Victoria, Australia,* [2]*CropMark, Christ Church, New Zealand*

E-mail: peter.moate@ecodev.vic.gov.au

Take home message Methane production and methane yield are not associated with the numbers of protozoa in ruminal fluid. Methane mitigation strategies reliant on reducing ruminal populations of protozoa are likely to be ineffective.

Introduction Ruminal protozoa are known to harbour methanogens and Hegarty (1999) proposed that nutritional strategies that decrease ruminal populations of protozoa may be effective at mitigating enteric methane emissions. We examined the associations between ruminal protozoal numbers and ruminal methane production, as well as methane yield when cows were offered diets containing a low (L) amount (3.0 kg DM/cow/d) of wheat or a high (H) amount (9.0 kg DM/cow/d) of wheat and also when the amount of wheat in the diet was reduced from H to L.

Material & methods Sixteen lactating Holstein dairy cows were allocated to two equal groups and individually fed freshly-harvested perennial ryegrass (*Lolium perenne*) supplemented with crushed wheat grain, 2.0 kg DM of canola meal and 0.2 kg DM of mineral mix. The experiment involved two sequential periods with Period 1 of 35 days duration and Period 2 of 14 days duration. During Periods 1 and 2, the cows in treatment LL were individually offered 16 kg DM/d of perennial ryegrass and 3.0 kg DM/d of crushed wheat. During Period 1, cows in treatment HL were offered 9 kg DM/d of perennial ryegrass and 9.0 kg DM of crushed wheat, while during Period 2, they were offered 16 kg DM/d of ryegrass and 3.0 kg DM of crushed wheat. Individual cow methane emissions were measured by the SF_6 tracer method (Moate *et al.,* 2017) on the last 5 days of Period 1 and the last 2 days of Period 2. Samples of ruminal fluid were collected on the last day of Period 1 and Period 2, and protozoa in ruminal fluid were counted, as described by Moate *et al.,* (2017). Data were analysed using REML in Genstat.

Results & discussion In Period 1, consistent with the findings of Moate *et al.,* (2017), cows fed the HL diet produced less ($P < 0.05$) methane, had lower ($P < 0.05$) methane yields and lower ($P < 0.05$) protozoal counts than cows fed the LL diet. However, in Period 2 when the wheat intake of the cows in the HL treatment had been reduced to the same as the cows in the LL treatment, their methane emissions and methane yields had recovered to be similar ($P > 0.05$) to those in the LL treatment, yet the protozoa numbers in ruminal fluid of cows in the HL treatment were still much lower ($P < 0.05$) than those in the LL treatment.

Table 1 Influence of the amount of wheat in the diet of dairy cows on means of methane emissions, methane yields and ruminal protozoa.

Treatment	LL		HL		SED[1]	SED[2]
Period	1	2	1	2		
Pasture DMI (kg/cow/d)	15.6	15.8	8.8	14.5	-	-
Wheat DMI (kg/cow/d)	2.9	3.0	8.7	3.0	-	-
Total DMI (kg/cow/d)	20.6	20.8	19.5	19.5	-	-
CH_4 (g/cow/d)	443^b	438^b	311^a	415^b	27.4	13.7
CH_4 (g/kg DMI)	22.0^b	22.1^b	16.1^a	21.3^b	1.21	0.70
Log_{10}(Total protozoa/ml)	5.84^b	5.48^b	4.7^a	4.6^a	0.36	0.17
Total protozoa (10^3/ml)	692^b	302^b	50^a	40^a	-	-

Means in the same row followed by different superscripts differ significantly $P < 0.05$

[1]SED = standard error of the difference of treatments within periods

[2]SED = standard error of the difference between periods within treatments

Conclusion These findings show that in dairy cows consuming perennial ryegrass and wheat grain, there wass not a direct association between numbers of protozoa in ruminal fluid and methane emissions or methane yield.

Acknowledgements This research was supported by grants from Dairy Australia and Agriculture Victoria.

References

Hegarty RS 1999. Australian Journal of Agricultural Research 50, 1321-7.

Moate PJ, Williams SRO, Jacobs JL, Hannah MC, Beauchemin KA, Eckard RJ, and Wales WJ 2017. Journal of Dairy Science 100, 7139-7153.

Adaptation of goat rumen fluid to the fermentation of fungi treated wheat straw

Eli Ratni[1], Anton S. M. Sonnenberg[2], Wouter H. Hendriks[1,3], John W. Cone[1]

[1]*Animal Nutrition Group, Wageningen University & Research, Wageningen, The Netherlands,* [2]*Plant Breeding, Wageningen University & Research, Wageningen, The Netherlands,* [3]*Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*

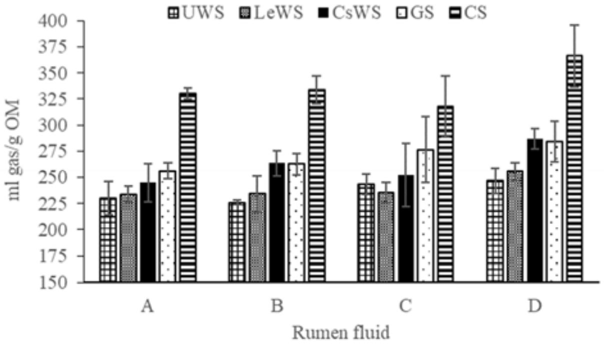
E-mail: eli.ratni@wur.nl

Take home message Adaptation of rumen fluid improves the fermentation of fungi treated wheat straw.

Introduction Lignin in wheat straw limits the degradation of the carbohydrates by rumen bacteria (Sarnklong *et al.*, 2010). Therefore, high lignin biomass does not result in high rumen fermentation. The degradation of straw in the rumen can be increased by pre-treating it with the white rot fungi, *Lentinula edodes* (Le, Shiitake) and *Ceriporiopsis subvermispora* (Cs). It is not known how adaptation of the rumen of goats to fungi treated wheat straw influences the *in vitro* fermentation of feed components.

Material & methods Chopped non-organic wheat straw was colonized by the two fungi for 7 weeks. Eight fistulated non-lactating Saanen goats were individually housed. The control ration (A) consisted of 45% grass silage (GS), 45% corn silage (CS), and 10% soybean meal. In the 3 other rations 30% of the control ration was replaced by untreated wheat straw (UWS) (ration B), *L. edodes* treated wheat straw (LeWS) (ration C), and *C. subvermispora* treated wheat straw (CsWS) (ration D). The goats were fed twice a day for 4 weeks in a partial crossover study. During the first week, all goats were fed with the control ration (A), without straw in it. Then the content of untreated and treated wheat straw was gradually increased to 30%. After 4 weeks, 0.5 L rumen fluid was collected from each goat for *in vitro* gas production analysis and organic matter digestibility of the untreated wheat straw, the fungi treated wheat straws and the grass and corn silages. Gas production (ml/g OM) was recorded using automatic equipment as developed by Cone *et al.,* (1996). Organic matter degradation (%) was determined after 72-h incubation in rumen fluid.

Results & discussion The gas production caused by fermentation of CsWS was slightly higher than that of LeWS and UWS, independent of the origin of the rumen fluid (Fig. 1). The level of gas production of the Cs treated wheat straw was the same as that of the grass silage (GS), showing that the fungal treatment makes the straw a good ruminant feed. Moreover, it is shown that the origin of the rumen had an effect ($P<0.0001$) on the total gas production of all feedstuffs. Using rumen fluid from the animals fed with CsWS (D) resulted in a higher fermentation ($P<0.05$) than using the rumen fluid from the other animals. Fig. 2 shows the relationship ($r=0.94$) between lignin content of the feed samples and the organic matter degradation ($P<0.05$) after 72 h incubation in the 4 different rumen fluids.



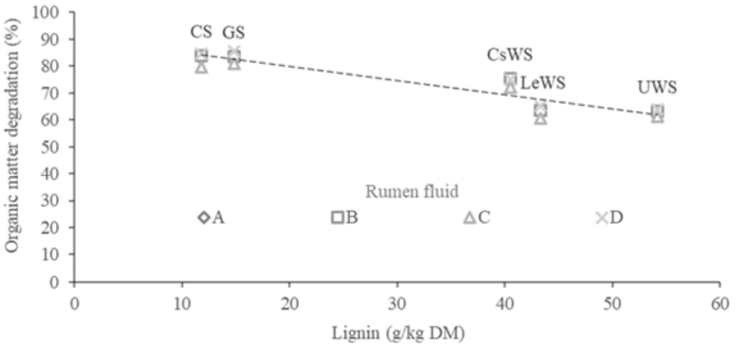


Figure 1 Total gas production (ml/g OM) of feedstuffs used by *in vitro* incubation in different rumen fluids (A, B, C, and D) of goats.

Figure 2 Correlation between organic matter degradation and lignin content of feedstuffs in different rumen fluids (A, B, C, and D).

Conclusion Feeding rations with different kinds of wheat straw resulted in adaptation of the rumen fluid of goats, resulting in differences in rumen fermentation of the different feed ingredients.

Acknowledgements The authors wish to thank the University Fund Wageningen, which was sponsored by Deka, ForFarmers, and Victam.

References

Cone JW, van Gelder AH, Visscher GJW and Oudshoorn L 1996. Animal Feed Science and Technology 61, 113-128.
Sarnklong C, Cone JW, Pellikaan W and Hendriks WH 2010. Asian-Australasian Journal of Animal Science 23, 680-692.

Rumen bacteria in sheep: first evidence of host's genetic control

Annabelle Meynadier[1], Beatrice Gabinaud[1], Regis Tomas[2], Jean-Marie Menras[2], Geraldine Pascal[1], Francis Enjalbert[1], Charlotte Allain[1], Christel Marie-Etancelin[1]

[1]*GenPhySE, Université de Toulouse, INRA, INPT, ENVT, Castanet Tolosan, France,* [2]*La Fage, INRA, Saint-Jean & Saint-Paul, France*

E-mail: a.meynadier@envt.fr

Take home message: Some low-frequency bacterial genera in the rumen may be modulated by sheep genes.

Introduction The microbiota of herbivorous animals plays a central role in the nutrition of its host: it directly affects its health and ability to produce both products of interest for humans and undesirable products such as methane. Studies on ruminal microbiota point out a significant effect of the "host", but very few publications have reported results concerning the impact of host genetics on the composition of ruminal microbiota. On sheep, Rowe *et al.,* (2015) showed that the first component of a principal component analysis of the ruminal bacterial community was heritable (h^2 of 0.24). Thus, we proposed to study the genetic determinism of bacterial relative abundances of sheep rumen microbiota.

Material & methods In 2015 and 2016, 369 dairy Lacaune ewes raised indoor at the INRA Experimental Farm of La Fage, were sampled for rumen fluid through a gastroesophageal tube and a vacuum pomp on 4 different days. These ewes were adult animals (at least 2nd lambing) at about 3 months of milking and belonged to 4 different lines (lines divergently selected on somatic cells count or on milk persistency). They were fed with a 93% hay-silage based diet. Ruminal metagenome was sequenced using 16s rRNA gene with Illumina Miseq technology at the Genomic Platform (INRA, Toulouse, France). Bio-informatic analysis of the microbiota sequences were implemented with FROGS pipeline to obtain relative abundances of bacteria (Escudié *et al.,* 2017). Fixed effects included in the genetic analyses of relative abundances were the "lines", the "parity" and the "day of sampling" effects. Heritability estimates of the square root of relative abundances were computed in single trait using the VCE 6.0 software (Neumaier and Groeneveld, 1998).

Results & discussion FROGS pipeline allowed clustering the 4,944,307 informative sequences into 2,135 OTUs, which represented 247 bacteria taxa: 140 different genera, belonging to 50 families, 31 orders, 17 classes and 9 phyla (Figure 1). The main representative bacteria phyla were *Bacteroidetes* and *Firmicutes* (50.8 and 38.0%, resp.). At the phylum scale, heritability estimates ranged from 0 (for *Bacteroidetes* and *Firmicutes)* to 0.17 (for *Spirochaetae*). At the genera scale, heritability values varied from 0.00 to 0.49 with a standard error of 0.11 on average: 22% of genera had heritability higher than 0.1 (Figure 2). Estellé *et al.,* (2015) on Large White pigs obtained 50% of the genera with heritabilities higher than 0.1, but on 63 selected bacterial genera .The most heritable genera (h^2>0.25) with coherent data distributions were *RuminococcaceaeUCG002*, *Lachnospira, Atopobium* and *Oscillospira* which also were taxa with low abundances.

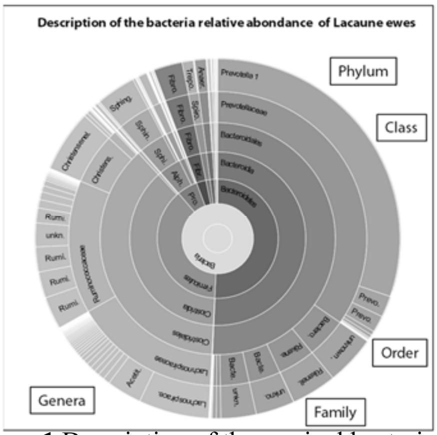


Figure 1 Description of the ruminal bacteria relative abondance of mid-lactation Lacaune ewes receiving a forage based diet.

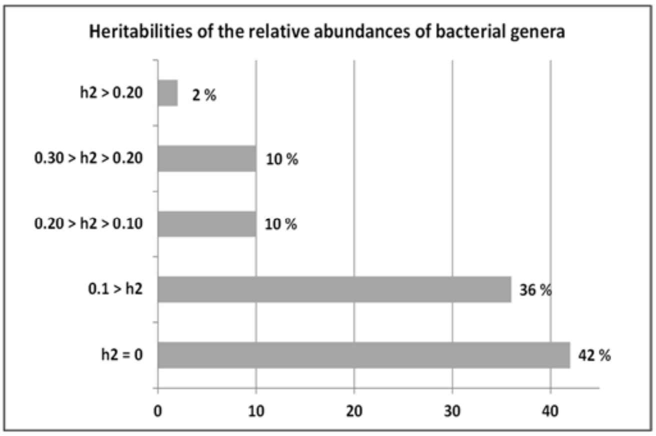


Figure 2 Heritabilities of the relative abundances of ruminal bacteria genus of Lacaune ewes.

Conclusion We confirm that bacterial abundances are, for some of them, heritable. Highly represented bacterial taxa that aggregated many subgroups appeared to be weakly heritable, but some quantitatively minor taxa, that could shape ruminal function, are partly controlled by host genetics.

Acknowledgements This work was supported by grants from INRA-PHASE and INRA-GA divisions.

References

Escudié F, Auer L, Bernard M, Mariadassou M, Cauquil L, Vidal K, Maman S, Hernandez-Raquet G, Combes S and Pascal G 2017, Bioinformatics, btx791.
Estellé J., Mach N., Larzul C., *et al.,* 2015. 23th Plant and Animal Genome, San Diego (USA), 10-14 January.
Neumaier A and Groeneveld E 1998. Genetics Selection Evolution 30, 3–26.
Rowe SJ, Kittelmann S, Pinares-Patiño CS, Wood GR, Dodds KG, Kirk MR, Ganesh S, Hickey SM, Janssen PH and McEwan JC 2015. New Zealand Society of Animal Production, 75, 67-69.

Individual sPLS on ruminal bacterial community of 2 lambs responding differently to a high starch diet to discriminate potential clusters producing *trans*10-18:1 and *trans*11-18:1 fatty acids

Annabelle Meynadier[1], Laurent Cauquil[1], Francis Enjalbert[1], Monica Costa[2], Susana P. Alves[2], Rui J.B. Bessa[2]

[1]GenPhySE, Université de Toulouse, INRA, INPT, ENVT, Castanet Tolosan, France, [2]CIISA, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal

E-mail: a.meynadier@envt.fr

Take home message Enhance quality of ruminant products by controlling fatty acid metabolism and microbiota in the rumen.

Introduction Actual ruminant production practices to face the competitive economic environment involve increased intensification, making use of high productive animals and high energy density diets (i.e. rich in starch and/or lipids). However, when submitted to these intensive production strategies, ruminants are prone to production diseases as subacute ruminal acidosis and to metabolic disturbances as the *trans*-10 shift. The *trans*-10 shift is an alteration of the pathways of unsaturated fatty acids biohydrogenation (BH) in the rumen where the *trans*-10 18:1 (*t*10-18:1) replaces the *trans*-11 18:1 (*t*11-18:1) as major BH intermediate resulting in an alteration of quality of products for consumers, like lamb and beef. The *trans*-10 shift is assumed to be a reflex of a modification of ruminal microbiota, but the exact nature of such microbial shift and the microbes responsible for the extensive t10-18:1 accumulation remain to be elucidated. The purpose of the present experiment was to induce the *t*10 shift in to 2 rumen cannulated rams using a high-starch and high-fat diet, and relate the rumen concentration of *t*10-18:1 and *t*11-18:1 with rumen microbiota.

Material & methods Two rumen fistulated rams were housed in metabolic cages and adapted to a *t*10-shift inducing diet composed of 82% dry matter (DM) of a wheat based concentrate containing 4 % of sunflower oil) and 18% DM of grass hay, during 29 days. Rumen contents were collected daily before and 3h after the morning meal. Once a week, rumen contents were collected every 1h30 from 9h30 to 20h00. Fatty acids from freeze-dried rumen samples were prepared and analysed by gas chromatography. Samples from days 8 and 10 were were sequenced using 16s rRNA gene with Illumina Miseq technology at the Genomic Platform (INRA, Toulouse, France). Bio-informatic analysis of the microbiota sequences were implemented with FROGS pipeline to obtain relative abundances of bacteria (Escudié *et al.,* 2017). A sparse Partial Least Square (sPLS) analysis was performed using R package mixOmixs (Lê Cao *et al.,*) integrating microbiota and fatty acids dataset.

Results There were 2 clusters correlated positively with *t*10-18:1 and *t*11-18:1 content in the rumen of animals 1 and 2: cluster 104 (*Bacteroidetes*; *Bacteroidia*; *Bacteroidales*; *Porphyromonadaceae*; unknown genus; unknown species) and cluster 159 (*Bacteroidetes*; *Bacteroidia*; *Bacteroidales*; *Prevotellaceae*; *Prevotella* 1; unknown species). From results, rumen *t*10-18:1 content was strongly correlated with cluster 104 (r=0.83) and to a lesser extent with cluster 159 (r=0.46), when *t*11-18:1 content was more correlated with cluster 159 (r=0.58) than with cluster 104 (r=0.47).

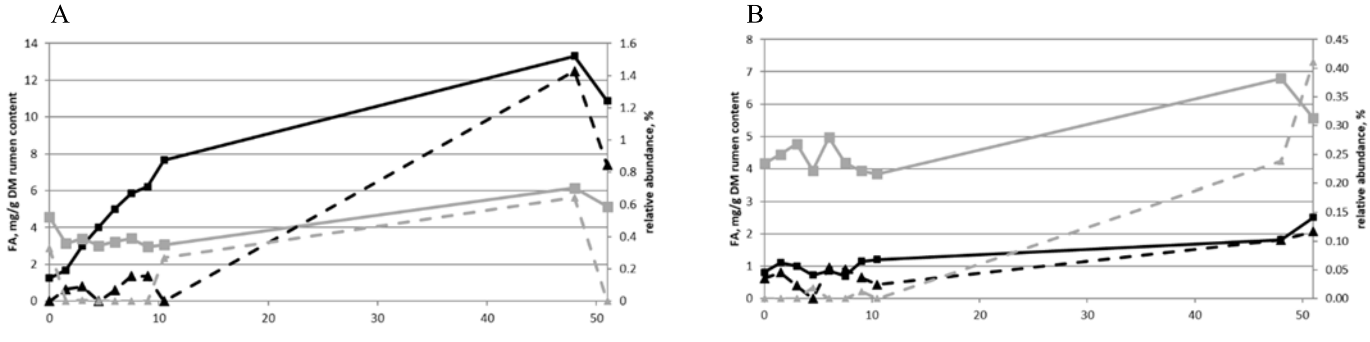


Figure 1 Animal 1 (A) and animal 2 (B) ruminal response to a high starch high fat diet: *t*10-18:1 (■) and *t*11-18:1 (■) rumen concentration, high positively correlated clusters: clusters 104 (▲) and 159 (▲).

Conclusions The sPLS analysis is a multivariate projection-based method that allows the selection of relevant variables from both data sets which covary (i.e. 'change together') across all samples. This approach showed two new potential bacteria that could be implicated in ruminal BH shifts.

References

Escudié F, Auer L, Bernard M, Mariadassou M, Cauquil L, Vidal K, Maman S, Hernandez-Raquet G, Combes S and Pascal G 2017, Bioinformatics, btx791.

Lê Cao K-A, Rohart F, Gautier B, Bartolo F, Gonzalez I and Déjean S mixOmics: Omics Data Integration Project. R package version 6.0.0. 2016

Characterizing the pan and core rumen bacteria across individuals of a large cohort of dairy cows

Mingyuan Xue[1], Huizeng Sun[1,2], Xuehui Wu[1], Diming Wang[1], Jiakun Wang[1], Leluo Guan[1,2], Jianxin Liu[1]

[1]Institute of Dairy Science, Zhejiang University, Hangzhou,Zhejiang, China, [2]Department of Agricultural, Food & Nutritional Science, University of Albert, Edmonton, Alberta, Canada

E-mail: myxue@zju.edu.cn

Take home message A total of 33 bacterial genera consisted a core rumen microbiota and contribute to the rumen microbial variation of 334 dairy cows.

Introduction Dairy cows converting indigestible plant substrates into milk are mainly attributed to rumen bacterial fermentation. Recent molecular based microbial identification has advanced our understanding of rumen microbiota. There exists high inter-animal variation in rumen bacterial communities (Mullins *et al.,* 2013). Whereas, knowledge is still limited on the inter-animal variation in a large cohort of dairy cows with least external variables. This study was conducted to explore the variations in rumen bacterial communities among individuals in a large cohort of dairy cows and to uncover the pan and core bacterial communities.

Material & methods A total of 334 Holstein dairy cows (parity = 3.08 ± 1.14, DIM = 159 ± 34) housed at a commercial dairy farm were selected. Cows were fed a same diet with a ratio of forage-to-concentrate at 45:55. Milk composition and milk yield were recorded. Rumen contents of all the cows were collected by an oral stomach tube for analysis of volatile fatty acids (VFA) and 16S rRNA gene sequencing of V3 and V4 regions. Operational taxonomic units (OTUs) were clustered with a 97% identity threshold, and taxonomy was assigned using the latest Greengenes database. The core rumen bacterial microbiota was defined as genera observed in 100% of the samples, and pan-microbiota was defined as a total observed richness in all the samples. The contribution of bacterial genera to microbiome variation was plotted on the two first principal coordinates analysis dimensions.

Results 16S rRNA gene amplicon sequencing of V3-V4 region by illumina Hiseq revealed the rumen microbiome to be diverse, containing 422 bacterial genera from 14 phyla (pan-microbiota) (Figure 1A). A core bacterial microbiota was identified, consisting of 33 genera from six phyla (Figure 1B). The variation in rumen microbiome was driven by the abundant core taxa, with *Prevotella*, YRC22 and *Shuttleworthia* showing the largest contribution (Figure 2).

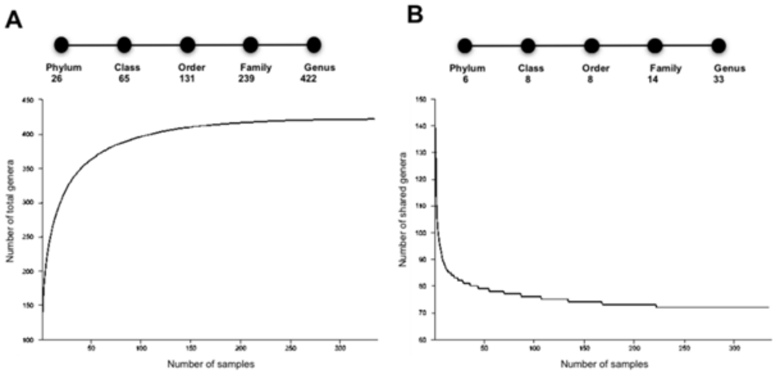


Figure 1 Distribution of pan (A) and core (B) rumen bacterial genera.

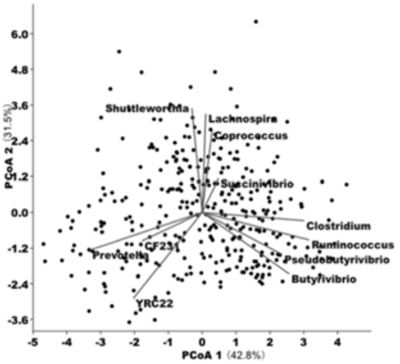


Figure 2 Principal coordinates analysis based on genus-level Bray-Curtis dissimilarity.

Conclusions In conclusion, this study explored the rumen bacterial microbiome composition of a large cohort of dairy cows using 16S rRNA gene amplicon sequencing. Our results revealed that a core bacterial component existed, in spite of the variation in their abundance among individuals, suggesting that a common group of bacteria are playing essential roles in rumen microbiome profiling.

Acknowledgements The research described in this communication was supported by grants from the National Natural Science Foundation of China (No. 31472121 to J.X.L and 31729004 to L.L.G. and J.X.L.), and by the China Agriculture (Dairy) Research System (CARS-36 to J.X. L.).

References

Mullins CR, Mamedova LK, Carpenter AJ, Ying Y, Allen MS, Yoon I, Bradford BJ 2013. Journal of Dairy Science. 96, 5872-5881.

383

The inhibitory action mode of nitrocompounds to rumen methanogenisis: A comparison of nitroethane, 2-nitroethanl and 2-nitro-1-propanol

Zhen-Wei Zhang[1], Lei Yu[2], Yan-Lu Wang[1], Wei-Kang Wang[1], Xue-Meng Si[1], Hong-Jian Yang[1], Sheng-Li Li[1]

[1]State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing, China, [2]China Animal Disease Prevention and Control Centre, Beijing, China

E-mail: qingyibushuo@163.com

Take home message More research about the inhibition action mode will ultimately provide a scientific, concrete reference for the practical use of nitrocompounds with the aim to reduce methane emission in ruminant animals.

Introduction Methane emission in ruminant animals is well known as greenhouse gas to global warming. In the methanogenic process, methyl–coenzyme M reductase (MCR) is a key enzyme responsible for hydrogen-electron transfer, and coenzyme F_{420} is involved as electron carrier in both catabolic and anabolic redox reactions. More recently, as potential anti-methanogenic additives, the relatively innocuous nitrocompounds including nitroethane (NE), 2-nitroethanl (NEOH) and 2-nitro-1-propanol (NPOH), etc. have attracted great attention since they can serve as electron acceptors to reduce CH_4 production (Anderson *et al.,* 2006). However, it is not clear until now how the use of these compounds would affect the above coenzymes during the inhibition of ruminal methanogenesis (Zhang *et al.,* 2018). Therefore, the present study attempted to investigate possible inhibition action mode of NE, NEOH and NPOH with *in vitro* rumen batch cultures.

Material & methods A hay-rich substrate (500 mg) consisted of 80% alfalfa hay and 20% maize meal was weighed into 120 ml glass bottles containing 0 (control), 10 mM NE, 10 mM NEOH, 10 mM NPOH following the addition of 50 mL medium (pH 6.85; Menke and Steingass, 1988) and 25 mL filtrated rumen fluid collected from three rumen-fistulated Holstein cows. The bottles in five replicate per treatment were connected to airbags to collect fermentation gases and anaerobically incubated at 39°C for 72 h. The batch culture was repeated four runs. After the incubation, fermentation gases and culture fluids were sampled for methane, ammonia N, microbial protein (MCP), volatile fatty acids (VFA) determination and gene expression of mcrA determined by real-time PCR, and coenzyme F_{420} activity determined by a fluorometric method and expressed as fluorescence intensity. Statistical analysis was perfomed using the GLM procedure of SAS (1999). Least square means were compared with a multiple comparison test (Tukey/Kramer), and significance was declared at $P<0.05$ unless otherwise noted.

Table 1 The effect of nitrocompounds addition on *in vitro* rumen fermentation

Items	Control	NE	NEOH	NPOH	S.E.M	*P* value
Final pH	6.88^{a}	6.82^{c}	6.83^{bc}	6.85^{ab}	0.018	0.003
ammonia N, mM	33.6	33.3	32.0	33.2	0.73	0.424
MCP, mg/ml	0.82^{c}	0.94^{b}	1.03^{a}	0.93^{b}	0.05	0.001
total VFA, mM	110	109	112	114	2.2	0.446
acetate, (%, molar)	60.9^{a}	58.8^{b}	58.3^{b}	58.0^{b}	0.29	0.002
propionate, (%, molar)	21.0^{b}	22.2^{a}	22.3^{a}	22.3^{a}	0.17	0.016
butyrate, (%, molar)	10.1^{b}	11.3^{a}	11.0^{a}	11.2^{a}	0.16	0.023

Results After the incubation, final pH in culture fluids was decreased by both NE and NEOH addition in comparison with the control. The nitrocompound addition in comparison with control did not alter ammonia N and total VFA production, but significantly increased MCP concentrations by 14%, 25% and 13% in NE, NEOH and NPOH, respectively. Compared with the control, the addition of these nitrocompounds significantly decreased molar acetate proportion and increased molar proportions of propionate and butyrate though no differences occurred among the three compounds. The about results suggested that the nitrocompound addition shifted rumen fermentation towards less acetate production and more propionate and butyrate production. As shown in **Fig. 1**, NE, NEOH, and NPOH addition in comparison with the control decreased methane production by 96%, 97%, and 40% ($P<0.05$). Meanwhile, both the *mcrA* gene expression and coenzyme F_{420} content were also decreased with the nitrocompound addition. More interestingly, methane production was positively correlated with coenzyme F_{420} content (r=0.74, $P<0.01$) and *mcrA* gene expression (r=0.88, $P<0.01$), suggesting that inhibition efficiency varies depending on nature of these nitrocompounds.

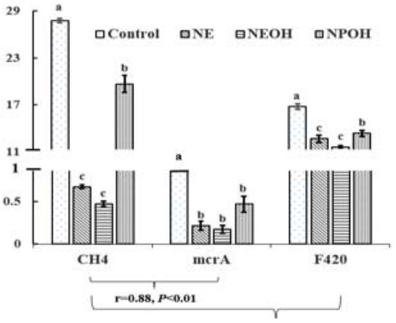


Figure 1 The effect of nitrocompounds on CH_4 production, *mcrA* gene expression and coenzyme F_{420} activity.

Conclusions The nitrocompound addition shifted rumen fermentation towards less acetate production and more propionate and butyrate production. Both NE and NEOH presented similar effectiveness to inhibit CH_4 production, and the lowest inhibition occurred for NPOH. The nitrocompounds would inhibit rumen methanogenisis by decreasing the coenzyme activities of MCR and F_{420}.

Acknowledgements The study was funded by the National Natural Science Foundation of China (Project no. 31572432).

References

Anderson RC, Carstens GE, Miller RK, Callaway TR, Schultz CL, Edrington TS, Harvey RB and Nisbet DJ 2006. Bioresource Technology 97, 2421–2426.
Zhang Z-W, Cao Z-J, Wang Y-L, Wang Y-J, Yang H-J and Li S-L 2018. Animal Feed Science and Technology 236, 107–114.
Menke KH and Steingass H 1988. Animal Research and Development, 28, 7-55.

Effect of varying sources of protein in glucogenic dairy diets on *in vitro* rumen microbial nitrogen yield

Behnaz Eyni, Mohsen Danesh Mesgaran, Alireza Vakili, RezaValisadeh

Ferdowsi University, Mashhad, Iran

E-mail: behnaz_eyni@yahoo.com

Take home message *In vitro* microbial protein synthesized in the rumen is influenced by the source of protein of glucogenic diets to meet the requirement of the dairy cows.

Introduction Dietary crude protein supplies amino acids (AA) for the dairy cows to sustain their need for maintenance and milk production. Amino acids that reach the small intestine of the ruminant are mainly originated from microbial cp (MCP) synthesized in the rumen, the dietary protein that escapes ruminal degradation, and the endogenous protein (Van Duinkerken *et al.,* 2011). Among these sources, microbial protein is considerable because of the AA pattern related to animal production, intestinal digestibility, and AA utilization. Thus, it is important to maximize the MCP synthesis in the rumen. Therefore, the objective of this study was to measure the effects of lipogenic diets on microbial protein production.

Material & methods The glucogenic diets including as ground barley+ soybean meal (BSB), ground barley + xylose protected soybean meal (BXPS), steam-flaked barley+ soybean meal (SFBSB), steam-flaked barley + xylose protected soybean meal (SFBXPS). An *in vitro* gas production technique was carried out to determine the rumen microbial yield as described by Grings et al (2005). The time of half-maximal gas production ($t_{1/2}$) was calculated after the first 96 hours of the gas run. Then, a second incubation was performed with the diets and were stopped at the diet-specific half time of gas production and the microbial N production was determined following the equation and using the N-balance equation, given as follows: Microbial N Production at $t_{1/2}$ = Diet N + ΔNH_3 – N - NDFN at $t_{1/2}$. The conversion of dietary N to microbial N (MN/DN) was determined from the value of microbial nitrogen divided by dietary nitrogen. The concentration of ammonia-N per dietary nitrogen (ammonia/DN) and ammonia-N+MN per dietary nitrogen (ammonia+MN/DN) were measured as the rate of conversion of N to ammonia-N and microbial-N in the rumen.

Results & discussion *In vitro* ruminal ratios of microbial nitrogen yield (MN/DN), ammonia-N (A/DN) and ammonia-N+microbial nitrogen to dietary nitrogen concentration (A+MN/DN) are demonstrated in Table 1. Highest MN/DN and lowest A/DN ratios were observed in SFBSB ($P< 0.05$). It has been indicated that moister heat-processed starch and grain could result in a greater proportion of propionic acid being produced during the fermentation in the rumen and could help to produce a higher amount of microbial protein. The results were in agreement with the previous study regarding the decrease in the ammonia-N concentration in steam-flaked grains (Fu-qiang *et al.,* 2015). Diet containing steam flaked barely and XPS tend to obtain less A +MN/DN ratio than those of the other ($p < 0.05$).

Table 1 *In vitro* ruminal ratios of microbial nitrogen yield (MN/DN), ammonia-N (A/DN) and ammonia-N+ microbial nitrogen to dietary nitrogen concentration (A+MN/DN).

	BSB	SFBSB	BXPS	SFBXPS	SEM
MN/DN	0.4^{bc}	0.59^{a}	0.38^{c}	0.43^{b}	0.02
A/DN	0.45^{a}	0.27^{c}	0.43^{a}	0.34^{b}	0.02
A+MN/DN	0.85^{a}	0.86^{a}	0.81^{b}	0.78^{c}	0.01

Conclusion The conversion of dietary nitrogen to microbial nitrogen was higher in diets with steam-flaked grains than that of the ground grain diets. Microbial protein synthesis is supposed to be energy-dependent and the processing of the grain can help to supply the energy. Thus, A good synchronization of SF grains with protein sources is the result of the highest MN/DN value.

References

Fu-qiang Q, Fei W, Li-ping R, Zhen-ming Z, Qing-xiang M and Yu-hong, B. 2015. Journal of Integrative Agriculture 14, 949-955.

Grings EE, Blummel M, and Sudekum, KH 2005. Animal Feed Science and Technology123-124, 527-545.

Van duinkerken G, Block MC, Bannin AK, Cone JW, Dijkstra J, Van vuuren AM and Tamminga S. 2011. The Journal of Agricultural Science 49, 351–367.

385

Effect of level and supplementation time of exogenous cellulase enzyme on *in vitro* rumen fermentation parameters of barley straws

Yasaman Ahmadi Bonakdar, Alireza vakili, Mohsen Danesh Mesgaran

Department of Animal Science, Faculty of Agriculture Ferdowsi University of Mashhad, Mashhad, Iran

E-mail: yasamanbonakdar@ymail.com

Take home message Improvement of *in vitro* ruminal digestion of barley straws may be achieved by adding exogenous celluloses.

Introduction Exogenous fibrolytic enzymes supplementation has been suggested to improve the efficiency of fermentation and increase fibrous feeds degradation in ruminants (Lewis *et al.,* 1999). However, level and time of exogenous enzymes supplementation are not fully specified. The objective of the present study was to investigate the effect of exogenous cellulase enzyme supplementation at the beginning or 12 h prior to incubation on rumen fermentation characteristics of barley straw using *in vitro* gas production technique.

Material & methods Samples barley straw were treated with four levels of cellulase (0.0, 2.5, 5 and 7.5 g/kg of DM) at the beginning of (A) or 12 hours prior to incubation (B). Cumulative volumes of the produced gas at 2, 4, 8, 12, 16, 24, 48, 72 and 96 h of incubation were recorded. *In vitro* disappearance of dry matter (IVDMD), neutral detergent fiber (DNDF) and acid detergent fibre (DADF) were also measured after 96 h incubation.

Results & discussion The enzyme applied to barley straw at the rate of 5 or 2.5 g /kg of DM in both A and B improved cumulative gas production from the soluble and insoluble fraction as well as fractional fermentation rate (Figure 1). The highest values of IVDMD, DNDF and DADF were observed when the straw samples were supplemented with 5 g/kg of DM at the beginning of incubation or 2.5 g/kg of DM at 12 h prior to incubation (Table 1).

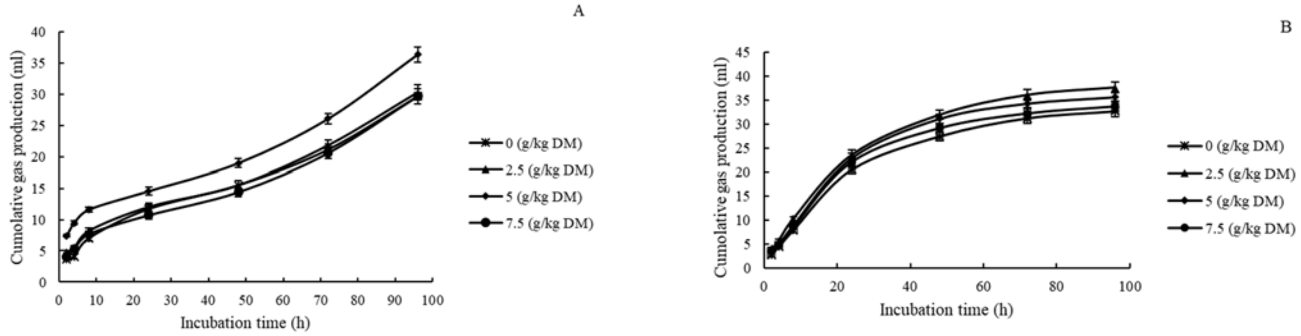


Figure 1 Effect of cellulase supplementation at the beginning of (A) or 12 h prior to incubation (B) on *in vitro* gas production parameters of barley straw.

Table 1 The effect of different levels and time of cellulase supplementation on percentage of *in vitro* dry matter (DMD), neutral detergent fiber (NDFD) and acid detergent fiber (ADFD) disappearance of barley straw after 96 h incubation.

Supplementation time	Enzyme concentration (g/kg DM)				SEM	P values
	0	2.5	5	7.5		
At the beginning of incubation						
DMD	50.95	56.65	53.80	53.45	1.31	0.05
NDFD	32.18^{c}	58.09^{a}	40.18^{b}	39.08^{b}	0.83	<0.01
ADFD	39.63^{b}	41.65^{a}	39.78^{b}	35.72^{c}	0.277	<0.01
12 h prior to incubation						
DMD	54.28^{c}	85.91^{a}	60.75^{b}	45.16^{c}	1.89	<0.01
NDFD	37.55^{c}	58.36^{a}	56.97^{ab}	56.43^{b}	0.47	<0.01
ADFD	41.10^{c}	47.94^{a}	46.71^{ab}	45.56^{b}	0.38	<0.01

Conclusion Exogenous cellulase improves *in vitro* rumen fermentation characteristics of barley straws depending on enzyme level and time of supplementation, ^{a,b,c} Means with different letters are significant

References

Lewis GE, Sanchez WK, Hunt CW, Guy MA, Pritchard GT, Swanson BI and Treacher RJ 1999. Journal of Dairy Science 82, 611–617.

Development of FibroChip, a functional DNA microarray as a targeted metatranscriptomic tool to monitor carbohydrate-active enzyme gene expression by rumen microbiota

Sophie Comtet-Marre[1,2], Frederique Chaucheyras-Durand[2], Pascale Mosoni[1], Ali Bayat[3], Pierre Peyret[1], Evelyne Forano[1]

[1]*INRA-UCA, Clermont-Ferrand, France,* [2]*Lallemand SAS, Blagnac, France,* [3]*Luke, Jokioinen, Finland*

E-mail: evelyne.forano@inra.fr

Take home message FibroChip microarray is a reliable and high-throughput tool to gain a focused insight on active rumen microorganisms involved in fibre degradation.

Introduction Biotic and abiotic factors influence the efficiency of fibre degradation in the rumen of ruminants, which can ultimately affect animal productivity and health (Chaucheyras-Durand *et al.,* 2012). Up to now, knowledge gaps remain on how the activity of rumen fibrolytic microorganisms is modulated at the transcriptomic level. Therefore, a functional DNA microarray targeting genes encoding key enzymes involved in cellulose and hemicellulose degradation by rumen microbiota was designed. The FibroChip was used to analyse the transcriptome of the rumen cellulolytic bacterium *Fibrobacter succinogenes* and to characterise the expression of the carbohydrate active enzyme (CAZyme) gene repertoire of the rumen microbiota of a dairy cow fed a mixed diet based on grass silage.

Material & methods 392 nucleic sequences encoding glycoside hydrolase families (GH5, GH9, GH10, GH11, GH43, GH48) and carbohydrate esterase families (CE1, CE6) were collected. They originated from 41 bacterial, 4 protozoal and 10 fungal species. Probes were designed within the coding sequence of each catalytic domain using the HiSpOD software and the GoArrays strategy (Dugat-Bony *et al.,* 2011; Rimour *et al.,* 2005). FibroChip demonstrated sensitivity and specificity, as well as explorative potential, as shown by hybridising PCR products and genomic DNA. *Fibrobacter succinogenes* S85 was cultivated for 6 h with cellobiose (Clb) or 50 h with wheat straw (WS) as substrate. The total rumen sample was previously analysed by RNA-sequencing (Comtet-Marre *et al.,* 2017). Extracted RNAs were mRNA enriched by rRNA subtraction (Comtet-Marre *et al.,* 2017). RNA was then labelled and hybridised for 17 h at 65°C. Fluorescence intensities (I) of probes were normalised using Signal-to-Noise Ratio (SNR). For each probe triplicate, the calculation was: SNR= median ($I_{probe}/I_{local\ background}$). Positive hybridisation was considered significant for probes having a SNR > 6 for *F. succinogenes* and > 2 for rumen sample. A gene was considered as expressed when 65% of probes (there were on average 9 probes per gene) were positive. The SNR value for a specific gene was then calculated as the average of the SNR of all probes targeting that gene.

Results & discussion *F. succinogenes S85 experiment.* All the CAZyme genes of *F. succinogenes* S85 (in total 31 genes) targeted by the array were expressed with either Clb or WS as growth substrate. Nine genes were up-regulated (log2 ratio > 2) on WS relative to Clb. The most expressed genes with WS encode a GH11, two GH5 and the GH9 cellulase EGB, previously characterised (Béra-Maillet *et al.,* 2000). Several of the highly expressed genes encode enzymes that have not been biochemically characterised yet, indicating they need further studies.

Total rumen sample experiment. The most abundant transcripts detected by the FibroChip encoded GH43, GH5 and GH10 enzymes. *Bacteroides*, *Fibrobacter* and *Ruminococcus* were the major contributors, while fungi and protozoa represented 8.1% and 6.7% of total detected transcripts, respectively. Compared to RNA-seq data on the same rumen sample, the FibroChip gave quite similar results for families CE1, CE6, GH9 and GH10. However, a discrepancy was observed for the other 4 families. Adding sets of probes targeting new sequences would thus be necessary. Interestingly, the FibroChip data highlighted the important, probably still under-estimated, role of eukaryotes in rumen fibre degradation, which is in line with the metatranscriptomic analysis.

Conclusion The FibroChip would be applicable to study temporal variations in CAZyme gene expression profiles in the rumen according to diet composition, environmental changes or supplementation of feed additives. This monitoring could be applied in nutritional experiments involving a large number of animals, and would allow understanding better how the fibre-degrading activity of rumen microbiota is controlled and modulated. Furthermore, targeted (meta)transcriptomics may give opportunities to identify which genes or proteins deserve further characterisation.

Acknowledgements This work was supported by grants from the ANRT, the Auvergne Region and funding from Lallemand.

References

Béra-Maillet C, Gaudet G, Forano E 2000. Biochimica et Biophysica Acta 1543, 77-85.
Chaucheyras-Durand F, Chevaux E, Martin C and Forano E 2012. Probiotic in Animals 119-152.
Comtet-Marre S, Parisot N, Lepercq P, Chaucheyras-Durand F, Mosoni P, Peyretaillade E, Bayat AR, Shingfield K, Peyret P and Forano E 2017. Frontiers in Microbiology 8, 67.
Dugat-Bony E, Missaoui M, Peyretaillade E, Biderre-Petit C, Bouzid O, Gouinaud C, Hill D and Peyret P 2011. Bioinformatics 27, 641–648.
Rimour S, Hill D, Militon C and Peyret P 2005. Bioinformatics 21, 1094-1103.

Effect of using RumenBuff plus® on *in vitro* duodenal utilizable crude protein of two glucogenic diets

Behnaz Eyni, Mohsen Danesh mesgaran, Foad Garajian

Ferdowsi university, Mashhad, Iran

E-mail: behnaz_eyni@yahoo.com

Take home message Dietary buffers have been added to dairy cattle diets in an attempt to improve ruminal fermentation.

Introduction When animal production increases, additional protein must be supplied from dietary protein that passes the rumen undegraded to meet the animal's protein requirement. Therefore, it is important to maximize amino acid reach in the small intestine. Administration of a dietary buffers improve protein digestibility wich could increase milk yield, milk fat percentage, and optimize DMI in the lactating cow (Iwaniuk and Erdman, 2015). There are little data available about the effect of buffers on nitrogen flow to the small intestine. We hypothesized that exogenous dietary buffers, such as RumenBuff plus®, can be added to high grain diets and improve rumen fermentation and prevent acidosis. Therefore, the objective of our study was to measure the effects of exogenous dietary buffers in glucogenic diets on nitrogen flow to the small intestine of dairy cows.

Materials & methods Two glucogenic diets based on barley grain (treatment 1 without additive) and (treatment 2 glucogenic diet based on barley grain + RumenBuff plus®) were analyzed for uCP using *in vitro* gas production technique. The procedure of *in vitro* CP utilization in duodenum was carried out using the gas production system (Edmunds *et al.*, 2012) in four replicates and three runs. The utilization of CP in duodenum was calculated as the difference between the N concentrations in the sample plus the ammonia concentration in the blank minus the ammonia-N concentration produced from the sample divided by the sample weight, using the following calculation: uCP (g/kg DM) = $(NH_3N_{blank} + N_{sample} - NH_3\,N_{sample})/(\text{weight (mg DM)}) \times 6.25 \times 1000$, where the N_{sample} is N added to the bottles.

Results & discussion In 8 and 24 hours after incubation, uCP tended to be higher in treatment containing RumenBuff plus®. Each factor affecting the quantity of the microbial protein leaving the rumen has the ability to influence uCP (Bach *et al.*, 2000). Probably, the positive effect of RumenBuff plus® on rumen fermentation is the result of increasing microbial nitrogen flow to the small intestine. Utilizable crude protein after 48 h of incubation was not significantly affected by treatments.

Table 1: Effect of treatments on utilizable crude protein (uCP) after 8, 24 and 48 h of incubation.

	Time (h)	treatment*		SEM
		1	2	
uCP(g/kg DM)	8	151.5^b	219.4^a	20.9
	24	122.2^b	131.5^a	16.6
	48	75.7	75.8	2.4

* treatment 1: glucogenic diet without addition, treatment 2: glucogenic diet with Rumen Buff plus®

Conclusion Results indicate that Rumen Buff plus ® could be used in dairy cow's ration to improve fermentation and increase nitrogen flow to the small intestine.

References

Bach A, Huntington GB, Calsamiglia S and Stern MD 2000. Journal of Dairy Science 83, 2585–2595.
Edmunds B, Sudeku KH, Spiekers H, Schusterc M and Schwarzd FJ 2012. Animal Feed Science and Technology 175, 106– 113.
Iwaniuk ME and Erdman RA 2015. Journal of Dairy Science 98, 1–13.

Dynamic changes of main rumen microflora in sheep supplemented with molasses-urea

Shuyuan Xue, Changqing Li, Alatengdalai

Inner Mongolia Academy of Agriculture and Animal Husbandry Sciences, huhhot, China

E-mail: lcqeg@126.com

Take home message Molasses-urea could increase the number of rumen bacteria and improve the nutrition intake of sheep.

Introduction Sheep digest and utilize nutrients through the effects of microbes in their rumen. Sucrose and monosaccharides in molasses are the most easily absorbed by animals. Their active ingredients are able to improve the productive performance, reproductively and immunity of sheep (Meng *et al*., 1995). Adding urea into feedstuff is considered an effective way of supplementing proteins in the diets of ruminants (Wan *et al*., 2009). Emmanuel (2016) reported that the urea content in molasses-urea can increase fodder intake by ruminants, so we can optimize the growth and nutrition metabolism of microbes in the rumen of ruminant animals supplemented with molasses and urea. However, further study is required on how molasses-urea promotes rumen fermentation, through supplementation of molasses-urea in feedstuff. In this experiment, quantitative RT-PCR was used to detect the main representative bacteria we discovered, which included *Ruminococcus albus* (*R. albus*), *Ruminococcus flavefaciens* (*R. flavefaciens*), *Fibrobacter succinogenes* (*F. succinogenes*), *Anaerovibrio lipolytica* (*A. lipolytica*) and *Selenomonas ruminantium* (*S. ruminantium*) in the rumen of sheep. The purpose of this was to research the dynamic changes in the main rumen microflora and ruminal fermentation in sheep that had diets supplemented with molasses-urea.

Materials & methods Eight sheep were selected and divided into two groups (a control group and a treatment group), and only the treatment group animals were supplied with molasses-urea for ad libitum consumption. Rumen fluid was collected every 2 h. The population of majority bacteria were investigated by real-time PCR.

Results & discussion The rumen bacteria of sheep in the experimental group increased significantly（P<0.5）after supplying molasses-urea (Table 1). The kinetics of all bacterium numbers were similar to that of *S. ruminantium*. The quantity of bacterium in each group reduced gradually after feeding, reaching the minimum quantity 2h after intake. Then it rose step by step and achieved maximum quantity at 8h after intake, before returning to the normal level that was detected before intake (Fig 1). The *R. albus* and *A. lipolytica* in the experimental group, at each time period, were notably higher than that detected in the control group（P<0.5), other bacteria only showed a significant difference at 4～8 h. These results showed the average quantity of protozoa in the experimental group was 22.6×10^4/ml, which was 18.8% higher than that of control group. The difference of these results was significant（P<0.05).

Table1 Effects of molasses-urea on rumenfermentation, protozoa and bacteria copies of sheep.

Items	Control group	Test group
R. albus	0.24±0.08	1.08±0.11**
F. succinogenes	3.35±0.55	3.71±0.89**
R. flavefaciens	2.26±0.35	3.23±0.82**
A. lipolytica	2.45±0.41	3.07±0.32**
S. ruminantium	2.68±0.44	3.08±0.49*

Note: Values with double asterisk superscripts in the samerow means extremely significant difference（P＜0.05, while an asterisk superscripts means significant difference（P＜0.01.

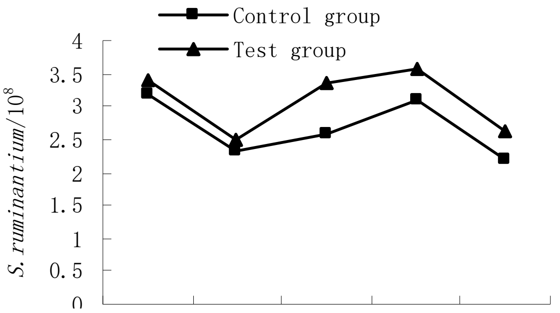


Figure 1 Effects of molasses-urea on rumen bacteria copies of sheep.

Conclusion Based on consistent diet and management conditions, the molasses-urea could maintain the pH of rumen in sheep and improve the concentration of ammonia nitrogen and MCP yields. However, beyond this it was also able to increase the quantity of microbes in rumen, therefore promote a productive and conducive environment inside the rumen. Consequently, it improved the utilization of low quality roughage and the nutrition intake of sheep.

Acknowledgements The present study was supported by the National Nature Science Foundation of China (31460615).

References

Emmanuel N, Patil NV, Bhagwat SR, Lateef A, Xu K and Liu HN 2015, Animal Nutrition. 4, 356-361.
Meng Q, Kerley MS and Belyen RL 1995. Journal of Animal Science, 73（Suppl 1, 262).
Wan GD, Li CL and Pan ZR 2009. Pratacultural Science, 26, 123-125.

Effect of dietary addition of EPA, DPA and DHA on rumen bacterial community in cows and ewes. An *in vitro* approach

David Carreño[1], Álvaro Belenguer[1], Eric Pinloche[2], David Yáñez-Ruiz[3], Pablo G. Toral[1], Gonzalo Hervás[1], Neil R. McEwan[2,4], C. Jamie Newbold[2,5], Frutos Pilar[1]

[1]Instituto de Ganadería de Montaña (CSIC-ULE), León, Spain, [2]IBERS, Aberystwyth University, Aberystwyth, UK, [3]Estación Experimental del Zaidin (CSIC), Granada, Spain, [4]Robert Gordon University, Aberdeen, UK, [5]Scotland's Rural College, Edinburgh, UK

E-mail: p.frutos@csic.es

Take home message Dietary addition of EPA, DPA, and DHA altered the abundance of several bacterial genera, such as *Prevotella*, *Hallella*, *Paraprevotella* and *Succiniclasticum*, that might participate in rumen biohydrogenation.

Introduction The role of marine lipids as modulators of rumen biohydrogenation (BH) of unsaturated fatty acids (FA) is likely to be due to the impact of their n-3 polyunsaturated FA on the microbiota (Toral *et al.*, 2017). However, the effect of individual FA, namely 20:5 n-3 (EPA), 22:5 n-3 (DPA), and 22:6 n-3 (DHA) has rarely been studied and it is still uncertain which rumen bacteria are involved in the BH process. Moreover, despite interspecies differences in the rumen bacterial composition (Toral *et al.*, 2016), we are not aware of any reports comparing the effects of these FA in cows and sheep. Therefore, rumen inocula from these species were used to examine *in vitro* the effect of EPA, DPA and DHA on the bacterial community. A concomitant study had analysed their influence on ruminal fermentation and BH (Toral *et al.*, 2017).

Materials & methods Batch cultures of rumen microorganisms were conducted using inocula from 2 ruminant species (*i.e.*, 2 cannulated cows and 2 cannulated ewes fed a TMR with a forage:concentrate ratio 50:50) and 4 treatments (control –the TMR without supplementation–, and the TMR plus 2% DM of EPA, DPA or DHA). Incubations lasted for 24 h and were repeated on 3 consecutive days. DNA was extracted from freeze dried samples and bacterial 16S rRNA (V1-V2) amplicon was analysed by Ion Torrent PGM next generation sequencing (de la Fuente *et al.*, 2014). The effect of animal species and treatments on the taxa's relative abundance was analysed by ANOVA, with the MIXED procedure of the SAS 9.4.

Results & discussion Only effects on bacteria that were previously suggested to be somehow related to rumen BH are reported here. Most changes were comparable in both species, but some groups shifted with EPA and/or DHA only in cattle (*e.g.*, increases in *Hallella*, *Ruminococcus* and *Ruminobacter*) or only in sheep (increments of *Paraprevotella* and decreases in *Oscillibacter*). *Prevotella* abundance was reduced by all treatments, especially EPA and DHA, in the two animal species (Figure 1A). This genus has been related to 18:0 formation (Huws *et al.*, 2011), which is consistent with observations by Toral *et al.* (2017). Abundances of *Hallella* in cattle and *Paraprevotella* in sheep increased similarly with EPA and DHA (Figure 1B). These microorganisms form succinate, a precursor of propionate for *Succiniclasticum* spp., whose abundance raised with all treatments in cattle and with DHA in sheep (Figure 1C). A putative association between propionate formation and a shift in BH pathways was previously speculated in Toral *et al.* (2017) and suggest that bacteria related to propionate metabolism might play a part in those BH routes.

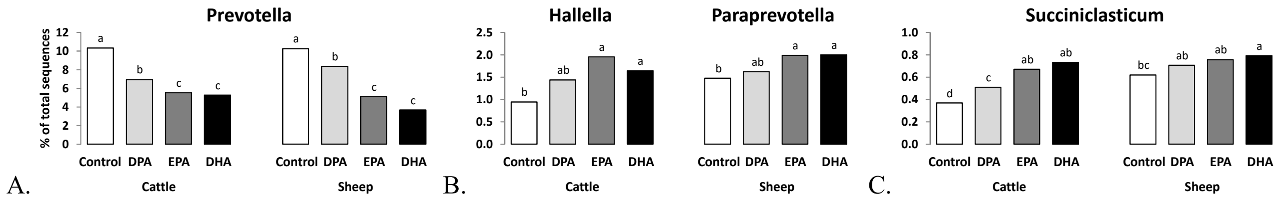


Figure 1 Relative abundances of *Prevotella* (A), *Hallella* and *Paraprevotella* (B), and *Succiniclasticum* (C) in ruminal digesta of cattle and sheep after 24 h-incubations without FA supplementation (Control) or with DPA, EPA or DHA.

Conclusion Dietary addition of 2% DM of EPA, DPA and DHA in *in vitro* batch cultures alter some bacterial genera potentially involved in lipid metabolism, such as *Prevotella*, *Hallella*, *Paraprevotella* and *Succiniclasticum*. Most changes were comparable in sheep and cows, but there were also variations exclusive to each ruminant species. The effect of DPA was less pronounced than that of EPA or DHA.

Acknowledgements Support by the Spanish MINECO (AGL2014-54587 and AGL2017-87812) and the European Regional Development Fund is acknowledged. D. Carreño benefits from a FPI and P.G. Toral from a Ramón y Cajal research contract.

References

De la Fuente G, Belanche A, Girwood SE, Pinloche E, Wilkinson T and Newbold CJ 2014. Plos One 9, 1-13.
Huws SA, Kim EJ, Lee MRF, Scott MB, Tweed JKS, Pinloche E, Wallace R J and Scollan ND 2011. Environmental Microbiology 13, 1500-1512.
Toral PG, Bernard L, Belenguer A, Rouel J, Hervás G, Chilliard Y and Frutos P, 2016. Journal of Dairy Science 99, 301-316.
Toral PG, Hervás G, Carreño D, Leskinen H, Belenguer A, Shingfield KJ and Frutos P 2017. Journal of Dairy Science 100, 6187-6198.

Proteomic analysis provides insights into the molecular basis of rumen papillae growth during adaptation of sheep to high concentrate diets.

Lei Xu[1], Weiyun Zhu[1], Chen Dai[2], Yue Wang[1], Shengyong Mao[1].

[1]Jiangsu Key Laboratory of Gastrointestinal Nutrition and Animal Health, Laboratory of Gastrointestinal Microbiology, Department of Animal Science and Technology, Nanjing Agricultural University, Nanjing, China, [2]College of Life Sciences, Nanjing Agricultural University, Nanjing, China

E-mail: njauxulei@163.com

Take home message These findings may provide new insights into understanding the molecular adaptive mechanisms of the rumen epithelium (RE) to high-grain (HG) diet feeding, which may contribute to developing technologies or feeding strategies in feedlot sheep and cattle.

Introduction Previous studies showed that RE adaptation to HG diets entailed morphological adaptations associated with tissue proliferation (Goodlad R, 1981, Steele MA *et al*., 2001). However, to date, little information is available on the changes in the expression of genes and proteins participating in the biological processes and cellular components in RE adaptation to HG diet. Thus, the objective of this study was to evaluate proteomic alterations in the RE during adaptation of sheep to HG diets using LC-MS/MS label-free methods.

Materials & methods A total of 20 sheep were divided into four groups based on age and body weight. All sheep were fed a forage-based diet containing 96.4% hay and 3.6% of a mineral and vitamin premix for at least four weeks. Five sheep in one of the 4 groups were slaughtered (control, CON, n = 5) before the day on which the rest of the sheep's diet was switched to a high-grain diet containing 40% forage and 60% concentrate mix. The transition to HG diet lasted 4 days (HG diet was increased 15% by 3.5% of BW per day gradually) until fed with 60% concentrate mix. One group was slaughtered on d 7 (HG7; n= 5), d 14 (HG14; n = 5), and d 28 (HG28; n = 5) after beginning the HG diet, respectively. All animals and experimental procedures involved this study were licensed by the Animal Care and Use Committee of Nanjing Agricultural University (SYXK(Su)2015-0656). Comparisons between groups were analyzed by one-way ANOVA, taking $P < 0.05$ as significant according to Tukey's multiple comparison Test. All data were analyzed using polynomial contrast to evaluate whether treatment resulted in linear, quadratic, or cubic patterns.

Results & discussion Results showed that the ruminal pH linearly decreased ($P < 0.01$) with feeding HG diet. Total rumen VFA concentration increased (linear, $P < 0.01$) by feeding the HG diet and was greater on days 14 and 28 than on day 0 ($P < 0.01$). All of the length, width and surface of the rumen papillae were enlarged ($P \leqslant 0.01$) after 14d of HG diet feeding. In total, 146 significant differential proteins were identified with < 0.05 FDR and > 1.5 VIP-value by label free LC-MS/MS. These proteins were classified into three clusters according to their expression trends. Interestingly, 56 significant differential proteins were selected based on biological process and KEGG pathway, which were classified into four categories: cell growth regulation, actin cytoskeleton regulation, cell junction or adhesion regulation, and metabolic regulation. Furthermore, 29 candidate proteins corresponding genes were further verified by real-time PCR. Results showed that the relative transcript level of Prostaglandin I2 synthase (PTGIS), Alpha-crystallin B chain (CRYAB), Integrin subunit alpha5 (ITGA5), Integrin beta (ITGB1), Filamin A (FLNA), Protein phosphatase 1 regulatory subunit 12A (PPP1R12A), Integrin linked kinase (ILK), Valosin-containing protein (VCP) and Signal transducer and activator of transcription (STAT3) in the function 1 matched well with the comparative proteomic data. These proteins were regulated by HG feeding, indicating that the rumen epithelium growth associated with cell cycle regulation, apoptosis block and proliferation increasement. The relative transcript level of Actin-related protein 2/3 complex subunit 4 (ARPC4), Actin-related protein 2/3 complex subunit 5 (APRC5L), Profilin (PFN2), Actinin alpha 1 (ACTN1) and Actinin alpha 4 (ACTN4) in the function 2 and 3 was also matched well with the comparative proteomic data. These five proteins might protect the rumen epithelium barrier function and restore the integrity of the cell junctions. The expression of genes in function 4 including Succinyl-CoA, 3-ketoacid-coenzyme A transferase (OXCT1), UDP-glucuronosyltransferase (UGT1A1), ATP-dependent 6-phosphofructokinase (PFKM), Dolichyl-diphosphooligosaccharide- protein glycosyltransferase 48 kDa subunit (DDOST) and Protein disulfide-isomerase (P4HB) were consistent with the proteomics data as well. These results suggested that the quality of our proteomics data is acceptable.

Conclusion These results demonstrated sequential changes in rumen fermentation and rumen papillae size caused by molecular mechanism involved in cell growth regulation, actin cytoskeleton regulation, cell junction, cell adhesion regulation and metabolic regulation during adaptation of sheep to HG diets.

Acknowledgements This work was supported by the National Natural Science Foundation of China (No.31572436) and China Natural Science Foundation of China (No. 31372339).

References

Goodlad R 1981. Quarterly Journal of Experimental Physiology 66, 487-499.
Steele MA, Vandervoort G, AlZahal O, Hook SE and Matthews JC 2011. Physiological Genomics 43, 308-316.

391

Effect of infusing with nitrate on ruminal fermentation and the bacterial flora in goats

Lin Miao, Gong Shuai, Gong Xiaoxiao

Yangzhou University, Yangzhou, Jiangsu, China

E-mail: 1104615502@qq.com

Take home message Large amount of nitrate intake in the rumen for one time has a great influence on rumen fermentation.

Introduction Nitrate has received much attention in recent years due to its methane inhibitor properties. Nitrate is reduced to ammonia through nitrite. However, nitrite assimilated into the bloodstream oxidizes the ferrous hemoglobin to methemoglobin, which will cause methemoglobinemia, and even death. This happens mostly when animals are fed with a high level of nitrate without adaptation. Previous research has highlighted that *Selenomonas ruminantium* plays a very significant role in nitrate metabolism (Yang *et al.*, 2016). How the microbial community responds to the different methods of nitrate intake needs further study.

Materials & methods Four goats (body weight = 25 ± 5 kg) with permanent rumen fistulas were chosen as the donor animals for rumen fluid. Potassium nitrate was added at 0.8g/kg of diet (DM basis) for 14 days following the protocol from Lin *et al.* (2011). Animals were fed twice daily (09:00 and 17:00), and had free access to water. During the infusion period, rumen concents were removed from each goat through the ruminal fistula and infused with potassium nitrate solution (accounted for 0.8g/kg of diet) every day before the morning feed. Rumen fluid samples were obtained at 1 h after morning feed on days -1 (pre, prior to infusion) and 1, 2, 3, 4, 5, 6 days after infusion. The NO_3^-, NO_2^-, NH_3-N and pH were determined. The bacterial 16S rRNA V4 fragments from pre, d1, d3 and d6 were analyzed and calculated in Mothur. Data within the normal distribution were analyzed by one-way analysis of variance (ANOVA), and Tukey's test was used when the F-test was significant. Statistical analysis was performed with SAS 9.2 (SAS Institute).

Table 1 Effect of nitrate on rumen fermentation profile.

	Pre	d1	d2	d3	d4	d5	d6	*P*-value
NO_3, mmol/L	0.177±0.04^{b}	0.458±0.35^{ab}	0.268±0.07^{ab}	0.415±0.14^{ab}	0.43±0.23^{ab}	0.767±0.15^{a}	0.225±0.08^{b}	0.01
NO_2, μmol/L	0.107±0.19	0.680±0.34	0.615±1.16	0.547±0.73	0.395±0.50	0.190±0.33	0.350±0.25	0.90
NH_3-N, mmol/L	3.40±1.81^{ab}	3.87±0.62^{a}	3.75±0.39^{a}	2.99±0.88^{ab}	2.21±0.57^{abc}	1.34±0.64^{bc}	0.83±0.40^{c}	0.003
pH	6.56±0.34	6.89±0.22	6.97±0.14	6.79±0.12	6.83±0.11	6.87±0.09	6.73±0.20	0.16

Results Nitrate concentration fluctuated during the experimental period (P=0.01; Table 1). When infusing with nitrate, nitrite concentration and pH were not affected, however, ammonia concentrations declined (P=0.003). During infusion with potassium for 6 days, the bacterial structure was distinctly different from prior to infusion, suggesting that artificial infusion with potassium nitrate at 0.8g/kg of the diet modified the bacterial community (Figure. 1).

Conclusion In goats, rumen contents artificially infused with potassium nitrate solution at 0.8g/kg of diet improved nitrite reduction rate and ammonia utilization, and changed the bacterial community.

Acknowledgements This work was supported by China Agriculture Research System (CARS-36).

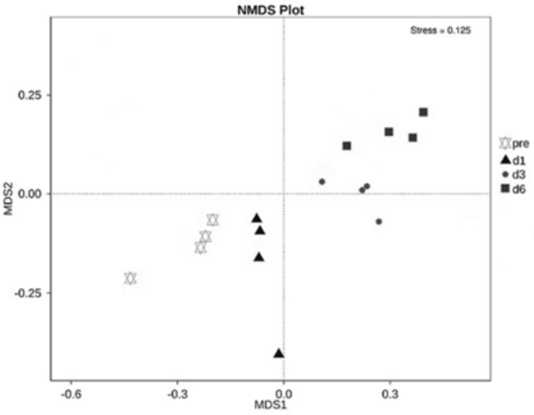


Figure 1 NMDS analyse of bacterial structure before infusion (pre) and during 6 days of infusion (d1, d3, d6).

References

Yang CJ, Rooke JA, Cabeza I and Wallace RJ 2016. Frontiers in Microbiology 7, 132.
Lin M, Schaefer DM, Guo WS, Ren LP and Meng QX 2011. Asian-Australasian Journal of Animal Science 24, 471-478.

Effect of additives from different nature on barley fermentation in diets for intensive beef production in an *in vitro* semicontinuous system

Zahia Amanzougarene, Susana Yuste, Antonio de Vega, Manuel Fondevila

Faculty of veterinary, Department of food science and Animal Nutrition, Zaragoza, Spain

E-mail: zahiaagro@yahoo.fr

Take home message Additives such as grape condensed tannins and fatty acids may reduce barley acidification potential.

Introduction The acidosis that often occurs in intensive beef fattening systems might be avoided by the use of feed additives to manipulate ruminal fermentation, maximizing the efficiency of feed utilization and consequently increasing ruminant productivity. This study evaluates the effect on rumen fermentation of the supplementation of additives under high concentrate feeding conditions, using barley as a reference substrate, in *in vitro* semicontinuous system.

Materials & methods Five additives from different natural sources included at doses previously established were used supplementing barley as a reference feed used in intensive feeding diets: grape condensed tannins (GCT, 20 mg/g); the essential oils cinnamaldehyde (CIN, 0.06 mL/g) and eugenol (EUG, 0.12 mL/g); linoleic acid (LIN, 0.03 mL/g) and a medium chain fatty acids mixture (MFA, 4 mg/g). Supplemented barley was included as control (CTR). Fermentation kinetics were determined *in vitro* in a semicontinuous system (Fondevila and Pérez-Espés, 2008, Prates *et al*., 2010) under a poorly buffered medium from 0 to 6 h, and allowing pH to rise to 6.5 from 8 to 24 h. Rumen contents from intensively fed beef calves was used as inoculum (20 ml/100 ml). Along the incubation, a volume of medium was extracted immediately after gas measurement and replaced anaerobically by incubation solution (without microbial inoculum) to simulate an approximate liquid turnover rate of 0.08/h. Gas production and incubation pH were recorded from 2 to 24h. Results were analysed by ANOVA, with the incubation series as block and the bottles as the experimental unit.

Results & discussion The pattern of pH incubation (Figure 1) shows that using of poorly buffered solution from 0 to 6 h and more buffered from 8 h onwards allowed to mimic the daily *in vivo* rumen pH fluctuation. The mean incubation pH was 6.43 ±0.16 at 0 h and the minimum was reached after 6 h incubation. Among treatments, no pH differences were recorded in the first 4 h (P>0.05) and the minimum pH was of lower magnitude with GCT (5.99) and lowest with CIN (5.80; P<0.05). Throughout all the incubation period, pH with GCT rose up to 0.2 unit higher than CTR (P<0.05) and reached a maximum at 20 h (pH 6.48; P<0.05), whereas with CIN lower pH values than CTR were always recorded (P<0.05). In terms of gas production (Figure 2), differences among treatments were recorded during the whole incubation period (P<0.001). Except at 6 h, the volume of gas with CTR was highest (P<0.05). From 2 to 16 h CIN recorded the lowest volume of gas (P<0.05), whereas at 20 and 24 h differences between GCT, CIN, and EUG disappeared (P>0.05).

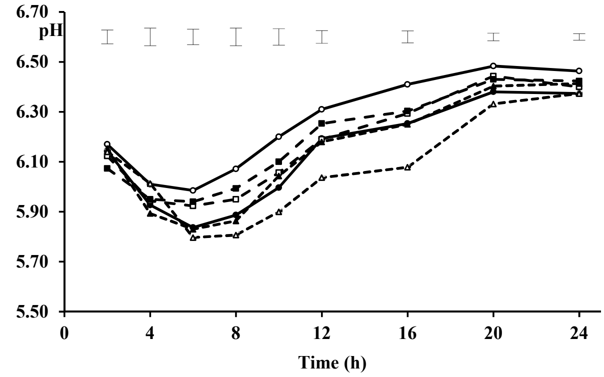


Figure 1: Pattern of incubation pH with CTR (●), GCT (○), CIN (△), EUG (▲), LIN (■), and MFA (□) Upper bars show standard error of means (n=3)

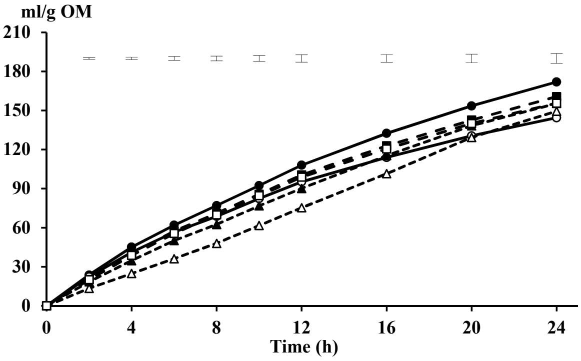


Figure 2: Pattern of the volume of gas produced with CTR (●), GCT (○), CIN (△), EUG (▲), LIN (■), and MFA (□). Upper bars show standard error of means (n=3)

Conclusion GCT as additive binds to barley components, reducing *in vitro* fermentation, at the time it may positively affect ruminal environment by maintaining a higher pH than barley alone. In contrast, CIN promoted a drop on pH but also reduced barley fermentation. Other additives tested (LIN, MFA and EUG) did not affect fermentation or environmental conditions.

Acknowledgements Spanish Government (project AGL2013-46820-P), Government of Aragon and European Social Funds.

References

Fondevila M, and Pérez-Espés B 2008. Animal Feed Science and Technology 144, 196–211.
Prates A, de Oliveira JA, Abecia L and Fondevila M 2010. Animal Feed Science and Technology 155, 186-193.

Bacterial DNA quantification and ^{15}N as methods to estimate microbial growth: a comparative study

Jairo García-Rodríguez[1], María Dolores Carro[2], Sergio Fernández-Prieto[1], Secundino López[1,3], María José Ranilla[1,3]

[1]Universidad de León, León, Spain, [2]Universidad Politécnica de Madrid, Madrid, Spain, [3]Instituto de Ganadería de Montaña ULE-CSIC, Grulleros, León, Spain

E-mail: mjrang@unileon.es

Take home message Microbial growth in the rumen could be estimated by quantification of total bacterial DNA.

Introduction ^{15}N is an external marker widely used for estimating microbial protein synthesis (MPS) in ruminants. Bacterial DNA (BDNA) has been also proposed as a potential internal marker to assess MPS, but studies comparing both procedures are limited. The objective of this study was to compare values of microbial growth in Rusitec fermenters determined using either ^{15}N or total BDNA concentration, and to assess if both procedures detect similar differences between diets in solid, liquid and total digesta.

Materials & methods Two independent Rusitec trials were carried out to assess microbial growth and rumen fermentation of four different diets containing 50:50 forage:concentrate. In trial 1, the concentrate was formulated either with maize (M) or with maize and citrus pulp (CP), and in trial 2 the forage in the diets contained either barley straw (BS) or olive cake (OC). In each trial, four Rusitec fermenters were used in a cross-over design in two 14-day incubation periods (giving four replicate fermenters per diet). From day 10 to 14, a solution of $^{15}NH_4Cl$ was added to the artificial saliva following the procedure described by Martínez *et al.* (2010). On days 13 and 14, samples of solid and liquid digesta were taken to estimate MPS in each digesta phase and for measuring BDNA by qPCR. Data were analysed as a mixed model, with diet, incubation period and their interaction as fixed effects and fermenter as a random effect. The relationship between values of MPS determined by ^{15}N and BDNA concentrations was assessed by Pearson's correlation coefficient.

Results & discussion In trial 1, there were no differences ($p > 0.05$) between M and CP diets in MPS estimated by ^{15}N in any digesta phase (solid, liquid and total; Table 1). Similarly, no differences between diets were found ($p > 0.05$) in BDNA concentrations. In trial 2, MPS was greater for BS diet compared with OC diet in the solid phase, but the opposite was detected in the liquid phase and there were no differences ($p > 0.05$) between diets in total MPS values. In contrast, BDNA concentrations were similar ($p > 0.05$) for both diets in both digesta phases. When using data from both trials (n=16), there was a positive and significant relationship between the MPS values and the amount of BDNA in each fermenter both in the liquid phase ($r=0.762$; $p < 0.001$) and in total digesta ($r=0.409$; $p > 0.05$), but no correlation ($p > 0.05$) was detected in the solid contents. These results are in agreement with those observed by Mateos *et al.* (2017) in Rusitec fermenters fed different diets.

Table 1 Microbial protein synthesis (MPS) estimated using ^{15}N as a marker and bacterial DNA concentrations (BDNA) in Rusitec fermenters fed diets containing maize (M) or citrus pulp (CP) in trial 1, and barley straw (BS) or olive cake (OC) in trial 2.

Trial 1					
	Digesta phase	M	CP	SEM	p =
MPS, mg microbial N/d	Solid	180	200	7.71	0.14
	Liquid	137	116	7.92	0.15
	Total	317	316	6.45	0.92
BDNA, mg DNA/d	Solid	2.51	3.14	0.23	0.13
	Liquid	3.11	2.30	0.47	0.29
	Total	5.62	5.44	0.34	0.73

Trial 2					
	Digesta phase	BS	OC	SEM	p =
MPS, mg microbial N/d	Solid	199	173	6.89	0.05
	Liquid	107	119	2.65	0.03
	Total	306	292	5.87	0.16
BDNA, mg DNA/d	Solid	4.25	3.61	0.99	0.67
	Liquid	2.11	1.06	0.40	0.14
	Total	6.36	4.67	0.96	0.28

Conclusion Both methods detected similar differences between diets in total microbial growth but differed when considering microbial growth either in solid or liquid phase of the fermenters. More studies are warranted to confirm BDNA quantification as a routine method for estimating microbial growth in the rumen.

Acknowledgements Funding from the Spanish Ministry of Economy and Competitiveness is gratefully acknowledged (Projects AGL2016-75322-C2-1-R and AGL2016-75322-C2-2-R).

References

Martínez M E, Ranilla MJ, Tejido ML, Ramos S and Carro MD 2010. Journal of Dairy Science 93, 3684-3698.
Mateos I, Ranilla MJ, Saro C and Carro MD 2017. Animal 11, 1939-1948.

The design of an automated trace gas recording system for *in vitro* microbial fermentation based on differential pressure transmitter technology

Hong-Jian Yang

State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing, China

E-mail: yang_hongjian@sina.com

Take home message The AGRS designed in the present study can be applied in gut fermentation exploration associated with not only animal science but also human food science.

Introduction *In vitro* gas production technique (IVGPT) has been extensively applied to determine feed digestion characteristics and the kinetics of fermentation in ruminant animals (Theodorou *et al*., 1994). Since the 1990s, in order to overcome time-consuming and laborious disadvantages of traditional syringe method, researchers in UK, USA and the Netherlands have developed some manual/ /semi-automated/fully automated gas production devices based on pressure sensor/switch. However, the pressure buildup in these systems are all too high (> 4.5 kPa), which would affect normal microbial growth and metabolic activity during microbial fermentation. The high pressure can also easily cause gas leakage, and further affect accuracy of gas amount determination The present study aimed to design and build a novel trace gas recording system (AGRS) based on differential pressure transmitters to overcome the shortcomings of the existing devices.

Materials & method
Differential micro-pressure transmitters, electromagnetic valve and data acquisition card were incorporated in the AGRS system. As shown in Figure 1, the system consists of (1) a temperature-controlled incubator, (2) an industrial computer equipped with two analog voltage and digital switch data acquisition and signal conditioning cards, (3) four sets of 16-channel gas recording units as shown in lower right 3D picture equipped with differential pressure transducers with a range of 0-300 Pa against atmosphere and electronic solenoid valves, (4) sixty-four glass bottles (120 mL) as fermenters with butyl rubber stoppers and Hungate's screw caps, which can be easily connected to gas inlets of each 16-channel gas recording unit with transfusion tubes and needles. Microsoft Visual Basic language under Visual Studio 2008 was applied to develop the operation-friendly software to enable experimental design setting, transduce setting, signal diagnosis, inoculation timing, gas leakiness checking, real-time gas curve displaying (Figure 2), and data exporting for 64 channels of cumulative gas production against different incubation time.

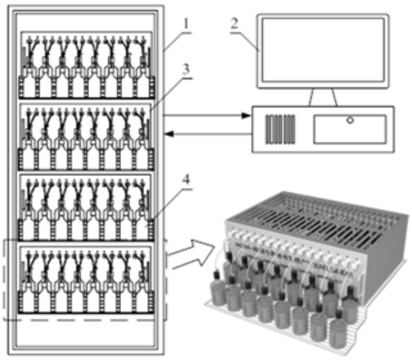


Figure 1. The 64-channel AGRS.

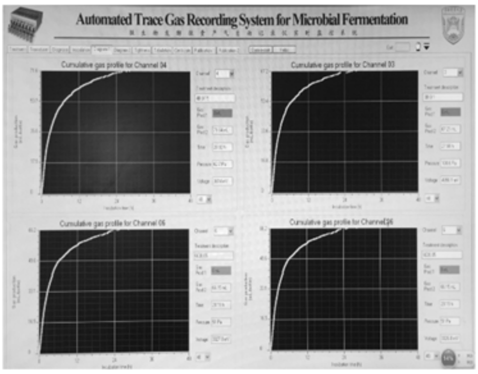


Figure 2 Real-time cumulative gas recording.

Results & discussion A ration (500 mg) consisted of 40% corn silage and 60% concentrate was anaerobically incubated with 50 mL medium (pH 6.85) and 25 mL filtrated rumen fluid collected from three rumen-fistulated Holstein cows. The fermentation was done in triplicate for each channel under different upper differential pressure (DP) setting as shown in Table 1. As a result, mean actual DP builtup of 32 to 293 Pa were detected, and corresponding gas production (GP) of 0.048 to 0.463 mL per venting was recorded. Average coefficient of variations (CV) among 64 channels for DP detected and GP recorded per venting were 2.2% and 2.7%, respectively. The optimal upper pressure range of 50-250 Pa was recommended depending on substrate fermentability. The system has been accepted and applied in Yangzhou University, Zhejiang Univerisity in China for feed evaluation and University of Queensland (Brisbane, Australia) for gut fermentation studies in food science.

Table 1 The differential pressure (DP) detected and gas production (GP) recorded per venting.

Upper DP (Pa)	DP detected		GP recorded	
	Mean (Pa)	CV (%)	Mean (mL)	CV (%)
30	32	5.7	0.048	6.7
50	54	5.4	0.083	5.6
70	75	3.8	0.116	4.5
90	92	1.3	0.144	3.6
110	115	2.5	0.182	2.5
130	138	2.9	0.216	2.4
150	152	1.2	0.240	2.2
170	171	0.4	0.270	2.0
190	199	2.5	0.313	2.8
210	216	2.0	0.343	2.1
230	237	0.9	0.373	1.2
250	254	1.1	0.403	1.2
270	276	1.1	0.435	1.2
290	293	0.4	0.463	1.0
Average	-	2.2	-	2.7

Conclusion The novel 64-channel AGRS system based on differential pressure transducer application was designed and built in China. It can sensitively measure trace amount of GP (0.05 ~0.50 mL) with quite low variation among different fermentation channels. The system is increasingly accepted and applied in a broad aspect of scientific research.

References

Theodorou, MK, Williams, BA and Dhanoa, MS 1994. Animal Feed Science and Technology 48, 185-197.

Some results of study on species of rumen ciliate protozoa of Mongolian lamb

Purevtsogt Dorjgoo, Zolzaya Majigsuren, Demberel Shirchin, Dugersuren Jamyan

Institute of Veterinary Medicine, Ulaanbaatar, Mongolia

E-mail: dpurevtsogt@yahoo.com

Take home message Rumen protozoa contribute significantly to the digestion of ruminants as anaerobic fermentative microorganisms.

Introduction Rumen ciliates play enormous roles in digestive functions of ruminants as fermentation microorganisms, as well as they are important for nutrition of host animals, protect carbohydrates from the attack of bacteria and improve rumen metabolism via digestion of bacteria. Besides of representing approximately 50% of rumen biota, ciliates degrade approximately 20% of proteins of all proteins received by their own host animal, which digests 91% of feed nutrients in its own stomach, and 7.5 to 15% of total lipids are produced by ciliates. Ciliates are also an important source of unsaturated fatty acids, neutralize toxins of poisonous plants, cleanse and destruct some toxins in digestive tract and stabilize the staphylococci counts in order to decrease some harmful lactic acids produced.

Materials & methods The present study was undertaken relying on sheep flock of a herder family in Ugtaaltsaidam soum of Tuv aimag and analyses of samples were performed by using premises of Laboratory of young animal physiology and pathology of Institute of veterinary medicine. Twelve clinically healthy lambs were selected in this study, rumen cud samples were taken by intubation from digestive tract contents, fixated in 10% buffered formalin, stored in $+4^0$C during shipping and used for counting ciliates and species identification in the laboratory. Counts and morphology of ciliates were determined by use of Goryaev's chamber, light microscopes of Nihon E600 and BK 1000 models, digital camera for micro photos and computer with Microsoft windows 10 ultimate software, and identification of species was made according to method of identification of microorganisms or classification described by Dehority BA (2005) and Baraka TA (2012), and the materials were documented by photography using above equipments. Mean differences in the count of ciliate protozoa were analyzed by the un paired t-test

Results & discussion Ciliates of digestive tracts of lambs at approximately 3 weeks of age belong to Entodinium spp only and count of ciliates was $42.14 \pm 7.04 * 10^3$ per ml of rumen cud. Further, total protozoa (ciliates) count reached $227.14 \pm 37.9 * 10^3$, when lambs are at 1.5 months of age, and such genera as Diplodinium spp and Isotricha spp were detected. During two months old age, when lambs growth and forestomach functions are intensified and feed utilization is improved, count of ciliates per ml of rumen cud was $324.14 \pm 18.5 * 10^3$, the generic and species composition increased and the genera, including Epidinium spp and Dasytricha spp were found. These ciliates belong to orders *Entodiniomorpha* and *Holotricha*. Studies demonstrated ciliates are found in the rumen contents of Mongolian lamb since 15 days of age, the count and species compositions of the ciliates are tended to increase with aging of lambs, especially increase intensively between 45 and 60 days of lamb age, when they start to eat fodders.

Conclusion Dominant genera of ciliates counted in the rumen cud of half month-old age lambs are *Entodinum* spp and increase of their counts with lamb aging resulted in expansion of generic compositions enriched by Diplodinum spp (16.97%) and Isotricha spp (2.47%) at a month of age, and Epidinium spp (1.08%), Ophryoscolex spp (2.16%) and Dasytricha spp (0.8%) at two month of lamb age.

Ciliates of 6 genera belonging to 2 orders were identified from rumen cud of pasture-raised lambs and preparation of manuscript of hand book containing album of color photos illustrating genera and species of ciliates relying on our study materials and other literatures can be used for teaching and advertisement.

Acknowledgements This research was supported from the lab of Young Animal Physiology and Pathology, of The Institute of Veterinary Medicine, Mongolia.

References

Baraka TA 2012. Journal of American Science, 2012/8

Dehority BA 2005. Ciliate protozoa. In Methods in Gut Microbial Ecology for Ruminants (eds. H.P.S. Makkar and C.S. McSweeney), pp. 67–78. Springer-Verlag, Berlin/Heidelberg.

Dugersuren J and Demberel SH 2001. Scientific Publications 6, 33-35,

Purevtsogt D, Demberel SH and Dugersuren JJ 2015. Mongolian Veterinary Science and Technology 1, 99-102. UB 2015

Hobson PN and Stewart Cs 1997 The Rumen Microbial Ecosystem. Springer Netherlands, Dordrecht

In vitro assessment of goat's saliva on rumen fermentation.

Juan Manuel Palma-Hidalgo, Alejandro Belanche, A. Ignacio Martín-García, David R. Yáñez-Ruiz

Estación Experimental del Zaidín (CSIC), Granada, Spain

E-mail: juanm.palma@eez.csic.es

Take home message Inclusion of saliva from goats promotes changes in rumen fermentation pattern *in vitro*.

Introduction Saliva, together with drinking water, represents the main liquid input into the rumen as its flow, depending on the diet, can range between 4.22 and 9.05 L/day in sheep (Durić *et al*., 1994). The different components of the ruminants' saliva (i.e. bicarbonates and phosphates as buffer, immunoglobulins and cytokines) have been suggested to modulate the composition and activity of the rumen microbial ecosystem and may explain the differences observed across individuals (Fouhse *et al*., 2017). However, the extent to which the saliva exerts such control is yet unknown. The aim of this work was to test *in vitro* the effect of gradual replacement of buffer with goats´s saliva on rumen fermentation. Filtered and non-filtered saliva was also used to account for the potential effect of microbial cells collected with saliva.

Materials & methods Four Murciano-Granadina goats were used as saliva donors. Saliva was sampled prior feeding for several days by swabbing the mouth with absorbent sponges. Sponges were then centrifuged at 190 g for 10 mins in order to collect the saliva. Half the saliva was further centrifuged at 16300 g for 5 mins and filtrated through 0.45 µm pore size to remove microorganisms. Filtrated (FS) and non-filtrated saliva (NFS) samples were pooled separately for each animal and stored at -80 ºC. A 24 h *in vitro* incubation was performed in Hungate tubes with a total volume of 6 ml containing 2 ml of fresh rumen fluid and 4 ml of a mixture of saliva and bicarbonate buffer. Four different proportions of saliva (0%, 16%, 33% and 50% of the total volume) were used in a 4×4×2 experimental design. Substrate consisted of concentrate (75 mg) and hay (75 mg) both ground at 1mm. Gas production was measured at 2, 4, 7, 10 and 24 h during the incubation. Incubation pH was measured at 0 and 24 h. At the end of the incubation, samples were taken to determine the concentration of NH_3-N and volatile fatty acids (VFA). A multifactorial ANOVA was performed considering the saliva/buffer proportion and saliva filtration as fixed factors and the animal as a random effect.

Results & discussion Filtrated saliva promoted a higher VFA but lower NH3-N (P<0.001 and P<0.01; respectively) concentration in comparison with NF saliva (Table 1). However, total gas production was unchanged as a result of filtration. The removal of protozoa after filtration may explain the differences in NH3-N concentrations (Newbold *et al*., 2015). Increasing the proportion of saliva had a substantial effect in all fermentation parameters. In general, the concentration of NH3-N and VFA increased and the Ac/Prop ratio decreased in relation to the increasing proportion of saliva (P<0.05; P<0.001; P<0.05; respectively). This suggests that the salivary components might foster a higher bacterial activity and a lower fibre degradation, which may be related to the drop in the incubation pH. Further studies need to be conducted to elucidate the individual contribution of different salivary components to such effects.

Table 1 Effect of using different proportions of non-filtrated (NFS) and filtrated (FS) saliva on *in vitro* rumen fermentation.

	Filtration		Proportion of saliva				SEM	*P* value		
	NFS	FS	0%	16%	33%	50%		F	P	FxP
Initial pH	6.94	6.89	6.85	6.90	6.98	6.94	0.039	ns	ns	ns
Final pH (after 24h)	5.50	5.50	5.63^{a}	5.51^{b}	5.47^{b}	5.40^{c}	0.012	ns	***	*
Gas Production, mL	22.4	22.8	23.9^{a}	23.9^{a}	22.3^{b}	20.2^{c}	0.284	ns	***	ns
NH_3-N, mg/dL	35.1	29.1	28.7^{c}	29.8^{bc}	34.3^{ab}	35.3^{a}	1.642	**	*	ns
Total VFA, mM	214	232	212^{b}	221^{a}	228^{a}	229^{a}	2.829	***	***	ns
Acetate, %	61.4	61.0	62.6^{a}	61.9^{a}	60.8^{b}	59.7^{c}	0.287	ns	***	*
Propionate, %	20.9	21.1	19.9^{c}	20.7^{bc}	21.4^{ab}	22.0^{a}	0.293	ns	***	ns
Butyrate, %	14.3	14.7	14.4^{bc}	14.1^{c}	14.5^{b}	15.0^{a}	0.095	**	***	***
Ac/Prop	2.95	2.90	3.14^{a}	2.99^{b}	2.85^{c}	2.72^{c}	0.047	ns	***	ns

*** P<0.001; ** P<0.01; * P<0.05; ns: not significant

Conclusion Our results show that the inclusion of saliva promotes changes in the ruminal fermentation and reduces gas production *in vitro* suggesting modulatory effects that need to be further studied.

Acknowledgements We acknowledge funding from MECD (FPU16/01981) and MINECO (AGL2017-86938-R).

References

Durić M, Zhao Y, Ørskov ER and Chen XB 1994. Experimental Physiology 79, 823-830.
Fouhse, JM, Smiegielski, L, Tuplin, M, Guan, LL and Willing BP 2017. Frontiers in Microbiology 8, 1-9.
Newbold CJ, de la Fuente G, Belanche A, Ramos-Morales E and McEwan NR 2015. Frontiers in Microbiology 6, 1-14.

In vitro evaluation of two new synthetic nitrocompounds antimethanogenic activity

Rabah Arhab[2], Asma Kheddouma[1], Antonio Ignacio Martín-García[3], Abdelmalek Bouraiou[4]

[1]*Faculty of Nature and Life Sciences, University of Abbes Laghrour, Khenchela, BP: 1252 Route de Batna Khenchela, 40004, Algeria,* [2]*Faculty of Nature and Life Sciences, Larbi Ben M'Hidi University, Oum El Bouaghi, Route de Constantine, Algeria,* [3]*Estación Experimental del Zaidín (CSIC),, Camino del Jueves s/n, 18100 Armilla, Granada, Spain,* [4]*4Unité de Recherche de Chimie de l'Environnement et Moléculaire Structurale (CHEMS), Université Frères Mentouri, Constantine 1, Algeria*

E-mail: arhabrabah@yahoo.fr

Take home message Molecular vectorization in animal nutrition is a new tool used in reducing ruminal methane production.

Introduction Nitrocompounds moieties have been largely used as methane modulators. But their major constraint is their risk of causing methemoglobinemia in livestock (Zhenming *et al*., 2012). However, recent work suggests that the risk of ruminal nitrite accumulation may be alleviated by co-supplementation with nitrite-reducing bacteria or decreasing the rapidity of nitrate reduction in the rumen by feeding nitrate with a slow-release coating (Raphélis-Soissan *et al*., 2017). This study aimed to evaluate *in vitro* the antimethanogenic activity of two new nitrocompounds: ABLE 244 (13-(4-nitrophenyl)-3,4-dihydro-2*H*-indazolo [1,2-b] phthalazine-1,6,11(2*H*, 13*H*)-trione) and ABLE 245 (16-(4-nitrophenyl)-1,16-dihydrophthalazino [2',3':1,2] pyrazolo [4,3-a] carbazole-9,14 dione) comparatively to two commercial nitrocompounds: Nitrophenol (NIP) and 5-Nitrobenzimidazol (5-NBZ)

Materials & methods ABLE 244 and ABLE 245 were synthesized at the Crystallography Laboratory (UMC1, Algeria). Media culture (0.3 g of oats hay plus 40 ml of artificial saliva) was supplemented with 0, 2, 8 and 12 mM of ABLE 244, ABLE 245, NIP and 5-NBZ. Gas pressure is recorded after 2, 4, 6, 12 and 24h. At the end of fermentation, methane production and volatile fatty acids profile were determined by gas chromatography.

Results & discussion Total gas production (GP) was decreased by near 30% with NIP (12 mM) compared to control (Figure 1, P <0.05**).** However, 5-NBZ, ABLE 244 and ABLE 245 did not affect gas production. NIP and 5-NBZ markedly inhibited CH_4 production by more than 60% when compared to control for 12 mM concentration. For synthetic compounds, only ABLE 244 reduce methane production (13.9%, Figure 1). VFA production in the *in vitro* ruminal cultures were altered by nitrocompounds added, and those alterations varied among the concentrations tested (Table 1). However, all compounds caused a decrease in acetate production. This situation is corroborated to the increase in propionate concentration.

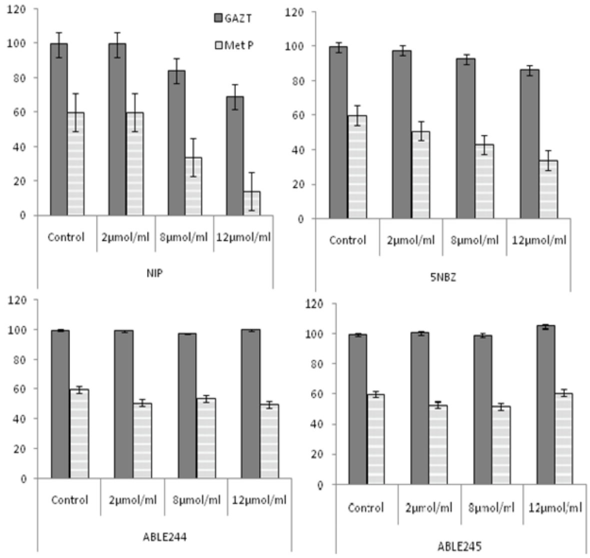


Figure 1 Total gas (GAZT) and methane (Met P) productions (ml/g DM) after 24h *in vitro* ruminal microorganisms culture.

Conclusion This study shows that ABLE 244 or ABLE 245 did not have a remarkable CH_4-inhibiting activity. We hypothesize that these long chain nitrocompounds are not easily degradable by the ruminal flora.

Table 1 Effects of nitrocompounds on volatile fatty acid (VFA) profile after 24 h of incubation.

		Dose (mM)					
Item	Compound	0	2	8	12	SEM	*p* value
VFAt (mM)	NIP	102^{ab}	138^{a}	99.1^{ab}	83.4^{b}	5.53	0.087
	5-NBZ	102	119	105	96.3	4.70	0.461
	ABLE244	102	110	104	102	2.66	0.810
	ABLE245	102	113	104	102	2.85	0.729
Acetate (%)	NIP	62.3	62.0	59.9	60.5	0.66	0.253
	5-NBZ	62.3^{a}	61.3^{a}	58.8^{ab}	57.7^{b}	0.49	0.006
	ABLE244	62.3	61.5	61.8	61.1	0.36	0.352
	ABLE245	62.3	61.6	62.1	61.8	0.44	0.853
Propionate (%)	NIP	17.8^{a}	17.6^{a}	19.9^{ab}	21.1^{b}	0.52	0.030
	5-NBZ	17.8^{a}	18.6^{ab}	21.3^{ab}	22.8^{b}	0.43	0.002
	ABLE244	17.8	18.0	18.1	18.5	0.25	0.359
	ABLE245	17.8	18.2	18.0	18.1	0.26	0.722
Isobutyrate (%)	NIP	1.66^{ab}	1.76^{a}	1.56^{ab}	1.20^{b}	0.06	0.024
	5-NBZ	1.66	1.76	1.56	1.70	0.05	0.829
	ABLE244	1.66	1.70	1.70	1.80	0.06	0.478
	ABLE245	1.66	1.63	1.70	1.73	0.06	0.648
Butyrate (%)	NIP	13.1	13.3	13.9	13.8	0.30	0.326
	5-NBZ	13.1	13.2	13.6	13.2	0.32	0.839
	ABLE244	13.1	13.5	13.4	13.5	0.22	0.573
	ABLE245	13.1	13.4	13.4	13.3	0.22	0.802
Isovalerate (%)	NIP	3.20^{a}	3.36^{ab}	3.20^{a}	2.46^{b}	0.09	0.027
	5-NBZ	3.20	3.20	2.96	3.00	0.08	0.322
	ABLE244	3.20	3.40	3.20	3.30	0.10	0.921
	ABLE245	3.20	3.30	3.10	3.20	0.10	0.843
Valerate (%)	NIP	1.93^{a}	1.86^{ab}	1.36^{ab}	0.70^{b}	0.07	0.000
	5-NBZ	1.93^{a}	1.76^{ab}	1.66^{b}	1.56^{b}	0.02	0.001
	ABLE244	1.93^{a}	1.80^{ab}	1.73^{b}	1.70^{b}	0.02	0.015
	ABLE245	1.93^{a}	1.73^{a}	1.56^{b}	1.66^{ab}	0.03	0.018
A:P ratio	NIP	3.50	3.52	2.99	2.90	0.12	0.065
	5-NBZ	3.50^{a}	3.30^{ab}	3.03^{ab}	2.80^{b}	0.10	0.039
	ABLE244	3.50	3.41	3.42	3.30	0.06	0.356
	ABLE245	3.50	3.38	3.45	3.41	0.07	0.783

References

Raphélis-Soissan V, Nolan JV, Godwin I.R, Newbold JR, Perdok HB and Hegarty RS 2017. Journal of Animal Feed Science and Technology 229, 57–64.

Zhenming Z, Zhongtang Y and Qingxiang M 2012. Bioresource Technology 103, 173–179.

Effect of *Eucalyptus globulus* leaves extracts on rumen fermentation, methanogenesis, *in vitro* degradability and protozoa population

Rabah arhab[2], Amina Boussada[1], Serena Calabro[3], Raffaella Grazioli[3], Maria Ferrara[3], Nadia Musco[3], Monica Isabella Cutrignelli[3]

[1]Department of Veterinary Medicine, Institute of Veterinary and Agricultural Sciences, Batna 1 University, 05000, Batna, Algeria, [2]Department of Natural and Life Sciences, Faculty of Exact Sciences and Natural and Life Sciences, Larbi Ben M'Hidi University, 04000, Oum El Bouaghi, Algeria, [3]Department of Veterinary Medicine and Animal Production, University of Napoli Federico II, Via F. Delpino 1, 80137, Napoli, Italy

E-mail: arhabrabah@yahoo.fr

Take home message *Eucalyptus globulus* is known for its essential oils. However, this work shows that it contains other bioactive substances that have antimethanogenic activities.

Introduction In livestock production systems chemical feed additives are commonly used as methane inhibitors. However, most of these practises have been banned for their toxicity problems for animals and indirectly to human health. Consequently, Effort has been devoted to exploit natural products (plant secondary metabolites) which play important roles in plants to guarantee normal growth development and defence against infection and injury. The objective of this study was to evaluate the effects of three extracts (*i.e.,* ethyl acetate, n-butanol, aqueous) rich on phenolic compound of *Eucalyptus globulus* on *in vitro* fermentation characteristics, rumen methanogenesis and protozoa population.

Materials & methods Three lyophilised extracts (ethyl acetate, n-butanol, aqueous) of *Eucalyptus globulus* plants with four dosages (0, 50, 75, 100 mg) were tested using the *in vitro* gas production technique (IVGPT) in two consecutive gas runs using as substrate a diet for ruminant (1g) and as *inoculum* a buffered rumen liquor from cow (85 ml). *In vitro* fermentation characteristics (*i.e.,* gas, degradability, end-products) were determined after 24 h of incubation, as well as methane production and protozoa count. Tested diet and *Eucalyptus globulus* plants were also characterised for chemical composition.

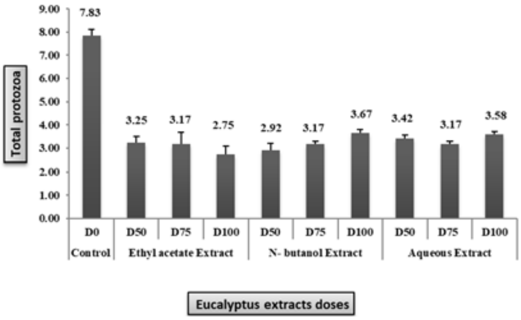


Figure 1 Effect of *E. globulus* extracts on total protozoa counts (No. x 10^5) after 24 h *in vitro* incubation.

Results & discussion Compared to the control, methane production was significantly lower for ethyl acetate extract ($P<0.05$) but higher for n-butanol and aqueous extracts (Table 1). Production of $N\text{–}NH_3$ was lower in all *Eucalyptus extracts* ($P<0.05$). Propionate production ($P<0.05$) increased for ethyl acetate and n-butanol extracts, whereas no effect was registered for pH and VFA, for all Eucalyptus extracts. Ethyl acetate extract decreased *in vitro* OMD ($P<0.05$) whereas for n-butanol and aqueous extracts were comparable to the control (Table 1). Protozoa quantification indicated that the protozoa population decreased ($P<0.05$) for all extracts in comparison with the control (Figure.1).

Table 1 Effect of *E. globulus* extracts on the *in vitro* fermentation characteristics and end-products after 24 h of incubation.

	Control	Ethyl acetate extract			n-butanol extract			Aqueous extract				
Parameters	0 mg	50 mg	75 mg	100 mg	50 mg	75 mg	10 mg	50 mg	75 mg	100 mg	S.E.M	P-value
GP24, ml/g	102.2^{a}	108.8^{a}	113.3^{a}	121.5^{a}	109.9^{a}	132.9^{a}	139.4^{a}	132.8^{a}	112.7^{a}	116.0^{a}	2.95	0.26
OMD, %	62.97^{a}	58.64^{ab}	54.46^{b}	52.15^{bc}	59.24^{a}	60.01^{a}	57.14^{a}	64.83^{a}	65.99^{a}	60.40^{a}	0.76	<0.001
CH_4, ml/g	13.07^{a}	2.15^{b}	1.22^{bc}	0.86^{c}	13.2^{a}	15.83^{a}	16.59^{a}	16.60^{a}	13.98^{a}	15.25^{a}	1.01	<0.001
pH	6.72^{a}	6.75^{a}	6.75^{a}	6.76^{a}	6.71^{a}	6.70^{a}	6.70^{a}	6.68^{ab}	6.66^{a}	6.70^{b}	0.01	<0.001
$N\text{-}NH_3$, mg/l	51.05^{a}	27.36^{b}	$20.52^{b.c}$	19.22^{c}	19.52^{b}	32.84^{ab}	21.82^{b}	33.71^{b}	32.30^{b}	29.62^{ab}	2.06	<0.001
tVFA, mmol/g	74.98^{a}	69.30^{a}	63.64^{a}	69.30^{a}	73.42^{a}	69.14^{a}	77.07^{a}	69.27^{a}	79.02^{a}	74.36^{a}	0.985	0.07
Acetate, mmol/g	44.25^{a}	36.81^{ab}	35.55^{b}	38.81^{ab}	43.66^{a}	39.86^{a}	45.16^{a}	40.28^{a}	47.26^{a}	44.58^{a}	0.78	0.004
Propionate, mmol/g	20.19^{a}	23.22^{a}	21.07^{a}	23.82^{a}	21.66^{a}	21.13^{a}	22.79^{a}	19.58^{a}	22.21^{a}	20.60^{a}	0.334	0.178
Butyrate, mmol/g	7.73^{a}	7.25^{a}	5.35^{b}	4.80^{b}	6.29^{b}	6.28^{b}	7.20^{ab}	7.12^{a}	7.32^{a}	7.04^{a}	0.165	<0.001
A/P ratio	2.21^{a}	1.59^{b}	1.69^{b}	1.63^{b}	2.02^{a}	1.88^{a}	1.98^{a}	2.06^{a}	2.13^{a}	2.17^{a}	0.044	<0.001

Conclusion The three different *Eucalyptus globulus* extracts (ethyl acetate, n-butanol and aqueous) tested in the present investigation appear to have different effects on the *in vitro* rumen fermentation studied, due to the nature, activity and concentration of their active compounds. In particular, the ethyl acetate extract, due to its aglyconic and mono-glycoside flavonoids, seems to be the best modulator of the *in vitro* fermentation because of its capacity to stimulate gas production with minimizing methanogenesis, in terms of methane and protozoa. However, since this extract also negatively affected organic matter degradability and volatile fatty acids production, various levels of the extract should be tested in order to find out a suitable dose to get maximum returns without adversely affect the nutritive value.

Acknowledgements: The present study was supported by the Italian Ministry of education, University and Research (individual research funding Serena Calabrò).

Effectiveness of using microbial solution on silage against spoilage microorganisms

Giuseppe Copani[1], Nina Milora[1], Zhigang Zhu[1], Bea Nielsen[1], Keith Bryan[1], Gabriele Rocchetti[2], Francesca Ghilardelli[2], Antonio Gallo[2]

[1]Chr. Hansen, Hoersholm, Denmark, [2]Department of Animal Science Food and Nutrition (DIANA), Universitá Cattolica del Sacro Cuore, Piacenza, Italy

E-mail: dkgico@chr-hansen.com

Take home message Microbial inoculant containing *Lactobacillus buchneri* and *Lactococcus lactis* is able to control yeast and mold growth in silage.

Introduction The reduction in nutritional value of silage exposed to air is driven by aerobic spoilage organisms, mainly yeast and mold. Microbial inoculants have been used in order to preserve nutrients, prevent dry matter losses and enhance aerobic stability (Contreras-Govea & Muck, 2006). *Lactobacillus buchneri* is well known for improving aerobic stability and converting lactate into acetic acid and 1,2-propanediol. Use of an inoculant containing a combination of homo-fermentative and hetero-fermentative lactic acid bacteria (LAB) resulted in positive effects on silage fermentation and increased aerobic stability in alfalfa silage (Copani *et al.*, 2017). The objective of this study was to evaluate the effectiveness of using a silage inoculant on microbial composition, aerobic stability and volatile compounds at different time points of fermentation in grass-legume mixture silage.

Materials & methods Six mini-silos for each treatment (grass-legume mixture) (n=3 vacuum bags and n=3 plastic buckets, 190 kg DM/m^3) were prepared as following: Control (CRT) with no inoculant or SiloSolve® FC (FC), containing 50% of *Lactobacillus buchneri* (LB1819; DSM22501/1k20738) and 50% of *Lactococcus lactis* (LLO224, DSM11037/1k2081) applied at a total dosage of 150,000 CFU/g of forage. Silage from the vacuum bags was used for microbiological enumeration (LAB, yeast and mold), pH and DM measurement after 2, 6 and 14 weeks of fermentation. The other three mini-silos were used to evaluate aerobic stability (AS) of the silo after air challenge. At the end of each fermentation time point (2, 6 and 14 weeks), the mini-silos were aerobically challenged for 7, 14 and 14 days, respectively. Meanwhile, microbial composition, pH and DM were also evaluated after AS. Acetate, lactate and propionate were determined by gas chromatography (GC). Volatile Organic Compounds (VOCs) were quantified by a headspace gas chromatography mass (HS-GC-MS). Data generated before and after AS were separately analysed in a completely randomized design with 2 x 2 factorial arrangement of treatments with GLM procedure in SAS, where the mino-silo was considered as experimental unit.

Results & discussion A significant increase of LAB was observed in the silages treated by the silage inoculant, before and after AS (treatment effect, $P < 0.05$). SiloSolve® FC significantly reduced the level of yeast at all fermentation times compared to the natural reduction in the CTR (treatment x day interaction, $P < 0.05$ before AS, whereas treatment effect, $P < 0.05$ after SA). A significant reduction in mold growth was observed before and after AS in silages treated with the inoculant after 2 weeks and 6 weeks fermentation (treatment x day interaction, $P < 0.05$). Overall, concentrations of acetic acid as well as VOCs related to heterolactic fermentations (i.e., 1-propanol, 3-methyl-butanal or propyl acetate) of mini-silos were higher in FC than CTR (treatment effect, $P < 0.05$). Conversely, greater levels of both ethanol and ethyl acetate were observed in CTR than FC mini-silos (treatment effect, $P < 0.05$). No differences were observed between treatments on aerobic stability. See Table 1.

Table 1 Silage characteristics and microbiological status, before and after aerobic challenge, on inoculated silages SiloSolve® FC (FC) or not Control (CRT) after 2, 6 or 14 weeks of fermentation.

Items	CTR			FC				*P*-value		
Weeks	2	6	14	2	6	14	s.e.m.	trt.	t.	trt.*t.
Before AS										
Lactic acid bacteria (log$_{10}$)	8.8	8.9	8.2	9.7	9.7	9.0	0.11	<.0001	<.0001	0.768
Yeast (log$_{10}$)	6.5	6.3	2.4	3.9	2.6	2.0	0.27	<.0001	<.0001	0.000
Mold (log$_{10}$)	4.0	4.0	2.0	2.0	2.0	2.0	0.001	<.0001	<.0001	<.0001
After AS										
Lactic acid bacteria (log$_{10}$)	9.98	9.98	9.05	10.72	10.01	9.98	0.079	0.001	<.0001	0.001
Yeast (log$_{10}$)	9.1	8.3	5.2	7.9	6.6	4.1	0.51	0.007	<.0001	0.836
Mold (log$_{10}$)	9.1	8.5	5.8	8.6	7.6	6.3	0.24	0.189	<.0001	0.022

s.e.m: standard error of the means; trt.: treatment; t.: time of ensiling

Conclusion This study shows the efficacy of SiloSolve® FC in improving fermentative and volatile compound profiles of grass-legume mixture silages, while controlling yeast and mold growth especially after a short period of fermentation.

References

Contreras-Govea FE and Muck RE 2006. Focus on Forage 8.4, 1-4.

Copani G, Groenlund Nielsen N, Bryan KA, Vrotniakiene V, Jatkauskas J and Witt KL 2017. Vth International symposium on forage quality and conservation.

Olive oil pomace affects *Anaerovibrio spp* abundance and increases polyunsaturated fatty acid in rumen liquor of sheep

Federica Mannelli[1], Alice Cappucci[2], Arianna Buccioni[1], Carlo Viti[1], Matteo Daghio[1], Francesco Pini[1], Marcello Mele[2]

[1]Dipartimento di Scienze delle produzioni Agro-alimentari e dell'Ambiente - University of Florence, Firenze, Italy, [2]Dipartimento di Scienze Agrarie, Alimentari, Agro-ambientali - University of Pisa, Pisa, Italy

E-mail: marcello.mele@unipi.it

Take home message Olive oil pomace is source of plant secondary metabolites that may modulate rumen metabolism and microbiota composition.

Introduction Olive oil pomace (OOP) is the main by-product of olive oil manufacturing and constitutes an important source of polyphenols. The use of OOP in ruminant feeding has been extensively studied since the '80s of the past century. Although OOP contained appreciable amounts of nutrients (mainly carbohydrates and lipids), the main constraint was the high content of lignin due to the presence of olive stones in the pomace. In the past few years there is a renewed interest in using stoned OOP from three- or two-phase extraction processes, because polyphenols (Pallara *et al*., 2014) are able to interfere with rumen microbiota and affect protein and lipid metabolism, with potential positive effect on meat and milk production. The aim of the present study was to evaluate the effect of two different kinds of stoned OOP rich in polyphenols on microbiota and fatty acid biohydrogenation in rumen liquor from dairy ewes fed diet supplemented with extruded linseed, as source of polyunsaturated fatty acids.

Materials & methods 24 multiparous ewes homogeneous for body weight and milk yield were randomly allotted into 3 experimental groups (8 animals per pen), The trial lasted 28 days, after 15 days of adaptation to the new diets. Diets were composed of chopped alfalfa hay administered ad libitum and 800 g per head and day of a concentrate formulated to contain the same amount of OOP (13.5 g/100g DM) and 10 g/100g DM of extruded linseed. Two kinds of OOP were chosen: one from a bi-phasic extraction process (OOP2) and one from a tri-phasic extraction process (OOP3). At the end of the experiment, rumen liquor (RL) samples were individually collected and analysed for fatty acid (FA) and dimethylacetal (DMA) profile and bacteria composition (sequencing the V3-V4 region of the 16S rDNA on Illumina Miseq with 300 bp paired end).

Results & discussion The content of rumenic acid (RA), alpha-linolenic acid (α-LNA) and some biohydrogenation intermediates of α-LNA were significantly higher in RL from sheep fed diet containing OOP, with the highest value for OOP2 diet (+44%). On the contrary, stearic acid content was significantly higher in RL from sheep fed control (C) diet (+25%). Significant differences ($p<0.05$) between control and OOP diets were found also for DMA profile. DMA13:0 resulted higher in RL from C group (+95%), whereas DMA18:0 was higher in RL from sheep fed OOP diets (+26%), irrespective to the type of OOP. DMA17:0 was six times higher in RL from sheep fed OOP3 diet if compared to RL from C diet, whereas the RL from OOP2 was intermediate. Previous studies found that DMAs could be associated with specific bacterial taxa; indeed, these molecules are present in bacterial membrane, especially of anaerobic species, thus they could be considered as biomarkers for microbial characterization (Alves *et al*., 2013). The most significant result on microbiota composition was about the *Anaerovibrio* genus, involved in the lipolysis process. The abundance of *Anaerovibrio spp*. was ten-fold reduced in the rumen microbiota of ewes fed OOP2 diet and, at a lesser extent (-80%), also in the rumen microbiota from ewes fed OOP3. The abundance of *Anaerovibrio spp.* was negatively related to DMA13:0 (-0.5836; $P = 0.0043$), to α-LNA (-0.6096; $P = 0.0025$) and to linoleic acid (-0.4795; $P = 0.0239$). Lipolysis is the rate-determining step for the biohydrogenation of polyunsaturated fatty acids (PUFA). Hence, a reduction of *Anaerovibrio spp*. concentration could be related to α-LNA accumulation in RL and, consequently, in milk fat.

Conclusion OOP could be an interesting ingredient for ruminant feeding, because it contains nutrients and appreciable amounts of polyphenols able to modulate rumen microbiota composition and biohydrogenation of PUFA. Interestingly, *Anaerovibrio spp*. in rumen microbiota was significantly affected by OOP, suggesting that the interference between olive polyphenols and biohydrogenation of PUFA could start with the inhibition of lipolysis, which, in turn, resulted in the accumulation of α-LNA in RL.

Acknowledgements
The research was funded by Tuscany Region Department, Project AGRIFOOD acronym NUTRIFOROIL.

References

Alves SP, Santos-Silva J, Cabrita AR, Fonseca AJ, and Bessa RJ 2013. PloS one. 8, e58386.
Pallara G, Buccioni A, Pastorelli R, Minieri S, Mele M and Rapaccini S 2014. BMC Veterinary Research 10, 271- 286.

Estimation of rumen microbial nitrogen supply using urinary purine derivatives excretion in merino sheep fed by peanut straw

Chusnul Hanim, Lies Mira Yusiati, Muhlisin Muhlisin

Faculty of Animal Science, Universitas Gadjah Mada, Sleman, Yogyakarta, Indonesia

E-mail: c.hanim@ugm.ac.id

Take home message Male and female Merino sheep have the same rumen microbial protein supply.

Introduction Small ruminants are important meat source for developing countries and are frequently associated with agriculture and crops production since the small ruminants are often fed with failed, surpluses or waste of agricultural crops; or anything could be found in grazing area, i. e. peanut straw (Budisatria IGS. *et al.,* 2007). This low quality of feeding system rise a question how is the microbial nitrogen supply of those animals, which are very important for ruminant productivity (Singh M. *et al.,* 2007). Cetinkaya *et al.* (2006) reported that purine derivative technique is one of the accurate method to measure microbial-N flow to intestine in ruminants. Therefore, this research was conducted to evaluate the purine derivatives excretion to estimate microbial protein supply in male and female merino sheep fed peanut straw basal diet.

Materials & methods Five male and five female Merino sheep aged ten to twelve months were fed peanut straw (crude protein 14.55%). All animals were kept in metabolism cages to separate feces from urine samples and were fed twice a day. The feeding trial was run for one-week collection period. During this time, urine was collected daily into plastic bucket placed under the cages and containing 10% sulfuric acid solution. At the end of collection period, the samples were analysis for purine derivatives (PD) (allantoin, uric acid, and xanthine-hypoxanthine) concentration. Allantoin and xanthine-hypoxanthine were determined by spectrophotometric method according to the procedure of Young and Conway (Chen and Gomes, 1992), while uric acid was determined by spectrophotometric method using a Kit. Microbial protein synthesis was estimated according to the equation $Y = 0.84X + (0.150\ W^{0.75}\ e^{-0.25X})$ proposed by Chen and Gomes (1992) with modifications in endogenous PD excretion for Merino sheep, where endogenous PD from Chen and Gomes's equation was replaced by 0.056 for male and 0.094 for female Merino sheep (Hanim *et al*., 2018). The efficiency of the microbial protein synthesis in the rumen was expressed as grams of microbial N per kilogram of digestible organic matter apparently digested in the rumen (DOMR).

Results & discussion There was no significant difference in PDexcretion between female and male Merino sheep. Total purine derivatives excretion and EMNS showed the same pattern, although DOMR in male sheep was higher than that of female. Proportion of allantoin:uric acid:xanthine-hypoxanthine in male and female Merino were 53.15:9.39:37.47 and 57.12:8.39:34.49, respectively.

Table 1 Purine derivative excretion and estimated microbial nitrogen supply of male and female Merino fed peanut straw.

Sex of sheep	Allantoin		Uric acid		Xanthine hypoxanthine		Purin derivatives		EMNS	DOMR	EMNS/ DOMR
	µmol/d	µmol/ $W^{0,75}$/d	µmol /d	µmol/ $W^{0,75}$/d	µmol/d	µmol/ $W^{0,75}$/d	µmol/d	µmol/ $W^{0,75}$/d	g/d	g/d	mg/g
Male	1608.68	105.15	284.19	18.45	1134.05	74.45	3026.91	198.05	2.27	367.45	6.14
Female	1597.07	110.94	234.71	16.43	964.43	67.81	2796.21	195.16	1.78	288.06	6.11
standard error	208.50	13.95	28.62	1.69	144.60	9.99	343.55	22.91	0.36	14.92	1.13
comparison with 0.5 [1]	ns	ns	ns	Ns	ns	ns	ns	ns	ns	*	ns

[1] Comparison with the theoretical 0.5 proportion of one way.

Conclusion The microbial protein supply in male and female Merino sheep were 6.14 and 6.11 mg/g, respectively.

Acknowledgements This study was supported by Ministry of Research, Technology and Higher Education.

References

Budisatriaa IGS, Udo HMJ, Eilers CHAM, Baliartia E and van der Zijpp AJS 2010. Small Ruminant Research 88, 16–22.
Chen XB and Gomes MJ 1992. HMJ. Occasional Publication of International Feed Resources Unit, Rowett Research Institute, Bucksburn Aberdeen, UK.
Cetinkaya N, Yaman S and Baber NHO 2006. Livestock Science 100, 91-98.
Hanim, C, Yusiati L and Muhlisin M 2018. Unpublished
Singh M, Sharma K, Dutta N, Singh P, Verma AK and Mehra UR 2007. Asian-Australian Journal of Animal Science 20,1567-1574.

Body mass index in lactating goats is more precise in predicting body lipids, measured by computer tomography, than BCS or body weight

Margrete Eknæs[1], Yves Chilliard[2], Knut Hove[1], Harald Volden[1]

[1]Norwegian University of Life Sciences, Ås, Norway, [2]UMR1213 Herbivores, Institut National de la Recherche Agronomique, Saint-Genès-Champanelle, France

E-mail: margrete.eknas@nmbu.no

Take home message BMI is better than BW or BCS to predict changes in body lipids in dairy goats.

Introduction In high-yielding goats, body lipid is mobilized when energy intake is insufficient to sustain lactation energy demands, thus channelling body reserves toward the mammary gland (Chilliard, 1993). To keep high-yielding goats healthy and prepare them for their next lactation, a careful monitoring of their nutritional status during lactation and dry period is of high importance. Body weight (BW) and body condition score (BCS) are common methods to evaluate nutritional status in goats, while body mass index (BMI) is used as a reasonable indicator of body fat in humans. The aim of this study was to assess these different methods against the measurement of body composition by use of computerized tomography (CT).

Materials & methods 30 multiparous Norwegian goats were studied from the beginning to the end of lactation for 230 days in milk (DIM). At 10, 30, 60, 90, 120, 200 and 230 DIM, BW (kg) was registrated, BMI [BW (kg)/neck height (m) x body length (m)] was measured as described by Tanaka *et al.* (2002), and BCS (0 to 5 points) was determined as described by Ngwa *et al.* (2007). Body tissue mass (carcass fat, visceral fat and protein-rich tissue) was determined by use of CT as described by Eknæs *et al.* (2006). Pearson correlation coefficients (Snedecor and Cochran, 1989) was calculated between adipose tissue and BW, BCS and BMI by using the PROC CORR procedure of SAS.

Results & discussion The average BW of goats was 53.7 ± 6.9 kg (N=208). Throughout the lactation cycle, proteinous tissue mass was conserved to a high degree (14.4 ± 1.6 kg). On the other hand, total body fat declined by 72% up to the 200th DIM. The total body fat (mean 6.1 ± 2.8 kg) showed low correlation to BW ($r=+0.26$; $p<0,001$; N=208) and BCS ($r=+0.17$; $p<0.05$) while its correlation to BMI was higher ($r=+0.44$, $p<0.001$). BMI was also better correlated to carcass than to visceral fat ($r= +0.52$ *vs.* $+0.37$, resp.; $p<0.001$, $p<0.001$).

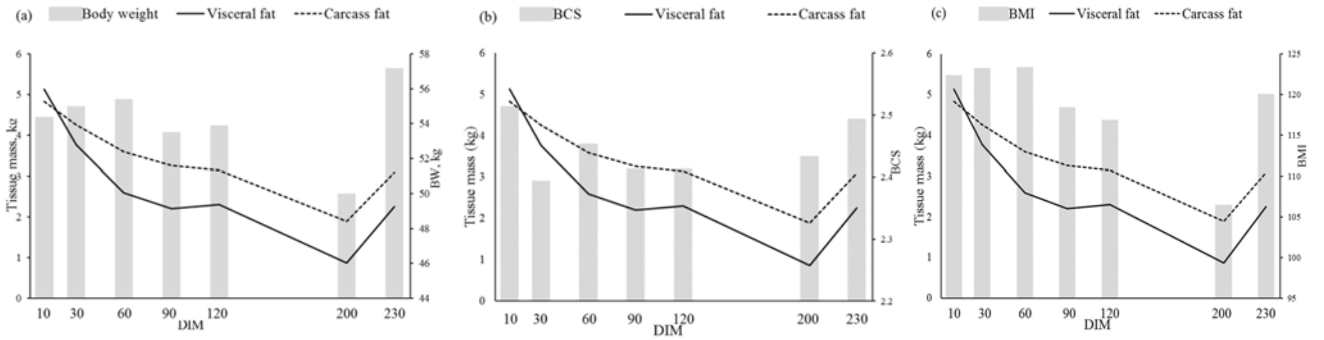


Figure 1 Relationship between adipose tissue and (a) body weight, (b) body condition score and (c) body mass index.

The low correlation (+0.26) of total body fat to BW was expected since BW varies with other components (mainly gut content, and to a lesser extend protein-rich tissues and bones). The low correlation to BCS could be due to the low amount of subcutaneous fat in dairy goats. The higher correlation to BMI suggests that, for a given body frame (or surface), the body fat is one significant part of BW variations.

Conclusion Of the parameters used to estimate body lipid reserves, the highest correlation ($r =+0.44$) was found between total adipose tissue mass and BMI, as this later parameter is integrating one part of the goat effect due to variations in animal frame.

Acknowledgements This study was financed by the Norwegian Research Council and TINE BA.

References

Chilliard Y 1993. Journal of Dairy Science 76, 3897-3931.
Eknæs M, Kolstad K, Volden H and Hove K 2006. Small Ruminant Research 63, 1-11.
Ngwa, AT, Dawson LJ, Puchala R, Detweiler G, Merkel RC, Tovar-Luna I, Sahlu T, Ferrell CL and Goetsch AL 2007. Small Ruminant Research 73, 27-36.
Snedecor, GWM and Cochran WG 1989. Statistical Methods, 8th ed. Iowa State University Press, Ames, IA, 503.
Tanaka T, Akaboshi Y, Inoue Y, Kamomae H and Kaneda Y 2002 Animal Reproduction Science 72, 185-196.

Two parsimonious software sensors for estimating the individual dynamic pattern of methane production by cattle

Rafael Muñoz-Tamayo[1], John Fredy Ramírez Agudelo[2], Sebastian Bedoya Mazo[2], Richard J. Dewhurst[3], Gemma Miller[3], Ricardo Rosero Noguera[2], Sandra Lucía Posada Ochoa[2], Helen Kettle[4]

[1]UMR Modélisation Systémique Appliquée aux Ruminants, INRA, AgroParisTech, Université Paris-Saclay, Paris, France, [2]Universidad de Antioquia - UdeA, Facultad de Ciencias Agrarias. Grupo de Investigación en Ciencias Agrarias -GRICA, Medellin, Colombia, [3]Future Farming Systems, SRUC, Edinburgh, UK, [4]Biomathematics and Statistics Scotland (BioSS), Edinburgh, UK

E-mail: rafael.munoz-tamayo@inra.fr

Take home message The dynamic pattern of methane production by cattle can be predicted by a simple model with only DMI or intake time as predictor variables. This model has potential applications for on-line monitoring.

Introduction Large efforts have been deployed for measuring methane emissions from cattle. For large scale applications, accurate and inexpensive methane predictors are required. In this work, we explored the integration of real-time data of animal feeding behaviour and an *in silico* model for predicting methane *via* a mathematical approach known as a software sensor. Existing dynamic rumen fermentation models contain a high number of parameters that require cautious calibration (Muñoz-Tamayo *et al.*, 2016), which make their application in practice difficult. As an alternative, we have developed a simple dynamic model that uses as predictor variables either the dry matter intake (DMI) or the intake time (IT).

Materials & methods Two data sets were analysed: D1: data from SRUC (United Kingdom) of CH_4, DMI and IT (recorded with load cells) from 18 steers (cross-bred Charolais and purebred Luing finishing beef cattle) receiving two contrasting diets consisting (g/ kg DM) of 500:500 and 80:920 forage to concentrate ratios (Troy *et al.*, 2015). D2: data from UdeA (Colombia) of CH_4 and an estimated IT (using an accelerometer) from one crossbred beef steers (Angus x Brahman) fed with a diet consisting of 600:400 forage to concentrate ratio. The IT estimation was derived from head position recordings: head tilted down = intake activity, head tilted up = no intake activity. For model construction, we explored different structures of Input-Output models *via* the Matlab® System Identification Toolbox (Ljung, 1997). We selected a parsimonious model with only two parameters, which are identifiable (Muñoz-Tamayo *et al.*, 2018).

Results & discussion Figure 1A displays the best example of fit for the SRUC data. The model predictors DMI and IT perform similarly. The Lin's concordance correlation coefficient (CCC) were in the interval [0.52 0.92] with an average of 0. 80 (DMI) and 0.77 (IT). When using the predictions on average daily basis (n=18), the CCC for was 0.96 (IT). On the basis of CCC reported in literature for methane prediction equations, our model performs very well. Figure 1B shows the model performance for the UdeA data (CCC = 0.57). This low value may be explained by the uncertainty of the IT estimation. On this basis, the accelerometer-based method requires further improvements to enhance methane predictions.

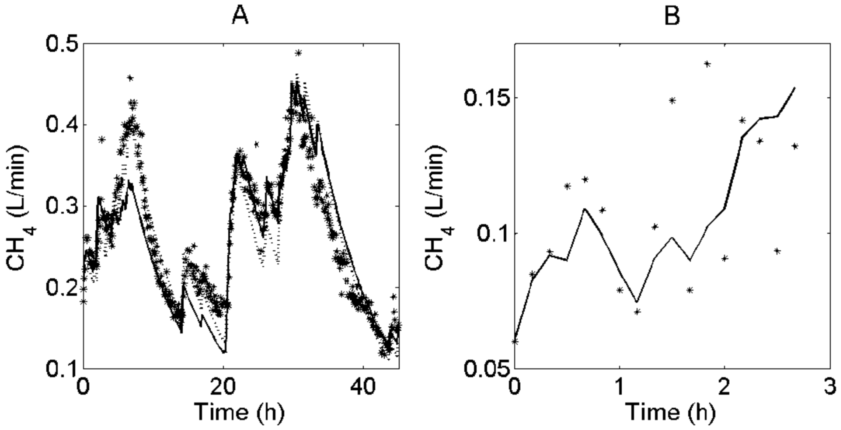


Figure 1 Experimental data of methane production (*) are compared against the predicted responses by the model using DMI (dotted line) and IT (solid line) as predictors. (A) Data from SRUC (B) Data from UdeA.

Conclusion We developed two software sensors for predicting methane emissions by cattle. The quality of the predictions depends on the quality of the measurements or estimations of DMI and IT. In a large-scale context, a software sensor based on IT offers an easier and economically viable alternative for methane prediction.

Acknowledgements This research is supported by a PhD grant of Colciencias. We thank Dr. John A. Rooke for helpful explanations on the respiration data from the SRUC the study.

References

Ljung L, 1997. MATLAB System Identification Toolbox: User's Guide, The Mathworks, Inc
Muñoz-Tamayo R, Giger-Reverdin S and Sauvant D 2016. Animal Feed Science and Technology 220, 1-21.
Muñoz-Tamayo R, Puillet L, Daniel JB, Sauvant D, Martin O, Taghipoor M and Blavy P 2018. Animal 12, 701-712
Troy SM, Duthie CA, Hyslop JJ, Roehe R, Ross DW, Wallace RJ, Waterhouse A and Rooke JA 2015. Journal of Animal Science 93, 1815-1823.

Benefits of behavioural characteristics in herbage intake estimation models for grazing dairy cows

Fredy Schori[1], Markus Rombach[1,2], Andreas Münger[1], Karl-Heinz Südekum[2]

[1]*Agroscope, Posieux, Switzerland,* [2]*Institute of Animal Science, Bonn, Germany*

E-mail: fredy.schori@agroscope.admin.ch

Take home message The inclusion of behavioural characteristics to estimate herbage dry matter intake brings some benefits, however behavioural characteristics alone are not sufficient to estimate individual herbage intake accurately.

Introduction Information about herbage dry matter intake (hDMI) is a core element of dairy cow management, even more pronounced in pasture-based feeding systems. Its knowledge helps to monitor the nutritional status and consequently to adapt the optional supplementation in accordance to the cow nutritional requirements. Furthermore, it enables the ranking of dairy cows in relation to their feed efficiency as well as to improve pasture and livestock management. Reliable routine estimation of the individual hDMI is not an easy task. Often the methods used for individual hDMI estimation are either expensive, time-consuming or impracticable at farm level. Estimation of hDMI using recorded ingestive behaviour data might be an elegant method, even more so as a variety of behavioural recorders exist and are more and more used at farm level.

The objective of this study was to develop models for individual hDMI estimation of grazing dairy cows and to quantify the estimation error of these models as well as the benefits of behavioural characteristics for hDMI estimation.

Materials & methods Data from four rotational grazing experiments on multi-species pastures, including treatments relative to supplementation, herbage mass and cow type, constituted the basis. These experiments took place in the western part of Switzerland from 2014 to 2016 between May and September and involved at all 94 dairy cows. Red Holstein and two different types of Holstein cows grazed between 16 and 19 h d^{-1} in these experiments. In total 130 seven-day measurements of hDMI (average 12.4 kg d^{-1}, 4.7 to 20.4) with the n-alkane double indicator technique (Heublein *et al*., 2017) as reference method were available. Behavioural data were collected simultaneously and processed using the RumiWatch System (RWS, Itin & Hoch GmbH, Liestal, Switzerland, Halter V 6.0, Converter 0.7.3.31). The RWS has been validated for grazing dairy cows by Rombach *et al.* (2018). In addition to the behavioural elements mentioned by Rombach *et al.* (2018) the used evaluation software (converter) allowed a differentiation of prehension bites and mastication chews during grazing. Due to technical issues 21 (16 %) records from the RWS could not be utilised for further evaluation.

The 53 variables available, describing pasture characteristics (e.g. herbage mass 589 to 2333 kg DM ha^{-1}), supplementation (e.g. amounts of chopped whole-plant maize silage 0 to 7.9 kg d^{-1} or concentrate 0 to 4 kg d^{-1}), cows (e.g. body weight 428 to 719 kg), cow performance (e.g. milk yield 14 to 38 kg d^{-1}) and behaviour (e.g. total eating time 441 to 742 min d^{-1}, total eating chews 31668 to 54174 n d^{-1}, grazing time 355 to 691 min d^{-1}, prehension bites 11037 to 40304 n d^{-1}, total eating chews at pasture 26225 to 48710 n d^{-1}) were thinned out based on statistical and nutritional-linked criteria. For the final reduction of predictors in the models the best subset regression approach was applied (R Core Team (2016), package leaps). As the sample size was too small to retain an independent validation dataset, the bootstrap cross-validation method was chosen (package rms).

Results & discussion Unfortunately, behavioural characteristics like number of prehension bites or grazing time, explained only 26 % or 35 %, respectively, of the hDMI variability. Thus, they are alone not sufficient for an accurate hDMI estimation. The variability in bite size (0.26 to 1.04 g DM) and intake rate (11.5 to 40.8 g min^{-1} grazing) between individual grazing dairy cows is too large. Consequently, other predictors than behavioural characteristics have to be included in the models for hDMI estimation. The root mean squared prediction errors (RMSPE) for different hDMI estimation models with 4 to 12 predictors were calculated. Models without information about supplementation and grazing behaviour exhibit a RMSPE between 2.1 to 2.3 kg d^{-1} depending on the number of predictors in the model. Inclusion of behavioural characteristics per day or during grazing like eating time or chews, prehension bites or rates, or eating chews head down reduced the error term by about 0.5 kg d^{-1}. If quantities of supplemental feed in the barn are available for modelling hDMI, the error term decreased again about 0.2 kg d^{-1}, to end at 1.4 to 1.6 kg d^{-1}. In this case, inclusion of behavioural information did not additionally improve the estimation accuracy. On the other hand, the inclusion of behavioural characteristics may be beneficial if individual hDMI is outside of the norm, caused for example by health problems, injuries, heat or other unusual circumstances.

Conclusion Eating behaviour characteristics alone are not sufficient to estimate individual hDMI accurately but combined with other predictors they bring some improvements. Prospectively, a larger dataset in terms of number of animals and herds, covering more breeds, different grazing systems, management patterns as well as behaviour recorders easier to handle at farm level would potentially increase the accuracy and robustness of the estimation models for individual hDMI.

References

Heublein C, Südekum KH, Gill F, Dohme-Meier F and Schori F 2017. Journal of Dairy Science 100, 1019-1036.
Rombach M, Münger A, Niederhauser J, Südekum KH and Schori F 2018. Journal of Dairy Science 101, 2463-2475.

Using carbon emissions, oxygen consumption, and energy retention estimates to calculate dietary energy partitioning and estimate ME intake by beef steers

Stacey Gunter[1], Corey Moffet[1], Colleen Burrus[2], Pablo Gregorini[3]

[1]United State Department of Agriculture, Agricultural Research Service, Woodward, Oklahoma, USA, [2]Southern Arkansas University, Magnolia, Arkansas, USA, [3]Lincoln University, Christchurch, New Zealand

E-mail: stacey.gunter@ars.usda.gov

Take home message Estimating ME intake by grazing cattle seems possible using respiration gas exchange estimates.

Introduction We hypothesized that CH_4, CO_2, and O_2 exchange estimates in breath clouds could be used as biomarkers to ultimately estimate DMI in grazing beef cattle when integrated into backward dietary energy partitioning calculations. Here, we aimed to estimate heat production (HP) through spot short-term measurements of gas consumption and emissions and the Brouwer equation (Brouwer, 1965), estimate energy retention (ER) via ADG, and estimate ME intake (NASEM, 2016).

Materials & methods Our experiment used 8 cross-breed beef steers (initial BW = 241 ± 4.1 kg) for a 77-d feeding period (June through August) to determine performance, respiration gas exchanges, ER, and HP. Steers were housed in a pen equipped with individual feed bunks and access controlled by Calan gates. Steers were randomly assigned to 1 of 3 treatments as a multiple of ME intake (MEx) at maintenance (7.43 Mcal of ME/d): 1.1 (1.1M, n = 3, DMI = 4.10 kg), 1.4 (1.4M, n = 2, DMI = 5.44 kg), and 1.8 (1.8M, n = 3, DMI = 7.41 kg). Diets fed were long-stemmed wheat hay (*Triticum aestivum* L.; 15% CP, 64% TDN) plus a daily supplement with 1.03 kg of alfalfa (*Medicago sativa* L.)/wheat middling pellets (15% CP) delivered via an automated head-chamber system (GreenFeed, C-Lock, Inc., Rapid City, SD USA) which measured CO_2 and CH_4 emission, and O_2 consumption. On d 1, 14, 35, 56, and 77 of the experiment, BW was recorded after a 17-h fast to adjust for gastrointestinal fill. Heat production (Mcal/d) was calculated from gas emissions and consumption [$HP = (3.866 \times CO_2\ (L/d) + 1.200 \times O_2\ (L/d) - 0.518 \times CH_4\ (L/d) - 1.431 \times N\ (g/d)) \times 1{,}000$; (Brouwer, 1965)] and urinary N output [g/d; 2.39×0.55 N intake (g/d) – $3.61 \times$ DMI (kg/d); (Dong *et al.*, 2014)]. Intake of ME was calculated by adding HP and to the ER estimates (NASEM, 2016). Dependent variables were analysed with the MIXED procedure (SAS Inst., Inc.; Cary, NC USA) and LSMEANS separated with linear (L) and quadratic (Q) for unequally-spaced treatments.

Results & discussion Average daily gain and ending BW increased linearly ($P < 0.01$) with increasing MEx. Likewise, CO_2 emissions and O_2 consumption increased linearly ($P \leq 0.01$) with MEx, but CH_4 did not. The urinary N excretion increased linearly ($P < 0.01$) from 1.1M to 1.8M (32, 40, and 52 g/d, SE = 0.6, respectively). Energy retention and HP increased linearly ($P < 0.01$) with increasing MEx. Hence, the predicted ME intake increased linearly ($P < 0.01$) from 1.1M to 1.8M (11.74, 12.62, and 15.30 Mcal/d, SE = 0.425, respectively).

Table 1 Performance, gas exchange, energy retention, and heat production by steer fed at three multiples of maintenance.

	Treatments				Contrast	
Item	1.1M	1.4M	1.8M	SE	L	Q
ADG, kg	0.12	0.29	0.65	0.078	< 0.01	0.55
Ending BW, kg	251	263	291	6.0	< 0.01	0.55
Gas exchanges, L/d						
CO_2 emission	1,957	2,090	2,274	65.7	0.01	0.97
CH_4 emission	227	237	237	11.1	0.41	0.69
O_2 consumption	2,384	2,460	2,775	99.2	0.01	0.49
ER, Mcal/d	0.34	0.77	2.03	0.221	< 0.01	0.33
HP, Mcal/d	11.40	11.84	13.26	0.425	< 0.01	0.53

Conclusion These results indicate that exchange estimates of CO_2, CH_4, and O_2 with an automated head-chamber system can be used to provide estimates of ME intake and HP for ruminants. Estimates of ME intake are greater than the ME requirements (8.2, 10.4, and 13.3 Mcal/d, respectively) suggested in the NASEM (2016) for this BW of cattle and ADG. Possible factors contributing to the discrepancy in ME intake could be the under estimation of the final shrunk BW (555 kg) needed to achieve a final body fat of 27.8% or a greater HP than previously planned because of an increased maintenance requirement resulting from the normally hot (average high of 34° C) summer days in that occur in northwest Oklahoma.

Acknowledgements This project was for by the United States Department of Agriculture, Agriculture Research Service.

References

Brouwer E 1965. Proceedings of the 3rd Symposium on Energy Metabolism.

Dong RL, Zhao GY, Chai LL and Beauchemin KA 2014. Journal of Animal Science 92, 4669-4681.

NASEM. 2016 Nutrient Requirements of Beef Cattle. 8th Rev. Ed. The National Academy of Science, Engineering, and Mathematics. Washington, DC.

An automated walk-over-weighing system for monitoring liveweight change of sheep at pasture

Eliel González-García[1], Moutaz Alhamada[1], Ana Clara Canto Souza[2], Zuzana Holubová[3], Sébastien Douls[4], Julien Pradel[4], Sara Parisot[4]

[1]INRA-SELMET, Montpellier, France, [2]Universidade Estadual de Londrina, Londrina, Brazil, [3]University, Praga, Czech Republic, [4]INRA-La Fage, Roquefort-sur-Soulzon, France

E-mail: eliel.gonzalez-garcia@inra.fr

Take home message: A daily, accurate, and fully automatic monitoring of individual liveweight of grazing sheep is feasible.

Introduction: Measurement of body weight (BW) is an established method for monitoring the performance of intensive and extensively managed farm animals. There are few reports of essaying automated BW recording of sheep (Brown *et al.*, 2012 & 2013). The objective of this work was to develop and evaluate an automated walk-over-weighing (WoW) system for a remote and close BW monitoring in grazing sheep.

Materials & methods: The prototype (Figure 1) was conceived to be light, mobile, autonomous in energy and resistant to outdoor conditions. It was designed in collaboration with Trutest (New Zealand) and Marechale Pessage (France) and has been evaluated between 2015 and 2017 in 3 consecutive experiments with Romane ewes at INRA Experimental Unit "La Fage" (43°54'54.52"N; 3°05'38.11"E; Roquefort-sur-Soulzon, France). Firstly, and indoor trial (IND), secondly a first grazing test under intensive grazing conditions (OUTin) and finally a first assay in an extensive rangeland (OUText) have been carried out. The IND trial calibrated the device under controlled conditions (González-García *et al.*, 2017). Twenty-four were fed good-quality hay and monitored for 1 month. The OUTin test was carried out in spring (April-June). A rotational grazing system was established in 1 ha (280 m^2 paddocks; 2-3 days stay) with 15 naïve ewe-lambs fed only the good-quality mix of herbage available at pasture. The OUText evaluated the prototype for the first time under real free-rangeland conditions during 1 month in winter (November-December). One hundred sheep (99 adult ewes at early pregnancy and 1 ram) grazed in a 15-ha paddock with native grasses and were supplemented silage (4.5 kg/head) and *ad libitum* hay. Water and mineral salts were always used as attractants for training ewes in a logical circuit to stimulate their individual voluntarily cross through the WoW device. For the 3 experiments the accuracy in the individual daily BW values recorded by the WoW prototype was evaluated by comparison with frequent static BW measured with a conventional weight scale balance. In parallel, we started to study the feasibility of estimating daily individual feed intake by using differences between BW measurements points during the day. An evaluation of the level of agreement between WoW and BW measured statically was made using Lin's concordance correlation coefficient (Lin, 1989).

Results & discussion The 100 % of ewes were well-adapted after 2-3 wk of familiarization with the WoW device. Thus, ewes voluntarily crossed several times (~ 6-8/ ewe/ d) and, overall, more than 65% of BW values recorded by the WoW in the three experiments were useful for interpretation. The rest of the observations (around 35%) were considered as outliers and removed from the database to be interpreted. Interestingly, more the conditions were extensive more concordant were the WoW BW values when compared with static BW. The Figure 1 illustrate the excellent fit of static *vs.* WoW BW on pooled grazing ewes groups (i.e. concordance correlation = 0.97).

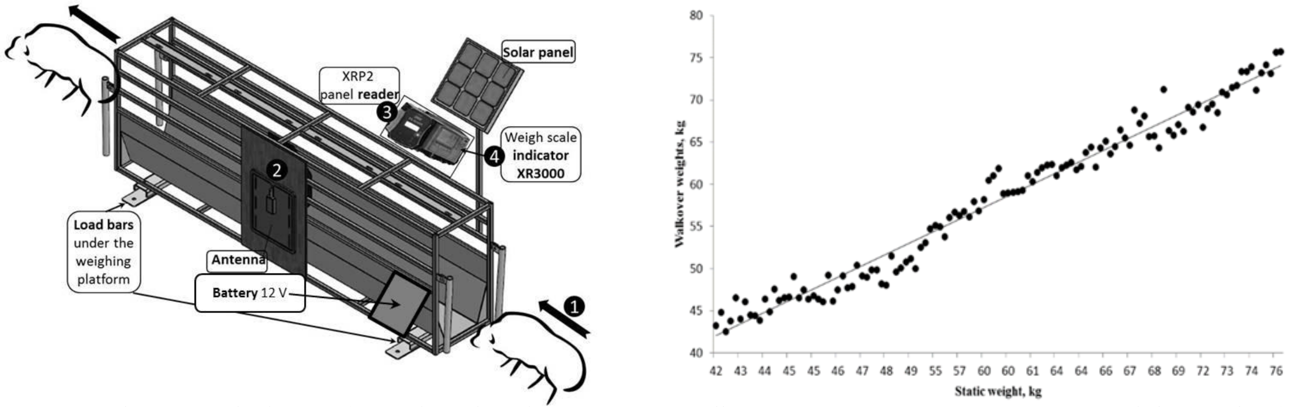


Figure 1 The WoW device (Left panel) and static BW versus walk-over BW (WoBW) measurements (right panel) of pooled groups of grazing Romane ewes weighed in spring and winter of 2017.

Conclusion: Our results demonstrate the feasibility of using this device as a promising option for the automatization of BW monitoring of sheep under a range of farming conditions. Its use would contribute to the animal welfare and to save labor without compromising the quality of BW measurement. Beyond the autoweighing objective, this experimental setting is also conceived for indirect estimation of daily individual feed intake from frequent BW variations during the day. Results from IND trial are promising and have been already reported (González-García *et al.*, 2017).

Acknowledgements: This work was supported by INRA-PHASE Division and the INRA-CSIRO Linkage Program.

References

Brown, D.J. Animal Production Science 2012
González-García, E. 2018. Animal (doi:10.1017/S1751731117002609).
Linn LIK 1989.Biometrics 45, 255-268.

Development and application of devices for the continuous monitoring of reticuloruminal motility

Nicholas Jonsson[1,4], Ivan Andonovic[2], Christopher Davison[2], Andrew Hamilton[2], Andrea Francesio[1], Will Tulley[3], Lorenzo Viora[1], Craig Michie[2]

[1]*University of Glasgow, Glasgow, UK,* [2]*University of Stratchclyde, Glasgow, UK,* [3]*Evidence Group, Penrith, UK,* [4]*Harbro Ltd, Turriff, UK*

E-mail: nicholas.jonsson@glasgow.ac.uk

Take home message The measurement of reticuloruminal contraction rate and amplitude is a potentially useful indicator of foregut health.

Introduction Reticuloruminal function is central to the digestive efficiency in ruminants. Previous work has shown that acidosis can lead to reduced strength and rate of reticuloruminal contractions. The continuous monitoring of reticuloruminal motility is therefore proposed as a route to providing useful information on the reticuloruminal function, potentially more economically and unambiguously than current pH boluses can. In order to validate the hypothesis, three studies were undertaken to a) determine whether a sensor system could be developed that reliably measures the rate and strength of reticular contractions, measured as frequency and amplitude of signals, b) determine the relationship between the rate and strength of reticuloruminal contraction and rumination and eating behaviour, and c) determine whether the rate and strength of reticuloruminal contraction is affected by diets high in soluble carbohydrates.

Materials & methods In Study-1 a prototype device comprising an accelerometer, battery and memory card inside a weighted plastic casing was used. A single device was placed in a restrained cow through a rumen canula, for approximately 30 minutes. While in place, real-time ultrasound was used to observe and record the frequency of reticuloruminal contractions. In Study-2, 3 rumen-canulated cows, 2 of which were loaded with an accelerometer and a tri-axis gyroscope and 1 with a hydrophone were monitored over a 24 h period. All cows were also fitted with collar-mounted accelerometers (Silent Herdsman) to characterise their activity type (ruminating, eating, inactivity, and 'other'). In Study-3, accelerometer-based boluses and pH boluses (smaXtec) were inserted in 6 cows in a Latin-square design and examined for the effects of low-starch-low-sugar (CONTROL), high-starch-low-sugar (STARCH) and low-starch-high-sugar (SUGAR) diets.

Results & discussion In Study-1, there was complete agreement between the trace generated and the observed rumen contractions in a restrained cow, with a mean inter-contraction period of 40 s. In Study-2, there was good agreement among the 3 devices and the duration of contractions varied significantly according to the cow's activity type, being 12.3 s when ruminating and 9.59 s when inactive ($p < 0.05$). In Study-3, STARCH and SUGAR both reduced the hourly median pH, hourly minimum pH and increased the standard deviation of pH (all $p < 0.0001$). Observations of reticuloruminal motility were affected by considerable noise caused by movements of the animal unrelated to reticuloruminal contraction (Figure 1). The mean inter-contraction period was 44.6 s. Neither STARCH nor SUGAR had a significant direct effect on the median value of the inter-contraction period ($p > 0.1$). STARCH significantly increased the amplitude of reticuloruminal contractions ($p < 0.005$) and tended to decrease the period ($p < 0.1$).

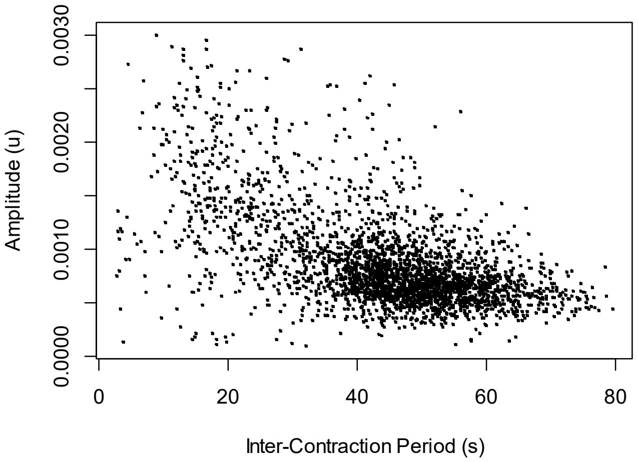


Figure 1 The relationship between the amplitude (size of the 3-D vector derived from accelerometer, reflecting the force of the contraction) and the period of contractions exhibited considerable noise but suggests that most of the likely true observations have periods of between 30 and 60 s.

Conclusion Devices to monitor reticuloruminal motility were developed, providing repeatable measures of contraction amplitude and period. The amplitude of the contraction was related to dietary input. Refinement of filtering algorithms is being undertaken to further improve the precision of estimates for the inter-contraction period and contraction amplitude.

Acknowledgement
Funding for this work was provided by the Universities of Glasgow and Strathclyde.

Large variability of intake of lick-block supplement among individual animals under different forage types in group-fed cattle

Jose Augusto Imaz[1,2], Lachlan Ingram[1], Sergio Garcia[1], Luciano Adrian González[1]

[1]*The University of Sydney, Sydney Institute of Agriculture, School of Life and Environmental Sciences, Sydney, NSW, Australia,* [2]*Instituto Nacional de Tecnología Agropecuaria, Capital Federal, Buenos Aires, Argentina*

E-mail: augusto.imaz@sydney.edu.au

Take home message The use of in-paddock technologies is a promising approach to understand individual animal response to lick-block supplements, offered as a free-choice.

Introduction Novel in-paddock technologies, such as electronic feeders (EF), allow measuring individual supplement intake and feeding behaviour of molasses and mineral blocks offered as free-choice to group-fed cattle. EF might also help to capture temporal changes in supplement intake as a result of forage type and grazing management (Titgemeyer *et al*., 2004). We studied the variability in lick-block supplement intake of individual animals as they grazed paddocks with different forage quantity and quality.

Materials & methods Twenty-seven Charolais x Angus steers and heifers (initial weight 192 ± 37 kg) rotationally grazed temperate pastures and oat crops (15 ha in 10 paddocks) during a 7-month trial from May to December (see Table 1). Animals were also fed supplementary hay of lucerne, oat and wheat during the last 72 days. An EF (Smartfeed) was installed at the only water point enclosed by a yard with an entry and an exit gate. A single lick block of cooked molasses was available inside the EF throughout the trial (40 kg; 4 Season Co. Pty Ltd, Creastmead, QLD). The EF records the electronic ID tag, date and time, feed consumed and duration of all visits. Animals were grouped into high (HG > 25 kg), medium (7 kg < MG < 25 kg) and low (LG ≤ 7 kg) intake groups based on the amount of supplement consumed during the entire trial. Supplement intake (g/hd/day) was analyzed using a mixed-effects linear regression model considering date as a repeated measure on each animal, and group, feed type and their interaction as fixed effects.

Results & discussion Supplement intake differed amongst intake groups (P-value Group <0.01) but this difference was not dependent on the type of forage available over time (P-value Group × Feed type > 0.05). Supplement consumption was greatest during hay feeding and lowest while Oat/Pastures feeding (P-value Feed type <0.01), showing more than 3-fold variability over time for MG animals (Table 1). LG included 2 non-feeder animals that never registered a visit.

Table 1 Mean ± SE of lick-block supplement intake (g/hd/day) of steers and heifers consuming different types of forages over time. Animals were assigned to groups of high, medium and low supplement intake. Feed type is ordered from the start (below) to the end of the experiment (above).

			Supplement intake group		
Feed type	Days	Average	High (n=10)	Medium (n=9)	Low (n=8)
Oat/Wheat hay	39	174 ± 14	293 ± 22	180 ± 22	48 ± 26
Lucerne hay	33	90 ± 48	190 ± 27	79 ± 27	0
Mix grazing Oat/Pastures	24	58 ± 36	121 ± 30	54 ± 30	0
Grazing Oat	36	73 ± 26	107 ± 26	89 ± 26	23 ± 70
Grazing Pastures	87	66 ± 17	130 ± 18	46 ± 20	22 ± 44
Average total intake		92 ± 14	168 ± 11	90 ± 11	19 ± 38

Conclusion Intake of lick-block supplements in group-fed cattle markedly differed between individual animals and the type of feed consumed. Electronic feeders allow monitoring supplement intake over time for all individuals under grazing conditions and identifying those animals with low supplement intake as well as using shifts in the supplement consumption to decide grazing strategies (e.g. moving animals to a fresh paddock). This may help to increase our understanding of factors affecting the intake of free-choice supplements in group fed cattle and improve nutritional management and productivity.

Acknowledgements This research was supported by grants from The University of Sydney.

References

Titgemeyer E, Drouillard C, Greenwood JS and Ringler JW 2004. Journal of Animal Science 82, 487-494.

Body temperature measured by thermal imaging of ewes supplemented with arginine under heat-stress conditions

Carla Manca[1], Marco Acciaro[1], Valeria Giovanetti[1], Gianpaolo Epifani[1], Salvatore Contini[1], Maria Gabriella Serra[1], Maria Dattena[1], Antonello Cannas[2], Fiammetta Berlinguer[3], Giovanni Molle[1]

[1]*Agris Sardegna, Sassari, Italy,* [2]*Dipartimento di Agraria, University of Sassari, Sassari, Italy,* [3]*Dipartimento di Medicina Veterinaria, University of Sassari, Sassari, Italy*

E-mail: cmanca@agrisricerca.it

Take home message Infrared thermography is capable of detecting body temperature variations and may represent a potential tool to predict distress conditions

Introduction Body temperature is an indicator of thermal balance and may be used to assess the adversity of the thermal environment. A rise of 1°C or less in rectal temperature is enough to reduce performance in most livestock species (Silanikove *et al*., 2000). Heat-stressed animals change their metabolism and physiology, with decrease in feed intake efficiency and utilization, reduced live body weight and impaired reproduction (Marai *et al*., 2007). The reproductive efficiency can be enhanced via supplementation with arginine (Saevre *et al*., 2011), which favours vascular perfusion of tissues. This work aimed to study the variation of body temperature as measured by thermal imaging of ewes submitted to arginine dietary supplementation under potential heat-stress conditions.

Materials & methods On June 12 (day -14) sixty late-lactating Sarda ewes, managed as a flock, were submitted to estrus synchronization and artificial insemination (AI, day 0). On day 12, the ewes were divided in two homogeneous groups on the basis of age, lactation stage, body weight, BCS, milk yield, body temperature and ovulation rate assessed on day 8. From day 14 to day 24 (treatment period) the groups were assigned to the following treatments: fed 100 g ewe^{-1} of a pelleted concentrate twice daily (group CON); fed 12 g of rumen-protected arginine blended with 85 g of the above pelleted concentrate and 15 g of a 20% (w/w) solution of molasses (group ARG). During the treatment period, the ewes were milked once at 8:00 and group-fed daily 0.5 kg ewe^{-1} of lucerne hay and 0.5 kg ewe^{-1} of ryegrass hay after the first concentrate meal and further 0.9 kg ewe^{-1} of ryegrass hay in the evening. On the same days meteorological data were downloaded from an indoor weather station on an hourly bases and temperature-humidity index (THI) and cumulative hours under severe heat stress at THI >75 (THI-hr) were computed (Peana *et al*., 2007). On day 14, 15, 16, 22 and 23 of the treatment period mean and maximum body temperature were detected at 15:00 using a thermal camera (ThermaCAM P25, FLIR Systems, Milan, Italy; 320 x 240 pixels, sensibility 0.08 °C) to record eye surface temperature (BT). On day 23 BT was measured at 7:00 (1 hr pre-meal), 9:00 (1 hr post-meal) and 15:00 (7 hrs post- meal). Body temperatures were analyzed by a PROC MIX of SAS inclusive of treatment (Tr), day of treatment (D) and their interaction as fixed effects and ewe as random effect. To investigate the effect of the hours of the days on the body temperature a further analysis of pre- and post-meal temperatures was run by a mixed procedure with Tr, measuring hour (Hr), their interaction as fixed effects and ewe as random effect. The relationship between THI and BT was studied by PROC REG procedure of SAS.

Results & discussion The mean values of the daily THI during the 10 d of the treatment period was 77 (max 81, min 65) and THI-hr totalled 152 hours. Data analysis across days showed that the Tr x D interaction affected body temperatures ($P<0.001$), with higher values in ARG than CON group, particularly on the second day of treatment for maximum BT and second and third day of treatment for mean BT. During the day, both Trt ($P< 0.05$) and Hr ($P<0.001$) affected maximum BT, with higher values in ARG than CON, and an increase from pre-meal to 7 hours post-meal, highly consistent with the expected diurnal variation. Mean BT was affected by Trt x Hr interaction ($P<0.001$) with higher pre-meal BT in ARG than CON group ($P<0.05$) and no differences in the subsequent measurements hours. These results suggest that the measuring time is a key factor to evaluate the effects of dietary or management treatments on body temperature. Overall, a significant positive relationship between mean THI and mean and maximum BT ($r^2= 0.68$; RMSE =1.56 and $r^2=0.79$; RMSE =1.26 respectively, $P<0.001$) was observed.

Conclusion Body temperatures of Sarda ewes, measured by thermal camera, was enhanced by an arginine-enriched supplement, due to an increase in vasodilatation and associated thermal dissipation under heat stress conditions. Moreover, the results confirm that BT tends to parallel with THI across days and during the day. Overall, our results suggest that the monitoring body temperature at least twice daily (pre- and post-meal) by thermal imaging represents an efficient method for an early detection of heat stress, thus favouring the early adoption of mitigation strategies.

References

Marai IFM, El- Darawany AA, Fadielc A and Abdel-Hafez MAM 2007. Small Ruminant Research 71, 1–12.
Peana I, Fois G, and Cannas A 2007. Italian Journal of Animal Science, 6:sup1, 577-579.
Saevre C, Meyer AM, Van Emon M L, Redmer DA, Caton JS, Kirsch J D, Luther JS, and Schauer CS 2011. NDSU Sheep Res. Rep. No. 52. North Dakota State Univ., Fargo. p. 13–16.
Silanikove N 2000. Livestock Production Science 67, 1–18.

Prediction of plasma non-esterified fatty acid concentration of dairy cows using milk fatty acids and mid-infrared spectroscopy

Tuomo Kokkonen[1], Päivi Mäntysaari[2], Sari Kajava[3], Terhi Mehtiö[2], Timo Latomäki[4], Laura Nyholm[4], Esa Mäntysaari[2], Martin Lidauer[2]

[1]University of Helsinki, Helsinki, Finland, [2]Natural Resources Institute Finland (Luke), Jokioinen, Finland, [3]Natural Resources Institute Finland (Luke), Maaninka, Finland, [4]Valio Ltd, Helsinki, Finland

E-mail: tuomo.kokkonen@helsinki.fi

Take home message Milk fatty acid composition determined by mid-infrared spectroscopy has potential to predict severe negative energy balance and excessive increase of plasma NEFA concentration in early lactation dairy cows.

Introduction High-yielding dairy cows are typically in negative energy balance during the early lactation, due to rapidly increasing milk production and insufficient feed intake. Severe negative energy balance increases the risk of production related diseases and negatively affects fertility, through delayed resumption of ovarian cyclicity. Individual energy balance is difficult to determine or predict, because measuring energy input and output is laborious and current feeding standards do not take into account the individual variation in energy requirements. Less labour-intensive methods are needed to predict individual energy status of large number of dairy cows for selection of animals with high feed efficiency but without excessive negative energy balance. Further, predicted energy status may be used as a tool to monitor and optimize feeding of early lactation dairy cows. The aim of the study was to evaluate the potential to predict plasma non-esterified fatty acid (NEFA) concentrations of dairy cows based on concentrations of individual milk fatty acids.

Materials & methods A database with 809 simultaneous measurements of plasma NEFA and milk fatty acid concentrations was built by collecting blood and milk samples from first and second parity cows at three research farms. Milk samples were collected during morning milking on lactation weeks 2 and 3 (two samples per week) and lactation week 20 (one sample). In herds where small amount of concentrate was fed during milking the blood samples were collected within 15 min after morning milking. In herd where no concentrate was fed during milking the blood samples were collected within 1 h after milking. Milk fatty acid composition was determined using mid infrared reflectance spectroscopy (MIR). Pearson correlation coefficients were calculated between individual fatty acid concentrations and plasma NEFA concentration to select the potential explanatory variables. SAS Mixed procedure was used to develop multiple regression models including the fixed effects of lactation day (days in milk, DIM) and concentration of individual long chain fatty acids (LCFA) in milk. In addition, the fixed effects of short and medium (MCFA) chain fatty acid concentrations, milk fat and protein concentrations, fat: protein -ratio and energy corrected milk yield were tested. Cow nested within the herd and lactation was considered as a random factor in the model, and effects of intercept and concentration of LCFA were included in the random statement of the model. Separate models were developed for first and second parity cows, due to the observed interaction between the effects of parity and LCFA concentration. The models were evaluated on the basis of Akaike's information criterion with correction and residual mean square error. Cross-validations were performed for each parity using two subsets, which were randomly constituted from calibration data.

Results & discussion Concentrations of LCFA (C18) were positively and MCFA (C10 to C14) negatively correlated with plasma NEFA concentration at lactation weeks 2 and 3 in both parities, whereas lower correlations were observed at lactation week 20. Average plasma NEFA concentrations were 0.60, 0.46 and 0.13 mmol/l at lactation weeks 2, 3 and 20 in first parity cows, and 0.47, 0.40 and 0.13 mmol/l in second parity cows. For the development of final models, only the data from lactation weeks 2 and 3 were used to address the period of negative energy balance and lipid mobilisation. Milk C18:1 cis9 concentration was the single best predictor of plasma NEFA concentration in both parities. Models including DIM and C18:1 cis9 explained 45% of the variation in first parity and 60% of the variation in second parity. Adding C14:0 concentration to the model improved coefficient of determination to 54% and 69% in first and second parity cows, respectively. Further addition of milk fat content in the model of first parity cows improved coefficient of determination to 58%, while the other studied factors did not significantly improve the models. In cross-validation, the coefficients of determination between predicted and observed plasma NEFA concentration were 0.37 for first parity cows and 0.36 for second parity cows. Root mean square error of cross-validation was larger for first than second parity cows (0.16 *vs.* 0.10 mmol/l). This might be due to factors other than energy status affecting plasma NEFA concentrations (e.g. sensitivity to stress related to animal handling during blood sampling). Lower rank of first lactation cows in a mixed group of cows of different parities may also subject them to larger variation of daily feed intake than older cows.

Conclusion These results demonstrate that prediction of plasma NEFA concentration using milk fatty acid composition determined by MIR spectra has potential to provide physiologically based information on energy status of dairy cows. The predicted values can potentially be used to differentiate between normal and excessive plasma NEFA concentrations.

Acknowledgements This research was funded by Finnish Ministry of Agriculture and Forestry, Valio Ltd, Faba co-op, VikingGenetics, Finnish Cattle Breeding Foundation and Raisioagro Ltd.

Functional classification of feed items in Pampa grassland based on their NIR spectrum

Júlio Cezar Rebés de Azambuja Filho[1,2], Magali Jouven[2,3], Denis Bastianelli[4], Philippe Haussoun[3], Olivier Bonnet[5], Paulo Cesar de Faccio Carvalho[1]

[1]UFRGS, Porto Alegre, Brazil, [2]SupAgro, Montpellier, France, [3]INRA, Montpellier, France, [4]Cirad, Montpellier, France, [5]Cerpam, Alpes de Haute Provence, France

E-mail: julioazambuj@gmail.com

Take home message: NIR spectrum can be used for a rapid classification of feed items consumed by ruminants, in heterogeneous grazing environments, which provides the basis for analysing and modelling forage intake at pasture.

Introduction The heterogeneous Pampa grasslands represent 70% of the nutritional supply of 18.4 million of domestic herbivores in Rio Grande do Sul state, south Brazil (IBGE, 2015). Due to poor animal performance at certain times of year, a number of farmers supplement their herds, without optimizing supplementation either in terms of timing or composition. In order to provide sound criteria to manage supplementation at pasture, it is important to assess how the Pampa ecosystem can meet the nutritional requirement of cattle, and thus to investigate animal foraging strategies. Given the diversity of Pampa vegetation, a first step is to group feed items (or "bites") into categories which can be interpreted in nutritional terms. The objective of this work was to produce a functional classification of bites based on their NIR (Near Infrared) spectrum.

Materials & methods The experiment was conducted at the University of Rio Grande do Sul (Brazil). Ten paddocks have been monitored since 1986 to test the impact of five forage allowances on the dynamics of the natural vegetation and the performance of 2-year-old heifers. In 2012, in each season, we hand plucked bites samples to simulate the cattle feeding behavior observed during continuous bite monitoring (Bonnet *et al*., 2015). A total of 1515 bite samples were collected, dried at 60ºC, weighed (providing bite mass) then ground to 0.5 mm and scanned for NIR spectrum (spectrometer Buchi NIRFlex 500). The spectra were pretreated (SNV, 2nd derivative) and submitted to a principal component analysis followed by a hierarchical classification using Ward's method (R software). An analysis of variance (Tukey test) was used to test the differences between groups. The nutritional characteristics (digestibility; protein, fibre and ash content) of each sample were predicted based on its NIR spectrum by the Belgian laboratory Wallonia research CRA-W.

Results & discussion The classification tree was cut to five groups, as lower level divisions produced less clear clusters. The five groups were associated with either nutritional or morphological characteristics (Table 1). Group 1 was dominated by *Luziola peruviana* species and included bites rich in proteins and minerals which suggest that such feed items may have a function in providing nitrogen to the ruminal microflora. Group 2 contained the most digestible bites, with lower fibre content and an intermediate bite size, made up of non-grass species and young grass leaves. Group 3 was an intermediate group in terms of protein and fibre content, providing small bites and thus low intake rate. Group 4 and 5 were rich in fibre and provided high bite mass; thus their main function might be to secure quantitative feed intake. Group 5 included almost all the bites from large tussocks or Apiaceae *Eryngium horridum*.

Table 1 Characteristics of the five "bite categories": *in vitro* Organic Matter Digestibility (OMD) g/kg organic matter; Crude protein (CP), Neutral Detergent Fibre (NDF) and Ash as g/kg dry matter; Bite mass (g DM) and Dominance (most frequent botanic type in each group). Letters refer to statistical difference (P<0.05) between groups.

Group (sample number)	OMD		CP		NDF		Ash		Bite mass		Dominant type of plant (Frequency in the samples, in %)
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	
1 (321)	537^{b}	58	147^{a}	33	687^{d}	35	102^{a}	16	0.09^{c}	0.1	Wetland grass and short grass (86%)
2 (135)	567^{a}	97	125^{b}	31	661^{e}	93	97^{b}	11	0.16^{cb}	0.1	Non grass sp. and short grass (77%)
3 (369)	447^{c}	46	92^{c}	14	740^{c}	26	88^{c}	9	0.11^{c}	0.09	Short Poaceae mix (78%)
4 (422)	309^{e}	40	58^{d}	13	783^{b}	22	68^{e}	8	0.27^{b}	0.2	Tussocks (72%)
5 (268)	338^{d}	71	59^{d}	23	812^{a}	47	72^{d}	13	0.69^{a}	2	Tall tussock or Apiaceae (63%)

Conclusion Classification of bites based on NIR spectrum enabled to produce groups of feed items, which seem to be interpretable in terms of function in the daily intake. The usefulness of such classification will be tested by applying it to the observed feeding choices of animals and in determining whether it can be used to describe seasonal feeding strategies.

Acknowledgements This work is part of a PhD thesis co-financed by the CAPES-COFECUB program Valorizar o Pampa.

References

Bonnet OJF, Meuret M, Tischler RT, Cezimbra IM, Azambuja JCR and Carvalho PCF 2015. Animal Production Science 55, 339-349.

IBGE, 2015. Website https://sidra.ibge.gov.br/tabela/3939

Plasma free amino acid concentrations can be used to determine if methionine is a limiting amino acid in lactating dairy cows

Charles Schwab[1], Nancy Whitehouse[2], Ryan Higgs[3], David Pacheco[4]

[1]University of New Hampshire, Durham, New Hampshire, USA, [2]University of New Hampshire, Durham, New Hampshire, USA, [3]Llenroc Ltd., Ohaupo 3881, New Zealand, [4]AgResearch, Grasslands Research Centre, Tennent Drive, Palmerston North 4442, New Zealand

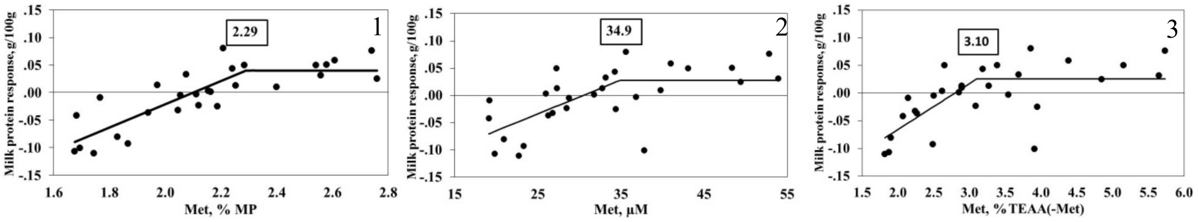
E-mail: charles.schwab@unh.edu

Take home message Plasma free amino acid (AA) concentrations can be a practical way to identify limiting AA in dairy cows.

Introduction Precision feeding is needed to optimize efficiency of nutrient usage, nutritional health and milk production. The optimal balance of absorbed AA for different stages of milk production remains to be established, but it is known that improvements in balance of absorbed essential AA (EAA) increases efficiency of use for metabolic functions. While there have been improvements in nutritional models, questions remain about their accuracy for predicting adequacy of AA supply and sequence of AA limitation, particularly with grazing cows and cows fed unconventional feedstuffs. Furthermore, measurement of duodenal flows for those situations is limited because of the technical challenges involved. It is known that increased absorption of EAA increases their concentrations in blood plasma and that increased absorption of the most limiting EAA increases content and yield of milk protein (Whitehouse *et al.*, 2017; National Research Council (NRC), 2001). Our hypothesis is that plasma free AA concentrations can be used to identify limiting AA and the extent of their limitation. Here, we test this hypothesis by using data from experiments conducted at the University of New Hampshire.

Materials & methods Milk protein and plasma free methionine (Met) data were used from 5 trials involving lactating Holstein cows fed corn-based, Met-deficient diets. Treatments were continuous infusion of increasing amounts of Met into the abomasum or feeding increasing amounts of Smartamine®M. The dataset consisted of 28 treatments means involving 139 individual cow observations. The NRC (2001) model was used to predict concentrations of Met in metabolizable protein (MP) for all treatments. Milk protein content and yield responses to supplemental Met were calculated as described in NRC (2001). Milk protein responses were regressed on model predicted concentrations of Met in MP and on different measures of expression of plasma sulfur AA using PROC NLIN in SAS (9.4). Relationships between these variables were described using a segmented rectilinear model which provides an objective, mathematically determined optimum supply.

Results & discussion The described Met treatments resulted in predicted Met concentrations in MP that ranged from 1.67 to 2.75% (see x-axis in Figure 1), which were positively correlated ($r^2 = 0.751$; graph not shown) with plasma Met concentrations that ranged from 19 to 54 µM. Regressions of milk protein content responses on Met in MP (Figure 1) resulted in a breakpoint estimate of 2.29%, a value similar to that determined for NRC (2001) with a different and much larger dataset. Regressions of milk protein content responses on concentrations of plasma Met (Figure 2) and Met as a percentage of total EAA (Figure 3) demonstrate a correlation between plasma Met concentrations and milk protein responses and indicate that an 'ideal' plasma EAA profile for lactating cows might eventually be estimated.



Figures 1-3. Milk protein content responses as a function of predicted Met in MP (1), measured plasma Met concentrations (µM) (2), and measured plasma Met concentrations expressed as a percentage of total EAA (3), respectively.

Conclusion Plasma free Met concentrations less than 35 µM and 3.10% of total EAA indicate Met as a limiting AA for lactating cows.

Acknowledgements This research was supported by grants from Adisseo N.A.

References

National Research Council. 2001. Nutrient Requirements of Dairy Cattle (7th Rev. Ed.) National Academy Press, Washington, DC.
Whitehouse NL, Schwab CG and Brito AF 2017. Journal of Dairy Science 100, 9585-9601.

Use of conceptual models to predict feed intake of cattle in tropical and subtropical environments

Christian Bateki, Uta Dickhöfer

University of Hohenheim, Institute of Agricultural Sciences in the Tropics, Stuttgart, Germany

E-mail: christian.bateki@uni-hohenheim.de

Take home message Feed intake of tropical cattle can be accurately predicted using conceptual semi-mechanistic models after their adaptation to tropical feeding conditions.

Introduction Dry matter intake (DMI) is the main determinant of ruminants' performance and nutrient use efficiency. Adopting and adapting temperate semi-mechanistic conceptual models (CM) for DMI prediction in (sub-)tropical cattle systems could help optimize and intensify production. While such CM are known to adequately predict DMI in temperate husbandry systems, it is unknown how accurate and precise they can be under typical (sub-)tropical husbandry conditions. Thus, our objectives were to identify and adjust two DMI CM to typical (sub-)tropical feeding situations and to evaluate their accuracy and precision in predicting DMI of stall-fed cattle using a meta-analysis.

Materials & methods The CM of Conrad *et al.* (1964) (C1) and Mertens (1987) (M1) were selected for their simplicity in reflecting physico-physiological DMI regulation. For C1, DMI is calculated as 0.0107LW/(1-D) and as DDM/D, where 0.0107 is the daily faecal output (g/kg LW), LW liveweight (kg), D digestible fraction, and DDM digestible DMI (kg/d). For M1, DMI is estimated 0.012LW/NDF and ME_{req}/ME_d, where 0.012 is the capacity of neutral detergent fibre (NDF) intake (g/kg LW), NDF the NDF concentration in the diet (g/kg DM), ME_{req} the animal's metabolizable energy (ME) requirements (MJ/d) and ME_d the dietary ME concentrations (MJ/kg dry matter). For both CM, the lowest DMI predicted from either of two equations was retained actual DMI. Moreover, using values reported in published studies, C1 was modified by increasing the faecal output rate to 0.0116LW to yield C2. For M1, NDF intake capacity was increased to 0.0135 g/kg LW and daily maintenance ME_{req} from 0.420 to 0.631 MJ ME/kg $LW^{0.75}$, while ME_{req} for gain were reduced from 0.0325 to 0.0243 MJ ME/g LW gain to yield M2. Lastly, for C3, C4, M3, and M4 the mean of the physically and physiologically predicted DMI rather than the lowest value was retained as actual DMI. The eight CM were then evaluated using a dataset summarizing results from 51 studies conducted under (sub-)tropical conditions with 231 dietary treatments. The mean bias (MB), root mean squared error of prediction (RMSEP), relative prediction error (RPE), and concordance correlation coefficient (CCC) were used to evaluate the CM's adequacy (i.e. accuracy and precision) (Tedeschi, 2006).

Results & discussion The C1 was more adequate than the M1 in predicting DMI of (sub-)tropical cattle, although both CM underestimated actual DMI (Table 1). Modifications of both CM reduced RPE, with M4 yielding the most accurate and precise predictions with lowest MB and RMSEP and highest CCC values. The CM C3 and C4 overestimated actual DMI, whereas CM M3 and M4 underestimated actual DMI, although their MB were much lower.

Table 1 Adequacy of eight models for prediction of dry matter intake in (sub-)tropical stall-fed cattle

Statistical measure	Conceptual models[†] C1	C2	C3	C4	M1	M2	M3	M4
Mean bias, kg/d	0.14	-0.22	-1.19	-1.54	2.30	1.28	1.25	0.07
RMSEP[*], kg/d	2.00	1.93	2.16	2.40	3.00	2.19	2.10	1.62
RPE[#], %	26.1	25.1	28.1	31.2	39.1	28.6	27.4	21.1
CCC[¶] (0 to 1)	0.85	0.86	0.83	0.81	0.69	0.83	0.83	0.91
Ranking[**]	3	2	5	7	8	6	4	1

[†]C1: Conrad *et al.* (1964), M2: Mertens (1987); C2, M2: with modified equation parameters, C3-4 and M3-4: mean of physically and physiological predicted dry matter intake. [*]Root mean squared error of prediction, [#]relative prediction error, and [¶]concordance correlation coefficient. [**]According to RMSEP

Conclusion Conceptual models from temperate husbandry systems are not valid to predict DMI of stall-fed cattle kept in (sub-) tropical environments. However, adjusting CM equation parameters greatly improves adequacy of DMI predictions.

Acknowledgements This research was funded by the Ellrichshausen Foundation.

References

Conrad HR, Pratt AD and Hibbs JW 1964. Journal of Dairy Science 47, 54–62.
Mertens DR, 1987. Journal of Animal Science 64, 1548 - 1558.
Tedeschi LO, 2006. Agricultural Systems 89, 225–247.

Optimizing dairy cows feeding in order to produce milk from high nutritional value with low environmental impacts

Eric Froidmont[1], Adeline Lefevre[1], Yvan Larondelle[2], Michel Focant[2]

[1]*Centre wallon de Recherches agronomiques, Gembloux, Belgium,* [2]*Université Catholique de Louvain, Louvain-la-Neuve, Belgium*

E-mail: e.froidmont@cra.wallonie.be

Take home message The improvement of the fine milk composition promoting better health of consumers and decreasing the environmental impacts in terms of nitrogen (N) and greenhouse gas emissions are two compatible objectives

Introduction Ruminant livestock is increasingly questioned in terms of its environmental incidence, according to the low feed efficiency of these animals and the methane emission from digestive fermentations. Milk production is also questioned for its impact on consumer's health, especially for its high levels of saturated fatty acids (FA) favouring cardiovascular diseases. We investigated the possibility of improving these aspects together through an optimal feeding of cows.

Materials & methods Two groups of 2 cows received a control diet (CTL) and an optimized diet (OPT) according to a cross over design. On a dry matter (DM) basis, CTL contained maize silage (7 kg/d), raygras silage (7 kg/d), soybean meal (3 kg/d), rapeseed meal (2 kg/d), pea (0.5 kg/d), barley (0.9 kg/d) and minerals. OPT was similar to CTL except that raygras silage was replaced by a mixed dactyl-clovers (white and red) silage, and that all the soybean meal was replaced by Nutex Excel [Dumoulin (B) - 2.3 kg/d, 32% extruded linseed, 18% extruded rapeseed, 22% wheat and 17% sunflower meal]. OPT also contained 10 g/d of vitamin E to prevent the drop of milk fat due to polyunsaturated FA (PUFA) supplementation. CTL diet was balanced for a milk production of 30 L of milk per day according to Dutch standards. Diets were similar in NE supply (32.3 Mcal/d), but differed in total DM offered (20.8 vs 20.1 kg/d), digestible protein (1858 vs 1458 g/d) and OEB (+332.8 vs -20.1 g/d) offered. Crude protein (17.0 vs 13.1% DM) and fat (4.1 vs 6.0%DM) contents also differed between OPT and CTL, respectively. Periods consisted of 21d for diet adaptation followed by 5d of measurements: milk production (5d), milk composition [fat and protein contents by MIR (5d), FA profile by GC (3d), vitamin B12, vitamin E (HPLC, 3d)], N balance (5d) and CH_4 emission by milk MIR prediction (3d, Vanlierde *et al*., 2015).

Results & discussion Compared to CTL, OPT decreased the energy corrected milk production by 4% (29.1 *vs* 28.0 kg/d, P=0.02) and milk protein content by 3% (3.28 *vs* 3.19 %, P = 0.002). Milk fat content (4.35%) did not differ while milk urea content largely decreased with OPT (291 *vs* 187 mg/L) due to the lower protein supply. OPT reduced the methane emission by 12 and 8% according to mode of expression (556 *vs* 491 g/d/cow, *P < 0.001*; 19.2 *vs* 17.6 g/kg of milk, *P < 0.001*). All parameters from N balance differed significantly (*P < 0.001*). N intake decreased by 25% with OPT (583 *vs* 438 g/d) while urinary (238 *vs* 129 g/d) and faecal (158 *vs* 152 g/d) N were reduced by 46 and 4%, respectively. In consequence, N efficiency (milk N/intake N) was improved by 23% with OPT (25.7 *vs* 31.5%). Compared to CTL, milk PUFA content increased by 62% (2.9 *vs* 4.7%) with OPT while saturated FA decreased by 15% (76.1 *vs* 64.5%). The ω6/ω3 ratio was lower with OPT (2.8 *vs* 1.1) that also largely increased (*P < 0.001*) linolenic and rumenic acids contents of the milk. With OPT, we also observed an increase of the milk vitamin E concentration (*P < 0.001*), due to the supplementation, and a tendency (*P = 0.1*) of higher amount of vitamin B12 in the milk (0.32 *vs* 0.47µg/100 g). Finally, the milk content of equol, an interesting polyphenol coming from digestive microbial transformation of isoflavones that are specific to legumes plants (Froidmont *et al*., 2016), was increased 8 fold (21 *vs* 173 µg/kg) with OPT.

Conclusion Despite a slightly lower milk production, OPT improved the nutritional value of the milk and, simultaneously, decreased largely methane emission and N excretion following minor feeding adaptations. The supply of PUFA by extruded rape and linseeds in OPT improved the milk FA profile and probably limited the activity of methanogens populations in the rumen (Martin *et al*., 2006). The satisfaction of animal's requirement with a lower DM intake also contributed to limit the methane emission by decreasing the amount of rumen fermentable organic matter. The respect of the animal protein requirements improved the N efficiency by decreasing N urine output, which probably limits NH_3 and indirect N_2O emissions. The higher proportion of local feedstuffs in OPT also contributed to limit the carbon footprint. At the end, the supply of grass silage rich in leguminous plants avoided the use of N fertilizers and increased equol in milk.

Acknowledgements This research was subsidised by SPW, DGO3, Namur, Belgium

References

Froidmont E, Daems F, Decruyenaere V, Dehareng F, Franckson D, Lefevre A, Ninane V, Romnee JM 2017. Fourrages 230, 141-146.

Martin CDP, Morgavi M, Doreau and Jouany JP 2006. Fourrages 187, 283.–300.

Vanlierde A, Vanrobays ML, Dehareng F, Froidmont E, Soyeurt H, McParland S, Lewis E, Deighton MH, Grandl F, Kreuzer M, Gredler B, Dardenne P and Gengler N 2015. Journal of Dairy Science 98, 5740-5747.

415

Effects of dietary energy and protein levels on structural properties and fermentation of rumen in weaned lambs

Xiaokang Lv[1,2], Minli Qi[1,2], Kai Cui[1,2], Yan Tu[1,2], Qiyu Diao[1,2], Naifeng Zhang[1,2]

[1]*Feed Research Institute of Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Key Laboratory of Feed Biotechnology of Ministry of Agriculture, Beijing, China*

E-mail: zhangnaifeng@caas.cn

Take home message Raising the dietary energy level can promote the rumen development of 120-day-old lambs, while the dietary protein level has little effect on rumen development.

Introduction Development of the rumen is critical for the utilization of dry feed for ruminants, especially the young ruminants. It has been reported that dietary restrictions affect the anatomic development of rumen (Sun *et al*. 2013) and rumen fermentation (Rabelo *et al*., 2003). Protein and energy, as the main nutrients, might have deep impact on the gastrointestinal development of lambs. The objective of this study was to explore the effects of dietary energy and protein levels on structural properties and fermentation of rumen in weaned lambs.

Materials & methods Sixty Hu lambs with initial BW of 14.95±0.56 kg were randomly assigned to receive one of four dietary treatments according to a 2×2 factors experimental design. Each group had four replicates with four lambs in each replicate. Lambs were fed pelleted starters with different protein (CP: HP: 16% *vs*. LP: 12%) and energy (ME: HE: 10.9 *vs*. LE: 8.6 MJ/kg) levels. Trial lasted for 60 days. Sixteen lambs were slaughtered at the end of experiment and the rumen morphology and fermentation parameters were analysed using GLM procedures of SAS 8.1. Duncan's Multiple Range Test was used to identify differences between specific treatments. Effects were considered significant when $P < 0.05$.

Results & discussion No differences were observed in DMI between groups ($P>0.05$) whereas the high dietary energy level significantly increased ADG ($P<0.05$) (Table 1). High energy starter reduced pH and increased total VFA and propionic acid concentration of rumen fluid ($P < 0.05$). But dietary protein levels had no significant effect on rumen fluid pH and VFA concentrations ($P > 0.05$). There was an interaction effect of energy and protein levels on the ammonia nitrogen content of rumen fluid ($P < 0.05$) that increasing the protein level enhanced the ammonia nitrogen content when the lambs were fed high energy diet. High energy starter increased the rumen weight and its percentages to the complex stomach of weaned lambs ($P < 0.05$). However, there was no significant effect of dietary energy and protein levels on the length and width of rumen papillae of lambs ($P > 0.05$).

Table 1 Effects of dietary energy and protein levels on rumen morphology and fermentation in lambs.

Items	HE		LE		SEM	*P* -value		
	HP	LP	HP	LP		En	Pr	En×Pr
DMI, g/d	1235	1260	1262	1275	13	0.2	0.1	0.7
ADG, g/d	249^{a}	226^{ab}	209^{b}	213^{b}	10	0.023	0.4	0.2
BW before slaughter, kg	28.7	28.2	26.1	24.0	0.9	0.080	0.5	0.6
pH	7.05^{b}	6.94^{b}	7.28^{a}	7.26^{a}	0.08	0.044	0.5	0.7
NH_3-N, mmol/L	19.0^{a}	10.6^{b}	9.0^{b}	11.8^{b}	1.2	0.1	0.3	0.026
TVFA, mmol/L	21.6^{a}	29.2^{a}	18.8^{b}	16.1^{b}	2.7	0.049	0.5	0.18
Acetate, mmol/L	13.8	17.8	13.1	11.3	3.2	0.2	0.7	0.4
Propionate, mmol/L	4.95^{a}	8.63^{a}	2.7^{b}	2.55^{b}	1.88	0.047	0.3	0.3
Butyrate, mmol/L	1.04	1.30	0.99	0.76	0.25	0.2	0.1	0.9
Rumen weight /g	664^{a}	609^{ab}	516^{b}	499^{b}	30	0.049	0.9	0.7
Rumen weight, % of complex stomach	69.3^{a}	68.0^{ab}	64.0^{b}	66.7^{ab}	0.1	0.027	0.6	0.2
Papillae length (μm)	994	976	839	1012	76	0.4	0.2	0.3
Papillae width (μm)	93.1	95.5	102.7	103.1	4.2	0.3	0.5	0.06

[a,b] Means within same row with the different superscript letter are significantly different ($P < 0.05$); En: energy effects, Pr: protein effects, En×Pr: interactions of energy and protein levels.

Conclusion Dietary energy level have great effect than dietary protein level on the rumen morphology and fermentation parameters of weaned lambs. Increasing dietary energy level can improve the growth performance, VFA concentration, and rumen development in weaned lambs.

Acknowledgements This work was supported by the Special Fund for Agro-scientific Research in the Public Interest (201403049) and the Earmarked Fund for China Agriculture Research System (CARS-39).

References

Rabelo E, Rezende RL, Bertics SJ and Grummer RR 2003. Journal of Dairy Science 86, 916-25.

Sun ZH, He ZX and Zhang QL 2013. Journal of Animal Science 91, 4336-4344

Effect of dietary NDF content on growth performance and rumen development of preruminant lambs

Biao Xie[1,3], Kai Cui[1,2], Shiqin Wang[1,2], Xiaokang Lv[1,2], Qiyu Diao[1,2], Naifeng Zhang[1,2]

[1]*Feed Research Institute of Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Key Laboratory of Feed Biotechnology of Ministry of Agriculture, Beijing, China,* [3]*Shanxi agricultrual university, Jinzhong, Shanxi, China*

E-mail: zhangnaifeng@caas.cn

Take home message Appropriate fibre content in starter feed is beneficial to the rumen development and growth performance of lambs.

Introduction Several studies have attempted to define the most appropriate solid feed for young ruminants. Low amount of forage is sufficient to foster an improving ADG (Castells *et al.* 2012) and a better rumen environment in calves (Castells *et al.* 2013) compared with young ruminants offered no forage. The better rumen environment and growth performance should be due to the positive changes of rumen development caused by the fibre of forage, which might be needed to prevent abnormal development of rumen papillae. Therefore, the objectives of this study were to evaluate the effects of different dietary NDF levels on rumen development in lambs during transition from liquid to solid feed.

Materials & methods One hundred *Hu* lambs (18 ± 2 days of age and 6.10 kg ± 0.10 kg of BW) were randomly assigned to 4 groups, each group had 5 replicates with 5 lambs each replicate. The lambs in the four groups were fed pelleted starter containing different NDF levels (10%, 15%, 20%, 25%, separately) from 20 to 90 days of age. Besides, all lambs were fed milk replacer (MR; 25.59 % CP, 12.60% Fat) from 20 to 60 days of age after a 3 days transition period. Lambs had *ad libitum* access to water and starter pellets. Twenty lambs (one per replicate) were slaughtered at the end of experiment and the rumen morphology were analysed using GLM procedures of SAS 8.1. Effects were considered significant when $P < 0.05$.

Results The ADG and total DMI were higher ($P < 0.05$) in lambs consuming starters containing 20% and 25% NDF compared with which containing 10% NDF. The percentage of reticulorumen to the complex stomach was linearly decreased and it was lower in lambs fed starter containing 25% NDF than that containing 10% and 15% NDF ($P < 0.05$). Compared with the lambs fed starter containing 10% NDF, the rumen keratin layer thickness and papillae width were decreased, whereas the rumen muscle layers thickness was increased in lambs consuming starters containing 15%, 20% and 25% NDF compared with which containing 10% NDF ($P < 0.05$).

Table 1 Effects of dietary NDF levels on rumen development of lambs.

Items	N10	N15	N20	N25	SEM	*P*-value
DMI, g/d	416.8^{c}	497.3^{bc}	569.1^{ab}	607.4^{a}	21.3	0.001
ADG, g/d	176.0^{c}	210.2^{b}	235.1^{ab}	247.3^{a}	6.0	0.001
Reticulorumen, g	626.50	592.50	488.33	476.50	27.23	0.122
Reticulorumen, % of complex stomach	83.41^{a}	81.78^{ab}	78.50^{bc}	74.28^{c}	1.01	0.002
Papillae length, µm	1628	1577	1623	1614	46	0.984
Papillae width, µm	353^{a}	309^{b}	297^{b}	308^{b}	6.52	0.008
Epithelium, µm	126.23^{a}	114.63^{a}	117.16^{a}	97.12^{b}	2.35	0.000
Keratin layer, µm	64.23^{a}	54.21^{b}	57.11^{b}	52.55^{b}	1.29	0.005
Muscle layers, µm	458.25^{c}	759.26^{a}	586.04^{b}	682.19^{ab}	18.40	0.000

$^{a–c}$ Means within a row with different superscripts differ ($P < 0.05$). N10, N15, N20 and N25: diets containing 10%, 15%, 10%, 15% and 20% of NDF (as-fed), respectively.

Conclusion In conclusion, appropriate NDF content is needed to maintain an abrasion factor to prevent abnormal development of rumen papillae. Providing with 20% and 25% NDF in lamb diet can promote growth performance and enhance the rumen development in young lambs.

Acknowledgements This work was supported by the Special Fund for Agro-scientific Research in the Public Interest (201303143, 201403049) and the Earmarked Fund for China Agriculture Research System (CARS-39).

References

Castells L, Bach A, Aris A, and Terre M 2013. Journal of Dairy Science 96, 5226-36.
Castells L, Bach A, Araujo G, Montoro C and Terre M. 2012. Journal of Dairy Science 95, 286-93.

The recording of grazing time of dairy goats is accurate using the Lifecorder Plus device

Rémy Delagarde[1], Marine Piriou[1], Alexia Charpentier[1,2]

[1]INRA Pegase, Saint-Gilles, France, [2]INRA Ferlus, Lusignan, France

E-mail: remy.delagarde@inra.fr

Take home message The use of the Lifecorder Plus is simple and allows to record accurately the grazing time of dairy goats at pasture.

Introduction The recording of daily grazing time in ruminants allows to better understand the sward-animal relationships and the behavioural constraints affecting daily intake at pasture. For grazing goats, very few portable devices have been validated. The Kenz Lifecorder Plus device (LCP, Suzuken Co. Ltd., Nagoya, Japan), based on an uniaxial accelerometer, originally developed for measuring human daily activity level, has been validated recently to record daily grazing time and pattern in dairy cows (Delagarde and Lamberton, 2015). The aim of this study was to compare grazing activity duration as recorded simultaneously by the Lifecorder Plus fitted on the goats's neck and by trained observers.

Materials & methods This validation study was conducted at the INRA experimental farm of Méjusseaume (Brittany, France) in 2015 and 2017 for a total of 173 hour × goat visual observations. The Lifecorder device is placed in a small box, attached to the goat's neck by means of a simple collar. Actual grazing, ruminating, and "other activities" were recorded by trained observers. The Lifecorder device records the average activity level for every 2-min period, with a range of possible values from zero (no activity) to 9 (maximal and permanent activity). Grazing activity was defined by an average activity level within a 2-min period greater than 0.5 as for dairy cows (Delagarde and Lamberton, 2015). Short periods of activity (2 or 4 min) as well as short intra-meal intervals (2 or 4 min) were not considered. Grazing activities were then summed per hour (for standardised statistical analyses) or per validation sequence (Figure 1). The grazing activity duration recorded by the Lifecorder Plus was compared with the actual grazing activity duration recorded manually by observers on a per hour basis, through the calculation of the mean prediction error which is the bias against the y=x axis (Bibby and Toutenburg, 1977), and though the calculation of sensitivity, specificity and accuracy (Visa *et al.*, 2011).

Results & discussion On average, at the hour scale, the correlation between predicted (by the Lifecorder, 52.0 min/h) and actual (by the observer; 50.1 min/h) grazing time was high ($R^2 = 0.87$), with a mean relative prediction error of 0.11, i.e. 5.4 min/h (Figure 1). There was no bias due to the slope (0% of Root Mean Square Error, RMSE), and a small over-estimation of grazing time (12% of RMSE due to the mean bias), the majority of the bias being due to random variation (88% of RMSE). At the scale of the observation sequence (mean duration: 162 min), the mean relative prediction error was only of 0.09, which indicates a good overall accuracy of the device. Sensitivity (proportion of true positive: 0.98) and accuracy (overall concordance: 0.96) were very high and showed that grazing activities were always well detected. Specificity (proportion of true negative: 0.74) was lower, indicating that some other activities were sometimes detected as grazing activity. These results indicate similar to greater prediction quality for recording daily grazing time in grazing goats when compared to the same device or other devices tested on grazing dairy cows. There is no similar published work on grazing goats.

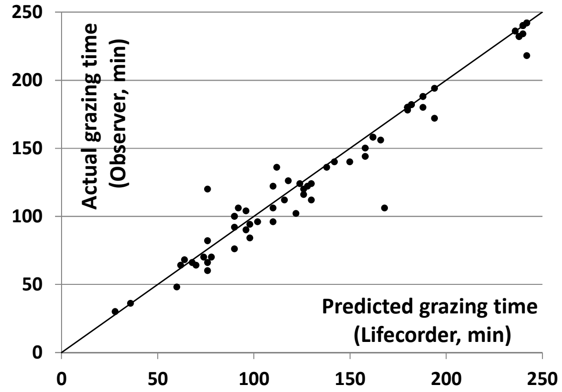


Figure 1 Relationship between grazing time recorded by the Lifecorder and by visual observation (69 sequences of 62 to 242 min of observation).

Conclusion The accuracy, sensitivity and specificity of the Lifecorder Plus to record grazing activities of dairy goats are high. This device can be used to record daily grazing time and nycthemeral pattern of grazing activities in goats at pasture.

Acknowledgements This research was supported by the french CASDAR project CAPHERB N°5546.

References

Bibby J and Toutenburg H 1977. Whiley, London
Delagarde R and Lamberton P 2015. Applied Animal Behaviour Science 165, 25–32.
Visa S, Ramsay B, Ralescu A and Van der Knaap E 2011. Proc. 22th Cognitive Science Conference, Cincinnati, Ohio, US.

Prediction of herbage mass in Campos grasslands based on herbage height

Martin Do Carmo[1], Geronimo Cardozo[2], Andrea Ruggia[3], Pablo Soca[4]

[1]*Universidad de la República, Montevideo, Montevideo, Uruguay,* [2]*Instituto Nacional de Investigación Agropecuaria, Treinta y Tres, Treinta y Tres, Uruguay,* [3]*Instituto Nacional de Investigación Agropecuaria, Las Brujas, Canelones, Uruguay,* [4]*Universidad de la República, Paysandu, Paysandu, Uruguay*

E-mail: martindocarmocorujo@gmail.com

Take home message Herbage mass of Campos grassland can be predicted (with medium prediction error) based on herbage height.

Introduction Measurement of herbage mass is crucial for management of stocking rate based on herbage allowance (Do Carmo *et al*. 2018) and as a state variable of grazing system related to ecosystem services (Ruggia *et al*. 2015). Campos grassland cover Uruguay, south Brazil and northeast of Argentina, and to extend the measurement of herbage mass for large paddocks and whole farms, methods have to be easy to apply for farmers and managers. Herbage height could be a proxy of herbage mass but require estimations of predictability. Predictability can be better estimated when data do not used to calibrate the equations is used to validate the model (Griggs and Stringer 1988). Our objective was to estimate the predictability of equations developed with data set from a large quantity of sites, years and botanical composition, to develop confident equations to estimate herbage mass in Campos grasslands.

Materials & methods Data sets from 12 sites (two experimental stations and ten commercial farms spread across Uruguay) and 29 plots covering a large spatial variability in soils and pasture composition was used to calibrate equations used in predictability by cross validation. Herbage height was measured with a ruler in cm in 5 points within each quadrat, where 80-90% of herbage concentrate, ignoring tall stalks (Stewart *et al*. 2001). Herbage mass was cut at ground level with manual or electrical scissors, including dead plus green herbage, and dried in forced air oven at 60 °C until constant weight. Dry matter in the quadrat of 50 x 50 cm was converted to kg/ha. Data set comprises samples from year 2007 to 2010 and from 2012 to 2015. Total number of samples were 3836, 1046 from winter, 1013 from autumn, 872 from spring, and 929 from summer. Data analysis of herbage mass included herbage height as a fixed effect (covariable) and season, year, and plot within site as random effects, (Mixed procedure of SAS). Half data of each season, (not from each site) was used for cross validation of the equations, see Table 1 (Griggs and Stringer 1988).

Results Equations differed between seasons, and herbage height was a significant to explain variation in herbage mass (Table 1). In spite of the high variability of sites and paddocks, slope coefficient was very stable around 300 kg per cm per ha, however predictability has medium prediction error, with a rank from 0 to 100% of error for all seasons and average RMSPE at Table 1. Average herbage mass and height were 2955, 2678, 2508, and 2508 kg/ha and 8.4, 7, 8, and 8 cm for autumn, winter, spring and summer respectively.

Table 1 Intercept and slope of the regression equations to estimate the herbage mass based on herbage height, and root mean square prediction error (RMSPE) for cross validation of herbage height-mass relationship for each season.

	Summer	Autumn	Winter	Spring
Samples to calibrate equation (n)	465	499	512	436
Intercept	391 (± 194)	228.5 (± 300)	355.6 (± 242.6)	521 (± 356)
Significance (p)	0.09	0.48	0.20	0.21
Slope (kg/cm)	264 (± 7.29)	313 (± 6.6)	325 (± 6.7)	320 (± 7.1)
Significance (p)	<0.0001	<0.0001	<0.0001	<0.0001
Samples for cross validation (n)	464	514	527	436
RMSPE (% relative to the true value)	30.8	28.9	30.2	28.7

Conclusion Predictability of herbage mass from herbage height has medium error. However, the value of kg per cm per ha constitute a valuable tool for manager and farmers who base the change of stocking rate on the herbage allowance management of each paddock. The slope value, was similar to the Australian standards for ruminants (Nutrient Requirements of Domesticated Ruminants, 2007, page 215) of 3 cm/ton/ha, thus seems to be a value reproducible for many grasslands around the world.

Acknowledgements This research was supported by FPTA 242, PA 11, PF 07, funds from INIA (Uruguay) and resources of UdelaR.

References

Do Carmo M, Sollenberger LE, Carriquiry M and Soca P 2018. Professional Animal Scientist 34, 32-41.
Griggs, T.C., Stringer, W.C. 1988. Agronomy Journal 80, 204-208.
Nutrient Requirements of Domesticated Ruminants, CSIRO 2007, 215.
Ruggia A, Scarlato S, Cardozo G, Aguerre V, Dogliotti S, Rossing W and Tittonell P 2015. 5th International Symposium for Farming System Design, Montpellier, France.
Stewart KEJ, Bourn NAD and Thomas JA 2001. Journal of Applied Ecology 38, 1148-1154.

419

Use of dietary neutral detergent fibre concentration to predict forage intake in New World camelids

Robert Van Saun

Pennsylvania State University, University Park, Pennsylavania, USA

E-mail: rjv10@psu.edu

Take home message Llamas and alpacas have a reduced neutral detergent fibre (NDF) intake capacity compared to other ruminants. To estimate forage intake capacity a suggested dietary guideline of 8 to 9 g NDF/kg BW is recommended.

Introduction The literature between South and North America has contrasting recommendations for dietary nutrient densities for llamas and alpacas in-spite-of use of the same nutrient requirement models (NRC, 2007; Lopez and Raggi, 1992). Underpinning this discrepancy is a difference in total intake expectations where the NRC describes maintenance dry matter intake (DMI) between 11 and 16 g/kg BW, whereas South American work suggests 15 and 17 g/kg BW for llamas and alpacas, respectively. The study objective was to summarize and model published research describing DMI of llamas and alpacas to determine if dietary NDF influences intake capacity.

Materials & methods A retrospective study was performed on summarized data from 9 published studies characterizing individual DMI of forage diets by llamas and alpacas. Daily DMI was quantified and characterization of the diet relative to crude protein (CP) and NDF, at a minimum, was provided. In these 9 studies data were derived from a total of 27 llamas and 38 alpacas. Intake comparisons were determined for 23 forage combinations. Intake was calculated on g/kg body weight (BW) and metabolic BW (g/kg$^{.75}$, MBW). In the experimental diets, forage CP and NDF ranged from 22 to 205 and 452 to 746 g/kg dry matter, respectively. Analysis of variance (ANOVA) and multiple regression modelling were used to define effect of species, dietary CP and NDF content, study and interactions on intake metrics. Study was confounded by diet composition thus regression modelling used only CP and NDF effects to model intake (g/kg BW or g/kg MBW).

Results & discussion Across studies DMI (1485 vs 721 g/d, $P<.0001$) and MBW intake (46.4 vs 35.2 g/kg MBW, $P=.0006$) were higher for llamas compared to alpacas. Comparison of observed llama and alpaca intakes (g/kg MBW) to expected intakes (Figure 1) shows the NRC model for DMI underestimates intake more than South American estimates (Lopez and Raggi, 1992). Observed intakes within species were highly variable given the wide range of forage quality fed. Least squared mean DMI (g/kg BW) for llamas (14.7 ± 0.6) and alpacas (13.0 ± 0.7) was not different. Least squared mean dietary NDF intake (g/kg BW) tended ($p=0.08$) to be higher for llamas (8.68 ± 0.31) compared to alpacas (7.61 ± 0.37). Regression modelling dietary NDF onto DMI showed an optimum relationship using a second order value for NDF similar to what was reported by Mertens (1997). Modelling DMI and NDF intake across species did not provide robust predictive models. Predictive relationship between dietary CP and NDF content (1st, 2nd and 3rd order variables) was low for DMI (g/kg BW; $r^2=.28$ vs .72) and NDF (g/kg BW; $r^2=0.24$ vs .60) in alpacas but high in llamas. Multiple regression modelling including dietary CP and NDF content as before and an interaction term between dietary CP and NDF content improved model predictive values for DMI (g/kg BW) for alpacas ($r^2=.69$) and llamas ($r^2=.92$). With the camelids noted ability to digest NDF more efficiently than ruminants, especially with poor quality forage, these results of lower NDF intake capacity are in agreement with the concept of lower feed intake coupled with slower rate of passage to facilitate microbial fermentation of fibre in camelid species.

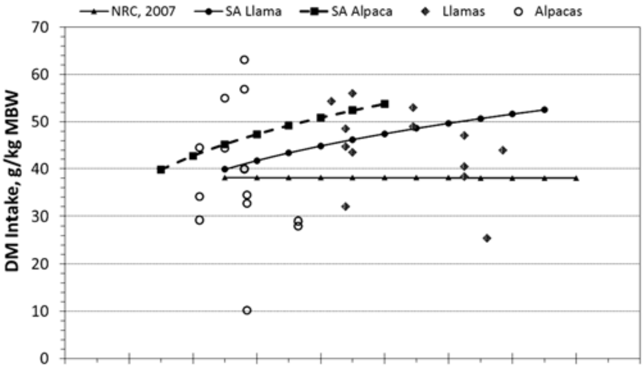


Figure 1 Observed llama and alpaca DMI for a range of diets compared to expected intakes across body weights based on NRC, 2007 and South American data (Lopez and Raggi, 1992).

Conclusion These preliminary findings suggest DMI capacity of camelids is controlled by dietary NDF content similar to what is observed in other ruminants, but to a lower capacity (8-9 g/kg BW) as result of documented slower rate of passage for dietary fiber. Llamas seem more sensitive to dietary NDF as an intake limiter compared to alpacas given the stronger relationship observed between NDF, CP and DMI capacity.

Acknowledgements The author appreciates efforts of all cited investigators for publishing their data used in this summary.

References

Lopez A and Raggi LA 1992. Archivos de Medicina Veterinaria XXIV, 121-130.
Mertens R 1997. Journal of Animal Science 64,1548-1558.
National Research Council. 2007. Nutrient requirements of small ruminants.

Appropriate dietary net energy level for the Chinese Holstein heifers aged 3 to 6 months

Yuan Li, Xiang Cui, Qiyu Diao, Kai Cui, Bing Wang, Yanliang Bi, Yan Tu

Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China

E-mail: tuyan@caas.cn

Take home message The most appropriate dietary net energy level for the heifers aged 3 to 6 months was 7.53 MJ/kg.

Introduction The importance of cow's energy and protein nutrition makes it a hot topic among dairy nutrition studies. Under the conventional dairy farm feeding model, the study of energy level is of great practical significance to the digestion and metabolism of nutrients in heifers (Albino *et al*, 2015). This study was conducted to investigate the effects of different dietary net energy and find the most suitable dietary net energy level for the Chinese Holstein heifers aged 3 to 6 months.

Materials & methods The Single factor experimental design was used. A total of 32 Chinese Holstein heifers aged about 98days (about 91kg) were randomly divided into 4 groups (A, B, C, D; n=8 each). Each group was subjected to 4 diets with different net energy level for lactation by adding rumen protected fat. The content of crude protein in the diets was 9.30%, the content of NE_L were 6.24(A), 7.04(B), 7.53(C)and 7.85MJ/kg(D) (dry matter basis), respectively, which was referred to the nutrition requirement of 125kg replacement heifers given by Feeding standard of dairy cattle in China (2004). Four heifers aged 181-187 days were randomly selected from each group to conduct the experiment of digestion and metabolism, 3 days for adaptation. Data referring to feed conversion ratio and energy utilization were analysed using a repeated measures One-way ANOVA. The VFA data were analyzed using the MIXED procedure of SAS software 9.1.3. Significant differences were accepted when $P<0.05$.

Results & discussion There was no difference on body weight (146.38,155.19,157.63 and 163.44kg, respectively) and average daily gain among 4 groups (Figure 1). But the feed conversion ratio (F/G) of heifers in D group was significantly lower than in other groups ($P<0.05$) (Figure 1). The intake of gross energy (GE) increased with the dietary energy level increasing ($P<0.05$), and the changes of ME and DE were the same as GE ($P<0.05$). There was no significant difference of ME/GE and DE/GE among the 4 groups ($P>0.05$) (Table1). The acetate/propionate value of heifers in D group was significantly lower than that in other groups, but the rumen microflora of 4 groups were with high similarity (Table 2).

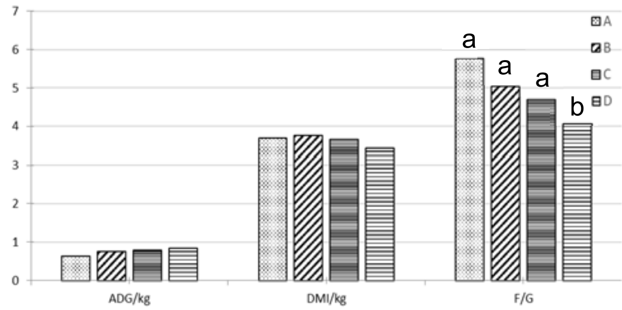


Figure 1 Effects of different dietary energy level on feed conversion ratio in heifers(ADG=average daily gain; DMI=dry matter intake; F/G= feed conversion ratio, feed/gain).

Table 1 Effects of different dietary energy level of diets on energy utilization.

Items	A	B	C	D	SEM	*P*
GE/(MJ/d)	96.33^c	100.13^b	108.69^a	112.27^a	1.74	<0.01
DE/(MJ/d)	66.96^b	75.19^a	81.35^a	79.20^a	1.69	<0.01
ME/(MJ/d)	57.48^b	65.41^a	70.91^a	67.87^a	1.58	<0.01
DE/GE/%	69.45	75.10	74.87	70.53	1.02	0.09
ME/GE/%	85.78	87.00	87.14	85.65	0.30	0.15

$^{a,\,b}$ Mean values within a row with different superscripts differ significantly ($P < 0.05$). The same as below.

Table 2 Effects of different dietary energy level on the VFA of rumen fluid in heifers of 181 days of age.

	Treatments					*P* value		
Items	A	B	C	D	SEM	T	D	T×D
Acetate (%Total VFA)	68.54^a	68.61^a	67.08^{ab}	64.78^b	0.5	0.03	0.29	0.69
Propionate (%Total VFA)	16.84^b	16.73^b	18.82^b	21.29^{ab}	0.44	<0.01	0.16	0.57
acetate/propionate	4.11^a	4.10^a	3.73^a	3.14^b	0.09	<0.01	0.41	0.64
Total VFA/(mmol/L)	35.93	31.68	36.61	33.18	1.48	0.90	0.89	0.98

Conclusion The dietary net energy at 7.53 MJ/kg can maintain the average daily gains of heifers (0.78 kg/d) and have the overall nutrition balance of heifers and digestion and metabolism levels. So, the most suitable dietary net energy level for the heifers aged 3 to 6 months was 7.53 MJ/kg.

Acknowledgements This research was supported by the Earmarked Fund for Beijing Dairy Industry Innovation Consortium of Agriculture Research System.

References

Albino RL, Marcondes MI and Akers RM 2015. Journal of Dairy Research, 82, 113-120.
Feeding standard of dairy cattle in China. 2004.

Nutrient digestibility in Algerian local young rabbits according to dietary energy

Dahia Saidj[1,2], Nassim Moula[3], Si Ammar Kadi[4], Hacina Ainbaziz[2], Manel Chirane[2], Hakima Mefti Korteby[1], Jean Luc Hornick[3]

[1]1Veterinary Sciences Institute Saad Dahleb University, Blida, Algeria, [2]2 Research Laboratory "Animal health and Production" High National Veterinary School, Alger, Algeria, [3]3 Faculty of Veterinary Medicine, Liège, Belgium, [4]4 Mouloud Mammeri University, Tizi Ouzou, Algeria

E-mail: dyhiasdj1@yahoo.fr

Introduction Balanced diet for fattening rabbits must cover fibre needs for preventing digestive disorders. This is crucial especially in the post weaning period because intestinal digestive maturity is not achieved yet. But reducing fibre content, which is equivalent to increasing digestible energy (DE), results in a reduction of voluntary intake (Gidenne, 2017) because rabbits adjusts their overall consumption according to the DE content of the diet and nitrogen intake expressed by the ratio of digestible protein / digestible energy (Gidenne, *et al.,* 2010). Therefore, the aim of the present work was to study the effect of increasing dietary energy content on nutrient digestibility by Algerian local young rabbits.

Materials & methods Apparent digestibility coefficients (ADC) of the experimental diets were assessed according to the European reference method described by Perez *et al.* (1995). Fifteen (15) young rabbits, 6 weeks old (891±109g), divided into three similar groups and housed individually in metabolic cage. Each group received *ad libitum* one of three diets formulated to obtain 2300, 2450 and 2600Kcal DE/kg DM respectively for L (Low), M (Medium) and H (High) diets and with the same crude protein level (15%). Diets formulated with corn grain (0, 12.5 and 32%), dehydrated alfalfa (35.4, 39.7 and 43.2%), barley grain (20.6, 18.6 and 7%), soybean meal (8, 11 and 13%) and wheat bran (32.5, 15 and 2%) respectively for diets L, M and H. Chemical diets and feces were analyzed. Apparent digestible energy was estimated according to the equation proposed by Battaglini and Grandi (1984). Data (LSM±SEM) were analyzed using SAS software (SAS, 2001) with GLM model. Results are significant at P<0.05.

Results Table 1 show the results of the effect of the DE content in diet on apparent digestibility coefficients on young Algerian local rabbit. Overall, our results of digestibility coefficient (%) showed that the digestibility of the three experimental diets meets the standards of digestibility in the growing rabbit. But digestibility differences were noticed for DM, ash, CF, NDF and apparent digestible energy were significantly higher in the group that received the diet with the highest level of energy. These differences were probably explained by the different proportions of ingredients used in the diets. The high energy diet contained more maize than other diets, and less wheat bran levels. Animals from H group received thus more resistant starch that could escape partially to digestion in small intestine. In that condition, highly fermentable starch was available in caecum and large intestine and might contributed to increase fiber digestibility and thus DE. Gidenne (2010) showed that in growing rabbits, when NDF decrease from 39 to 22% fiber intake decrease by 18g/d (58%) under the combined effect of lower fiber content and the decrease in diet intake, while the amount of starch ingested increases only by 6 g/d. El Tahan *et al* (2012) concluded that growing feeding rabbit with high energy diet containing up to 22.11% starch allowed to obtain the best growth performance and digestibility coefficients.

Table 1 Apparent digestibility coefficients of diets with increasing DE value measured on young Algerian local rabbit.

Digestibility Coefficient (%)	Diet			SEM	*p*-value
	L	M	H		
Dry Matter(DM)	69.72 ^{a}	71.88 ^{a}	76.23 ^{b}	1.00	0.002
Ether Extract (EE)	78.49	75.51	81.07	1.99	0.184
Crude Protein (CP)	79.64	78.31	80.72	1.28	0.439
Ash	73.00 ^{a}	74.47 ^{a}	80.09 ^{b}	1.20	0.003
Crude Fibre (CF)	18.83 ^{a}	16.91 ^{a}	27.39 ^{b}	2.16	0.011
NDF	43.01 ^{a}	51.19 ^{b}	69.92 ^{c}	1.19	<0.001
Apparent Digestibility of Energy	69.90 ^{a}	72.14 ^{a}	76.67 ^{b}	1.04	0.002

SEM: standard error of the mean, ^{a,b,c,} Means with different letters on the same row differ significantly (P<0.05).

Conclusion This study shows that, the use of diets with different energy content in diet of young local rabbit reared in Algeria's conditions, improved the indicators of digestibility for DM, ash, crude fibre, NDF and the apparent digestibility of energy without causing changes in the digestibility for EE and CP.

References

El Tahan HM, Amber KH and Morcy WA 2012. 10th World Rabbit Congress–September 3-6, Egypt, 501-505.
Gidenne T, Garreau H, Drouilhet L, Aubert C and Maertens L 2017. Animal Feed Science and Technology 225, 109-122.
Gidenne T, Lebas F and Fortun-Lamothe L 2010. Chapter 13. In de Blas C, Wiseman, J, "Nutrition of the rabbit" – CAB International Ed., pages 233-252.
Perez JM 1995. World Rabbit Science 3, 41−43.

ASKBILL: forecasting sheep carcase traits to better manage turnoff dates for producers and uncertainty of supply for processors

Naomi Duijvestijn[1], Lewis Kahn[1,2], Ian Johnson[2], James Rowe[2]

[1]*Faculty of Science, Agriculture, Business and Law, University of New England, Armidale, NSW, Australia,* [2]*Cooperative Research Centre for Sheep Industry Innovation, Armidale, NSW, Australia*

E-mail: lkahn3@une.edu.au

Take home message Forecasts of sheep carcase weight and fatness, in addition to growth rates, will be available for Australian producers and processors.

Introduction The Australian sheep supply chain is becoming increasingly sophisticated with feedback from processors to producers about carcase characteristics and disease information. Information about carcase traits is likely to be expanded in the near future as Dual Energy X-ray Absorptiometry units, able to measure lean meat yield of individual animals at line speed in abattoirs, become operational. While these advances enrich the supply of current information, they do not provide a forecast service through which processors and producers can better manage uncertainty of supply. In this regard, new decision support applications such as ASKBILL (Kahn *et al.* 2017), developed to provide forecasts of sheep wellbeing and productivity, can be extended to provide forecasts of carcase weight and fatness which can be the basis for payments.

Materials & methods ASKBILL is a web-based application developed by the Sheep CRC. The application contains a number of biophysical models adjusted on a daily time-step in response to user inputs, Australian sheep breeding values and weather forecasts. Turn-off forecasts are provided for a 90-day lead time and provide alerts based on progress against targets. The animal growth model is an extension of Johnson *et al.* (2012) that predicts growth of protein, fat, bone and wool (and hence live weight) in response to pasture and supplement. Extending the predictions of live weight to carcase components was enabled through reference to the Information Nucleus Flock data (Van der Werf *et al.* 2010). A generalised linear model including fixed effects as described by Gardner *et al.* (2015), random effects of sire and slaughter group, and a clustering of breeds, was used to estimate dressing percentage (DP) of sheep (< 1 year old) from final live weight and hot standard carcase weight (i.e. DP% = carcase weight ÷ live weight x 100). Carcase fat was estimated from a relationship determined by the regression of final live weight and carcase fat determined from computed tomography using Information Nucleus flock data (precision for predicting dissectable fat R2=0.718; RMSE=0.713; Gardner, Pearce, and Smith 2007). The effect of breed cluster and final live weight on forecasts of hot standard carcase weight and carcase fat are described.

Results & discussion DP adjusted for final live weight, differed ($p<0.0001$) among breed clusters (Table 1). Final live weight was a significant covariate ($p<0.0001$) and can be used to adjust breed cluster average DP by 0.055% per kg. The correlation between final live weight and carcase fat (Figure 1) was +0.81.

Table 1 Hot standard carcase weight (HSCW), dressing percentage (DP) and final live weight (LW) of lambs from different breed clusters.

Breed cluster	HSCW (kg)		Final LW (kg)		DP (%)	
	Mean	SE	Mean	SE	Mean	SE
Fine wool Merino	20.4	0.41	47.9	0.75	43.1^{a}	0.37
Maternal x Merino	22.1	0.34	50.0	0.63	44.5^{b}	0.30
Terminal	23.3	0.32	51.7	0.59	45.1^{c}	0.27

In each column, means with different superscripts differ significantly ($p<0.001$).

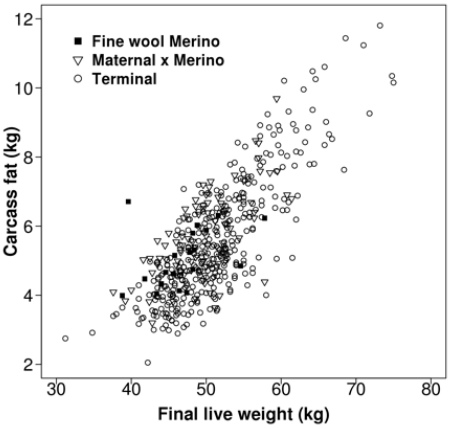


Figure 1 Association of final live weight and carcase fat of lambs from different breed clusters.

Conclusion Estimation of hot standard carcase weight and carcase fatness from breed information and predicted final live weight enables ASKBILL to provide up to 90-day forecasts of these traits. These forecasts will be of value for processors and producers allowing adjustment of feed supply, better meeting of market requirements, as a basis for payments and forward scheduling of lamb supply.

References

Gardner G, Pearce K and Smith N 2007. Project B.SGN.0115, Meat and Livestock Australia Ltd.
Johnson I, France J, Thornley J, Bell M and Eckard RJ 2012. Journal of Animal Science 90, 4741–4751.
Kahn LP, Johnson I, Rowe JB, Hogan L and Boshoff J 2017. Animal Production Science 57, 2257–2262.
Van der Werf JHJ, Kinghorn BP and Banks RG 2010. Animal Production Science 50, 998-1003.

Towards a Tier 3 methodology to calculate methane emission inventory for ruminants (cattle)

Maguy Eugene[1], Daniel Sauvant[2], Pierre Noziere[1], Damien Viallard[1], Khaled Oueslati[1], Michel Lherm[1], Etienne Mathias[3], Michel Doreau[1]

[1]INRA, Saint Genès Champanelle, Auvergne, France, [2]Agroparistech, Paris, France, [3]CITEPA, Paris, France

E-mail: maguy.eugene@inra.fr

Take home message Estimation of CH4 emissions from livestock for national inventories can be improved by a new and customizable methodology

Introduction According to French inventories, methane represented 12.9% of net GHG national emissions in 2014 (Citepa, 2015) and livestock is the main contributor to methane (CH4) emissions. It is thus important to determine with accuracy emissions from ruminants in the context of national GHG inventories, according to standardized international guidelines. The objective of the study is to propose and evaluate a new methodology, aiming to improve the accuracy of the French inventory of CH4 emissions, which complies with IPCC (2006) rules for a Tier 3 method.

Materials & methods Calculations were made for main animal categories, diets, production systems and for several manure management systems, determined from case studies, published surveys and on the expertise of extension services, resulting in an average CH4 emission corresponding to a representative animal category. Several steps are required 1) Calculation of the net energy (NE) requirements with a factorial approach (INRA, 2018); 2) Calculation of dietary NE content and DM intake to meet animal NE requirements, accounting for digestive interactions, 3) calculation of digested OM intake (DOMI) and of non-digested OM intake from DM intake, OM content and OM digestibility (DOM, corrected for digestive interactions). 4) Enteric CH4 emissions were calculated by applying a generic equation based on a large database (450 dietary treatments). Priority was given to a relationship with OM digestibility, consistent with the new French feeding systems (INRA, 2018), and to make consistent CH4 determination from both enteric and manure source, this latter being directly related to (1-OM digestibility). Enteric CH4 (expressed as g/kgDOMI), is predicted by feeding level (FL = DM intake in kg/100 kg LW) and dietary proportion of concentrate ($0 \leq PC \leq 1$): CH4 (g/kg DOMI) = $45.42-6.66 * FL+0.75 * (FL)^2 * 19.65 * PC-35,0 * (PC)^2-2,69 * FL * PC$ (rmse = 2.3 g/kg DOMI). Then enteric CH4 emission factor (EF) is calculated ($CH4 * DOMI^{-1} * DOMI$). 5) The amount of manure is estimated from OM intake – DOMI (kg/d) and from estimated urinary energy (0.04 x GE intake). Then manure CH4 EF is calculated as for IPCC (2006).

Table 1 Methane emission factor (INRA EF, enteric and manure, kg.head^{-1}.year^{-1}) of dairy and beef cattle for some animal categories calculated with INRA methodology, and comparisons with IPCC (2006) Tier 2 methodology.

	INRA EF			INRA/IPCC, %		
Animal category	Enteric	Manure	Total	Enteric	Manure	Total
Mature dairy cow, 650 kg BW, 6300 kg.year^{-1} milk yield	119.3	9.0	128.3	97	80	96
Mature beef cattle, 630kg BW, 1700 kg.year^{-1} milk yield	86.6	2.7	89.3	94	86	94
Replacement dairy heifers, 30 months old	40.9	1.4	42.3	114	87	113
Growing / fattening beef cattle post-weaning, slaughtered at 17 months	37.0	2.1	39.1	88	53	86
Feedlot-fed beef cattle (diets with > 90 % concentrates, slaughtered at 17 months)	13.2	3.3	16.5	114	22	96

Results & discussion Table 1 presents the ratio between INRA and IPCC Tier 2 methods for some animal categories in France. For enteric methane, according to the animal category, INRA EF represents between 88 and 114% of IPCC Tier 2 EF. The ratio is close to 100% for adult cows, which represent the major part of bovine heads. In France, feedlot animal's manure is managed outdoor, explaining the low INRA/IPCC manure ratio. INRA/IPCC ratio for enteric CH4 is very close to the ratio for total CH4, because enteric CH4 represents 93% of total emissions. The main differences with IPCC is the use of a robust equation (numerous data and diets), based on DOM to predict consistently CH4 from both enteric and manure source.

Conclusion This new and improved CH_4 estimation methodology, based on equations from a large-sized literature database, complies with IPCC rules for a Tier 3 method. Developed for French inventory, it can be customizable for other regions. Improvements can be reached, using in-country data (feeding and manure management systems, equations) and accounting for mitigation strategies amongst others.

Acknowledgements This research was supported by a grant from the French Ministry in Charge of Agriculture.

References

CITEPA, 2015 http://www.citepa.org/fr/activites/inventaires-des-emissions/secten.
IPCC, 2006. IPCC Guidelines for National Greenhouse Gas Inventories, Chapter 10. IGES, Japan.
INRA, 2018. Inra Feeding System for Ruminants. Wageningen Academic Publishers, Wageningen, the Netherlands, 640.

A meta-analysis to evaluate the accuracy of the Cornell Net Carbohydrate and Protein System model for prediction of energy and protein variables from dairy cow calorimetry studies

Sheila Andrew[1], Mariska Bartlett[6], Charles Sniffen[3], Christian Cruywagen[2], Ian Lean[4], Tom Tylutki[5]

[1]University of Connecticut Animal Science, Storrs, CT, USA, [2]University of Stellenbosch, Stellenbosch, Western Cape, South Africa, [3]Fencrest LLC, Holderness, NH, USA, [4]Scibus, Camden, NSW, Australia, [5]Agricultural Modeling and Training Systems, Groton, NY, USA, [6]Vitam International, Pretoria, Gauteng, South Africa

E-mail: mariska.bartlett@uconn.edu

Take home message A meta-analysis of documented animal calorimetry data showed that prediction of net energy balance from the Cornell Net Carbohydrate and Protein System was acceptable while prediction of crude protein balance was poor.

Introduction The accurate prediction of metabolisable energy balance (ME) and metabolisable protein balance (MP) in dairy cattle rations using ration modelling programs supports animal health, productivity and environmental management. The purpose of this study was to investigate the accuracy of the Cornell Net Carbohydrate and Protein System (CNCPS) version 6.55 ration modelling program using the AMTS platform for prediction of net energy (NE) and MP balances from respiration calorimetry (RC) in individual lactating Holstein cows from the Beltsville Agricultural Research Centre. As these predictions are normally based on averages of cow groups, this gave a unique opportunity to assess the accuracy of these parameters with individual cow data. Further, reducing dietary protein can reduce nitrogen-losses in manure and urine and increase nitrogen (N) utilization (Colmenero and Broderick, 2006). Thus, accurate predictions using ration models to balance ME and MP will advance animal health and productivity and economic and environmental sustainability.

Materials & methods Energy balance and N balance, transformed to crude protein (CP) balances, from RC data in 185 individual lactating Holstein cows across six experiments with a total of 17 treatment trials, were used as the benchmark data. Prediction of ME and MP balances from CNCPS were used to predict the RC energy and protein variables, respectively. All rations were corn silage or haylage forage-based with corn and soybean meal as the major concentrates. Mixed model analyses to test the prediction of RC NE and RC CP balances from CNCPS ME balance and MP balances, respectively, were undertaken (Stata version 15). The analyses allowed for random effect of trial and experiment. An unstructured covariance structure was used for measurements within cow. In order to evaluate the effect of experiment on concordance estimates, Rho and Pearson's correlation coefficients were transformed using Fishers' transformation and evaluated using meta-analysis (Lin, 1989; Giavarina, 2015). Lastly, Bland and Altman concordance value for differences between the mean near zero and non-significance imply concordance. Significance was determined at $P < 0.05$.

Results For the RC NE balance predictions, one observation was an outlier and was not included in the analysis. The model included days in milk (DIM) ($P = 0.001$), ME balance (coefficient 0.571; SE = 0.03; $P = 0.001$). The intercept was -1.15 (SE = 0.8) and the goodness of fit RMSE was 3. The overall Lin's concordance correlation rho was 0.803; 0.855 for Pearsons' correlations and the bias estimate was 0.939. The mean difference between ME measures was 1.575 with a correlation between difference and mean -0.494, which was non-significant, indicating poor fit. The estimate of rho controlling for experiment was 0.732 indicating that concordance was lower, a finding supported by heterogeneity ($I^2 = 69.1\%$) in the meta-analysis. For RC CP balance predictions, all observations were included in the analysis. The MP balance coefficient for the mixed model was 0.185 (SE = 0.04; $P = 0.001$) and the intercept was 25.37 (SE = 43.1). The DIM was not significant. The goodness of fit RMSE (141.0) indicated a poor fit of the model. The overall concordance correlation coefficients were 0.256 and 0.296 for rho and Pearson's, respectively. The mean difference between MP measures was 2.0 (SD = 295) with a correlation between the difference and mean -0.52 indicating a poor concordance. The estimate of rho controlling for experiment was 0.232 indicating that concordance was significant, a finding that was consistently poor as indicated by homogenous outcomes ($I^2 = 0\%$) across experiments observed in the meta-analysis.

Conclusion Results from this meta-analysis demonstrate that the ration modelling program, CNCPS, has a moderate predictive value for predicting RC NE balance from CNCPS ME outputs on an individual animal basis, with a significant mixed model coefficient and concordance correlation coefficients. Based on the individual cow biological variability, these estimates demonstrate an acceptable predictive ability for CNCPS to predict RC NE balance. In contrast, the prediction of RC CP balance from CNCPS MP outputs was poor, and this was consistent across experiments as evidenced by the significant concordance values. These studies provide opportunities to further refine ration modelling programs to improve ration formulation, animal productivity, and health and environmental management.

Acknowledgements Tom Tylutki and Mariann Fessenden at Agricultural Model and Training Systems (AMTS).

References

Colmenero JJO and Broderick GA 2006. Journal of Dairy Science 89, 1704–1712.
Giavarina D 2015. Biochemia Medica 25, 141–151.
Lin L 1989. Biometrics 45, 255–268.

Time-budget and location of activities in the paddock can be estimated from GPS-data

Lucile Riaboff[1,3], Nicolas Bédère[2], Sébastien Couvreur[2], Sébastien Aubin[1], Etienne Goumand[3], Aurélien Madouasse[4], Jacques Magnier[2], Alain Chauvin[4], Guy Plantier[1]

[1]ESEOTech – LAUM, Angers, France, [2]URSE, Ecole Supérieure d'Agricultures, Univ. Bretagne Loire, Angers, France, [3]Terrena Innovation, Ancenis, France, [4]BIOEPAR, INRA, Oniris, Nantes, France

E-mail: lucile.riaboff@eseo.fr

Take home message Cow time-budget and organization of activities in the paddock can be studied efficiently without laborious observation using GPS data and GPS-located resources.

Introduction Emerging tools like embedded GPS sensors could help to optimize pasture management (Manning *et al.*, 2017), with a better understanding of the use of the paddocks by the cows. This paper aims to study (1) whether embedded GPS sensors could predict the behaviour of dairy cows and (2) enable to study the organisation of their activities during the day and its association with pasture characteristics or other elements in the paddock.

Materials & methods Fourteen Hosltein cows were tracked with GPS sensors mounted on collars in a commercial dairy farm (Lion d'Angers, France). They grazed in temporary pasture, sown with a mixture of perennial ryegrass and white clover and were milked every day at 9 am and 6 pm. The distribution of cows on the pasture was studied for a whole day by computing the percentage of cows located in each 3*3 m area. In order to discriminate between resting, grazing and walking activities, behaviours were recorded by two observers for each cow successively, during the entire day. Distances and turning angles between each fixe were computed in order to calibrate a decision tree (rpart package, R). Behaviours were then predicted using the calibrated decision tree. A chi square test was performed in R to compare the time-budget per half day. The location of main activities in the field was also studied. To explore the potential link between location of predicted behaviours and resources distribution, samples of vegetation cover were collected and GPS-located. The percentage of dry matter was obtained and the ratio of perennial ryegrass, white clover and self-propagating plants were determined, other species were regarded as negligible. A multinomial analysis was performed with nnet and car packages in R to conclude on the relationship with behaviours.

Results The distribution of the cows in the paddock area was heterogeneous, with some areas avoided and others frequently visited (Figure 1a). A satisfactory calibration of the decision tree was obtained with a misclassification rate of the behaviours of 10%, as expected (de Weerd *et al.*, 2015). The time-budget was significantly different between each half day ($p < 0.0001$), with 50.4±6.2% of grazing (ranging from 40.0 to 58.8% among cows) and 49.6±6.2% of resting (ranging from 41.2 to 60.0%) in the morning *versus* 38.5±9.3% (ranging from 27.1 to 59.2%) and 61.5±9.4% (ranging from 40.3 to 72.9%) in the afternoon respectively. Cows seemed to organize their activities inside the paddock (e.g. more resting close to the drinking trough; Figure 1b). No significant relationship between behaviour and DM and floral distributions was observed.

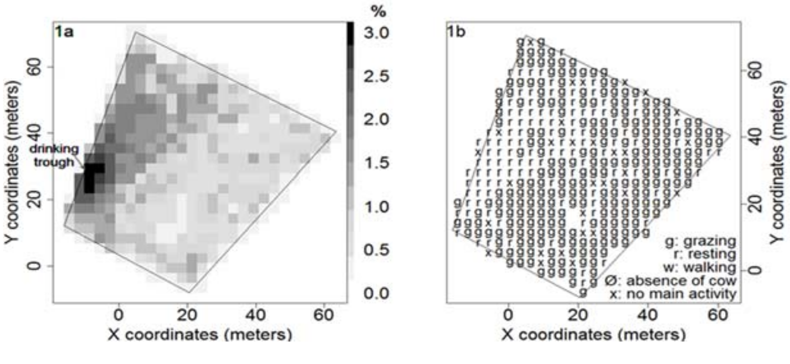


Figure 1 a) Distribution of cows (%) inside the paddock. The more the area was frequented the darker the square is. b) Dominant behaviour in the same 3*3m area predicted with a decision tree.

Conclusion The use of GPS embedded sensors highlighted a heterogeneous distribution of cows in the paddock, with some areas being preferentially used for resting or grazing. In this study, this organization of activities seemed to be influenced by structural elements in the paddock, like the drinking trough, rather than pasture characteristics.

Acknowledgements This study was funded by the region Pays-de-la-Loire within the framework of the Vagabond project.

References

Manning J, Greg C, Luciano G, Evelyn H, Andrew M and Lachlan I 2017. Agriculture 7, 45.
de Weerd N, van Langevelde F, van Oeveren H, Nolet BA, Kölzsch A, Prins HHT and de Boer WF 2015. Plos one 10, 1-17

Validation of BEHARUM device for recording grazing behaviour of dairy sheep

Mauro Decandia[1], Valeria Giovanetti[1], Giovanni Molle[1], Marco Acciaro[1], Mauro Mameli[3], Andrea Cabiddu[1], Rossella Cossu[2], Maria Gabriella Serra[1], Carla Manca[1], S. Piergiacomo Rassu[2], Corrado Dimauro[2]

[1]AGRIS Sardegna, Sassari, Italy, [2]Università degli studi di Sassari, Sassari, Italy, [3]Electronic Systems, Alghero, Italy

E-mail: mdecandia@agrisricerca.it

Take home message BEHARUM device provides accurate estimates of grazing activity in dairy sheep with restricted time access to pasture.

Introduction Monitoring feeding behavior of grazing animals is important to assess animal health and welfare and to manage the grazing system. Most of developed sensors have been used so far for measuring animal feeding behaviour in indoor systems while BEHARUM device can also monitor such behaviour in pasture-based conditions, as shown by a recent cross-validation (Giovanetti *et al.*, 2017). The aim of this study was to validate BEHARUM device to record sheep grazing behaviour using an independent dataset (external validation).

Materials & methods The study was conducted at Bonassai research station, in North-Western Sardinia, Italy (40°N, 8°E, 32 m a.s.l.). Six ewes, equipped with BEHARUM device, were allowed to graze monocultures or mixtures of berseem clover (*Trifolium alexandrinum* L.) and Italian ryegrass (*Lolium multiflorum* Lam.) for 6 hours day^{-1}. A short validation test (6 ewes x 2 hrs =12 hrs) was run, comparing BEHARUM behavior estimation with visual observations. The BEHARUM device includes a halter equipped with a three-axial accelerometer sensor and a force sensor positioned under the lower jaw of the animal. The sensor is connected to a microcontroller that samples the raw acceleration and force signals at a frequency of 62.5 Hz, convert them into digits and selects only three converted values per second and axis (Giovanetti *et al.*, 2017). Data recorded were processed as reported by Giovanetti *et al.*, (2017). Grazing and other activities were predicted, with an epoch time setting of 3 min, by a linear discriminant analysis that assigned new observations to the two behaviours, using a calibration equation previously set up by the same authors for dairy sheep. To validate the predicted behavior, direct observations were run. Three trained observers recorded the behaviour of two ewes each, monitoring the animals for 2 hrs during the grazing session, with a recording frequency of 3 min, distinguishing the dominant activity between grazing and other activities. We classified as grazing the activities that included the act of searching for food while walking with the head down, with or without evidence of biting, or standing still while biting and chewing either with the head down or up, and as other activities all activities not included in grazing. BEHARUM estimates were compared with corresponding observed data, and the device performance were evaluated as sensitivity, precision for each behavior. Overall accuracy and K coefficient (Landis and Koch, 1977) were also calculated.

Results & discussion As expected, grazing was the main activity observed during the test, averaging 90% of the total recording time. This confirms results by Molle *et al.* (2014) in ewes submitted to similar restricted time allocation to pasture. The disproportion between grazing and other activities affected the validation performance of BEHARUM. Actually, grazing behavior showed much higher sensitivity and precision than other activities. This caused a moderate rate of agreement between predicted and observed data (K=0.41) corrected for agreement that would be expected by chance, although the overall accuracy was good (Table 1). This result is in fact comparable to those sourced from cross-validation of BEHARUM (Giovanetti *et al.*, 2017) and another accelerometer based device (Alvarenga *et al.*, 2016).

Table 1 Performance in the validation of behavioural activities predicted on the basis of the accelerometer data.

	Sensitivity	Precision	Overall accuracy	Overall K
Grazing	94.2	93.9	89.3	0.41
Others activities	46.2	47.4		

Conclusion BEHARUM device confirmed its ability to accurately predict grazing behavior of sheep with a restricted allocation time to pasture. Further validation trials should be run to predict more behaviours with unconstrained time access to pasture.

Acknowledgements The Government of Sardinia (Italy) for the financial support (Projects CRP-17287 PO Sardegna, FSE 2007–2013 LR 7/2007).

References

Alvarenga FAP, Borges I, Palkovič L, Rodina J, Oddy VH and Dobos RC 2016. Applied Animal Behaviour Science 181, 91–99.

Giovanetti V, Decandia M, Molle G, Acciaro M, Mameli M, Cabiddu A, Cossu R, Serra MG, Manca C, Rassu SPG and Dimauro C 2017. Livestock Science 196, 42–48.

Landis J and Koch G 1977. Biometrics 33, 159–174.

Molle G, Decandia M, Giovanetti V, Manca C, Acciaro M, Epifani G, Salis L, Cabiddu A, Sitzia M and Cannas A 2014. Animal Production Science 54, 1233-1237.

Faecal near infrared reflectance spectroscopy in combination with animal variables to predict the dry matter intake of grazing dairy cows

Ben Lahart[1,2], Emer Kennedy[1], Sinead McParland[1], Tomas Condon[1], Norann Galvin[1], T.M. Boland[2], Frank Buckley[1]

[1]*Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland,* [2]*School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland*

E-mail: ben.lahart@teagasc.ie

Take home message The incorporation of animal variables into faecal NIRS equations can improve predictive accuracy of equations.

Introduction Near infrared reflectance spectroscopy (NIRS) of faeces has been proposed by many authors as a means to predict individual animal dry matter intake (DMI) however, previous equations have been developed solely with spectral information. The objective of this study was to assess the usefulness of biologically plausible, readily available predictors of intake such as parity, milk yield and body weight in concordance with faecal NIRS to predict the DMI of grazing dairy cows.

Materials & methods A faecal sample set comprising of 1178 samples with corresponding DMI values was available from four grazing experiments conducted at Teagasc, AGRIC, Moorepark, Fermoy, Co. Cork, Ireland. Individual cow DMI was estimated within each experiment using the n-alkane technique (Mayes *et al.*, 1986) as modified by Dillon and Stakelum (1989) Milk yield (MY) was recorded daily, whilst body weight (BW) was recorded once within a week of DMI measurement. Reflectance and absorbance values from each faecal sample were gathered using a FOSS-NIRSystem 6500 SYII (FOSS-NIRSystems, Silver spring, MD, USA). Partial least squares regression analysis (PROC PLS; SAS Institute Inc., Cary NC) was used to develop five separate equations using; 1) solely faecal NIRS wavelengths or 2) faecal NIRS wavelengths in conjunction with one additional animal predictor variables; parity, BW and MY. The accuracy of each equation was tested using both split-sample cross validation, whereby every 20thsample from the calibration data set was removed and predicted using data from the remaining calibration set, and external validation, whereby data from an entire experiment were retained from calibration and used to test the equation; external validation was undertaken 4 times on each equation, once for each experiment.

Results & discussion Faecal NIRS wavelengths as a sole predictor of intake performed moderately ($R^2 = 0.34$). All additional animal variables improved the accuracy of prediction and reduced the RMSE (Table 1). When just parity, BW and MY were used to predict DMI, accuracy of prediction was 0.37, thus faecal NIRS provides additional information to the prediction of DMI

Table 1 Fitting statistics[1] of cross- and external-validation prediction equations to predict DMI.

Cross-validation			Validation			
Variables	RMSE	R^2	Bias	RMSE	Slope (SE)	R^2
Faecal NIRS	2.29	0.36	0.12	2.16	0.87 (0.08)	0.34
Faecal NIRS & Parity	1.91	0.55	-0.001	1.81	0.82 (0.05)	0.54
Faecal NIRS & BW	1.93	0.54	0.07	1.89	0.91 (0.06)	0.54
Faecal NIRS & MY	2.03	0.48	0.1	1.73	0.98 (0.06)	0.58

[1]No = number of samples; RMSE = root mean square error; R^2= coefficient of determination between true and predicted values

Conclusion Although there is a relationship between faecal spectra and DMI, this relationship is not strong enough to form a solid prediction equation. However, the incorporation of biologically plausible predictors of DMI into NIRS equations can significantly improve equation accuracy.

Acknowledgements Funding from the Irish Government under the National Development Plan 2007-2013 through the Department of Agriculture Food and the Marine Research Stimulus Fund 13/S/496 RAPIDFEED is gratefully acknowledged.

References

Dillon P and Stakelum G 1989. Irish Journal of Agricultural Research 8**,** 104.
Mayes R, Lamb C and Colgrove PM 1986. The Journal of Agricultural Science 107**,** 161-170.

Effect of different levels of concentrate fed to Lacaune dairy ewes producing three different milk yields

Philippe Hassoun[1,2], Alain Hardy[3], Anne Tesnière[1,2], Jean Legarto[4], Catherine De Boissieu[4]

[1]INRA, Montpellier, France, [2]Univ Montpellier, Montpellier, France, [3]Lycée Agricole La Cazotte, Saint-Affrique, France, [4]Institut de l'Elevage, Castanet-Tolosan, France

E-mail: philippe.hassoun@inra.fr

Take home message Dairy ewes can be individually fed at their exact requirements rather than over fed in groups.

Introduction In France, dairy ewes are fed in large groups with one diet based on the average milk yield of the group (Hassoun and Bocquier 2010). Such a strategy tends to overfeed a high proportion of the ewes and to underfeed the most productive ones. Individual concentrate feeders are developing in several dairy ewe farms but their effect on milk production is not known. Therefore we conducted an experiment to compare these two feeding systems.

Materials & methods The experiment was carried out over 14 weeks in winter (2016-2017). One hundred and forty Lacaune multiparous ewes (42 days in milk) were selected to constitute two batches: control (C, 60 ewes) fed with the same level of concentrate (table 1) according to the system recommended by INRA; experimental (E, 80 ewes) fed according their body weight (BW) and milk yield (MY). Each batch was separated into three (C) or four (E) groups of 20 ewes based on their initial MY (L/d): High 3.7, Medium 3.2 and Low 2.7 and also mainly balanced (C vs E) on their milk fat (FC) and protein (PC) content and BW. In batch E, another L group (L0) received only a small amount of concentrate to cover its protein requirements. All ewes were fed ad libitum a mixed forage basal diet. Groups were not modified until the end of the experiment. The MY, FC, PC, urea content (UC) were measured every two weeks, BW and BCS monthly and the forages dry matter intake (DMI-F) weekly. All of the data were compared intra group using STATISTICA software, v10, for Windows (Statsoft 2010, www.statsoft.fr). The DMI-F and average daily gain were analysed with the Mann-Whitney nonparametric U test. The MY, FC, PC and UC were analysed with a one-way ANOVA.

Results & discussion The DMI-F (table 1) did not change except for EL and EL0 where the substitution effect was marked. This low effect of concentrate on forage DMI may be due to the daily distribution which was fractionated into 4 times. When concentrate amount decreased by 0.5 up to 1.0 kg DM/d, MY for M and L groups decreased only by 0.2 to 0.3 l/d. That means that the negative effect of excessive concentrate amount on forage digestibility (Nozière *et al.* 1996) is mitigated and part of the energy is allocated to ADG (table 1). FC increased in M and L groups and PC increased only in EM group. UC decreased or tended to decreased in all E groups, which means less nitrogen loss. Although there was a lower ADG for all E groups, body condition score was not different. Mikolayunas *et al*., (2008) found similar results with dairy ewes at pasture. Over the 91 days experiment, total MY was not different in H groups but 22 kg DM/ewe of concentrate were saved in EH. In groups M and L, MY was 27 and 18 L/ewe lower in batch E, but 45 and 59 kg DM/ewe of concentrate were saved respectively.

Table 1 Average forage (DMI-F) and concentrate (DMI-C) dry matter intake, milk yield (MY), milk fat (FC), protein (PC) and urea (UC) content and daily weight gain (ADG).

Group	CH	EH	CM	EM	CL	EL	EL0
DMI-F (kg/d)	2.5	2.6	2.3	2.2	2.1^{a}	2.3^{b}	2.4^{c}
DMI-C (kg/d)	1.4^{A}	1.2^{B}	1.4^{A}	0.9^{B}	1.4^{A}	0.8^{B}	0.4^{C}
MY (l/d)	2.5	2.5	2.3^{A}	2.0^{B}	2.0^{A}	1.8^{B}	1.7^{B}
FC (g/l)	72	73	73^{A}	79^{B}	74^{A}	81^{B}	80^{B}
PC (g/l)	59	60	60^{A}	64^{B}	62	64	62
UC (mg/l)	446	430	427^{a}	390^{b}	460^{A}	376^{B}	362^{B}
ADG (g/d)	47^{a}	25^{b}	71^{A}	35^{B}	83^{A}	72^{AB}	55^{B}

Values with different superscript letter are significantly different (A, B, C, $P<0.01$; a,b,c, $P<0.05$)

Conclusion Feeding high producing dairy ewes with good forage as basal diet at their exact requirements, slightly reduces MY but save significant amount of concentrate. This strategy can be recommended in these conditions (forages with a good nutritive value) at least for the highest producing ewes. For EM and EL groups, it could be useful to provide more energy than exact requirements. These results must be confirmed with other forages as basal diet and consequences on future reproductive performances must be analysed regarding the lower ADG observed for almost all E groups. An economic study must also be conducted in order to know whether such a strategy is economically valuable.

Acknowledgements This research was supported by grants from the CASDAR AUTELO project.

References

Hassoun P and Bocquier F 2010. In: Quae (Ed) Alimentation des bovins, ovins et caprins Versailles, France, p 123-138.

Mikolayunas CM, Thomas DL, Albrecht KA, Combs DK, Berger YM and Eckerman SR 2008. Journal of Dairy Science 91, 1477-1485.

Nozière P, Besle JM, Martin C and Michalet-Doreau B 1996. Journal of the Science of Food and Agriculture 72, 235-242.

Methionine supply during the periparturient period alters plasma amino acids and liver metabolism in dairy cows

Fernanda Batistel[1], Rainie R.C.S. Yambao[1], Claudia Parys[2], Yuan-Xiang Pan[1], Juan J. Loor[1]

[1]*University of Illinois, Urbana, USA,* [2]*Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany*

E-mail: batiste2@illinois.edu

Take home message Enhanced methionine supply in periparturient cows increased animal performance, synthesis of antioxidants in the liver, as well as oxidation of substrates through the TCA cycle.

Introduction In dairy cows, the high demand of nutrients for foetal growth and lactation around parturition is associated with a reduction in dry matter intake (DMI) leading to negative energy (Drackley, 1999) and amino acid (Bell *et al*. 2000) balance, with a consequent impact on hepatic metabolic activity. Methionine is an essential sulfur-containing amino acid associated with key physiologic events including synthesis of milk protein, antioxidants (taurine and glutathione), and S-adenosyl methionine (the universal methyl donor). We previously observed that methionine supply increased liver function, and decreased inflammation and oxidative stress in periparturient cows (Batistel *et al*., 2018); thus, we hypothesized that methionine supply would increase the synthesis of antioxidants and the oxidation of subtracts through the TCA cycle. The objective of this study was to investigate the effect of enhancing the methionine supply during the periparturient period on plasma amino acid concentrations and liver metabolism (TCA cycle, C1 metabolism, transsulfuration pathway and gluconeogenesis) in dairy cows.

Materials & methods Sixty multiparous Holstein cows were used in a block design and assigned to a control diet or the control plus ethyl-cellulose rumen-protected methionine (RPM) (Mepron, Evonik Nutrition & Care GmbH, Germany). Mepron was fed from −28 to 30 days relative to parturition at a rate of 0.09% and 0.10% of the DMI during the prepartum and postpartum period, respectively. That rate ensured that the ratio of lysine to methionine in the metabolizable protein was close to 2.8:1. Blood was sampled from the coccygeal vessel before the feeding in 15 cows per treatment at −14, +7, +21 and +30 days relative to calving date. Liver was sampled via puncture biopsy from 8 cows per treatment at −10, +10, and +30 days relative to parturition. Targeted metabolomics (LC-MS) was performed to quantify 32 metabolites related to the TCA cycle, C1 metabolism and the transsulfuration pathway. Activity of betaine-homocysteine methyltransferase (BHMT), methionine synthase (MTR) and cystathionine-β-synthase (CBS) also was measured. Messenger RNA expression of the enzymes involved in the TCA cycle, C1 metabolism, transsulfuration pathway and gluconeogenesis was measured by RT-PCR. The data were analyzed using the Mixed procedure of SAS considering block as random effect, and treatment, time and its interaction as fixed effect. Significance as declared at $P \leq 0.10$.

Results Compared with control, feeding RPM increased DMI in the prepartum period by 1.2 kg/day, DMI in the postpartum by 1.7 kg/day, milk yield by 4.1 kg/day, milk fat yield by 0.17 kg/day and milk protein yield by 0.20 kg/day. A treatment × time interaction was observed for the plasma concentration of asparagine ($p = 0.05$), cysteine ($p = 0.09$), and glycine ($p = 0.09$) due to its higher concentration in RPM-supplemented cows during the prepartum. Compared with control, the plasma from RPM cows had greater concentration of methionine ($p < 0.01$), lysine ($p = 0.03$), threonine ($p = 0.05$), leucine ($p = 0.09$), valine ($p = 0.04$), and phenylalanine ($p = 0.06$). Liver from RPM cows had greater concentrations of metabolites related to the TCA cycle (isocitric acid, $p = 0.06$; α-ketoglutaric acid, $P = 0.08$; and malic acid, $p = 0.09$), C1 metabolism (methionine, $p = 0.08$) and transsulfuration pathway (glutathione, $p = 0.05$; and taurine, $p = 0.10$) than control cows. Enzyme activity of CBS was greater ($p = 0.03$) in RPM supplemented cows compared with control. Compared with control, RPM cows had greater mRNA expression of genes related with the TCA cycle (*ACO2* [aconitase 2], $p = 0.05$; and *FH* [fumarase], $p = 0.04$), C1 metabolism (*MAT1A* [methionine adenosyltransferase], $p = 0.06$), transsulfuration pathway (*CBS*, $p = 0.07$), and gluconeogenesis (*PCK1* [phosphoenolpyruvate carboxykinase 1], $p = 0.09$).

Conclusion Results indicate that feeding RPM to achieve a lysine to methionine ratio of 2.8:1 during the periparturient period increased dry matter intake, milk production, milk fat and protein yields, blood amino acid concentration, oxidation of substrates in the liver as well as the synthesis of antioxidants (glutathione and taurine).

Acknowledgements Fernanda Batistel was supported by a fellowship from CAPES (Brazilian Ministry of Education, Brasilia, Brazil).

References

Batistel F, Arroyo JM, Garces CIM, Trevisi E, Parys C, Ballou MA, Cardoso FC and Loor JJ 2018 Journal of Dairy Science 101, 480-490.
Bell, AW, Burhans, WS and Overton TR 2000. Proceedings of the Nutrition Society 59, 119-126.
Drackley JJ 1999. Dairy Science 82, 2259-2273.

Dynamic feeding of dairy cows in automatic milking systems

Sabine Ferneborg, Egil Prestløkken

Norwegian University of Life Sciences, Ås, Norway

E-mail: sabine.ferneborg@nmbu.no

Take home message Feed quality has a larger impact on energy balance in early lactation than feeding regimen.

Introduction The increasing automation in modern dairy cow systems allows a detailed surveillance of feed intake, body weight and milk production on a day-to-day basis, which could be used to improve management and feeding of dairy cows. The NorFor system (Volden, 2011) for feeding of dairy cows was first introduced in 2006 and has since then been widely applied in the Nordic countries. The system is a semi-mechanistic model that predicts nutrient requirements for cattle based on a number of animal and feed characteristics. For a lactating dairy cow, a desired 305-d milk yield is used for ration calculation. Our hypothesis was that including daily milk yield and roughage intake in the calculation of concentrate ratios could better adapt the intake of concentrates to the actual need of the dairy cow, enabling a lower increase in body condition score throughout lactation.

Materials & methods 61 cows of the breed Norwegian Red were subjected to one of two feeding regimes; static or dynamic feeding together with early or late harvested silage (26 vs 36% DM; 579 vs 614 g NDF/kg DM; 6.75 vs 6.18 MJ NE/kg DM). Cows were in lactation number 2.3±1.36 (mean±sd, range 1-6) and entered the trial on d 5 of lactation. Cows were housed in a loose housing system with AMS. Feed intake was recorded using feeding troughs on weight cells. In the dynamic feeding regimen, requirements were based on the need for maintenance and lactation, and actual roughage intake using registrations from the last 7 days. In the static feeding regimen, feeding was based on a NorFor template based on lactation number and desired 305-d milk yield of 7500, 8500 and 9000 kg ECM for cows in 1st, 2nd and >2nd lactation respectively. Allocated concentrate amounts were adjusted weekly for the cows in dynamic feeding and bi-weekly for cows in static feeding. Silage was provided *ad lib*. Data were analysed using the mixed procedure in SAS 9.4 and the model accounted for feeding, harvest time, lactation number and their interactions as well as a random effect of cow.

Results & discussion There were no effects of feeding regimen on body condition score, however, early harvested silage resulted in a higher body condition score on DIM 50 and tended to result in a higher body condition score on DIM 75 (Table 1). Energy balance tended to be higher in the static feeding regimen at DIM 50, and cows on the static feeding regimen tended to consume more concentrate in early lactation (DIM 25, 9.1±0.21 vs 8.55±0.21 kg DM daily, p=0.0721). There was also a tendency for an interaction between harvest time and feeding on DIM 50, where dynamic cows fed late harvested silage had a lower energy balance, and static cows fed late harvested silage had a higher energy balance.

Table 1 Body condition score and energy balance at DIM 25, 50 and 75 of cows feed according to a static or dynamic feeding regime, with early or late harvested silage.

		Dynamic		Static			p-values		
	DIM	Early harvest	Late harvest	Early harvest	Late harvest	SE	Harvest time	Feeding	Harvest time*feeding
Body condition score	25	3.52	3.44	3.39	3.46	0.040	0.8902	0.1327	0.1150
	50	3.58	3.40	3.47	3.35	0.069	0.0347	0.2510	0.6120
	75	3.61	3.42	3.50	3.37	0.095	0.0763	0.3455	0.7839
Energy balance (%)	25	102.0	96.6	102.4	104.5	6.00	0.7835	0.4858	0.5264
	50	105.8	90.5	106.5	115.1	6.56	0.5935	0.0526	0.0623
	75	109.1	114.4	112.0	107.2	6.86	0.9661	0.7461	0.4470

Conclusion In early lactation, harvest time and feed quality has a larger impact on body condition score and energy balance than feeding regimen.

Acknowledgements This research was as a part of the project “New approaches to management and breeding, in automatic milking systems” (NRC Project No.: 244231) funded by The foundation for Research levy on Agricultural Products, TINE SA, DeLaval and Geno.

References

Volden, H. (ed) .2011. NorFor - The Nordic feed evaluation system: Wageningen Academic Publishers

Goat kids can be characterized on their pre-weaning feeding behaviour

Marjorie Cellier, Birte L. Nielsen, Alexandra Eymard, Christine Duvaux-Ponter

UMR Modélisation Systémique Appliquée aux Ruminants, INRA, AgroParisTech, Université Paris-Saclay, 75005, Paris, France

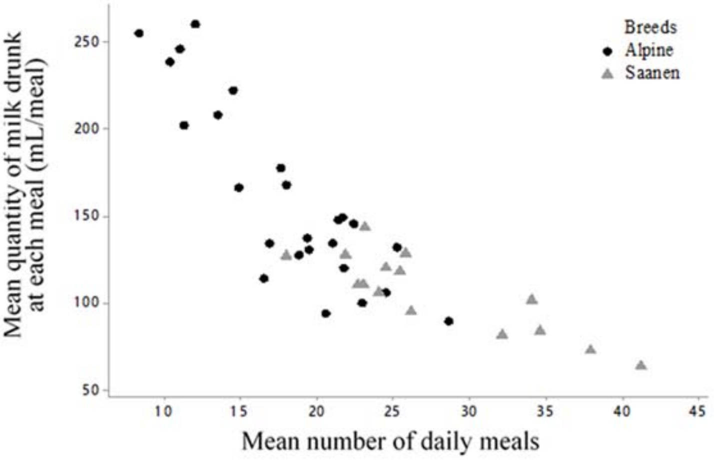
E-mail: marjorie.cellier@agroparistech.fr

Take home message Alpine and Saanen goats can be distinguished with regard to their pre-weaning feeding behaviour (milk intake). This simple criterion may give an early indication of the adult (feeding behaviour) phenotype of dairy goats.

Introduction The development of farming systems to comply with the societal demands for more sustainable animal production and more concern for animal welfare will require adaptation of farming techniques to changing and increasingly diverse environmental situations. It will also solicit the animal's ability to adapt. A key component to succeed in these conditions lies in the link between the adaptability of each animal in the herd, its production performance and its management. Preliminary measurements have shown that in adulthood and for the same diet, goats show significant individual variation in feeding behaviour (distribution of meals during the day, rate of intake, etc; Desnoyers *et al*., 2011, Duvaux-Ponter *et al*., 2013). We wanted to find out if a similar individual variation was detectable pre-weaning with a view to establish stability of feeding patterns over time.

Materials & methods Our study involved 40 female goat kids (25 Alpine goats + 15 Saanen goats) born in January and February 2017. They were placed in an automatic milk dispensing device at an average age of 26.6 days ± 7.69. They remained in this device during on average 10.8 days ± 1.37 (including a day of habituation). The device included two crates, each one with two teats, and *ad libitum* access to milk replacer. The kids were placed in groups of 12 to 14 individuals mixing the two breeds. The quantity of milk drunk at each visit (mL) and the number of daily visits were recorded via electronic identification. Visits separated by less than one minute were considered as a single meal (Levy and Alexandre, 1985). Mann-Whitney tests were used.

Results & discussion The kids displayed a large variation in individual feeding behaviour based on the average number of daily meals and the average quantity of milk drunk at each meal (mL/meal). A significant difference was found between the two breeds for these two variables ($P<0.01$; Figure 1). The kids could be assigned to three groups: a group where the individuals had few, but large intakes per meal (L: n = 7), a group where the individuals had many, but small intakes per meal (S: n = 5), and an intermediate group (M: n = 28) ($P<0.01$ for both variables). Only Alpine goats were found in group L, while only Saanen goats were in group **S**. Other variables did not vary between the breeds and among these groups: the total quantity drunk per day, the average age and the average live weight at the time of measurement, the age at weaning or the teat used ($P>0.05$).



Conclusion These results show a difference in milk feeding behaviour between the two goat breeds tested, with a pre-weaning individual variation in the quantity of milk drunk at each meal and in the number of daily meals. If feeding patterns are stable over time, this simple criterion may give an early indication of adult feeding behaviour phenotype of dairy goats, and allow early selection of goats by the farmer.

Acknowledgements The authors thank the staff of the INRA goat unit for taking care of the animals.

References

Desnoyers M, Giger-Reverdin S, Sauvant D and Duvaux-Ponter C 2011. Journal of Dairy Science 94, 842-852
Duvaux-Ponter C, Giger-Reverdin S, Tessier J, Ricard E, Ruesche J, David I and Bodin L 2013. Proceedings of European Federation of Animal Science (EAAP), 26-30 August 2013, Nantes, France.
Levy F and Alexandre G 1985. INRA/EDP Sciences 34, 181-192.

Cows collars and herd management system for remote managing of grazing beef herd

Arieh Brosh[1], Sinay Goldberg[1], Aviv Asher[2], Arieh Ben Yosef[1], Yehuda Yehuda[2], Haim Gorelik[2], Ran Malanud[1], Arnon Gat[1]

[1]*MOOnitor LTD, Bazra, Israel,* [2]*North R&D Migal, Kiryat Shmonah, Israel*

E-mail: ariehbrosh5@gmail.com

Take home message Cows' collars activities' data and cloud analysis provides all information needed for significant improvement of herd management.

Introduction Optimal herd management is based on adjustment of cows' diet quality and availability to cow needs. Attending to these needs, detecting oestrus and responding quickly to events of sickness, epidemic and extreme climate should increase significantly the production rate of grazed herds. Analysis of published data in conditions where forage biomass is above 800 kg DM per hectare was used to create the MOOnitor' system algorithm. In all published studies cows' heat production (HP) was measured by heart rate O_2 pulse method, cows' HP and entire energy balance variables were measured or estimated from cows HP as described by Brosh (2007). Analysis of the published data showed a significant correlation between cows' daily grazing hours forage quality and cows energy balance. Cows' events like calving, oestrus, lost calves (like weaning for example) and sickness should affect daily activities behaviour. Automatic monitoring of grazing cows' energy balance and events will provide the farmer with an efficient tool to increase herd production and profits. We present here validations of MOOnitor Ltd. system for monitoring cows' herd energy balance, forage quality, sickness, reproductive events and the identification of significant shortage of forage biomass.

Materials & methods MOOnitor Ltd. develops collars that use three axes accelerometers output calculated as sum of square and three thresholds for determining, every 4 sec, cows' daily activities: resting, grazing, walking, number of meals per day and mastication at rest (hours/day), i.e. mainly the rumination time. Cows' location is measured by GPS three times per day. The collar battery is recharged by solar panels. The data is transmitted by satellite, analyzed within MOOnitor's server and arrives the user via the cloud. For research purposes, the collars save activities and locations throughout the day. The system tests during more than 3 years along years' seasons, using several beef herds. System estimation of consumed forage quality were compared to those tested by NIRS analysis of fecal samples (Landau S. *et al*., 2018) and of cows' energy balance using changes of body condition score (BCS, scale 1-5). We record field biomass throughout the year and we examine MOOnitor's system capability to determine significant reduction in forage biomass. Sickness and reproduction events were identified by comparing grazing, walking and resting ratios changes along days comparing to its entire herd change

Results & discussion Energy retained of 25 mature non-lactating cows, average BW of 479 kg was estimated along 127 days by comparing BCS changes (start 2.86, end 3.42) to those calculated by MOOnitor's algorithm. When both estimations were translated to changes in body fat tissue weight (NRC 2001), i.e. mainly the empty BW changes, both estimations were almost identical, 39 kg by BCS changes to 39.3 kg by MOOnitor's algorithm. Actual consumed forage quality estimated by NIRS analysis of faecal samples (nine' seasons) was highly correlated ($R^2 = 0.82$, $P < 0.001$) to those estimated by MOOnitor's algorithm. When the collars' data logger is used, the daily pattern of cows' activities, including mastication at rest can be measured. Cows' daily activities changes throughout the year' seasons well represent forage quality as affected by the rain and herd events. Mathematic calculation based on cows' activities well identified the states where shortage of forage biomass (less than 500 kg DM/hectare) significantly increase grazing time while cows' energy balance become negative. Sickness and oestrus event were well identified, but about 30% errors (fouls positive) were found for oestrus events.

Conclusion This is the first hi-technology system designed for commercial beef herds. The system overcomes difficulties like communication and power in remote locations and harsh conditions. The system provides daily measureable accurate data about: herd energy balance, consumed pasture quality, low forage biomass and health problems. The management system adds scientific analysis to the data. Ranchers receive research quality data allowing very efficient herd management.

Acknowledgements We thank the beef herds' teams where we have developed and tested the collars and MOOnitor system: Kibbutz Gal-Ed, Tabash family, Kibbutz Kfar-Szold, Dabosh family in Kari Deshe-North R&D.

References

Brosh A 2007. Journal of Animal Science 85, 1213–1227.
Landau S, Dvash L, Yehuda Y, Muklada H, Peleg G, Henkin Z and Ungar E 2018. Animal, 12, 265-274.
NRC 2001. Washington (DC): National Research Council, National Academy Press.

433

The incisive arcade breadth, a relevant indicator of the bite mass for ruminants

Maryline Boval, Daniel Sauvant

INRA, Paris, France

E-mail: maryline.boval@agroparistech.fr

Take home message The incisive arcade breadth (IB), easily measurable using an inexpensive caliper is linked with body weight (BW) and explains variations in bite mass (BM).

Introduction BM is a key parameter in the forage acquisition, being the smallest scale in foraging and informing about larger scales. BM may vary at pasture according to sward characteristics as sward height, or herbage bulk density. However, BM depends also on animal traits, thus few relationships between IB and BW have already been proposed (Gordon, 1996). A quantitative review of the literature was carried out to obtain more generic relationships between IB, BW and BM, in order to provide useful and simple tools for monitoring nutrition and to better assess individual variations.

Materials & methods This work was performed from a meta-analysis of published data. The database comprised 6 publications, 27 experiments and 59 treatments (44 vs 15 with cattle vs sheep). The regressions were calculated intra-experiment for studies where IB varied significantly.

Results& Discussion

The IB(6.3±1.9, min=2.7, max=8.6 cm) is closely related to BW (327±214, min=26, max=618 kg):

$\mathrm{IB(cm)}=0.91\ \mathrm{BW}^{0.346}$ (n=59, nexp=27, RMSE=0.27) [1, Figure 1]

The allometry coefficient of this equation is <1 and is fairly similar to that of Gordon *et al* (1996). Besides, there is a close intra-experiment regression between BM (0.56±0.07, min=0.1, Max=1.2 g DM/bite) and IB:

$\mathrm{BM\ (g)} = 0.023\ \mathrm{IB}^{1.71}$ (n=49, nexp=22, RMSE=0.10) [2, Figure 2]

The power coefficient is > 1, stressing the advantage of having a larger IB, propitious for forage acquisition for grazers. It could be seen as a systematic advantage of grazing cattle, compared with sheep (Figure 2). In the same dataset IB is also related to the theoretical diameter of the bite and therefore to the bite area, but not at all with the bite depth, which is more impacted by sward height in grazing conditions. BM is largely dependent of the volume of the bite, which depends of the opening of the mouth and of the extending of the tongue.

Figure 1 Influence of Body Weight on incisive arcade breadth.

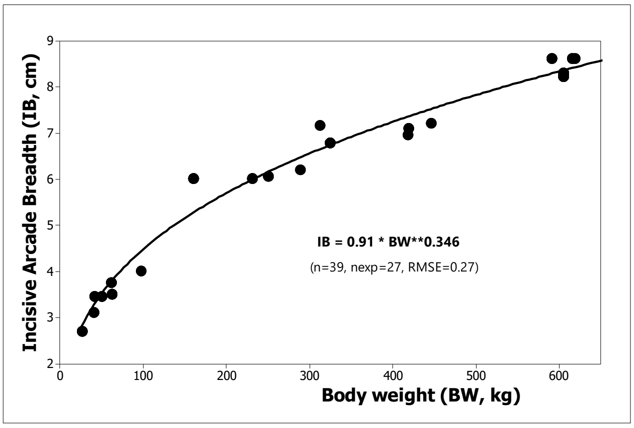
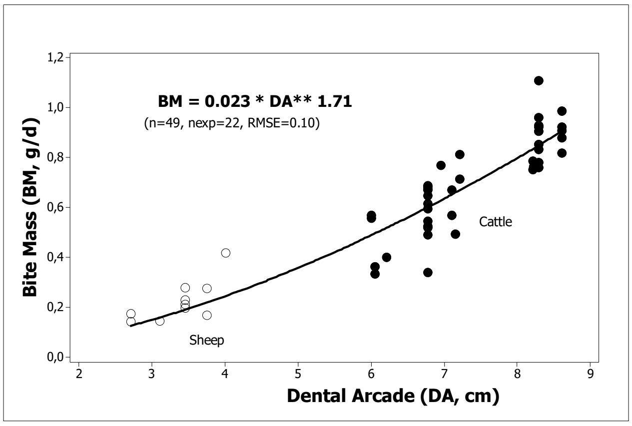


Figure 2 Influence of incisive arcade breadth on Bite Mass.



Conclusion The measurement of IB should facilitate the evaluation of BM since measuring IB is easy to implement and not expensive. BM depend also of sward characteristics (*i.e.,* sward height, herbage mass or bulk density) but the RMSE of the predictions by these sward characteristics are much higher than the prediction starting from IB. Moreover, IB is predictable from BW. Such a measure can help to better understand individual variations in the acquisition of forage, since we have noticed that the relationships between IB and BM vary significantly inter individual at pasture and likely indoor.

References

Gordon IJ, Illius AW and Milne JD 1996. Functional Ecology 10, 219-26.

Simulation of the variation in grass growth, milk production and residency time in a 40 hectare farm composed of either 6 or 40 paddocks

Elodie Ruelle[1], Luc Delaby[2], Deirdre Hennessy[1]

[1]*Teagasc, Fermoy, County.Cork, Ireland,* [2]*INRA, Saint-Gilles, France*

E-mail: elodie.ruelle@teagasc.ie

Take home message The variation in paddock residency time from 1-2 days to 9-12 days (due to the variation in paddock size) does not impact annual grass growth or farm milk production.

Introduction Management of pasture (such as fertilisation, cutting and grazing height), as well as weather, is an important factor influencing grass growth. The potential to increase grass growth and utilisation through advanced grassland management is receiving more attention in grass-based milk production systems. In Ireland, research has shown the importance of using small paddocks with a residency time of one day to maximise grass utilisation. This paper presents the impact of having either 6 (6.7 ha) or 40 (1 ha) paddocks on a 40 ha farm using the pasture based herd dynamic milk model (PBHDM) (Ruelle *et al*., 2015) linked with the MoSt grass growth model (Ruelle *et al*., 2018).

Materials & methods The PBHDM simulates individual cows and paddocks within a farm. The PBHDM allows the establishment of the impact of different management, farm characteristics and weather conditions on grass growth, milk production, silage making, and supplementation required. The model was used on a 40 ha farm, with either 100 cows and a moderate grazing intensity calculating the post grazing sward height (postGH) using the following equation: $4 - 0.1 \times \text{preGH} + 0.015 \times \text{preGH}^2$ (Ruelle *et al*., 2015) or 150 cows and a high grazing intensity calculating the postGH using the following equation: $3.5-0.1 \times \text{preGH} + 0.015 \times \text{preGH}^2$ (Ruelle *et al*., 2015) with either 6 or 40 paddocks.

Results & discussion Overall, variation in terms of milk production per cow grass intake, silage fed at grazing and, grass growth are similar between systems (Table 1). The amount of grass cut per ha is greater in the 6 paddocks situation (+1,125 kg DM/ha) when 150 cows are present but this difference is not present when only 100 animals are on farm. Pre-grazing height was similar for both system however the post grazing height was lower with 40 paddocks. This is due to the fact that the cattle change paddock only after milking (morning or evening) which leads to a tighter grazing when the paddocks are smaller.

Table 1 Impact of 6 or 40 paddocks in a 40 ha farm with either 150 or 100 cows in term of milk production, silage fed at grazing, grass intake, grass growth, silage produced, grass cut, and grazing management.

Number of cow	Grazing intensity	Number of paddock	Milk production (kg/cow)	Silage fed at grazing (kg DM/cow)	Grass intake (kg DM/cow)	Grass growth (kg/ha)	Silage produced (kg/ha)	Residence time (day)	Pre grazing height (cm)	Post grazing height (cm)
150	high	6	5,162	834	2,629	12,276	1,962	8.5	10.1	4.2
150	high	40	5,032	817	2,581	12,298	2,236	1.3	10.0	3.8
100	moderate	6	5,185	735	2,790	12,068	3,908	11.7	9.9	4.6
100	moderate	40	5,166	519	2,956	12,106	3,710	1.6	9.8	4.0

The model was able to recreate the variation in milk production of the cows due to the low variation in grazing height when the cows remained in the same paddock for several days. However, this variation did not impact overall cumulative milk production on the farm.

Conclusion Overall milk production and grass growth were similar from the 6-paddock farm and the 40-paddock farm.

Acknowledgements The authors acknowledge the funding from the Research Stimulus Fund 2011 administered by the Department of Agriculture, Fisheries and Food (Project 11/S/132).

References

Ruelle E, Shalloo L, Wallace M and Delaby L 2015. European Journal of Agronomy 71,106-114.
Ruelle E, Hennessy D, and Delaby L 2018. European Journal of Agronomy (accepted subject to correction)

Phenotyping milk fatty acid composition in dairy goats to study variations in rumen fermentation patterns and to predict the occurrence of acidosis

Sylvie Giger-Reverdin[1], Pablo Toral[2], Gonzalo Hervas[2], Pilar Frutos[2], Daniel Sauvant[1]

[1]INRA, AgroParisTech, Université Paris-Saclay, UMR Modélisation Systémique Appliquée aux Ruminants, Paris, France, [2]Instituto de Ganaderia de Montana (CSIC-University of Leon), Grulleros, Leon, Spain

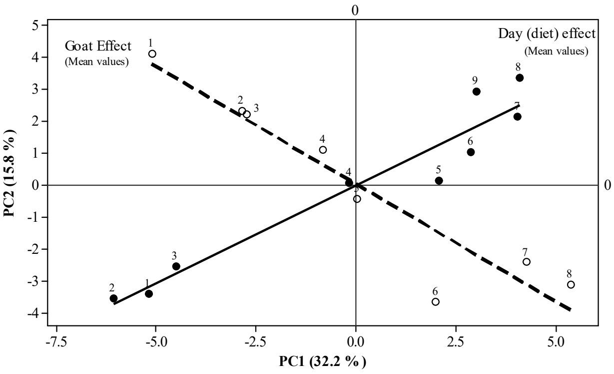
E-mail: sylvie.giger-reverdin@agroparistech.fr

Take home message Individual milk fatty acid composition is a good biomarker in dairy goats to detect status of rumen fermentation patterns. According to this purpose, it can be used to phenotype dairy goats susceptibility to acidosis.

Introduction Main factors of variation of milk fatty acid (FA) composition are linked to diet composition and physiological status. This trial aimed to study the between animal variation when they were moved from a low concentrate diet to a high one and test if milk FA profile could be a good biomarker of SARA (sub-acute rumen acidosis).

Materials & methods Eight rumen cannulated dairy goats adapted to a low concentrate diet (20 %) were switched to a higher concentrate diet (50 %). Samples of milk were taken individually on the morning for 2 days before the change (Days 1 and 2), the 4 days following the change (Days 3 to 6) and once weekly for 3 weeks (Days 7 to 9). Therefore, the day effect would represent the effect of the diet (i.e. before, during and after diet transition). 91 fatty acids (FA) were detected by gas chromatography. Rumen fluid was sampled simultaneously before the morning feed (T0) and 1, 2, 4 and 6 hours afterwards and analysed for pH and volatile fatty acid (VFA) composition. A principal component analysis (PCA) was used to describe the relationships among milk FA % which were pooled in groups according to their saturation degree and C chain length. Rumen fermentation data were considered as supplementary variates in this PCA.

Results & discussion Score plots of the observations (mean values for goats and mean values for days) on the first two components (48 % of variance explained) showed that there were significant goat and day effects, but almost independent effects: goat axis (between-animals variation) was perpendicular to day axis (between days variation, Figure 1).



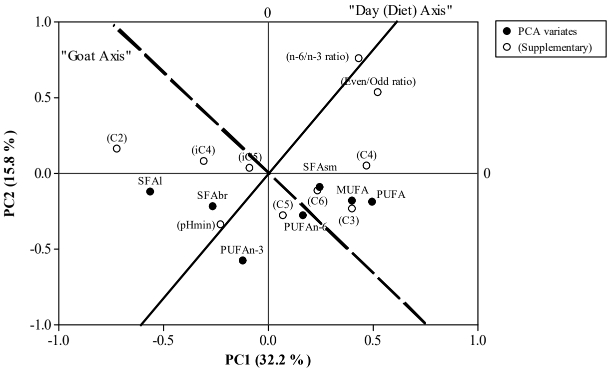


Figure 2 Score plots of the variates on the first 2 components of the PCA.

Score plots of the variates showed that the goat effect can be linked to an opposition between short saturated (SFAs), mono (MUFA) and polyunsaturated (PUFA) FA, on one hand, and long (SFAl) and branched SFA (SFAbr), on the other hand (Figure 2). Modification of FA profile after the dietary change began very rapidly. A steady state status was observed around one week after it. Concentration of odd FA decreased, while the even/odd ratio of SFA and the n-6/n-3 ratio increased with the high concentrate diet. Score plots of the supplementary rumen variates showed that differences between goats were mainly linked to rumen fermentation patterns. Rumen pH was opposed to even/odd and n-6/n-3 ratios. On the PC1, % of C2, iC4 and iC5 are opposed to % C3 and % C4 which corresponds to a more acidogenic profile. This also suggests that VFA profile was stronger affected than pH, probably due to differences in microbiota. Goats in the bottom right quadrant (6 to 8) seemed more susceptible to acidosis than goats in the up left quadrant (1 to 3). Thus, fatty acid composition of goat milk is linked to rumen fermentation patterns as previously reported in cows (Fievez *et al.*, 2012), with an important between-animal variation that can explain why some authors find a marked between-trial effect (Bhagwat *et al.*, 2012). Phenotyping animals on this trait could outline specific rumen fermentation patterns.

Conclusion This study suggests that milk FA composition detected by gas chromatography may be a biomarker of changes in rumen fermentation and subsequent SARA, and so phenotyping goats on that trait might be of practical interest.

Acknowledgements This research was partially supported by grants from Idele in the project CASDAR rfi AcID 1310. The authors thank J. Tessier and his colleagues from Inra-AgroParisTech experimental farm for taking care of the goats

References

Bhagwat AM, De Baets B, Steen A, Vlaeminck B and Fievez V 2012. Journal of Dairy Science 95, 3926-3937.
Fievez V, Colman E, Castro-Montoya JM, Stefanov I and Vlaeminck B 2012. Animal Feed Science and Technology 172, 51-65.

Interest and limits of two methods to characterize the individual variability in feeding behaviour of barn-dried hay measured continuously in dairy goats

Sylvie Giger-Reverdin, Daniel Sauvant

INRA, AgroParisTech, Université Paris-Saclay, UMR Modélisation Systémique Appliquée aux Ruminants, Paris, France

E-mail: sylvie.giger-reverdin@agroparistech.fr

Take home message The segmentation-clustering method applied to barn-dried hay dry matter intake measured continuously can better characterise individual feeding behaviour pattern than fitting data with a simple exponential model.

Introduction Barn-dried hay might be a good option to produce a palatable forage of good nutritional value with safer conditions than a field-dried hay. This study aimed to compare two methods to characterize individual variability in feeding behaviour of dairy goats, because this trait is highly variable and might be linked to performances in ruminants (Dado *et al.*, 1994).

Materials & methods Feeding behaviour was assessed on 24 dairy goats housed in individual crates and fed concentrates (1100 gDM/d) and *ad libitum* barn-dried hay (15 % refusals) twice a day for 7 days. Dry matter hay intake (DMIh) was weighted continuously. Individual cumulative hay dry matter intakes were fitted using an exponential model (DMIh = a $(1-e^{-bt})$). The data concerning the intake evolution after the afternoon allowance were also characterised by a segmentation-clustering method (Giger-Reverdin *et al.*, 2012). To describe the major interrelations across the variables, a principal component analysis (PCA) was performed on milk yield (MY), body weight (BW), DMIh between afternoon and morning milking (DMIAft), DMIh during 180st minutes after afternoon allowance (DMI 180), the ratio P180 = DMI180/DMIAft, number of eating bouts (Neb), intake and duration of the first eating bout estimated by the segmentation-clustering method (Int1steb and Dur1steb), parameters a et b, the initial rate of intake (ab) and residual mean square error (RMSE) of the equations.

Results & discussion Barn-dried hay was very palatable as total DMI was 26 % higher than a prediction based on body weight and milk yield (Sauvant *et al.*, 2018). Around 58 % of the total variance was explained by the first 2 components (PC) of the PCA (Figure 1). The short distances between the same parameters of the exponential model for the afternoon and morning curves showed that feeding behaviour was quite repeatable within a day for a given animal On the first PC axis, there was an opposition between on the right of the figure, the parameters characterising total intake and rate of intake, and on the left one, number of eating bouts and residual mean square error of the exponential model. This means that it has a better ability to fit meal kinetic with high intake and rate of intake and low number of bouts. On the second PC axis, there was an opposition between intake (upper part) and rate of intake (lower part). Animals with the highest intakes ate a lot during their first eating bout (or main meal) which was also of longer length (Dur1steb) what agrees with the literature.

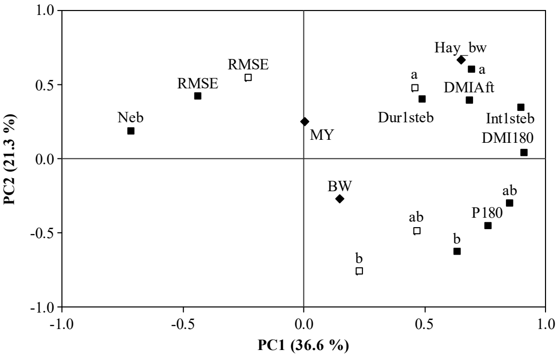


Figure 1 Projection of the variates on the first two principal components or PC (dark squares for afternoon data and open squares for morning ones).

The opposition between rate of intake and RMSE of the models and Neb pointed out that when the animals had a lot of eating bouts, the exponential model cannot characterize DMIh evolution between two distributions. At the opposite, the segmentation-clustering method is useful to better interpret data with the output of a vector of parameters which can explain, for example, the results of the exponential model: when the animals have a lot of eating bouts, the exponential model is of poor accuracy. Score plots of the individuals (not shown) pointed out that they were disseminated in the whole first two loading vectors plan what means that there was a high inter-individuals variability in feeding behaviour.

Conclusion. The segmentation-clustering method is a promising method to characterise individual pattern of feeding behaviour with cumulative dry matter intake obtained in the context of precision livestock farming in dairy ruminants.

Acknowledgments This research was supported by grants from the PSDR4 Grand Ouest FLECHE.

References

Dado RG and Allen MS 1994. Journal of Dairy Science 77, 132-144.

Giger-Reverdin S, Lebarbier E, Duvaux-Ponter C and Desnoyers M 2012. Computers and Electronics in Agriculture 83, 109-116.

Sauvant D and Giger-Reverdin S 2018. In INRA, 2018. INRA feeding system for ruminants, 339-374.

Evaluation of a dynamic mechanistic whole animal beef model: feed intake predictions

Virgilio Ambriz-Vilchis[1,2], Merryl Webster[2], Jennifer Flockhart[1], John Rooke[1]

[1]*Scotland's Rural College, Future Farming Systems, Edinburgh, UK,* [2]*BioSimetrics Ltd., Edinburgh, UK*

E-mail: virgilio.ambriz@sruc.ac.uk

Take home message Given an adequate description of the animal and consumed diets, the BSM-Beef model can successfully predict feed intake.

Introduction Mathematical computer modelling allows the simulation of feeding regimes and consequent performance of livestock. These models can aid in the formulation of diets at the farm level that will enhance performance, profitability and reduce wastage. To achieve this, accurate prediction of feed intake is paramount. Therefore, the objective of this study was to evaluate the accuracy of a whole animal dynamic mechanistic model to predict intake of beef steers fed a total mixed ration.

Materials & methods A trial was carried out at the Beef and Sheep Research Centre, SRUC, Scotland UK. Eighty cross-bred steers (Angus or Limousin, 40 of each) were housed in 8 pens of 10 animals each. Animals were offered a total mixed ration (TMR, Wholecrop barley 69.96%, barley 13.97% , distillers maize dark grains 13.87%, molasses 1.69% and minerals premix 0.50%) using electronic feeders (n = 4 per pen; HOKO, Insentec, Marknesse, The Netherlands) and individual feed intakes (kg fresh matter (FM) /steer/day) were recorded daily. A subsample of sixteen steers was selected to evaluate the accuracy of the mechanistic whole animal beef model (BSM-Beef, BioSimetrics Ltd., Edinburgh, UK) predictions of feed intake. Details of the animals (mean body weight (BW = 447 ± 14.2), condition score (BCS = 3), breed) and the TMR (composition, chemical and fermentation characteristics) consumed were recorded weekly and were used as inputs for BSM-Beef. The intake predictions obtained with BSM-Beef for each animal were compared to those obtained on-farm. To evaluate the predictions a linear mixed effect model (LMEM), a modified version for repeated measures of the limits of agreement (LoA) method (Bland and Altman, 2007) and the concordance correlation coefficient (CCC, Lin, 1989) were used. All statistical analyses were carried out using R (R Core Team, 2017).

Results & discussion Details of the animals and the feedstuff consumed were used as inputs to run BSM-Beef to obtain intake predictions per animal and were compared to measured feed intakes. Figure 1 shows the observed *vs*. the predicted intakes (FM kg/steer/d). Observed and predicted values were in good agreement; the equation line obtained with the mixed effect model running closely by the line of equality (LMEM intercept = 59 and residual = 88) and CCC = 0.78.When evaluated with the LoA method the relationship between observed and feed intake predicted by BSM-Beef showed a slight tendency of the model to over predict intakes in animals consuming less than 20 kg / FM / day (lower limit of agreement at -2.04) and it appears to have slightly under-predicted intake in animals consuming more than 20 kg/FM/day (upper limit of agreement at 2.13). Overall, the model gave acceptable feed intake predictions compared to consumption observed on-farm (mean 0.05).

Conclusion Given an adequate description of the animal and consumed diets, BSM-Beef acceptably predicts intake (+/- 2 kg/FM/day) of beef steers consuming TMR. Further work is required to test the model's ability to predict intake in other management regimes.

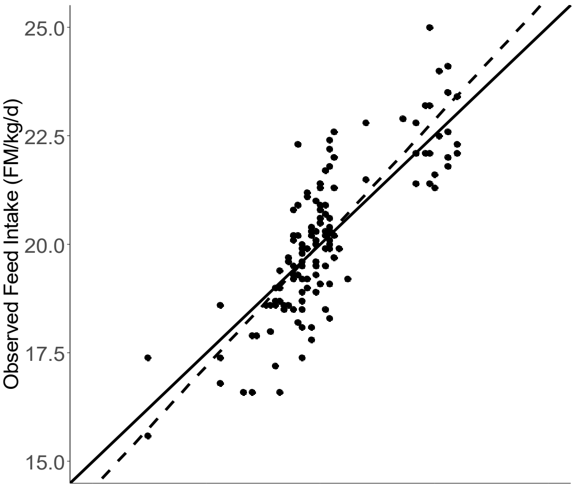


Figure 1 Observed vs. predicted feed intake. The solid line represents the line of equality and the dashed line the mixed effect model equation line.

Acknowledgements This research was supported by Innovate UK via Knowledge Transfer Partnership, SRUC and Biosimetrics Ltd.

References

Bland JM and Altman DG 2007. Journal of Biopharmaceutical Statistics 17, 571 – 582.
Lin LI 1989. Biometrics 45, 255 – 268.

A model of urinary nitrogen excretion: a way to assess diet protein value at individual level?

Pierre Nozière[1], Solveig Mendowski[1,3], Patrick Chapoutot[2], Daniel Sauvant[2], Gonzalo Cantalapiedra-Hijar[1]

[1]INRA, Theix, France, [2]AgroParisTech, Paris, France, [3]Valorex, Combourtillé, France

E-mail: pierre.noziere@inra.fr

Take home message The model of urinary nitrogen (Nur) excretion responses to diets, included in the renewed INRA feeding system for ruminants (INRA, 2018), could help to assess the protein value of diets from nitrogen (N) balance trials even at the individual level.

Introduction The estimation of diet protein values (PDI) for ruminants based on the *in situ* degradation rate of feedstuffs has also consequences on the estimation of the rumen protein balance (RBP), on the efficiency of PDI utilisation (PDIeff) by the animal and thus on the urinary N (Nur) excretion (Sauvant *et al.*, 2016). This is explicitly integrated in the renewed INRA (2018) feeding system for ruminants. We aimed to investigate, using an N balance trial on dairy cows fed high levels of proteaginous seeds, if this predictive model of Nur could be used to assess the PDI value of the diets (assuming observed = calculated Nur), even at individual level.

Materials & methods Eight dairy Holstein cows were used in a replicate 4×4 Latin square N balance trial (see Mendowski *et al.*, 2018). Cows were fed *ad libitum* iso-energy (1680 kcal NEL/kg DM) and iso-nitrogenous (146 g CP/kg DM) diets, differing in N source (40% of total CP in diets):proteaginous (faba bean or lupin):linseed blends (90:10) either raw (R) or extruded at 140°C (E1) or 160°C (E2), compared to soybean meal (SBM). Each cow received 50 g/d Metasmart® to avoid deficit in methionine. All calculations (mean±SD) were performed according to INRA (2018). The dietary PDI (91±7 g/kg DM) and RPB (6±6 g/kg DM) were calculated assuming N effective degradability (ED_6N, %) of 63 (SBM), 84 (R), 65 (E1), or 50 (E2). The PDIeff (0.75±0.07) was calculated as the ratio protein synthesis / available PDI. Protein synthesis (1231±145 g/d) accounted for milk (875±113) + endogenous faecal (284±39) + scurfs (10.4±0.4) + body retention (62±47). Available PDI supply (1660±279 g/d) accounted for PDI intake (1886±283) – endogenous urinary protein (226±15). The calculated Nur excretion (161±31 g/d) accounted for N from 0.79 x RPB (14.9±16.7) + N from PDI inefficiency (77.7±33.8) + microbial and non proteic N (20.2±1.2) + endogenous Nur (36.2±2.4), and was corrected for N balance default (Nbal) (0.47 x Nbal =12.5±11.5). The difference "calculated minus observed" Nur was assessed by paired-samples t-test, and by regression. Effects of animal, period and treatment on this difference were assessed by Anova.

Results Calculated and observed Nur were significantly correlated inter-treatment (P=0.056), inter-individual (P=0.001), or globally (P=0.009, Figure 1). The slope of the inter-treatment (0.876) and overall (0.860) relationships did not significantly differ from 1, but the inter-individual one was lower (0.501). The difference (20±31 g/d, P=0.001) between calculated (161±31) and observed (142±28) Nur was mainly explained by a period effect (period 1 > others, P=0.007) and at a lesser extent by an animal effect (P=0.07), and was irrespective of the diet (P=0.925). This difference was mainly related (P=0.001) to the N balance default (Nbal = 27±24 g/d).

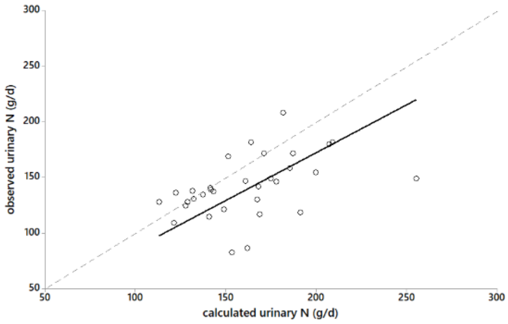


Figure 1 Observed vs calculated Nur individual losses in dairy cows.

Conclusion The present approach provides an evaluation of the integrated Nur excretion model based on dietary PDI, RPB and PDI efficiency concepts. Beyond the Nur variations across dietary treatments predicted by the model, the individual data are also satisfyingly ranked. However, the uncertainty related to the experimentally uncontrolled N balance default remains a limit of the present approach to assess the actual PDI value of diets at individual level.

Acknowledgements The data used have been obtained from an experiment conducted as part of the Proleval project, supported by Valorex and financed by Bpifrance.

References

INRA (2018). INRA Feeding System for Ruminants. Wageningen Academic Publishers, the Netherlands, 640 pp.

Mendowski S, Chapoutot P, Ferlay A, Chesneau G, Enjalbert F, Largeau V, Genestoux L and Nozière P 2018. ISNH 2018, submitted.

Sauvant D, Cantalapiedra-Hijar G, Delaby L, Daniel JB, Faverdin P and Nozière P., 2015. INRA Productions Animales, 28, 347-368.

439

Variations of global DNA methylation in Peripheral Blood Mononuclear Cells, in milk leukocytes and in milk epithelial cells in dairy cows: effects of micro-nutrient supplemented diet.

Helene Jammes[1], Maxime Gasselin[1], Marion Boutinaud[2], Audrey Prezelin[1], Perrine Debournoux[2], Emmanuelle Mariani[3], Jackie Zawadzki[4]

[1]UMR1198, INRA, Jouy en Josas, France, [2]UMR 1348 PEGASE, INRA, Saint Gilles, France, [3]XR-Repro, Coubon, France, [4]Pilardière Group, Saint-Mars-la-Réorthe, France

E-mail: helene.jammes@inra.fr

Take home message Nutrition changes could induce global epigenetic effects in different cell types.

Introduction Postpartum period is a critical period in dairy cows. This crucial time is marked by major physiological and metabolic changes that affect milk production, immune response and fertility. The nutrition is considered as one lever to provide a control of negative energy balance and a better general health status of the dairy cows (Drackley *et al*., 2014). Epigenetics provides molecular mechanisms (DNA methylation, post translational histone modifications, short and long non-coding RNAs) that affect genome activity and gene expression without DNA sequence modification (Schübeler, 2015). Moreover, epigenetic imprinting can also be triggered by environmental factors (pathogenic, nutritional, chemical and physical factors). This study aimed to evaluate the phenotypic effects and epigenetic imprinting of a micro-nutrient supplemented diet, distributed before and after calving in order to provide the metabolic statue and health in dairy cows.

Materials & methods Multiparous (n=17) and Primiparous (n=7) dairy cows were used in this study and randomly allocated to one of two dietary treatments (n=12 for each group): control diet (CTRL group) and diet with commercial nutrient supplementation (Genial®; SUPPL. group) for 4 weeks before calving and for 8 weeks afterwards. Before calving, the cows were fed ad libitum according to INRA guidelines (INRA 2007). Before calving, the diet contains 84.5% corn silage, 9% soybean meal, 2.5% straw, on a dry matter basis. After calving, the diet contains, on a dry matter basis, 52.5% and 64.7% corn silage, 24.7 and 15.5% non-mineral supplement, 2.5% straw for only the first 15 days of lactation and 8% soya oilcake, 10% alfalfa silage and 0.8% urea. The CTLR and SUPPL cows received after calving, 260 and 200 g classic mineral supplement for multiparous and primiparous cows respectively. The SUPPL cows received in addition 160 g for 4 weeks before calving and 250 g of Genial® during the first 60 days of lactation. Genial® is a nutrient supplementation enriched in organic selenium from yeast, in trace elements (zinc and copper), in a cocktail of minerals and in extracts from plants and microalgae containing □ carotene, vitamin A, D3, E and natural Superoxide dismutase, currently commercialized by PILARDIERE group (PILARDIERE, Saint-Mars-la-Réorthe, France). Milk production and composition (fat, protein and lactose contents, somatic cell count (SCC)), body weight (BW), body condition score (BCS), dry matter intake and health (calving score, metritis, mastitis) were recorded over the trial period. At D15 and at D60 post calving, blood and milk samples were collected from cows. From the milk samples, different components were analyzed. Mammary Epithelial Cells (mMEC) and milk leukocytes were purified and counted. The viability of both cell types was estimated. From blood, the Peripheral Mononuclear Blood Cells (PBMC) were purified and counted. As new phenotypic parameter, the global DNA methylation of the three different cell types (PBMC, mMEC and milk leukocytes) was measured by Luminometric Methylation Assay (LUMA).Statistical analysis: Zootechnical data were analyzed by ANOVA using the SAS (SAS Institute, 1999) MIXED procedure with the repeated statement using D15 and D60 of lactation as repeated effect and cows as subject. The effects of parity, diet, stages, and the interaction parity x stage, parity x diet, diet x stage and parity x diet x stage were tested.

Results & discussion Genial® provided an enhancement of BCS and BW without modification of dry matter intake and milk yield and composition. The supplement also improved calving conditions and reduced delay between calving and first observation of the cow heat (first service).

Global DNA methylation was measured by LUMA that provides the methylation level of pan genomic MspI/HpaII cleavage sites (Me-CCGG). Using 35 PBMC samples and matched 35 milk leukocytes and mMEC samples, Me-CCGG was found different between cell types (with median value of 65.4 % in mMEC, 68.3 % in milk Leukocytes and 74.6 % in PBMC, $P < 0.0001$). In PBMC, Me-CCGG was different between stages of lactation ($P = 0.021$) and modified by commercial nutriment supplementation for primiparous cows (LMS 72.4 % ± 0.9 and 75.4 % ± 0.8). In milk leukocytes, a parity x diet effect was observed on Me-CCGG ($P = 0.0054$). In mMEC, Me-CCGG was found not significantly different between parity, stage and diet groups. However, a stronger variability of Me-CCGG was observed in mMEC than in milk leukocytes and PBMC ($cv_{(mMEC)}=11.8$ %, $cv_{(milk\ leukocytes)}= 5.9$ % and $cv_{(PBMC)}= 2.2$ %).

Conclusion Our study shown that the cell specificity Me-CCGG was highly significant, suggesting the importance of global methylation level in regulation of cell differentiation. Moreover, the variations of Me-CCGG in response of different conditions analyzed (parity, lactation stages, diet) were also specific of the considered cell type.

Acknowledgements The authors thank the staff of the experimental dairy farm of Mejusseaume dairy farm, (UMR1348 IEPL Le Rheu, France) for the animal management.

References

Drackley JK and Cardoso FC. Animal 2014, 8 Suppl 1, 5-14
Schübeler D 2015. Nature 517, 321–326.

Feeding *Pistacia lentiscus* improves milk quality and curd properties in Damascus goats

Nurit Argov-Argaman[1], Oren Hadaya[1], Tzach Glasser[3], Hussein Muklada[2], Moshe Shemesh[2], Yan Landau[2]

[1]Hebrew Universty, Jerusalem, Israel, [2]Agricultural Research Organization, Rishon LeZion, Israel, [3]Ramat Hanadiv Nature Park, Zikhron Yaakov, Israel

E-mail: argov.nurit@mail.huji.ac.il

Take home message Feeding *Pistacia lentiscus* in confinement serve as a model in the investigation of the effect of brush land grazing on goat milk attributes.

Introduction The effect of pasture on milk composition and quality is the subject of many studies in recent years. Previously, we found a positive effect of tannin rich pasture on milk composition in grazing Damascus goats (Argov-Argaman *et al*., 2016;Hadaya *et al*., 2017).

Monitoring dietary consumption on pasture, revealed that *Pistacia lentiscus* is one of the major dietary components of grazing Damascus goats in Israel. *Pistacia lentiscus* is a tannin-rich brush which grows wild in the Mediterranean basin ecosystem. *Pistacia lentiscus* consists various secondary, bioactive metabolites, including flavonoids and anthocyanins, phenolic acids, triterpenoids, and tannins.

We hypothesized that *Pistacia lentiscus* is responsible for the positive effect of pasture on milk constituents in Damascus goats, and that the positive effect of grazing can be reproduced, under confinement, by replacing hay in confined dairy goats ration with *Pistacia lentiscus* as a source for forage.

Materials & methods Forty Damascus goats, 117 days in milk, were randomly assigned to four treatment groups (n=10): grazing (P), grazing with poly-ethylene glycol supplementation (PPEG), confined group fed vetch hay without (H) or with provision of clipped *P. lentiscus* ad-libitum (PIS) for two periods (A and B). Goats in the pasture groups, P and PPEG, grazed for 4h daily. Milk samples were collected to determine milk solids content, lipid composition, and curd properties. Also, feces was collected individually to determine dietary DMI, ADF, NDF, crude protein and tannin consumption (%) by fecal NIRS (Glasser *et al*., 2008). Differences between groups were tested by LSMean Tukey HSD multiple comparison test with treatment as fixed effect and goat as a random effect.

Results & discussion Groups did not differ in their DMI (gr/day) or in their milk yield (kg/day) in both periods, A and B ($P = 0.88$). The PIS diet increased milk protein content in period A (3.57 vs 3.93%; $P = 0.014$) and B (3.41 vs 3.77%; $P < 0.001$). In addition, the PIS group had 49 and 39% higher curd strength in period A and B, respectively, compared with P-group ($P = 0.1$ and $P < 0.01$, respectively). Goats in the PIS group produced significantly ($P < 0.05$) higher milk fat content versus H-group in period A (5.85 *vs.* 4.71%) and B (5.89 *vs.* 4.36%, respectively). In period B, PIS-group produced milk that was 48% richer in omega 3 than H-group ($P = 0.0001$), resulting in lower ratio of omega 6 to omega 3 (5 vs 8.19, respectively; $P < 0.0001$).

Conclusion Feeding system of dairy goats based on *P. lentiscus* enhance milk protein production, health attributes and improve the efficiency of cheese production.

References

Argov-Argaman N, Hadaya O, Glasser T, Muklada H, Dvash, L, Ronit Mesilati-Stahy R and Landau SY 2016. International Dairy Journal 58, 2-8.

Glasser T, Landau SY, Ungar ED, Perevolotsky A, Dvash L, Muklada H, Kababya D, Walker JW 2008. Journal of Animal Science 86, 1345-1356

Hadaya O, Glasser T, Muklada H, Dvash L, Mesilati-Stahy R, Landau SY and Argov-Argaman N 2017. Small Ruminant Research 153, 31-40.

Effects of feeding a *Moringa oleifera* rachis and twig preparation to dairy cows on their milk production and composition, and plasma antioxidants

Kai Cui, Tingting Zhang, Yan Tu, Qiyu Diao

Feed reseach institute of Chinese academy of agricultural sciences, Beijing, China

E-mail: cuikai@caas.cn

Take home message A 6% (w/w) *Moringa* supplement in cow feed increased the yield of their milk and positively altered its fat profile and improved the plasma antioxidant capacity of cows.

Introduction *Moringa oleifera Lam* is indigenous to sub-Himalayan tracts in north-west India, Pakistan, Bangladesh and Afghanistan and now widely distributed in most tropical regions. Moringa leaves are exceptionally rich in pro-vitamin A, vitamins B and C, iron, and several amino acids and have received attention for its effects on the health and commercial performance of goats, sheep, cows, and laying hens as a supplement to animal diets. However, little information is available concerning the effects of adding a preparation of twigs and rachises to the diets of animals. Therefore, for the work reported herein, we evaluated the effects of a moringa twig and rachis preparation on milk production, fatty acid (FA) composition, and plasma antioxidants of lactating Holstein cows.

Materials & methods Sixty-four multiparous Chinese Holstein dairy cows in mid-lactation stage (DIM=120±8, milk yield=31.9±1.2 kg day^{-1}) were randomly assigned to one of four dietary treatments, and each was offered a basal diet containing 0, 3%, 6% or 9% (w/w) of the moringa (*Moringa pterygosperma Gaertner*) supplement. All diets were isoenergetic, isonitrogenous, and met the nutritional requirements of lactating dairy cows. The cows were fed the experimental diets for 9 weeks, which included an initial 2-week adaptation period and were fed twice daily at 07.00 and 17.00 hours and milked two times daily at 06.00 and 16.00 hours. Milk samples were analysed for protein fat, lactose and total solid content by infrared spectroscopy according to the standard IDF 141C 2000, and somatic cell counts (SCC) by automatic counter. The concentration of milk urea nitrogen (MUN) was determined using the diacetyl monoxime binding assay described by Wang (2010). Blood samples were collected 3 h after feeding on day 1 of weeks 3, 5, 7 and 9. The plasma samples were used for the analysis of glucose (GLU), nonesterified FA (NEFA), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidise (GSH-Px) and total antioxidant capacity (TAOC). All results were analysed by the MIXED procedure that statistics as a randomised design with the treatments as the random factor and each cow as the fixed factor. The mean separation test was performed using the Tukey multiple range test. Orthogonal polynomial contrasts were then usedto determine linear and quadratic responses to moringa supplement levels.

Results Milk yield, fat concentration and energy corrected milk (ECM) increased in cows receiving the moringa supplement (quadratic, P <0.05). A 25% decrease in the SCC was observed for cows fed the 6% (w/w) moringa supplement compared with control ones (P <0.05, Table 1). The glutathione peroxidise concentration, plasma superoxide dismutase and total antioxidant capacity increased in the milk of cows fed the moringa supplement (linear and quadratic, P <0.05, Table 2).

Table 1. Effects of the moringa supplement on the DMI of the dairy cows, and their milk yield and composition.

Variable	Moringa supplement				SEM	*P*-value	
	0	3%	6%	9%		Linear	Quadratic
DMI, kg/d	20.6	20.9	20.7	19.3	0.53	0.09	0.08
Milk yield, kg/d	29.6^{b}	29.7^{b}	31.0^{a}	28.6^{b}	0.46	0.46	0.04
ECM	31.7^{b}	32.2^{b}	34.5^{a}	31.4^{b}	0.57	0.69	0.01
Milk composition, g kg^{-1}							
Fat	35.4^{b}	36.2^{b}	38.2^{a}	36.8^{ab}	0.66	0.04	0.03
Protein	36.5	37.0	36.6	37.0	0.31	0.49	0.78
Lactose	51.0	50.8	50.8	50.5	0.26	0.14	0.32
Total solid	130	131	132	131	0.80	0.13	0.11
Urea nitrogen	132	135	130	131	1.59	0.32	0.43
SCC, ×1,000/ mL	65.8^{b}	64.3^{b}	49.6^{c}	82.7^{a}	5.82	0.22	<0.01

Table 2. Effects of the moringa supplement on the plasma metabolic variables of the dairy cows.

Variable	Moringa supplement				SEM	*P*-value	
	0	3%	6%	9%		Linear	Quadratic
GLU, mmol/L	3.23	3.25	3.15	3.19	0.04	0.24	0.49
NEFA, µmol/L	111	125	112	120	4.41	0.46	0.66
MDA, nmol/mL	3.21	3.20	2.72	2.90	0.26	0.06	0.08
SOD, U/mL	64.0^{b}	65.8^{ab}	67.9^{a}	68.2^{a}	0.69	<0.01	<0.01
GSH-Px, U/mL	74.8^{b}	83.8^{a}	85.9^{a}	83.6^{a}	0.55	<0.01	<0.01
TAOC, U/mL	1.65^{b}	1.71^{b}	1.89^{a}	1.94^{a}	0.02	<0.01	<0.01

Conclusion Addition of a *Moringa rachis* and twig supplement to the feed of dairy cows increased their milk yield and milk fat content. The moringa supplement enhanced the plasma antioxidant capacity of the cows, which may help in disease prevention. A 6% (w/w) moringa supplement in cow diet was sufficient to result in perceptible changes. We conclude that supplementation with our moringa preparation is beneficial for milk yield and plasma antioxidant capacity.

Acknowledgements This study was supported by the Century Love International Investment Group Co. Ltd.

References

Chouinard PY, Le'vesque J, Girard V and Brisson GJ 1997. Journal of Dairy Science 80, 2913-2924.
Wang C, Liu HY, Wang YM, Yang ZQ, Liu JX and Wu YM 2010. Journal of Dairy Science 93, 3661-3670.

The farm-to-table continuum as an innovative trade-off approach for optimal management decisions in the beef sector: flavour clustering of young bull meat cuts.

Mohammed Gagaoua, Brigitte Picard, Valérie Monteils

Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, Theix, 63122, France

E-mail: mohammed.gagaoua@inra.fr

Take home message Powerfulness of decision trees for beef flavour categorization using rearing factors and carcass data.

Introduction Consumer studies had shown that once variation in beef tenderness is controlled, flavour becomes the most important driver of eating satisfaction (Killinger *et al.,* 2004). However, most of the studies have focused mainly on tenderness and little has been done on flavour. Accordingly, we hypothesise that a more thorough understanding of the farm-to-table continuum leads to produce consistent flavourful beef. Thus, this study on young bulls aims to use both machine learning and decision tools to predict and cluster beef flavour using rearing factors and carcass traits.

Materials & methods A total of 308 young bulls from the main French beef breeds were used in this trial. Data of animal characteristics [Age at slaughter], fattening period [Duration (days), Initial body weight (BW, kg), Final BW, %Forage, %Concentrate, dry matter intake (DMI) (kg DM/day), Energy intake (Mcal/day), BW gain (kg/d) and Feed efficiency (kg/kg DM)] and carcass characteristics [%Dressing, Carcass weight (kg), Carcass fat weight (kg), Carcass muscle weight (kg), EUROP conformation score and Fatness carcass score (1 – 5 scale)] were recorded (Soulat *et al.*, 2016; Gagaoua *et al.*, 2017). Meat samples aged for 14 days were assessed for beef flavour (0 – 10) using a sensory panel (Gagaoua *et al.*, 2016). For the identification of flavour classes, unsupervised learning methods (k-means and hierarchical cluster analysis (HCA)) were performed. Among them and based on Silhouette widths (*Si*), HCA gave the best results and lead to 3 clusters that were named flavourful (FLAV+), medium (FLAV=) and less flavourful (FLAV–). Then, the frequently used decision tree algorithms (C&RT, CHAID and QUEST) were performed to categorize the beef cuts using carcass and rearing factors based on HCA clusters. The best decision tree was obtained by Chi-squared Automatic Interaction Detection (CHAID) method.

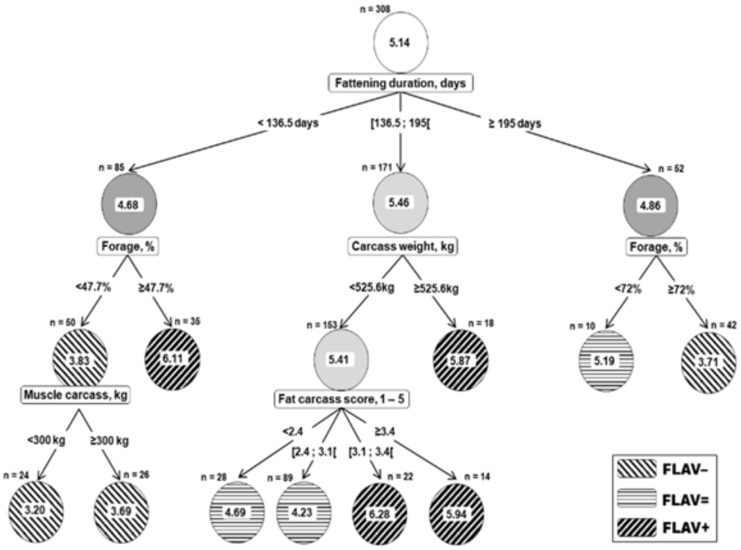


Figure 1 Best decision tree built using both carcass and rearing factors, which predicted 79.54% the HCA flavour's classes.

Results & discussion The prediction accuracy was 79.54% by retaining 5 variables: Fattening duration, %Forage, Carcass weight, fat carcass score and muscle carcass (Figure 1). In line to our results, recent studies showed that fattening duration has significant role in beef qualities (Soulat *et al.,* 2016; Picard *et al.,* 2017). The use of fattening duration combined with forage amount or with carcass weight are likely to categorize FLAV–, FLAV= and FLAV+ clusters and allows the identification of 54 FLAV+ cuts. Then, carcass weight <525.6kg with fatness scores greater than 3.1 identify the remaining 36 FLAV+. These results indicate the possibility to achieve the desirable beef flavour, using different strategies and combinations of both rearing factors and carcass characteristics. By doing so, decision trees would be easily implemented to categorize beef cuts for their flavour and propose strategies at the farm and slaughterhouse levels.

Conclusion These results showed the possibility to properly cluster young bulls' carcasses for their flavour potential using carcass characteristics and rearing practices factors. This would be beneficial at both the economic and consumer levels.

Acknowledgements This study is conducted under project S3-23000846 funded by ARA Region and FEDER.

References

Gagaoua M, Micol D, Picard B, Terlouw CE, Moloney AP, Juin H, Meteau K, Scollan N, Richardson I and Hocquette JF 2016. Meat Science 122, 90-96.
Gagaoua M, Monteils V, Couvreur S. & Picard B 2017. Journal of Agricultural and Food Chemistry 65, 8264-78.
Killinger KM, Calkins CR, Umberger WJ, Feuz DM and Eskridge KM 2004. Journal of Animal Science 82, 3294-301.
Picard B, Al Jammas M, Couvreur S, Pécot M, Aminot G, Leger S, De Koning L, Valais A and Bonnet M. 2017. In: Proceedings of the 63rd International Congress of Meat Science and Meat Technology, pp. 819-820, Cork, Ireland.
Soulat J, Picard B, Leger S and Monteils V 2016. Journal of Animal Science 94, 1712-26.

Saliva samples as non-invasive proxies to determine the rumen 18-carbon fatty acid composition of dairy cows suffering or not from subacute ruminal acidosis

Lore Dewanckele[1], Longhui Jing[1], Barbara Stefanska[2], Bruno Vlaeminck[1,3], Wilfried Van Straalen[4], Ant Koopmans[4], Veerle Fievez[1]

[1]Laboratory for Animal Nutrition and Animal Product Quality, Ghent University, Ghent, Belgium, [2]Poznan University of Life Sciences, Poznan, Poland, [3]Research Group Marine Biology, Ghent University, Ghent, Belgium, [4]Schothorst Feed Research B.V., Lelystad, The Netherlands

E-mail: lore.dewanckele@ugent.be

Take home message Saliva samples seem not to be a good alternative to predict the rumen fatty acid composition in dairy cows. However, a direct comparison study is needed to confirm this.

Introduction High starch diets or a low rumen pH can affect milk fat yield and composition through alterations in ruminal biohydrogenation (BH) of 18-carbon fatty acids (FA; Harvatine *et al.*, 2009). To investigate the interaction between diet, rumen metabolism, and milk fat, it is of interest to assess the rumen FA composition. Rumen sampling can be carried out by oral intubation, by rumenocentesis or via a ruminal cannula. However, these methods are less desirable in terms of animal welfare and impractical for sampling large numbers of animals. As ruminants regularly regurgitate large amounts of rumen material to their oral cavities, oral samples may be an alternative to rumen sampling. In this respect, research revealed that buccal swabs may be used as a non-invasive sampling for analysis of rumen microbial communities (e.g. Tapio *et al.*, 2016). This study aimed at investigating the saliva FA composition of dairy cows which were subjected to a subacute ruminal acidosis (SARA) challenge by increasing the amount of rapidly fermentable carbohydrates (RFCH) in the diet.

Materials & methods Nine Holstein-Friesian dairy cows were fed a total mneuixed ration *ad libitum* with a limit of residual feed intake to prevent cows from feed selection. The experiment lasted for 28 days and was divided in 4 periods based on the RFCH proportion in the diet: i/ adaptation to the experimental diet (d0-4), ii/ low RFCH (d5-18), iii/ increasing RFCH (incr; d19-24) and iv/ high RFCH (d25-28). To increase the RFCH proportion, a standard concentrate was gradually and partly replaced by a concentrate high in RFCH during the increasing period to remain at a constant level during the high period. The reticular pH was measured every 10 minutes using a SmaXtec Premium bolus (SmaXtec animal care GmbH, Austria). Cows were divided into 2 groups based on their reticular pH behaviour during the increasing period: tolerant (n = 4) and sensitive (n = 5) cows. Saliva was collected using a sponge on days 13, 14, 20, 21, 22, 25, 27 and 28 to analyse proportions of 18-carbon FA. Data were analysed using the MIXED procedure of SAS.

Results & discussion Sensitive cows suffered from SARA throughout the whole experiment (pH < 6 for more than 6 h/d; Neubauer *et al.*, 2017), whereas tolerant cows did not. Proportions of 18:0 and 18:1 in saliva were higher ($P = 0.047$) and lower ($P = 0.004$), respectively, in tolerant cows compared with sensitive cows (Table 1). This might indicate inhibition of the last BH step in sensitive cows, resulting in higher amounts of intermediates. Based on differences in pH, and in milk and plasma FA composition (data not shown), higher *trans*-10 and lower *trans*-11 proportions were expected i/ during the high period in both groups and ii/ in sensitive cows compared with tolerant cows. However, this was not observed in saliva.

Table 1 Reticular pH parameters and proportions of 18-carbon FA (g/100 g C18) in saliva from tolerant and sensitive cows to increasing amounts of RFCH in the diet during different periods.

	Tolerant (n = 4)			Sensitive (n = 5)				*P*-value		
Item	Low	Incr	High	Low	Incr	High	SEM[1]	Group (S)	Period (Pe)	S × Pe
Time pH < 6 (h/d)	0.04	< 0.01	0.01	7.54	10.99	6.99	0.876	< 0.001	0.090	0.082
18:0	32.32	34.08	32.25	27.01	23.10	27.64	4.187	0.047	0.937	0.683
18:1	31.38	32.27	32.35	35.14	37.82	36.48	1.806	0.004	0.639	0.871
trans-11[2]	8.15*	3.95	4.76	10.24*	2.24	3.24	1.711	0.703	0.018	0.260
trans-10[2,3]	3.52	0.94	1.75	4.49	0.87	1.73	1.137	0.729	0.128	0.444

[1] SEM, standard error of the mean. [2] *trans*-11, *trans*(*t*)-11 18:1 + *cis*(*c*)-9, *t*11 CLA, + *t*11*c*15 18:2 + *c*9*t*11*c*15 CLnA; *trans*-10, *t*10 18:1 + *t*10*c*12 CLA + *t*10*c*12*c*15 CLnA. [3] Reported *P*-values are based on the log transformation of the respective parameter. * Means within a row annotated with this symbol differ significantly from the other periods ($P < 0.05$).

Conclusion Proportions of particular saliva FA (i.e. 18:0 and 18:1) were different between sensitive and tolerant cows. However, proportions of total *trans*-11 and *trans*-10 intermediates in saliva did not reflect differences which were expected based on reticular pH and milk and plasma FA proportions. This suggests that saliva, collected using the current sampling procedure, seems to not be a good alternative to predict the rumen FA composition of dairy cows. However, direct comparisons between rumen and saliva samples are needed to confirm these results.

Acknowledgements PhD research of Lore Dewanckele is financed by the Special Research Fund of Ghent University.

References

Harvatine KJ, Boisclair YR and Bauman DE 2009. Animal 3, 40-54.

Neubauer V, Humer E, Kröger I, Braid T, Wagner M and Zebeli Q 2017. Journal of Animal Physiology and Animal Nutrition 102, 343-349.

Tapio I, Shingfield K J, McKain N, Bonin A, Fischer D, Bayat AR, Vilkki J, Taberlet P, Snelling TJ and Wallace RJ 2016. PLoS ONE. 11, e0151220.

Exploring the effects of dietary lipid content and digestibility on lipophilic contaminants transfer from feed to milk in dairy cows: insights from a physiologically-based toxicokinetic modelling approach

Sylvain Lerch[1], Olivier Martin[2], Agnès Fournier[1], Jérôme Henri[3]

[1]*Université de Lorraine / INRA, Vandoeuvre-lès-Nancy, Lorraine, France,* [2]*INRA / AgroParisTech, Paris, Ile-de-France, France,* [3]*Anses, Fougères, Bretagne, France*

E-mail: sylvain.lerch@univ-lorraine.fr

Take home message A physiologically-based toxicokinetic (PBTK) model describes finely the fate of lipophilic contaminant in dairy cows. It allows handling of the complex interaction between cow nutrition and physiology, and contaminant properties when assessing the risk of contaminant transfer from feed to milk.

Introduction Livestock production faced several lipophilic pollutants (e.g. dioxins, polychlorinated biphenyls…) contamination incidents which compromise consumers' confidence and induce social distress for farmers. To face such hazards, integrative approaches should be developed for assessing the risk of on-farm contamination of ruminant milk and meat. Besides, the fate of lipophilic contaminant in ruminant should be understood and described finely by taking into account both animal physiology and nutrition, and contaminant properties. The aims of this study were to set up a mechanistic physiologically-based toxicokinetic (PBTK) model describing the fate of lipophilic contaminant in dairy cows and further explore the effects of dietary lipid content and digestibility on the feed to milk transfer rate, depending on contaminant lipophilicity and metabolism clearance rate.

Materials & methods The PBTK model consist of two sub-models, one describing dairy cow physiology, and the other contaminant toxicokinetic. The physiological sub-model is based on the dynamic dairy cow model of Martin and Sauvant (2007). It describes digestion processes and dairy cow performance (milk production and body fat dynamics) over lactation cycle depending on milk production potential. Empty body is further divided into blood, liver, perfused and deep adipose tissues, and "other tissues and organs" compartments. In the toxicokinetic sub-model, contaminant is absorbed from digestive contents to blood, distributed by blood to tissues and organs, and eliminated by liver metabolism (clearance rate parameter), or milk or faeces excretions (Figure 1). Transfers between compartments are limited based on blood perfusion rate (MacLachlan, 2009), except those between digestive contents and blood, and perfused and deep adipose tissues, which are diffusivity-limited based on fugacity (McLachlan, 1994).
Dynamic simulations were performed with Modelmaker 4.0 for a cow producing 30 kg/d of raw milk at lactation peak and receiving a contaminated diet (constant concentration) from calving up to 150 days in milk (DIM). Feed to milk transfer rate was derivate from the carry over rate (COR = contaminant excreted in milk / contaminant oral intake) for every combinations between contaminant lipophilicity (octanol/water partition coefficient: log K_{ow} of 6, 7.5 or 9), liver clearance rate (0, 50 or 500 L/h), lipid supplementation level (0, 2.5 or 5% of added oil in total DMI) and digestibility (vegetable or mineral oils, 0.7 or 0 digestibility, respectively).

Results The use of the PBTK model allows to explore the interaction between dairy cow physiology and contaminant properties. Simulations suggest that increase in log K_{ow} from 6 to 9 decreases COR by more than 20-fold, due to diffusive resistance at the absorption step, whereas increase in liver clearance rate from 0 to 500 L/h decreases COR by more than 10-fold. Moreover, increase in fecal lipid excretion due to lipid supplementation reduces COR until 4.5-fold in the case of 5% mineral oil in total DMI for moderately lipophilic (log $K_{ow} \leq 7.5$) and poorly-metabolized (clearance rate ≤ 50) contaminants (Table 1).

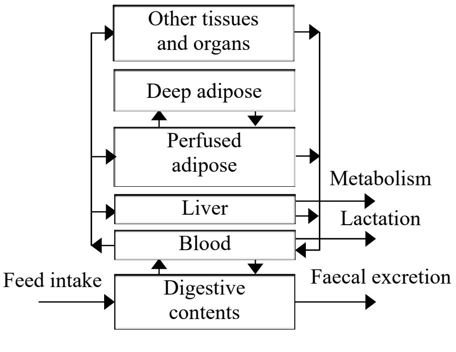
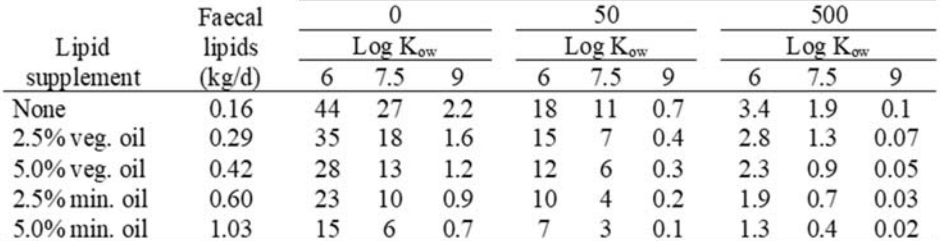


Figure 1 The PBTK model describing lipophilic contaminants fate in dairy cow.

Table 1 Model simulations of carry over rates (COR, %) from feed to milk depending on contaminant lipophilicity and liver clearance, and dietary lipid content and digestibility[1].



		Liver clearance rate (L/h)								
		0			50			500		
	Faecal	Log K_{ow}			Log K_{ow}			Log K_{ow}		
Lipid supplement	lipids (kg/d)	6	7.5	9	6	7.5	9	6	7.5	9
None	0.16	44	27	2.2	18	11	0.7	3.4	1.9	0.1
2.5% veg. oil	0.29	35	18	1.6	15	7	0.4	2.8	1.3	0.07
5.0% veg. oil	0.42	28	13	1.2	12	6	0.3	2.3	0.9	0.05
2.5% min. oil	0.60	23	10	0.9	10	4	0.2	1.9	0.7	0.03
5.0% min. oil	1.03	15	6	0.7	7	3	0.1	1.3	0.4	0.02

[1] Contaminant exposure from 0 to 150 DIM, COR reported are 140 to 150 DIM averages

Conclusion Use of a mechanistic PBTK model suggest that lipophilic contaminant transfer rate from feed to milk could be decreased by dietary supplementation with non-absorbable mineral oil.

Acknowledgements This research was supported by grants from the INRA-PHASE Division and the GALA association.

References

MacLachlan DJ 2009. Food Additives and Contaminants 26, 692-712.
Martin O and Sauvant D 2007. Animal 1, 1143-1166.
McLachlan MS 1994. Environmental Science and Technology 28, 2407-2414.

Effects of starch-rich or lipid-supplemented diets that induce milk fat depression on lipid metabolism and methane emissions in Holstein lactating dairy cows

Adeline Bougouin, Cécile Martin, Michel Doreau, Emilie Tixier, Anne Ferlay

Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, Saint-Genès-Champanelle, France

E-mail: adeline.bougouin@inra.fr

Take home message Diets inducing milk fat depression result in very different milk fatty acid profile with similar enteric methane emissions in dairy cows.

Introduction To meet energy requirements of high yielding dairy cows, the supplementation with dietary lipids, or the use of diets with high level of starch from concentrate and/or maize silage, have become common practices for dairy producers (Jenkins and McGuire, 2006). However, these nutritional strategies imply low dietary fibre content and can lead to milk fat depression (MFD). The objectives of this experiment were to study the effects of 4 concentrates with different MFD potentials on milk fatty acid (FA) profile, and on enteric methane (CH_4) emissions in dairy cows fed maize silage-based diets.

Materials & methods Four multiparous lactating Holstein cows (body weight = 621 ± 12 kg, Days in milk = 61 ± 11 days, and milk yield = 30.7 ± 1.8 kg/day) were used in a 4 × 4 Latin square design with 4 periods of 28 d. Four dietary treatments, potentially leading to MFD, were assigned based (dry matter basis) on 56% maize silage, 4% hay, and 40% concentrates rich in: (1) saturated fatty acids (SFA) from Ca-salts of palm oil [Palm]; (2) starch from maize grain and wheat [Starch]; (3) monounsaturated FA (MUFA; *c*9-C18:1) from extruded rapeseed [RS]; and (4) polyunsaturated FA (PUFA; C18:2n-6) from extruded sunflower [SF]. Intake and milk production were measured daily and milk composition and FA profile at the end of each experimental period. Methane production was measured for the last 5 days of each period when cows were housed in open-circuit respiration chambers. Individual daily data were averaged per period before analysis using mixed-effect models with cows as random effect, period and treatments as fixed effects.

Results & discussion Dry matter intake (DMI), milk production, fat and protein contents, and CH_4 emissions (Table 1) were similar among the 4 diets. The milk fat content was negatively correlated to *t*10-C18:1 (r = -0.77; data not shown). We observed a higher SFA concentration with Palm and Starch in comparison to RS and SF ($P < 0.01$; Table 2), and lower milk MUFA and *t*10-C18:1 ($P < 0.001$), while *t*11-C18:1 remained unchanged among diets. Milk total *trans*-FA concentration was greater for SF than for Palm and Starch, with RS being intermediate. Milk total PUFA concentration was the greatest for SF. Milk C18:3n-3 content was higher for RS than SF (data not shown). The MFD seems more severe with SF and RS. Indeed, in agreement with Ferlay *et al.* (2017), we observed with these diets lower numerical milk fat content, a decrease in milk SFA concentration, and a stronger shift from *t*11-C18:1 to *t*10-C18:1 in milk in comparison to Palm and Starch. The SF diet increased more milk *trans*-FA, *t*10-C18:1 and PUFA contents than RS, indicating that rumen biohydrogenation pathways of PUFA differ between these two diets.

Table 1 DMI, milk production and composition, and daily methane emissions in dairy cows fed 4 MFD-inducing diets.

Diets	Palm	Starch	RS	SF	SEM	*P*-value
DMI (kg/d)	18.5	18.4	18.6	18.5	0.65	0.88
Milk yield (kg/d)	25.2	27.3	27.3	27.5	2.19	0.24
Milk fat (%)	2.99	2.94	2.43	2.00	0.332	0.15
Milk protein (%)	3.29	2.93	3.13	3.22	0.170	0.25
CH_4 (g/d)	374	346	354	349	36.0	0.58

Table 2 Milk concentrations of fatty acids (g/100 g total FA) from dairy cows fed 4 MFD-inducing diets.

Diets	Palm	Starch	RS	SF	SEM	*P*-value
ΣSFA	58.2^a	60.6^a	49.0^b	42.4^b	2.41	<0.01
ΣMUFA	35.4^b	32.1^b	42.8^a	46.3^a	2.05	0.01
Σ*trans*-FA	9.0^c	8.4^c	13.7^b	19.3^a	1.24	<0.01
*t*10-C18:1	2.4^c	2.9^c	5.1^b	8.6^a	0.62	<0.01
*t*11-C18:1	1.5	1.3	1.4	2.7	0.54	0.21
ΣPUFA	4.5^b	5.2^b	5.1^b	8.3^a	0.51	<0.01

Conclusion Diets inducing MFD led to numerically different milk fat contents, which were strongly negatively correlated to *t*10-C18:1 concentration, without modifying CH_4 emissions. Milk FA profile from cows fed RS showed better nutritional value with the decrease in SFA content and the higher C18:3n-3 content.

Acknowledgements This study was supported by Adisseo France SAS, Agrial, APIS-GENE, Deltavit, DSM Nutritional Products AG, IDELE, INRA, Lallemand, Moy Park Beef Orléans, Neovia, Techna France Nutrition, Valorex, and the ANRT with a Cifre grant.

References

Jenkins T C and McGuire M A 2006. Journal of Dairy Science 89, 1302–1310.
Ferlay A, Bernard L, Meynadier A and Malpuech-Brugere C 2017. Biochimie 141, 1-14.

Effects of forage to concentrate ratio on the lactational performances of high- and mid-yielding dairy ewes in early-lactation

Abdelaali Elhadi[1], Gerardo Caja[1], Ahmed A. K. Salama[1,2], Miriam Mendivil[3], Eduardo Durán[3], Elena Albanell[1]

[1]*Universitat Autonoma de Barcelona, Bellaterra, Barcelona, Spain,* [2]*South Dakota State University, Brookings, South Dakota, USA,* [3]*Universidad Nacional Autonoma de Mexico, México D.F., Mexico*

E-mail: gerardo.caja@uab.es

Take home message Concentrate fed to dairy ewes, at rates between 30 and 60%, did not modify milk production nor composition and only affected to body reserves. Optimal rate should depend on desired body condition score of the ewes and price of feedstuffs.

Introduction Milk yield and milk composition are negatively correlated in sheep (Pulina *et al.*, 2006), as in other species. Increasing the rate of concentrate in the diet of dairy ewes allow increasing daily energy intake to cope with requirements, but it might reduce milk fat content whereas increases body fat deposition (Bocquier & Caja, 2001). However, when level of concentrate exceeds 50% DM of the diet, subacute rumen acidosis is expected, which impairs fiber digestion and compromise milk yield and composition (Sutton & Morant, 1989). The effect of forage:concentrate ratio (F:C) on milk quality has been studied extensively in cattle and goats, while it received less attention in dairy ewes (Bocquier & Caja, 2001). The objective of this work is to study the lactational response of dairy ewes to different levels of concentrate (70:30 to 40:60) in the diet of high and mid-yielding dairy ewes.

Materials & methods A total of 72 dairy ewes of 2 breeds, differing in milk yield and composition but similar in frame, were used after the weaning of the lambs. Breeds were Manchega (MN, n = 36, 70.0 ± 1.3 kg BW) and Lacaune (LC, n = 36, 71.5 ± 1.7 kg BW). Ewes were in early-lactation (65 ± 1 DIM) and allocated in 12 groups of 6 ewes by breed, sheltered in straw/wood-chips bedded pens, milked ×2-daily in a 2×12 parlor with electronic milk meters (Delaval, Tumba, SE) and fed a TMR ad libitum and 0.1 kg corn grain at each milking. Experimental design was a factorial 2×3×2 (breed×diet×duplicate). Dietary treatments were done by adding concentrate (0, 0.5 or 1 kg/d, as fed) to a basal diet, and were: HF (high-forage, 70:30; 16.5% CP, 60% NDF, 1.79 Mcal), MC (medium concentrate, 55:45; 15.7% CP, 52% NDF, 1.82 Mcal) and HC (high concentrate, 40:60; 15.2% CP, 45% NDF, 1.84 Mcal; on DM basis). The HF diet was fed to all groups during a pre-experimental period of 4 wk, to be used as covariate, followed by the experimental period (wk 5 to 8). Milk recording was done at each milking and milk was sampled (wk 3 and 7) for composition (NIR system; Foss, DE). Data were analyzed using Proc MIXED of SAS v.9.4 (SAS Inst. Inc., Cary, USA).

Results No differences in total intake were detected by treatment (Table 1), but forage intake decreased 28 and 37% in LC and MN ewes, respectively, by F:C ($P < 0.05$). Milk yield varied by breed (MN *vs.* LC, 1.46 ± 0.09 *vs.* 2.12 ± 0.22 kg/d; $P < 0.001$), but no yield nor composition vary by treatment ($P = 0.967$ to 0.238). Nevertheless, milk fat content decreased numerically in HF *vs.* HC in both breeds (MN, −8%, $P = 0.238$; LC, −5%, $P = 0.530$). No effect was detected between HF and MC diets. Body weight and BCS markedly increased in the LC ewes (HF-MC *vs.* HC, 2.7 *vs.* 4.6 kg BW; $P = 0.015$), but not in the MN ewes. No effect of concentrate was detected on feces consistency, although urinary pH tended to decrease (MN, $P = 0.235$; LC, $P = 0.095$) and fecal pH decreased ($P < 0.001$) when the concentrate increased.

Table 1 Effects of forage to concentrate ratio in two breeds of dairy ewes in early lactation.

Breed	Item	70:30	55:45	40:60	SEM	*P* =
Lacaune	Total intake, kg DM/d	2.96	3.00	3.08	0.19	0.823
	Forage intake, kg DM/d	2.01^{a}	1.76^{ab}	1.45^{b}	0.10	0.060
	Milk, kg/d	2.17	2.10	2.10	0.22	0.967
	Fat, %	6.60	6.61	6.25	0.25	0.530
	Protein, %	5.76	5.81	5.81	0.13	0.951
Manchega	Total intake, kg DM/d	2.59	2.54	2.58	0.10	0.912
	Forage intake, kg DM/d	1.74^{a}	1.38^{b}	1.10^{b}	0.07	0.016
	Milk, kg/d	1.43	1.41	1.54	0.09	0.532
	Fat, %	8.14	8.35	7.46	0.38	0.238
	Protein, %	6.29	6.66	6.30	0.22	0.393

Conclusion The use of concentrate, between 30 and 60%, had no effect on milk yield and milk composition of dairy ewes, producing remarkable BW gains during lactation. On the feeding practice, the final decision on the amount of concentrate to be used will be dependent on the feedstuffs prices and the already achieved BCS of the ewes.

Acknowledgements Project MINECO Spain (AGL2015-69435-C3-3-R).

References

Bocquier F and Caja G 2001. INRA Production Animal 14, 129-140.
Pulina G, Nudda A, Battacone G, Cannas A 2006. Animal Feed Science and Technology 131, 255-291.
Sutton JD and Morant SV 1989. Livestock Production Science 23, 219-237.

Comparison of milk polar lipid composition of cows and goats fed various lipid supplements

Hélène Fougère[1], Carole Delavaud[1], Sylvain Emery[1], Justine Bertrand-Michel[2], Laurence Bernard[1]

[1]INRA, Saint-Genès-Champanelle, France, [2]I2MC, INSERM, Université Paul Sabatier, Toulouse, France

E-mail: helene.fougere@inra.fr

Take home message Among milk polar lipids, phosphatidylcholine is differently affected by diets in cows and goats.

Introduction In milk, lipids are secreted in the form of fat globules with a core mainly composed of neutral lipids (triacylglycerides; 98%) and a membrane of polar lipids (PL) (about 1%, mainly phosphatidylethanolamine (PE), phosphatidylcholine (PC), sphyngomyelin (SM), phosphatidylserine (PS), phosphatidylinositol (PI) and ceramide (Cer)). The interest in the milk PL fraction considerably increased due to its potential health benefit and functional properties (Contarini *et al.,* 2013). The whole milk lipid fraction is modulated by addition of lipids to the diet with species-specific responses but the impact of nutrition and ruminant species on the PL fraction is not well documented. The objective of this study was to determine the composition in milk PL in cows and goats fed diets supplemented or not with various lipids sources.

Materials & methods Twelve Holstein cows and 12 Alpine goats were fed a basal diet (45% forage + 55% concentrate) not supplemented (CTL) or supplemented with corn oil and wheat starch (COS; 5% diet dry matter (DM)), or marine algae powder (MAP; 1.5% diet DM) or hydrogenated palm oil (HPO; 3% diet DM) in a replicated 4X4 Latin square design with 28-d experimental periods. Milk samples were collected over 2 consecutive milkings on d24 of each experimental period. Subsamples were pooled by species, period and diets (n=16 per species) and lipid extracted for PL determination by liquid chromatography-ElectroSpray Ionization-QQQ. Data were subjected to ANOVA using the MIXED procedure of SAS with statistical model including species, experimental diet and interaction between species and diets, as fixed effects and animal pool as random effect.

Results Irrespective of diets, milk fat of cows was richer in PE and PC compared to goats (+ 22% and + 40% respectively), whereas milk fat of goats contained more Cer compared to cows (+ 72%). MAP diet decreased PI in both species (mean - 21%; $p < 0.001$) compared to control. In cows, COS diet increased PI (+ 31%, $p < 0.01$) and PC (+ 52%; $p < 0.001$) compared to control whereas these effects are lower in goats (+ 6 and +23% respectively; NS). Due to a high individual variability, in particular among goats, SM was not significantly affected by species or diets even though higher numerical values were obtained in goats compared to cows (Table 1).

Table 1. Relative abundance of polar lipid classes in milk of cows and goats fed diets without additional lipid (CTL), or with addition of corn oil and starch (COS), or marine algae powder (MAP), or hydrogenated palm oil (HPO) (arbitrary units determined as the abundance relative to an internal standard per PL class)

Sum per class	Cows				Goats				p-value[1]			
	CTL	COS	MAP	HPO	CTL	COS	MAP	HPO	SED[2]	Sp	D	Sp × D
Σ PS	24.6	30.0	22.0	32.0	30.3	32.4	24.2	32.7	1.63	0.275	0.019	0.833
Σ Cer	2.04	2.40	2.20	3.04	3.50	5.63	3.30	4.39	0.30	0.006	0.050	0.182
Σ PE	52.1	55.8	59.3	57.3	42.6	44.7	51.0	45.7	1.69	0.005	0.139	0.953
Σ PI	19.2	25.2	16.9	25.1	25.5	26.9	18.1	28.7	1.13	0.094	<0.001	0.175
Σ SM	15.2	21.5	13.8	16.1	36.4	28.4	13.9	23.6	3.65	0.133	0.308	0.488
Σ PC	24.7^{bc}	37.5^{a}	21.4^{bc}	28.8^{b}	17.6^{c}	21.7^{bc}	18.0^{c}	21.0^{bc}	1.19	0.002	<0.001	0.011

$^{a\text{-}c}$Means within a row not sharing a common superscript differ ($P < 0.05$) due to species by diet interactions.
[1]Probability of significant effects due to species (Sp), experimental diet (D), and their interaction (Sp x D).

Conclusion This study is the first one reporting data of PL composition of milk fat in two ruminant species under similar dietary conditions. We provided evidence of differences of PL classes responses according to species and diets. These data, together with analysis of individual molecular PL species, will contribute to better understand the determinant of milk fat secretion processes and control milk fat composition to improve its nutritional quality.

Acknowledgements Lipidomic analysis were performed in Metatoul Platform (Toulouse - France). This research was financed by APIS-GENE under the Nutrilip project.

Reference

Contarini G and Povolo M 2013. International Journal of Molecular Sciences 14, 2808-2831.

Alterations in ruminal biohydrogenation pathways and milk fatty acid composition during diet-induced milk fat depression in lactating cows

Heidi Leskinen, Laura Ventto, Piia Kairenius, Tomasz Stefanski, Kevin Shingfield, Johanna Vilkki

Natural Resources Institute Finland (Luke), Jokioinen, Finland

E-mail: heidi.leskinen@luke.fi

Take home message Under the conditions of this experiment, diet-induced milk fat depression in lactating cows was not clearly associated with biohydrogenation intermediate *trans*-10, *cis*-12 conjugated linoleic acid. The shift in ruminal biohydrogenation towards the *trans*-10 pathway in milk fat depression was accompanied with increases in *trans*-10 18:1 and *trans*-10, *cis*-15 18:2 as well as *trans*-9, *cis*-11 conjugated linoleic acid in omasal digesta and milk fat supporting the theory that these intermediates or other additional mechanisms have a role in the regulation of milk fat synthesis in the bovine mammary gland.

Introduction Diet-induced milk fat depression (MFD) in lactating cows has been attributed to the formation of specific biohydrogenation intermediates of fatty acids in the rumen that directly inhibit lipogenesis (Bauman and Griinari, 2003). However, the mechanisms regulating milk fat synthesis in the mammary gland are not well defined. *Trans*-10, *cis*-12 conjugated linoleic acid (CLA) is the only biohydrogenation intermediate known to inhibit milk fat synthesis, but it is uncertain if its increased ruminal production is the sole explanation of MFD.

Materials & methods Four Nordic Red cows in mid-lactation and fitted with rumen cannulas were used in a 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments and 35-d experimental periods. Treatments were formulated to cause changes in ruminal lipid metabolism and milk fat synthesis and comprised total mixed rations based on grass silage with low (L, 65:35, forage:concentrate, FC) or high (H, 35:65) concentrate diets supplemented with either 0 g (treatments L and H, respectively) or 50 g/kg dry matter of sunflower oil (SO; treatments LSO and HSO, respectively). Milk yield and composition and milk fatty acid composition were determined daily. Spot samples of digesta entering the omasal canal were collected four times daily at 3-h intervals from d 22 to d 24 to obtain a representative sample of the entire feeding cycle. The associations between the progress of MFD, the flow of specific fatty acid intermediates at the omasum and the proportions of these intermediates in milk fat over time were studied to unravel the processes that activate MFD.

Results & discussion HSO treatment induced MFD and lowered (FC×SO interaction $P < 0.01$) milk fat yield from 20.2 to 31.9% relative to H, L and LSO (Figure 1) with concomitant shift in ruminal biohydrogenation. The flow of *trans*-10, *cis*-12 CLA at the omasum was greater ($P < 0.01$) on both H and HSO compared with L and LSO implicating that the availability of *trans*-10, *cis*-12 CLA for absorption was not the only factor causing MFD. Contrary to this, MFD on the HSO treatment was accompanied by an increase in *trans*-10, *cis*-12 CLA in milk fat compared with other treatments (FC×SO $P < 0.05$). Relative to other treatments, HSO resulted in the formation of *trans*-10 18:1 and *trans*-9, *cis*-11 CLA in the rumen, causing increased proportions of these intermediates also in milk (FC×SO $P < 0.05$). The previous evidence that *trans*-10 18:1 and *trans*-9, *cis*-11 CLA inhibit milk fat synthesis is inconsistent, but the present results support the theory that these intermediates have a role in the regulation of milk fat synthesis. Biohydrogenation product *trans*-10, *cis*-15 18:2 was found in omasal digesta and milk fat from HSO providing more support to the existence of alternative pathway of 18:3n-3 metabolism in the rumen that is pronounced in cows fed diets causing MFD.

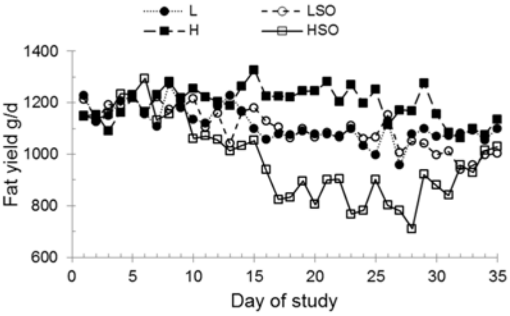


Figure 1 Temporal changes in milk fat yield. Cows were fed the experimental diets for 26 d (dotted line) followed by 9 d of washout.

Conclusion MFD was associated with shift in ruminal biohydrogenation towards the *trans*-10 pathway and increases in *trans*-10 containing intermediates in milk. Ruminal *trans*-10, *cis*-12 CLA formation was not accompanied by MFD, suggesting that other biohydrogenation intermediates, such as *trans*-10 18:1, *trans*-9, *cis*-11 CLA and *trans*-10, *cis*-15 18:2, or additional mechanisms contribute to the regulation of fat synthesis in the bovine mammary gland.

Acknowledgements This research was supported by the Finnish Ministry of Agriculture and Forestry, Helsinki, Finland, Valio, Ltd, Helsinki, Finland, Raisio Science Foundation, Raisio, Finland and the August Johannes and Aino Tiura Agricultural Science Foundation, Helsinki, Finland.

References Bauman DE and Griinari JM 2003. Annual Review of Nutrition 23, 203–227.

Adipose tissue transcriptome during transition period in dairy cows with low and high negative energy balance

Namya Mellouk[1], Christelle Ramé[1], Eric Briant[1], Patrice Humblot[2], Theodoros Ntallaris[2], Delphine Naquin[3], Joëlle Dupont[1]

[1]INRA-CNSR-Université François Rabelais Tours-IFCE, Nouzilly, France, [2]Division of Reproduction-Department of Clinical Sciences-Swedish University of Agricultural Sciences, Uppsala, Sweden, [3]Institute for Integrative Biology of the Cell-CNRS-CEA-Université Pari Sud-Université Paris-Saclay, Gif sur Yvette, France

E-mail: namya.mellouk@inra.fr

Take home message Variation in the Energy Balance is linked to regulation of genes involved in lipid metabolism in adipose tissue in dairy cows.

Introduction In dairy cows, genetic selection for milk production within the past 50 years has led to metabolic disorders which may contribute to decrease reproductive performance in dairy cows (Lucy *et al*., 2001). In dairy cows, the energetic cost of milk production during early lactation is greater than energy consumed resulting in a prolonged period of negative energy balance (NEB). The NEB can lead to a mobilization of 50 to 60 kg of fat during early lactation. This effect on adipose tissue is associated with changes in the expression of several key lipid metabolism actors and alterations in hormone profile, including adipokines (e.g leptin, adiponectin and chemerin) which in turn could influence fertility (Mellouk *et al*., 2017). The objective of this study is to investigate the effect of a high and low NEB on biological mechanisms that contribute to adipose tissue functions through the use of RNA-sequencing technology.

Materials & methods We performed a study, included in a European project (Prolific), with two groups of primiparous Holstein dairy cow. The first group is composed of twenty-two Holstein dairy cows fed with a high energy diet (HE) calculated to provide 35 kg of milk per cow per day. The second group includes eighteen Holstein dairy cows fed with a low energy diet (LE) determined to enable a production of 25 kg of milk per cow per day. Adipose tissue biopsies were made at -4, 1 and 16 weeks peripartum (pp) during the first lactation for each animal. We calculated EB for each animal and then we isolated total RNA from adipose tissue samples of three animals with high NEB and three animals with low NEB at -4; 1 and 16 weeks pp. We then performed RNA sequencing in collaboration with a French transcriptomic platform (Imagif, Gif sur Yvette, France). Comparison of the differential genes list and gene networks was made using Ingenuity Pathways Analysis (IPA®).

Results & discussion We have found 977 genes differentially expressed between -4 weeks and 1 week pp and 31 genes differentially expressed between 1 week pp and 16 week pp in low NEB cows. In high NEB cows, a total of 79 genes were found differentially expressed between -4 week pp and 1 week pp and 9 genes were differentially expressed between 1 week pp and 16 week pp. In other hand, 214 genes are differentially expressed at 1-week pp and 510 genes at 16 week pp, between low NEB and high NEB cows. The IPA analysis revealed that most of these genes are involved in lipid metabolism pathway especially fatty acid metabolism. We also noted that a part of the genes is involved in fatty acid metabolism was adipokines.

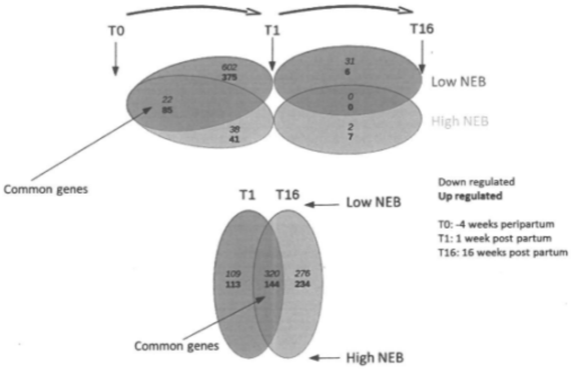


Figure 1 Venn diagram representation of gene differentially expressed according to the time of lactation (energy mobilization) or the status of the EB.

Conclusion Regarding to these results, we showed that the energy status in Holstein dairy cows influences gene expression, particularly those of adipokines involved in fatty acid metabolism in adipose tissue of Holstein dairy cows.

Acknowledgements The research leading to these results has received funding from the European Union Seventh Framework Program (FP7/2007-2013) under the grant agreement n°311776

References

Lucy MC 2001. Journal of Dairy Science 84, 1277-1293.
Mellouk N 2017. Journal of Dairy Science 100, 8518-8533.

The effect of lactation stage on fatty acid profile and sensory properties of Etawah Crossbred goat milk

Yuni Suranindyah, Adiarto Adiarto, Budi Prasetyo Widyobroto, Tridjoko Wisnu Murti Tridjoko Wisnu Murti, Sulvia Dwi Astuti Sulvia Dwi Astuti, Rochijan Rochijan

Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia

E-mail: yuni.suranindyah@ugm.ac.id

Take home message Different stages of lactation significantly affected milk fatty acid profiles of Etawah Crossbred Goat.

Introduction One element that caused goat milk to be less favored by people in Indonesia is the impression of "goaty flavor". According to Kouřimská *et al.* (2014), the specific flavor of goat's milk was formed by short and medium chain fatty acids, consisting of caproic (C6: 0), caprylic (C8: 0) and capric (C10: 0) acids. There was different effects of lactation stage on goat milk short-chain fatty acids. According to Strzałkowska *et al.* (2009), maximum content of short- chain fatty acids in goat milk was reached at the start of lactation, the opposite was observed by, Soryal *et al.* (2005), the highest content of those was reached both at the start and the last stages of lactation. Little was known about the effect of lactation stage on milk composition and the relationship with sensory properties of goat in smallholder. The present study aimed to describe the fatty acid profile which caused goaty flavor and sensory traits of Etawah Crossedbred goat milk in the different lactation stages.

Materials & methods The study used 24 lactating Etawah Crossedbred goats at dairy farms, which were similarity managed at Sleman, Yogyakarta, Indonesia. Milk samples were collected from goats in early (2nd to 3rd) , mid (4th to 5th) and late (longer than 7th) lactation. Milk samples were analyzed in the laboratory for determination of fatty acid profile and sensory properties (taste and flavor) according to methods of gas chromatography and panels tests, respectively. Data were statistically analyzed using ANOVA, and a Chi-Square test.

Results & discussion The study showed there was different fatty acid profiles at the different stages of lactation. The average content of total caproic, caprylic and capric acids in mid and late lactation was higher ($P<0.01$) than in early stage, with value of 10.50, 11.75 and 8.56%, respectively (Table 1). Similar result was found for the content of the sum of caprylic and capric acids and the score of milk sensory. Data agree with Kouřimská *et al.* (2014) that showed that short and medium chain fatty acids determined goaty flavour. In this study longer stage of lactation caused higher content of fatty acids producing goaty flavour and there was significant contribution of caprylic and capric acids. Chilliard *et al.* (2003) indicated that branched- chain fatty acids and high *butyric acid* contents constituted milk flavour. In this study, there was no effect of lactation stage on butyric acid. High score of goaty flavour was in line with high content of caprylic and Capric acids in goat milk.

Table 1. Milk composition, fatty acid profile and sensory properties of goat milk at the different lactation stages.

	Lactaton stage		
Fatty acid (% of total fatty acid)	Early	Mid	Late
Butyric acid (C4:0)	3.82 ± 1.58	3.49 ±2.20	3.69 ± 0.81
Caproic acid (C6:0)	0.93 ± 0.13	1.00 ± 0.08	1.02 ± 0.18
Caprylic acid (C8:0)	1.57 ± 0.27^{a}	1.77 ± 0.25^{ab}	1.98 ± 0.23^{b}
Capric acid (C10:0)	6.06 ± 0.93^{a}	7.74 ± 1.83^{ab}	8.75 ± 1.56^{b}
Medium-chain fatty acids (C8:0-C10:0)	7.63 ± 1.19^{a}	9.51 ± 2.05^{ab}	10.73 ± 1.76^{b}
Total of Caproic, Caprylic and Capric acids	8.56 ±1.31^{a}	10.50 ± 2.02^{ab}	11.75 ± 1.68^{b}
Sensory properties score (flavor, taste)	1.58 and 1.42	1.63 and 1.58	1.67 and 1.52

a, b = in the same row showed significant difference ($P<0.05$), score 1= no flavor; 2 = goaty flavor

Conclusion Medium-chain fatty acid content and goaty flavour of Etawah Crossedbred goat milk in mid and late lactation were higher than in early lactation.

Acknowledgements Faculty of Animal Science Universitas Gadjah Mada, Indonesia for supporting fund in the study.

References

Chilliard Y, Ferlay A, Rouel J and Lamberet G 2003. Journal of Dairy Science 86, 1751-1770

Kouřimská LE. Vondráčková E, Fantová M, Nový P, Nohejlová L and Michnová K 2014. Scientia Agriculturae Bohemica 45, 162–169

Soryal K, Beyene FA, Zeng S, Bah B and Fesfai K 2005. Small Ruminant Research 58, 275-281.

Strzałkowska NA, Jóźwik E, Bagnicka J, Krzyżewski K, Horbańczuk B, Pyzel JO, Horbańczuk A 2009. Animal Science Papers and Reports. 27, 311-320

Inclusion of aerial part and condensed tannins extract from *Cistus ladanifer* L. in lamb diets – Effect on fatty acid composition of subcutaneous fat

Olinda Guerreiro[1,2], Susana P. Alves[2], David Soldado[1], Liliana Cachucho[1], Alexandra Francisco[3], José Santo-Silva[3], Rui J. B. Bessa[2], Eliana Jerónimo[1,4]

[1]Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL) / Instituto Politécnico de Beja (IPBeja), Beja, Portugal, [2]Centro de Investigação Interdisciplinar em Sanidade Animal (CIISA), Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal, [3]Instituto Nacional de Investigação Agrária e Veterinária, Polo de Investigação de Santarém (INIAV-Fonte Boa), Vale de Santarém, Portugal, [4]Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM), Universidade de Évora, Évora, Portugal

E-mail: olinda.guerreiro@cebal.pt

Take home message *Cistus ladanifer* condensed tannins extract improves the fatty acid profile of lamb subcutaneous fat

Introduction Modulation of the ruminal biohydrogenation (BH) has been appointed as an effective approach to reduce saturation in ruminant fat and increase its contents in healthy fatty acids (FA) like polyunsaturated fatty acids (PUFA) and conjugated linoleic acid isomers (CLA). Plants and plants extracts rich in condensed tannins (CT) has been shown to be able to modulate the ruminal BH of dietary unsaturated FA (Vasta and Bessa, 2012). *Cistus ladanifer* L. (rockrose) is a shrub quite abundant in Mediterranean region containing high levels of CT. The aim of the study was to evaluate the effect of two levels of aerial part and CT extract from *C. ladanifer* in lamb diets on FA composition of subcutaneous (s.c.) fat.

Materials & methods Thirty crossbred Merino Branco ram lambs with an average body weight of 19.8 ± 1.86 kg (mean ± s.d.) were randomly assigned to individual pens, and six pens were attributed to each diet. Four diets were formulated considering two levels of *C. ladanifer* CT (1.25 and 2.5% of CT) and two ways of CT supply (*C. ladanifer* aerial part composed by leaves and soft stems *vs.* CT extract obtained from *C. ladanifer* aerial part). Diet without CT sources was also prepared. The five diets were: L – basal diet composed of dehydrated Lucerne supplemented with soybean oil (60 g/kg); CL1.25 – L plus 125 g/kg *C. ladanifer*; CL2.5 – L plus 250 g/kg *C. ladanifer*; Ex1.25 – L plus 20.5 g/kg *C. ladanifer* CT extract; and Ex2.5 – L plus 41 g/kg *C. ladanifer* CT extract. All diets were isoenergetic. Crude protein content was 162, 147, 133, 154 and 155 g/kg DM in L, CL1.25, CL2.5, Ex1.25 and Ex2.5 diets, respectively. The trial lasted for 35 days after an adaptation period of 7 days. Subcutaneous fat lipids were extracted and FA were transesterified and analysed according to Oliveira *et al.* (2016). Data were analysed using the Mixed procedure of SAS, considering diet as main effect.

Results The 18:1 *cis*-9 was the major FA present in s.c. fat (239-294 mg/g total FA, Table 1), followed by 16:0 (213 mg/g total FA) and 18:0 (182-223 mg/g total FA). The 18:1 *cis*-9 and 18:0 in s.c. fat were affected by dietary treatments (P<0.05), with higher levels of 18:1 *cis*-9 in L diet than in CL2.5 and Ex1.25 diets and lower levels of 18:0 in L diet than in CL2.5 and Ex2.5 diets. Vaccenic acid (18:1 *trans*-11) was the predominant biohydrogenation intermediate in s.c. fat (54.1-96.7 mg/g total FA), presenting higher content in Ex1.25 diet than in all other diets (P<0.001). The CLA isomer, 18:2 *cis*-9, *trans*-11 was higher in L and Ex1.25 diets when compared with other diets (P<0.001; 17.2 *vs.* 12.6 mg/g total FA). The content of 18:3*n*-3 was lower in L diet than in CL1.25, Ex1.25 and Ex2.5 diets (P=0.011). Totals of saturated FA (509 mg/g total FA) and PUFA (53.5 mg/g total FA) were not affected by treatments.

Table 1 Effect of *Cistus ladanifer* (CL) and *Cistus ladanifer* condensed tannins extract (Ex) on fatty acid composition fatty (mg/g of total fatty acids) of subcutaneous fat.

Fatty acids[1]	Diets L	CL1.25	CL2.5	Ex1.25	Ex2.5	*P*-value
16:0	214 ± 4.3	214 ± 6.4	211 ± 6.9	215 ± 5.9	209 ± 6.0	0.949
18:0	182 ± 6.9^{b}	199 ± 10.2^{ab}	223 ± 11.1^{a}	200 ± 9.5^{ab}	216 ± 9.6^{a}	0.028
18:1 *cis*-9	294 ± 7.0^{a}	278 ± 10.4^{ab}	239 ± 11.4^{c}	253 ± 9.6^{bc}	270 ± 9.8^{ab}	0.004
18:1 *trans*-11	72.6 ± 4.67^{b}	54.1 ± 6.91^{c}	70.9 ± 7.55^{bc}	96.7 ± 6.42^{a}	67.6 ± 6.53^{bc}	0.001
18:2 *cis*-9, *trans*-11	17.3 ± 0.78^{a}	12.3 ± 1.16^{b}	12.5 ± 1.27^{b}	17.0 ± 1.08^{a}	13.1 ± 1.10^{b}	<0.001
18:2 *n*-6	37.0 ± 2.41	43.7 ± 3.56	37.3 ± 3.89	40.3 ± 3.30	42.0 ± 3.36	0.467
18:3 *n*-3	8.37 ± 0.379^{b}	10.3 ± 0.56^{a}	9.00 ± 0.612^{ab}	9.47 ± 0.520^{a}	10.5 ± 0.53^{a}	0.011
SFA	495 ± 8.0	506 ± 8.7	532 ± 9.2	501 ± 7.9	512 ± 8.0	0.083
PUFA	49.1 ± 2.78	58.2 ± 4.12	50.3 ± 4.50	53.6 ± 3.82	56.5 ± 3.89	0.295

[1]Adjusted for total lipids content; SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; Values are means ± standard error of the mean. Means within a row with different superscript letters are significantly different (P<0.05)

Conclusion Dietary supplementation with 1.25% of *C. ladanifer* CT extract resulted in the highest 18:1 *trans*-11 contents in s.c. fat, suggesting the higher production of this *trans*-FA during ruminal BH than in other diets. Despite the higher availability of 18:1 *trans*-11 to endogenous synthesis of the 18:2 *cis*-9, *trans*-11, in Ex1.25 diet the 18:2 *cis*-9, *trans*-11 content was similar to L diet, which may be due to downregulation of Stearoyl-CoA desaturase (SCD) activity. The SCD activity is intrinsically related with fat deposition level (Bessa *et al.*, 2015), which was low in the present work (Guerreiro *et al.*, 2018, unpublished data). *Cistus ladanifer* CT extract might be a good approach to enhance the nutritional value of ruminant fat, but further studies are needed to ensure the endogenous synthesis of 18:2 *cis*-9, *trans*-11.

Acknowledgements "CistusRumen" project (ALT20-03-0145-FEDER-000023) funded by Alentejo2020 through the FEDER, and FCT by research grants of O. Guerreiro (SFRH/BD/84406/2012) and S.P. Alves (SFRH/BPD/76836/2011).

References

Bessa RJB, Alves SP and Santos-Silva J 2015. European Journal of Lipid Science and Technology 117,1325-1344.
Oliveira MA, Alves SP, Santos-Silva J and Bessa RJB 2016. Animal Feed Science and Technology 213, 64-73.
Vasta V and Bessa RJB 2012. Dietary Phytochem and Microbes. pp263-284

Use of near infrared reflectance spectroscopy and multivariate analysis as a tool for discrimination of suckling lambs according to their geographical origin

Marco Acciaro[1], Carla Manca[1], Corrado Dimauro[2], Marco Caredda[1], Valeria Giovanetti[1], Giuseppe Scanu[1], Giampaolo Epifani[1], Mauro Decandia[1], Giovanni Molle[1]

[1]*AGRIS Sardegna, Olmedo (SS), Italy,* [2]*Università di Sassari, Sassari (SS), Italy*

E-mail: macciaro@agrisricerca.it

Take home message NIR spectroscopy combined with multivariate techniques is able to differentiate suckling lamb meat according to their geographical origin.

Introduction The traceability of food, because of the various food scares concerning meat and the greater health awareness, is considered an essential element for the consumers. Currently, classification of meat products, according to the geographical origin, is based on various analytical methods that are time-consuming and require complex pre-treatment (Sun *et al.* 2012). In contrast, the use of near infrared spectroscopy (NIRS), coupled with chemometrics, is a promising way to obtain, quickly, at low cost and in a non-destructive way, trustworthy results (Zhao *et al*. 2013). In this preliminary study, the feasibility of NIRS combined with a chemometric method for quickly tracing the geographical origin of lambs was investigated.

Materials & methods Lamb carcasses (N =313), collected from different geographic areas (Sardinia, Sa 230 carcasses and Sicily, Si 61 (Italy) and Macedonia, Ma 22), were scanned in the diffuse reflectance mode between 4000–10000 cm^{-1}, by applying 4 cm^{-1} resolution. NIRS spectra were collected from the surface of carcasses in coincidence with the 12th thoracic vertebrae, using a NIRFlex N-500 spectrometer equipped with Fiber Optic Solids (Büchi Labortechnik AG, Flawil, Switzerland). Each sample was scanned in triplicate and the mean of three measurements was used for subsequent data analysis. The 313 spectra were exported and analysed using Linear Discriminant Analysis (LDA) to test the hypothesis of whether geographical origin of lambs (groups) could be traced back on the basis of the available set of predictors (wavelengths, cm^{-1}). Data were randomly split in two subsets of 221 and 92 samples, respectively as training and validation sets, The training database was first submitted to a stepwise analysis, specifically conceived to select the subset of variables that best discriminate groups. The selected variables were then submitted to LDA. The effective separation between groups was measured with the Mahalanobis' distance and the corresponding Hotelling's T-squared test (Dimauro *et al.*, 2013). The Total Error Rate (TER) was expressed as the total number of misclassifications over the total sample size, estimated in both the training and validation set (Biffani *et al.*, 2015). All statistical analyses were developed by using the R software (R version 3.3.2).

Results Out of 1501 initial wavelenghts that form the spectrum of each lamb, only 20 were retained at the end of the stepwise analysis. The LDA pointed out the existence of three distinct groups, as evidenced by the Hotelling's T-squared test, highly significant for all the Mahalanobis' distances ($p < 0.0001$), with 4.07% and 8.70% TER for the training and validation sets, respectively. Errors in assignment more frequently involved lambs from Sicily and Sardinia as confirmed by the Mahalanobis' distances between the three geographic areas (Table 1), where the lower distance was between the Sardinian and Sicilian lambs. Figure 1, in line with the Mahalanobis distances, shows a clear separation between Sa and Ma groups and Si and Ma groups, whereas Sa and Si groups were partially overlapped (Table 1). This result is partially expected taking into account that the lambs raised in Sardinia and Sicily are probably submitted to closer environmental conditions and the Sicilian breed is sometimes crossbred with the Sarda one.

Table 1 Mahalanobis' distances among group centroids of geographical areas

Lambs origin	Sardinian	Sicilian	Macedonian
Sardinian			
Sicilian	0.100		
Macedonian	0.943	0.679	

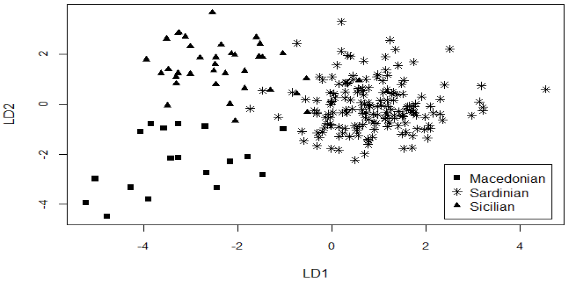


Figure 1 Score plot of the two linear discriminant functions (LD1 and LD2) of 221 lambs from 3 geographic areas (Sardinia, Sicily and Macedonia).

Conclusion. These results confirm the potential of NIRS fingerprints, combined with the LDA chemometric methods, to differentiate, in a rapid and non-destructive way the carcasses of suckling lambs from Italy and Macedonia regions. Further analyses will be needed to confirm these results and to identify the most relevant compounds that allowed us to discriminate the different geographical origin of lambs.

References

Biffani S, Dimauro C, Macciotta N, Rossoni A, Stella A and Biscarini F 2015. Genetics Selection Evolution 47, 4.
Dimauro C, Cellesi M, Steri R, Gaspa G, Sorbolini S, Stella A and Macciotta NPP 2013. Animal Genetics 44, 377–82.
Sun S, Guo B, Wei Y and Fan M 2012. Food Chemistry 135, 508–514.
Zhao H., Guo B., Wei Y and Zhang B 2013. Food Chemistry 138, 1902–1907.

Tall fescue, a possible alternative to timothy silage in dairy cow rations

Gaëtan Tremblay[1], Anne-Marie Richard[2], Rachel Gervais[2], Gilles Bélanger[1], Édith Charbonneau[2]

[1]*Agriculture and Agri-Food Canada, Québec, QC, Canada,* [2]*Université Laval, Québec, QC, Canada*

E-mail: gaetan.tremblay@agr.gc.ca

Take home message For lactating dairy cows, tall fescue silage or haylage is a good alternative to timothy silage and feeding mixed grass:lucerne silages rather than sole grass species silages is beneficial.

Introduction Tall fescue (*Schedonorus phoenix* (Scop.) Holub) might be an interesting alternative to timothy (*Phelum pratense* L.) in rations of dairy cows of eastern Canada because of its high nutritive value, its drought and heat resistance, and its good summer regrowth. Tall fescue, however, is not always considered an option because of its perceived inadequate palatability (Easton *et al.*, 1994).

Materials & methods We evaluated the effect of (1) replacing timothy (cv AC Alliance) with tall fescue (cv. Courtenay), offered as sole forage or in association with lucerne (*Medicago sativa* L., cv Genoa); and (2) feeding tall fescue as silage (35% of dry matter, DM) or haylage (55% DM). Dietary treatments consisted of modifying the forage portion of the diet (70:30 forage:concentrate) as follows: (T) 100% timothy silage; (TF) 100% tall fescue silage; (T + L) mixed timothy 55%:lucerne 45% silages; (TF + L) mixed tall fescue 55%:lucerne 45% silages; (TF-H) 100% tall fescue haylage. Fifteen Holstein cows were randomly assigned to treatments in a triple 5 × 5 Latin square design. Treatment periods lasted 21 d with the last 3 d used for data and sample collection. The five dietary treatments were compared using four preplanned contrasts (Table 1).

Results Grass species did not affect DM intake (DMI, 23.5 kg/d), milk yield (27.5 kg/d), and milk fat concentration (4.21 g/100 g) (Sp. preplanned contrast, Table 1). Feeding mixed grass:lucerne silages resulted in higher DMI (24.3 *vs* 22.6 kg/d) and milk yield (28.3 *vs* 26.7 kg/d), but in lower milk fat concentration (4.15 *vs* 4.27 g/100 g) than feeding sole grass species silages (Mix. preplanned contrast). Milk protein concentration was not affected by grass species when mixed with lucerne silage (3.49 *vs* 4.46 g/100 g), but was higher with timothy than tall fescue silage when offered as sole grass species (3.53 *vs* 3.40 g/100 g). Dry matter intake was higher, but milk yield and milk fat concentration were similar and milk protein concentration tended to be lower, when tall fescue silage was fed as compared with tall fescue haylage.

Table 1 Dietary treatment effects on dairy cow performance.

	Treatment[1]					Preplanned contrast, *P* value			
	T	TF	T + L	TF + L	TF-H	Sp.[2]	Mix.[3]	Sp. × Mix.[4]	TF *vs* TF-H[5]
DMI, kg/d	22.6	22.6	24.7	23.9	20.8	0.36	<0.01	0.28	<0.01
Milk yield, kg/d	26.1	27.1	28.6	28.0	26.8	0.62	<0.01	0.11	0.58
Milk fat, g/100 g	4.28	4.27	4.17	4.13	4.23	0.55	<0.01	0.65	0.53
Milk protein, g/100 g	3.53	3.40	3.49	3.46	3.44	<0.01	0.52	<0.01	0.06

[1]T: Timothy silage; TF: Tall fescue silage; T + L: Timothy + lucerne silages; TF + L: Tall fescue + lucerne silages; TF-H: Tall fescue haylage.

[2]Grass species: Timothy versus Tall fescue silages [(T and T + L) *vs* (TF and TF + L)].

[3]Sole grass species silages versus Mixed grass:lucerne silages [(T and TF) *vs* (T + L and TF + L)].

[4]Grass species × Mixed grass:lucerne silages interaction.

[5]Tall fescue silage versus Tall fescue haylage (TF *vs* TF-H).

Conclusion Results show that tall fescue silage represents a good alternative to timothy silage for lactating dairy cows and confirm the beneficial effect of feeding grass species silage in association with lucerne silage. Using tall fescue haylage instead of silage reduced DMI but did not affect milk yield.

Acknowledgements Financial support from “Action concertée Fonds de recherche du Québec – Nature et technologies (FRQNT), Ministère de l’Agriculture, des Pêcheries et de l’Alimentation du Québec (MAPAQ), and Novalait Inc.” is acknowledged. The “Centre de recherche en sciences animales de Deschambault” also contributed to the project.

Reference

Easton HS, Lee CK and Fitzgerald RD 1994. New Zealand Journal of Agricultural Research 37, 405-417.

Effect of milking time, temperature and diet nitrogen level on milk composition of dairy cows and on some milk properties (freezing point, lipolysis and heat stability)

Catherine Hurtaud, Ruben Riosa, Sophie Brégeron, Angèle Suzanne, Nadège Edouard

PEGASE, INRA, Agrocampus Ouest, 35042 Rennes, France

E-mail: catherine.hurtaud@inra.fr

Take home message Cows that received a diet with high nitrogen level produced milk with less lipolysis.

Introduction The originality of this experiment was to take interest in the native properties of the milk which are decisive for future milk consumption or transformation: lipolysis, heat stability and freezing point. We tested the impact of feeding restriction (nitrogen level of diet) and climate parameters (ambient temperature) associated with milking time (morning or evening) on milk composition, structure and native properties.

Materials & methods The experiment, performed in closed and controlled experimental rooms, followed a double Latin 4 x 4 square design, using 4 Prim'Holstein dairy cows, during 4 periods of 3 weeks each and using 4 treatments. We compared 2 levels of nitrogen in the diet (a diet poor in degradable nitrogen,13.5% crude protein, named N-, and a diet rich in degradable nitrogen, 17.5% crude protein, named N+) crossed with 2 levels of ambient temperature (18°C during the whole day, T- or a diurnal peak of 28°C for 7 hours, T+). The first week was used as an adaptation period to the diet (N+ or N-). The temperature factor (T+ or T-) was applied during the second and third weeks. Milk was sampled during 2 days of the second week at each milking (morning and evening).

Results & discussion Milking time had no effect on milk protein content, but milk fat content increased in evening milk (+1.5 g/kg, Table 1). Evening milk contained more unsaturated fatty acids (+0.9 units percent) and had an increased activity of Δ9-desaturase (increase of c9C14:1/C14:0 and c9C16:1/C16:0 ratios). Ferlay *et al.* (2010) found the same results. This difference between morning and evening milking may be related to the intervals between the milkings or to the difference of activity of dairy cows in the day or in the night. In evening milks, freezing point was higher (+0.08 °C), heat stability was lower (-0.6) and lipolysis was higher (+0.42 mEq/100 g fat). The increased lipolysis could be explained by the longer interval between evening and morning milking (14 h) than between morning and evening milking (10 h), as suggested by Bachman *et al.* (1988) who did not find any difference in lipolysis levels between morning and evening milks taken at identical milking intervals (12/12 h).

Table 1 Effects of temperature and nitrogen level on milk physical properties.

	T-		T+		RMSE	T effect	N effect	N×T effect
	N-	N+	N-	N+				
Milk fat globule size, µm	3.89	3.92	3.87	3.97	0.154	0.795	0.079	0.335
Milk casein size, nm	141.6	146.2	139.2	133.1	10.11	<0.001	0.726	0.016
Heat stability	9.8	9.9	10.6	10.1	0.56	0.062	0.528	0.256
Freezing point, °C	-0.527	-0.527	-0.525	-0.531	0.0014	0.360	0.015	0.030
Lipolysis, mEq/100 g fat	0.85	0.75	0.89	0.69	0.162	0.891	0.012	0.345

Temperature had no effect on milk fat and protein contents. T+ decreased milk casein size. T+ significantly increased C7:0, C10:0, C11:0, C14:0, C15:0. T+ tended to increase milk heat stability (+0.4, P=0.062), and had no effect on freezing point and lipolysis. The lack of effect of temperature on lipolysis is surprising because there is often a peak of lipolysis in summer. It could be due to problems with the room temperature regulation system and therefore lower mean temperatures than expected. Nitrogen level had no effect on milk fat and protein contents and on milk fatty acid composition. N+ significantly decreased milk calcium content (-28.5 mg/kg). N+ tended to increase milk fat globule size (+0.06 µm, P=0.079) and had no effect on milk casein size. N+ had no effect on milk heat stability but decreased freezing point especially with T+ (0.06 °C) and lipolysis (-0.15 mEq/100 g fat). This result on lipolysis agreed with Salih and Anderson (1979) who found that diets with high levels of total nitrogen content (18% vs 9%) resulted in a decrease in lipolysis.

Conclusion Diets with high levels of nitrogen seemed to decrease lipolysis. This rather original result should be confirmed with new experiments using more animals. The effects of temperature were disappointing. We confirmed that lipolysis is higher in evening milk with no explanation at that time.

Acknowledgements This research was supported by grants from Ademe (Cortea Urea project) and by Gala association.

References

Bachman KC, Hayen MJ, Morse D and Wilcox CJ 1988. Journal of Dairy Science 71, 925-931.
Ferlay A, Martin B, Lerch S, Gobert M, Pradel P and Chilliard Y 2010. Animal 4, 627-640.
Salih AMA and Anderson M 1979. Journal of Dairy Research 46, 623-631.

455

Milk yield and composition, and milk fatty acid profile in Holstein dairy cows fed a pomegranate peel extract

M. J. Abarghuei[1], Y. Rouzbehan[1], A.Z.M. Salem[2], M.J. Zamiri[3]

[1]Tarbiat Modares University, Tehran, Iran, [2]Facultad de Medicina Veterinariay Zootecnia, Universidad Autónoma del Estado de México,, México, Mexico, [3]Department of Animal Science, College of Agriculture, Shiraz University,, Shiraz, Iran

E-mail: rozbeh_y@modares.ac.ir

Application Dietary supplementation of pomegranate peel extract (PPE) increased milk yield and improved the milk fatty acid profile in Holstein dairy cows.

Introduction Public concern about the use of antibiotics in livestock rations to alter the rumen microbiota has led to the use of natural replacements such as plant secondary metabolites (PSM). These latter were found to have a positive effect on ruminal fermentation parameters, by manipulating bacterial populations involved in ruminal biohydrogenation altering the fatty acid profile of ruminant-derived food products such as milk (Cabbidu *et al*., 2009; Turner *et al*., 2005). Pomegranate peel (PP) is a by-product of pomegranate juice extraction and contains PSM such as polyphenolic compounds, primarily punicalagin and ellagitannins, which can improve ruminal fermentation parameters and inhibit ruminal biohydrogenation of polyunsaturated fatty acids. Therefore, we hypothesized that the inclusion of PP extract (PPE) into the diet would improve milk yield and fatty acid profile. This experiment was carried out to assess the effect of administering three concentrations of water-extracted PPE on milk yield and composition, milk efficiency, and milk fatty acid profile in dairy cows.

Materials & methods Sun-dried PP was extracted at 1g PP/ml of water. The peel was soaked in tap water at 40ºC for 72 h in a closed tank. The contents were then filtered, and the filtrate was stored at 4ºC for further use. Different levels of PPE were mixed with the concentrate part of the diet. Four Holstein cows were used in a 4x4 Latin square design with 28-d periods and 4 treatments which contained 45:55 forage to concentrate ratio and either PPE0 (no extract), PPE400 (400 ml PPE/cow/d), PPE800 (800 ml PPE/cow/d) or PPE1200 (1200 ml PE/cow/d). Dry matter intake (DMI), milk yield and composition were measured (Table 1). Data were analysed according to a MIXED procedure. The model included the treatments and periods as the fixed effects and cow as a random effect.

Results Milk yield, milk fat and protein yield (kg/d), and milk efficiency were increased by inclusion of PPE800 in the diet. Milk from cows receiving PPE800 had significantly lower ω6 fatty acids/ω3 fatty acids ratio and higher content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) than milk from cows that received no PPE.

Table 1 Dry matter intake (DMI, kg/w$^{0.75}$), milk yield (4% FCM, kg/d), milk composition, milk efficiency (kg/kg), and milk fatty acid profile (g/100 g of total fatty acids) of Holstein cows fed PPE supplemented diets.

	Diets					*P*-Value	
	PPE0	PPE400	PPE800	PPE1200	SEM	Linear	Quadratic
DMI (kg/w$^{0.75}$)	4.20	3.84	3.77	4.03	0.135	0.667	0.271
Milk yield	25.2^{b}	28.4^{ab}	29.9^{a}	26.6^{ab}	1.34	0.360	0.049
Fat yield (kg/d)	1.00	1.13	1.12	1.04	0.030	0.439	0.012
Protein yield (kg/d)	0.96^{b}	1.07^{a}	1.11^{a}	1.02^{ab}	0.027	0.119	0.012
Milk efficiency	1.187^{c}	1.372^{ab}	1.383^{a}	1.255^{bc}	0.058	0.435	0.035
Fatty acid							
C18:0	3.46^{a}	2.41^{b}	2.29^{b}	2.83^{ab}	0.219	1.33	0.083
C18:3 *c* (n-3)	0.11^{cb}	0.10^{b}	0.13^{a}	0.11^{b}	0.004	0.046	0.274
C20:3 n-3	0.03^{a}	0.007^{b}	0.03^{a}	0.02^{a}	0.003	0.764	0.023
C20:4 n-6	0.05^{a}	0.02^{b}	0.04^{a}	0.05^{a}	0.004	0.067	0.011
C22:0	0.01^{a}	0.00^{b}	0.01^{a}	0.01^{a}	0.002	0.012	0.014
DHA (C22:6 n-3)	0.006^{a}	0.06^{a}	0.04^{a}	0.01^{b}	0.008	0.575	0.009
EPA (C20:5 n-3)	0.001^{b}	0.05^{a}	0.03^{a}	0.01^{b}	0.007	0.707	0.012
ω6 fatty acids/ω3 fatty acids	7.00^{a}	2.98^{b}	4.20^{b}	6.88^{a}	0.559	0.872	0.001

EPA: eicosapentaenoic acid. DHA: docosahexaenoic acid. Milk efficiency: kg of milk yield/kg of DMI.

Conclusion The results suggest that PPE800 level could improve milk yield and composition, milk efficiency, and the milk fatty acid profile in dairy cows.

References

Cabbidu A, Molle G, Decandia M, Spada S, Fiori M, Piredda G and Addis M 2009. Livestock Science 123, 230-240.

Turner SA, Waghorn GC, Woodward SL, Thomson NA 2005. Proceedings of the New Zealand Society of Animal Production 65, 283-289.

Preliminary data mining of downregulated genes in lactating ewes showing *trans*-10 *cis*-12 CLA- or fish oil-induced milk fat depression

Aroa Suárez-Vega[1], Beatriz Gutiérrez-Gil[1], Pablo G. Toral[2,1], Gonzalo Hervás[2], Juan José Arranz[1], Pilar Frutos[2]

[1]*Universidad de León, León, Spain,* [2]*Instituto de Ganadería de Montaña (CSIC-ULE), León, Spain*

E-mail: p.frutos@csic.es

Take home message Comparison of genes downregulated in lactating ewes showing CLA- or fish oil-induced milk fat depression highlights the involvement of several genes related to mammary lipogenesis.

Introduction Milk fat depression (MFD) is a phenotype shown in dairy sheep supplemented with both marine lipids (fish oil, FO) and *trans*-10 *cis*-12 CLA (an antilipogenic conjugated linoleic acid isomer). Nutrigenomic research on MFD has been mainly focused on the evaluation of candidate genes associated with lipid metabolism. However, the application of high-throughput RNA sequencing approaches to elucidate the functional regulation of complex phenotypes has proven to be very useful as this methodology evaluates all the transcripts present in the studied tissue (Suárez-Vega *et al.*, 2017). This study focuses on common downregulated genes when the mammary gland transcriptome of sheep suffering from fish oil- or CLA-induced MFD is compared to that from control animals.

Materials & methods We used 14 lactating Assaf ewes fed a total mixed ration without supplementation (n=4; Control animals without MFD), supplemented with 2.4% FO (n=4; FO-MFD) or supplemented with 1% of rumen-protected *trans*-10 *cis*-12 CLA (n=6; CLA-MFD). Compared to the Control, decreases in milk fat concentration averaged 36% in FO-MFD and 30% in CLA-MFD. The protocol for milk sampling and total RNA extraction was previously described by Suárez-Vega *et al.* (2015). Independent RNA-Seq bioinformatics workflows were followed to obtain the differentially expressed genes between FO-MFD and Control, and between CLA-MFD and Control. Samples were aligned to the Oar_v.3.1 ovine reference genome using the Oar_v.3.1_r88 annotation with STAR v.2.4.0 (Dobin *et al.*, 2013). The quantification step was performed with RSEM v.1.3.0 (Li and Dewey, 2011), and DESeq2 (Love *et al.*, 2014) was used for differential expression analyses. Genes with a p-adjusted < 0.05 were considered as differentially expressed (DEG). In order to elucidate key genes controlling the MFD phenotype, we looked for common downregulated DEGs identified in the two comparisons: FO-MFD *vs.* Control, and CLA-MFD *vs.* Control.

Results & discussion A total of 237 differentially expressed genes (80 of them downregulated) were found between FO-MFD and Control, whereas 1,528 DEGs (271 of them downregulated) were detected between CLA-MFD and Control. The transcriptomes of the MFD ewes fed diets containing either FO or *trans*-10 *cis*-12 CLA had only 20 downregulated genes in common. After the functional enrichment analysis, all DEGs were clustered in fifteen GO terms. The highest enriched terms were “acyl-CoA metabolic process” (FDR=8.40E-03) and “acid-thiol ligase activity” (FDR=2.79E-05), in the GO-Biological Process and GO-Molecular Function categories, respectively. Among the common downregulated genes, we found genes associated with the activation of acetoacetate to acetoacetyl-CoA (*AACS*), activation with CoA (*ACSS2*, *ACSS3*), de novo synthesis (*ACACA*), and desaturation (*FADS2*) of fatty acids, which suggests that the reduction of milk fat content was mediated by their downregulation. Some genes related to cholesterol synthesis (*MVD, LSS*) were also downregulated.

Conclusion Fish oil- and *trans*-10 *cis*-12 CLA-induced MFD in lactating sheep is underpinning by the downregulation of genes mainly related with mammary lipogenesis.

Acknowledgements This work was supported by the Spanish Ministry of Economy and Competitiveness (MINECO; AGL2014-54587-R and AGL2015-66035-R). P.G. Toral and B. Gutierrez-Gil benefit from Ramón y Cajal research contracts. Cofunding by the European Regional Development Fund is also acknowledged.

References

Dobin, A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M and Gingeras TR 2013. Bioinformatics 29, 15–21.
Li B and Dewey CN 2011. BMC Bioinformatics 12, 323.
Love MI, Huber W and Anders S 2014. Genome biology 15, 550.
Suárez-Vega A, Gutiérrez-Gil B, Klopp C, Robert-Granie C, Tosser-Klopp G, Arranz JJ 2015. Scientific Reports 5, 18399.
Suárez-Vega A, Toral PG, Gutiérrez-Gil B, Hervás G, Arranz JJ and Frutos P 2017. Scientific Reports, 7, 45905.

Effect of feeding cold-pressed sunflower cake on dairy cow milk and rumen fatty acid profiles

Idoia Goiri, Raquel Atxaerandio, Izaro Zubiria, Roberto Ruiz, Aser Garcia-Rodriguez

Neiker, Vitoria-Gasteiz, Spain

E-mail: aserg@neiker.eus

Take home message Cold-pressed sunflower cake modifies milk FA profile in dairy cows obtaining thus a healthier milk.

Introduction In these last years a significant research effort has been directed towards modifying milk fat composition in order to increase the concentration of fatty acids (FA) with positive effects on human health. The use of polyunsaturated FA (PUFA)-rich cold pressed sunflower (SUN) cake (170 g fat kg^{-1} DM) could increase the concentrations of nutritionally interesting FA on milk, yielding a product with a healthier FA profile, such as it has been reported with the addition of sunflower oil (Shingfield *et al*., 2008). Therefore, the objective of the present study was to study the effect of SUN on ruminal FA biohydrogenation and milk FA profile of dairy cows.

Materials & methods 10 dairy cows were used in a crossover design with two treatments (CTR, SUN) and two 63-day experimental periods. Cows were fed total mixed diets. Concentrates were CTR and SUN (230 g kg^{-1} sunflower cold pressed cake), formulated to provide similar amounts of energy, protein and fat. Cows were milked twice daily at automatic milking system, samples of milk were taken for FA analysis. Ruminal samples were obtained using an oesophageal tube and analysed for FA analysis using gas chromatography. Data were analysed using the MIXED procedure of SAS, with fixed effects of concentrate, sequence of treatments and period. Treatment means were separated using a Bonferroni adjustment.

Results & discussion SUN did not affect total saturated FA, but increased C18:0 and decreased C16:0 in rumen and milk (Table 1). SUN increased total monounsaturated FA (MUFA) and Σ *trans* MUFA in rumen, but only increased Σ *trans* MUFA in milk. SUN did not increase Σ PUFA and 18:2n-6 in rumen, but an increase in milk was observed. Σ CLA and *cis*-9, *trans*-11 CLA were not affected by treatment in rumen while SUN increased these FA in milk. This could be explained with the observed increase in trans-11 18:1 in rumen with SUN, which is recognized to be the precursor of endogenous synthesis of CLA via stearoyl-CoA desaturase in ruminant tissues (Palmquist *et al*., 2005). SUN increased n-6 FA, n-3 FA and n-6 FA:n-3 FA in ruminal contents while it only increased n-6 FA in milk.

Table1 Mean effects of feeding sunflower on rumen and milk FA composition of lactating cows.

FA (g kg^{-1} FA)	Rumen				Milk			
	CTR	SUN	SEM	P-value	CTR	SUN	SEM	P-value
16:0	219.4	156.6	5.36	<0.0001	307.1	290.8	6.43	0.0346
18:0	440.1	513.2	7.26	<0.0001	114.8	122.4	2.76	0.0253
Σ SFA	769.4	762.4	8.34	0.4212	672.8	663.3	7.47	0.2376
cis-9 18:1	42.5	40.0	3.71	0.5118	229.4	229.6	6.50	0.9832
18:2n-6	25.0	23.9	2.71	0.6943	17.1	19.3	0.52	0.0036
trans-11 18:1	48.3	55.6	2.61	0.0233	14.4	19.0	1.21	0.0055
cis-9, trans-11 CLA	4.7	4.30	0.80	0.5893	6.5	8.8	0.59	0.0045
Σ MUFA	169.4	189.4	5.88	0.0093	285.4	289.6	7.13	0.5705
Σtrans MUFA	107.1	126.0	3.30	0.0004	25.2	30.5	1.26	0.0031
Σ PUFA	47.7	50.9	4.00	0.4463	33.2	38.1	1.35	0.0065
Σ CLA	9.1	8.0	0.95	0.3063	8.1	10.6	0.69	0.0071
Σ n-6 FA	0.80	1.8	0.18	0.0007	19.4	21.7	0.56	0.0038
Σ n-3 FA	6.6	8.9	0.97	0.0509	5.7	5.9	0.31	0.5584
n-6 FA:n-3 FA	0.12	0.21	0.024	0.0058	3.67	3.86	0.164	0.2794

SFA: saturated fatty acid, MUFA: mono unsaturated fatty acid, PUFA: polyunsaturated fatty acid

Conclusion Concentrate formulated with SUN (230g kg^{-1}) affected ruminal PUFA biohydrogenation, resulting in a different ruminal FA profile. Concomitantly milk FA profile was modified by SUN, obtaining milk with higher PUFA and CLA contents.

Acknowledgements This research was supported by RTA2015-00022-C03-02.

References

Palmquist DL, Lock AL, Shingfield KJ and Bauman DE. 2005. Advances in Food and Nutrition Research 50, 179-217.
Shingfield KJ, Ahvenjärvi S, Toivonen V, Vanhatalo A, Huhtanen, P and Griinari JM 2008. British Journal of Nutrition 99, 971-983

Effect of red clover silage or soybean meal intake by dairy cows on milk equol concentration

Adeline Lefèvre[1], Michel Focant[2], Frédéric Daems[1], Yvan Larondelle[2], Eric Froidmont[1,2]

[1]*Centre wallon de recherches agronomiques, Gembloux, Belgium,* [2]*Université catholique de Louvain, Louvain-la-Neuve, Belgium*

E-mail: a.lefevre@cra.wallonie.be

Take home message Red clover silage is more efficient than soybean meal supplementation to enrich the bovine milk in equol.

Introduction Isoflavones are one of the major classes of phytoestrogens which can bind to estrogen receptors and provide estrogenic or antiestrogenic effects (Setchell *et al*. 2002). After ingestion by the cows, these molecules can be transferred to milk or be transformed by some micro-organisms present in the rumen and in the intestines. Equol, one of these microbial metabolites, is more bioactive than its precursors (daidzein and formononetin) and would have diverse pharmacological properties such as anticarcinogenic or antioxidant activities (Setchell *et al*. 2002). Soybean meal and red clover are important sources of, respectively, daidzein and formononetin in ruminant feedstuffs. This study compared 2 balanced diets for which the supply of these feedstuffs were maximized, on the efficiency to enrich the bovine milk in equol.

Materials & methods 2 groups of 2 cows received 2 iso-dry matter (DM), iso-nitrogen and iso-net energy diets with the same forage:concentrate ratio according to a cross over design. On a DM basis, the first diet (SBM) contained maize silage (9kg/d), ray grass silage (7kg/d), soybean meal (2.7kg/d) and dried sugar beet pulp (1.3kg/d), The other diet (RC) contained also maize silage (9kg/d) but ray grass silage was replaced by red clover silage (7kg/d), and the concentrates contained corn gluten meal (0.95kg/d), dried sugar beet pulp (2.05kg/d) and rapeseed meal (1kg/d). The control diet (SBM) contained only soybean meal as a source of isoflavones while these molecules were only supplied by red clover silage in the other diet (RC). Periods consisted in 15 d for diet adaptation followed by 5 days of measurements: feed intake, milk yield, fat and protein milk (by infrared milk analyzer), milk equol content (method validated by Daems *et al*. 2015). Feed samples were collected to determine the contents of daidzein and formononetin (Daems *et al*., 2016) of each diet and their individual intake.

Results & discussion Feed intake was slightly lower with SBM diet (19.1 *vs.* 19.9 kg DM/j). Only soybean meal (667mg/kg DM daidzein) and red clover silage (764 and 190 mg/kg DM formononetin and daidzein respectively) contained equol precursors. Due to the amounts incorporated in diets (7.0 kg DM red clover silage *vs.* 2.7 kg DM soybean meal) total intake of isoflavones was higher in RC diet (6557 mg/d *vs.* 1890 mg/d – n=4 - $P<0.001$). Diet had no effect on milk production or on fat and protein contents. But consequently, to the intake of isoflavones, the equol content in milk was higher with RC diet than with SBM diet (282.3 µg/kg *vs.* 46.9 µg/kg – n=4 - $P<0.001$). Furthermore, efficiency of isoflavones transformation into equol was also better when cows fed RC diet (1.15 vs 0.70 µg equol/mg intake of precursors – n=4 - $P<0.001$). We also observed a pronounced animal effect on the isoflavones intakes and on the equol content ($P<0.001$).

Conclusion In our experimental conditions, feeding cows with red clover silage or soybean meal as the only source of isoflavones had no effect on milk yield and fat and protein milk contents. But due to its higher isoflavone content, the improved efficiency of their transformation into equol and its ability to be incorporated in higher amount than soybean meal in cattle feeding, red clover silage appeared to be more adapted for increasing equol content in milk than soybean meal.

Acknowledgements This research was subsidised by SPW, DGO3, Namur, Belgium.

References

Daems F, Romnée JM, Lognay G and Froidmont E 2015. Dairy Science and Technology 95, 303-319
Daems F, Romnée JM, Rasse C, Froidmont E, Heuskin S and Lognay G 2016. Chromatographia 79, 711-725
Setchell KDR, Brown NM and Lydeking-Olsen E 2002. Journal of Nutrition 132, 2577-2584

459

Effects of the inclusion of pomegranate by-products in sheep diet on *in vitro* ruminal biohydrogenation

Antonio Natalello[1,2], Gonzalo Hervás[2], Pablo G. Toral[2], Giuseppe Luciano[3], Bernardo Valenti[1], Alejandro G. Mendoza[2], Mariano Pauselli[3], Alessandro Priolo[1], Pilar Frutos[2]

[1]*University of Catania, Catania, Italy,* [2]*Instituto de Ganadería de Montaña (CSIC-ULE), León, Spain,* [3]*University of Perugia, Perugia, Italy*

E-mail: p.frutos@csic.es

Take home message Pomegranate by-products can be used to enhance the content of potentially health-promoting fatty acids in ruminant meat and milk. Specific effects may vary depending on the by-product used.

Introduction The demand for pomegranate (*Punica granatum* L.) juice has led to an increase in the amount of its by-products, which are rich in bioactive compounds. Seeds contain a high concentration of conjugated linolenic acids (CLnAs; especially punicic acid, *c*9*t*11*c*13 18:3), while residual peels and pulps include a considerable amount of tannins. Therefore, different pomegranate by-products might differently affect the ruminal fatty acid metabolism. This study investigated the effect of including three pomegranate by-products in sheep diet on *in vitro* rumen biohydrogenation (BH) of fatty acids (FA), with the final aim of increasing the content of potentially health-promoting FA in ruminant-derived products.

Materials & methods Three cannulated ewes were used as donors of inocula for batch cultures of rumen microorganisms. Four diets were incubated at 39°C for 24 h: a total mixed ration (TMR; control, C), and, on a DM basis, 80% TMR plus 20% of either pomegranate seeds (PS), or pomegranate peels and pulps (PPP) or whole pomegranate by-product (WPB). Incubations were repeated on 3 different days (runs). The FA composition of the digesta was examined by gas chromatography following Shingfield *et al.* (2003) and Toral *et al.* (2017), but with a lower (40ºC) acid-methylation temperature. Data were analysed by one-way ANOVA using the MIXED procedure of SAS, with a model that included the fixed effect of diet and the random effect of run. Means were adjusted for multiple comparisons using Bonferroni's method.

Results & discussion The higher proportion of Δ11,13 CLA and vaccenic acid (VA, *t*11 18:1) in the PS treatment, with an intermediate value in WPB, suggest that they derive from saturation of the CLnAs present in the pomegranate seeds (>60% of total FA). On the other hand, the concentration of rumenic acid (RA, *c*9*t*11 CLA) did not differ between PS, WPB and C treatments, but was significantly greater in PPP, which may suggest that BH was limited in this treatment (Table 1). This is in line with the inhibitory effect of tannins on ruminal biohydrogenation (Vasta and Luciano, 2011; Carreño *et al.*, 2015). The content of these phenolic compounds in PPP (44.7 g tannic acid equivalents/kg DM; compared with 2.4 in the control), could have affected the activity of ruminal microorganisms involved in the biohydrogenation of dietary FA. This hypothesis seems to be supported by the high proportion of linoleic (*c*9*c*12 18:2) and linolenic (18:3n-3) acids found in the PPP treatment. The fact that VA concentration was similar in C and PPP suggests that pomegranate tannins, at least under the conditions of this *in vitro* trial, would mainly impair the first steps of rumen BH.

Table 1 Fatty acid composition of the ruminal digesta (g/100 g FA) after 24 h *in vitro* incubations.

	Diet					
	C	PS	PPP	WPB	SED	P-value
*t*11 18:1	4.54^{c}	6.88^{a}	4.64^{c}	5.11^{b}	0.0833	<0.001
*c*9*c*12 18:2	1.70^{c}	1.11^{d}	2.33^{a}	1.92^{b}	0.0525	<0.001
*c*9*t*11 CLA	0.093^{b}	0.092^{b}	0.162^{a}	0.130^{ab}	0.0141	0.007
Σ Δ11,13 CLA	0.043^{c}	0.694^{a}	0.059^{c}	0.324^{b}	0.0267	<0.001
18:3n-3	0.382^{b}	0.214^{c}	0.440^{a}	0.382^{b}	0.0157	<0.001
*c*9*t*11*c*13 18:3	-	0.431^{a}	-	0.217^{b}	0.0419	0.036
Other Δ9,11,13 18:3	-	1.852^{a}	0.141^{c}	0.757^{b}	0.1641	<0.001
Σ PUFA	2.82^{c}	5.02^{a}	3.73^{b}	4.26^{ab}	0.2088	<0.001

All treatments containing pomegranate by-products showed a higher content of total PUFA than the control, the greatest value being observed in the PS treatment, which was likely due to the amount of CLnAs in the incubated seeds. Tannins in PPP and WPB probably protected dietary PUFA from ruminal BH.

Conclusion The inclusion of pomegranate by-products in sheep diets increased the content of potentially beneficial fatty acids in the ruminal digesta. These lipids might then be transferred to ruminant-derived products.

Acknowledgements This study was supported by the VATC-20160394 research project (IGM, Leon).

References

Carreño D, Hervás G, Toral PG, Belenguer A and Frutos P 2015. Animal Feed Science and Technology 202, 42-51.
Shingfield K, Ahvenjärvi S, Toivonen V, Ärölä A, Nurmela K, Huhtanen P and Griinari JM 2003. Animal Science 77, 165-179.
Toral PG, Hervás G, Carreño D, Leskinen H, Belenguer A, Shingfield KJ and Frutos P 2017. Journal of Dairy Science 100, 6187-6198.
Vasta V and Luciano G. 2011. Small Ruminant Research 101, 150-159.

Sensory characterization of lamb meat from animals fed combining pasture and total mixed ration

María José Urioste[1], Lucia Antunez[2], Victoria Fariña[1], Analía Pérez-Ruchel[1], Gonzalo Fernández Turren[3], José Luis Repetto[3], Cecilia Cajarville[1], José María Arroyo[1]

[1]Departamento de Nutrición Animal, Instituto de Producción Animal, Facultad de Veterinaria, Universidad de la Republica, Ruta 1 km 42.5, San José, Uruguay, [2]Facultad de Química, Universidad de la República, Montevideo, Uruguay, [3]Departamento de Bovinos, Instituto de Producción Animal, Facultad de Veterinaria, Universidad de la Republica, Ruta 1 km 42.5, San José, Uruguay

E-mail: chemaarroyo@gmail.com

Take home message Dietary energy source affected the sensory characteristics of lamb meat from animals raised on combined diets (pasture + total mixed ration).

Introduction Forage-based feeding systems are central in South America for raising lambs. This production system has lower feeding cost and is well perceived by consumers who demand healthy products produced in a 'natural' way (Fraser *et al.,* 2004). It has been reported that consumers generally prefer meat from animals raised on high concentrate diets which are higher in intramuscular fat content and, therefore, more tender (Font i Furnols *et al.,* 2009). In this context, the aim of this study was to assess the sensory characteristics of lamb meat from animals raised on different regime diets: total mixed rations (TMR) and a mix of pasture and TMR.

Materials & methods Eighteen castrated male lambs Corriedale x Ile de France (BW = 29.5 ± 2.1 kg) were assigned by stratified randomization to 3 dietary treatments. Two dietary treatments (Mix_A and Mix_F) involved a mixed diet of alfalfa pasture plus TMR formulated with different energy sources. The energy source used to prepare TMR for Mix_A (amylaceous) was a mix of cereals (ground corn and wheat grain) whereas, defattened corn germ was used to prepare TMR for Mix_F (fibrous). Both TMRs were isoproteic (CP =160 g/kg DM) and isoenergetic, with a starch content of 314 and 141 g/kg DM for Mix_A and Mix_F, respectively. The third treatment (C) consisted of a mix of both TMR in a 1:1 ratio. In both mixed diet treatments, TMRs were offered at 75% of the potential intake and the lambs had additionally access to alfalfa pasture (CP = 226, and NDF = 366 g/kg DM) during 8 h/day. In treatment C, TMR was offered *ad libitum* but, lambs had no access to pasture. After 44 days the lambs were slaughtered and the *longissimus dorsi* of each one was collected and immediately stored at -20º C.

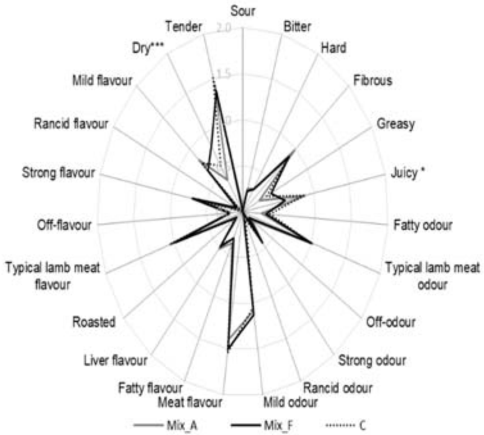


Figure 1. Spider chart summarizing the mean RATA score of each sensory attribute for each lamb meat sample.

The sensory characteristics of lamb meats were assessed using Rate-all-that-apply (RATA) questions (Figure 1). Previous to the sensory evaluation, the lamb meats were cooked following a standardized protocol. The study was carried out with a semi-trained panel in two separate sessions, involving the assessment of nine samples each. The semi-trained panellist received the lamb meat and a list of 23 sensory terms. They were asked to indicate whether the terms from the RATA list applied to describe the lamb meat, and if they did, to rate their intensity using a 3-point structured scale with anchors "low", "medium" or "high". Analysis of variance was performed on the ratings of each attribute considering dietary treatments as fixed effect. Data was analysed using R (R Core Team, 2017).

Results & discussion Regardless of the dietary treatment used for animal feeding, the lamb meats were mainly characterized for being fibrous and tender as well as for their typical lamb meat odour and flavour. Meats were also characterized by their mild odour and meat flavour. However, significant differences among samples were identified in the intensity of two texture attributes: juicy ($P<0.05$) and dry ($P<0.01$). In particular, the lamb meat from animals fed with Mix_F was described to be dryer relative to those from the other two dietary treatments (0.83 *vs.* 0.41 and 0.58 for Mix_A and C, respectively) as well as less juicy (0.59 *vs.* 0.88 and 0.85 for Mix_A and C, respectively). Although exploratory, this result suggests that feeding lambs with diets mainly based on a fibrous energy source caused a significant effect on the texture characteristics of meat.

Conclusion The sensory characteristics of lamb meats from animals fed combining pasture and TMR formulated with an amylaceous energy source were similar to those from a classic TMR feeding system. On the contrary, the use of a fibrous energy source caused a negative impact on texture characteristics.

Acknowledgements This work was supported by the ANII funded project Innovagro FSA_1_2013_1_12561.

References

Font i Furnols M, Realini CE, Guerrero L, Oliver MA Sañudo C, Campo MM, Nute GR, Cañeque V, Álvarez I, San Julián R, Luzardo S, Brito G and Montossi F 2009. Meat Science 81, 196-202.

Fraser MD, Speijers MHM, Theobald VJ, Fychan R and Jones R 2004. Grass and Forage Science 59, 345–356.

NRC 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. Washington, DC: The National Academies Press.

R Core Team 2017. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.

Individual variation in the extent of milk fat depression in dairy ewes: rumen fermentation and biohydrogenation of fatty acids

Pilar Frutos[1], Gonzalo Hervás[1], Álvaro Belenguer[1], Alejandro G. Mendoza[1], Federica D'Aniello[1,2], Stefano Sanna[1,3], Pablo G. Toral[1]

[1]Instituto de Ganadería de Montaña (CSIC-ULE), León, Spain, [2]University of Pisa, Pisa, Italy, [3]University of Sassari, Sassari, Italy

E-mail: p.frutos@csic.es

Take home message Individual variation in the severity of milk fat depression cannot be explained by a single mechanism (*e.g.*, changes in rumen VFA or biohydrogenation metabolites) but most probably by a complex combination of factors.

Introduction Dairy ewes fed diets supplemented with marine lipids show large individual variations in the extent of milk fat depression (MFD) but reasons behind this variability are still uncertain. In a previous study (Frutos *et al.*, 2017), we were not able to demonstrate that differences in the milk concentration of some fatty acids (FA), particularly antilipogenic FA, and in transcript abundances of candidate genes involved in mammary lipogenesis explained individual variations in fish oil-induced MFD severity. It is known that MFD is related to active biohydrogenation (BH) intermediates that are produced under certain feeding conditions that alter rumen function (Bauman and Griinari, 2001). Therefore, we hypothesized that differences in the processes of ruminal fermentation and BH of unsaturated FA would account for the individual response in the susceptibility to the low-fat milk syndrome.

Materials & methods We used 15 lactating Assaf ewes fed a total mixed ration supplemented with 0 (control; n=5) or 20 g of fish oil/kg DM [10 animals were selected out of 22 receiving this supplemented diet and were divided in two groups: ewes showing a strong MFD (RESPON+, n=5) or a slight MFD (RESPON−, n=5); see Frutos *et al.* (2017) for details]. After 36 days on the diets, samples of rumen fluid were collected through stomach tube for pH, ammonia and volatile FA (VFA) concentrations, and for lipid analysis. Ammonia was determined by colorimetry, and VFA and biohydrogenation intermediates by gas chromatography. Data were analysed with the MIXED procedure of SAS 9.4 using orthogonal contrasts [namely, Control *vs.* (RESPON− and RESPON+), and RESPON− *vs.* RESPON+].

Results & discussion After 5 weeks on diets, the decrease in milk fat content, compared with the control, averaged 7.7% in RESPON− and 25.4% in RESPON+. Feeding marine lipids affected all rumen fermentation parameters (pH, ammonia and VFA) but only changes in VFA concentrations were linked to MFD severity ($P \leq 0.05$). Thus, the total VFA content (in mmol/L) fell from 117 in the control to 107 in RESPON− and 92 in RESPON+. Similar decreases were observed in acetate (74 *vs.* 68 *vs.* 58 mmol/L) and propionate (23 *vs.* 21 *vs.* 18 mmol/L) contents for control, RESPON− and RESPON+, respectively, while butyrate concentrations showed comparable reductions in both supplemented groups and molar proportions remained stable. A role of VFA on MFD has been consistently dismissed (see review by Bauman and Griinari, 2001). In fact, despite acetate is the main substrate for *de novo* synthesis of FA in dairy ruminants, it was widely accepted that its supply did not affect milk fat yield. Nevertheless, this has been recently challenged by Urrutia and Harvatine (2017) and would merit further research. Concerning BH metabolites, dietary fish oil had a strong effect on 18:0, with ruminal concentration in animals displaying MFD averaging only 15% of the control value ($P < 0.001$) but without significant variation between RESPON− and RESPON+. Only few minor FA (*e.g.*, *cis*-6+7 16:1 or 17:0 *anteiso*) differed between these treatments ($P < 0.05$). Most *cis* and *trans* 18:1 isomers (*e.g.*, *cis*-9 or *trans*-11 18:1) were favoured in rumen digesta from supplemented ewes, but no differences were found in relation to variability in responsiveness to MFD-inducing marine lipids. As expected, most demonstrated or putative antilipogenic FA (*e.g.*, *cis*-9 16:1, 10-oxo-18:0, *cis*-11 18:1, *trans*-10 *cis*-12 CLA, *trans*-10 *cis*-15 18:2, which coeluted with *trans*-11 *cis*-15 18:2, or 22:6n-3) showed a higher concentration in ewes fed the fish oil-supplemented diet ($P < 0.05$). On the contrary, surprisingly, none of them differed significantly in relation to MFD intensity (*i.e.*, between RESPON− and RESPON+). These results agree with those observed previously about changes in milk FA profile and mRNA abundances of lipogenic genes (Frutos *et al.*, 2017) and suggest that most probably the individual variability in the extent of diet-induced MFD will rely on a complex combination of factors.

Conclusion Rumen concentrations of VFA that are precursors of milk FA, mainly acetate, show lower values in animals with a strong MFD (*i.e.*, in RESPON+). Quite the opposite, changes in rumen BH metabolites show very little variation in ewes displaying different degrees of the syndrome, which would rule out their role as main responsible for MFD severity.

Acknowledgements This work was supported by the Spanish MINECO (AGL2014-54587-R). P.G. Toral benefits from a Ramón y Cajal research contract. Cofunding by the European Regional Development Fund is also acknowledged.

References

Bauman DE and Griinari JM 2001. Livestock Production Science 70, 15-29.
Frutos P, Toral PG and Hervás G 2017. Journal of Dairy Science 100, 9611-9622.
Urrutia N and Harvatine KJ 2017. The Journal of Nutrition. 147 763-769.

How to handle trade-offs between animal performances and sensory and nutritional beef qualities?

Marie-Pierre Ellies-Oury, Muriel Bonnet, Gonzalo Cantalapiedra-Hijar, Marie-Madeleine Mialon, Dominique Gruffat, Brigitte Picard

Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès-Champanelle, France

E-mail: marie-pierre.ellies@inra.fr

Take home message Increasing the energy in diet of finishing Charolais young bulls promotes daily gain, improves carcass composition, fat development and meat nutritional value at the expense of sensory quality.

Introduction To increase their economic return, breeders of late-maturing animals try to shorten production cycles by increasing the energetic value of finishing diets. This feeding practice, which aimed to increase animal performances and average daily gain may affect carcass fatness and meat qualities. The challenge was to identify, thanks to a clustering of variables (Ellies-Oury *et al.,* 2016), phenotypes related to animal performances, meat nutritional value and meat sensory quality that could be modulated simultaneously, in order to manage trade-offs among these traits.

Materials & Methods Twelve Charolais young bulls were fattened with a control or a high-energy diet for up to 750 kg corresponding to a slaughter age of 548 ± 40 days (Mialon *et al.,* 2015). Control and high-energy diets were based on barley straw and concentrated (containing either 1500 or 2000 kcal of net energy per kilogram of dry matter). Animal performances, meat quality traits and nutritional value of *Longissimus thoracis (LT)* muscle were described by 17, 39 and 24 variables, respectively. A clustering of variables was used (ClustOfVar method; Chavent *et al.,* 2012) to establish a total of 11 clusters (4 clusters for animal performances data set; 4 clusters for meat quality traits data set; 3 for nutritional value of meat data set) associated with 11 synthetic indexes (Ellies-Oury *et al.,* 2016). A second clustering, performed on these synthetic indexes, establishes the proximities between the 3 data sets (animal performances, meat nutritional value and meat sensory) and the way to obtain the better trade-off depending on animal diets.

Results & discussion The two finishing diets appears clearly opposed on the two first axis of the Principal Component Analysis (explaining respectively 38,3 % and 18,2 % of the variance). As expected, the high-energy diet induces higher average daily gain, fattened carcasses (fatness score; weight and part of fat in the carcass, weight of fat in the 5th quarter) and higher marbling development in LT (lipids, total fatty acids [FA]) but also a higher growth development than the control diet. High-energy diet led to LT muscles with lower ratio of polyunsaturated FA / saturated FA and lower n-6/n-3 ratio.

The clustering of variables indicates that lower score for sensory descriptors of juiciness, tenderness, flavour are associated with lower abundancy in some proteins considered as tenderness and marbling biomarkers, such as FHL1 (Four and a half LIM domains 1) and TUBA4A (Tubulin alpha-4A chain). On the contrary, some proteins representative of fast glycolytic properties (Activities of metabolic enzymes such as Lactate Dehydrogenase and PhosphoFructoKinase, Myosin heavy chain-I, α-enolase 1, β-enolase 3) appears more abundant. This is in agreement with previous results indicating that in French beef breeds, the LT is more tender when it contains lower abundance of fast glycolytic proteins (Picard *et al.,* 2014).

Conclusion The clustering of variables is an interesting method to evaluate the interactions existing between animal performances and nutritional and sensory qualities of meat. This approach provides an effective tool for integrating different concepts in order to improve simultaneously animal performances and meat quality.

Acknowledgements McKey Food Services, Valorex, and the Regional council of the French "Centre" for "Défiviande" project; together with the staff of INRA-UMR1213 INRA-UE232, INRA-UERT (Theix) and Institut Curie RPPA platform.

References

Chavent M, Kuentz V, Liquet B and Saracco J 2012. Journal of Statistical Software 50, 1-16

Ellies-Oury MP, Cantalapiedra-Hijar G, Durand, D, Gruffat D, Listrat A, Micol D, Ortigues-Marty I, Hocquette J.F, Chavent M, Saracco J and Picard B 2016. Meat Science. 122, 163-172.

Mialon MM, Renand G, Ortigues-Marty I, Bauchart D, Hocquette JF, Mounier L, Noël T, Micol D and Doreau M 2015. Journal of Animal Science 93, 319-333.

Picard B, Gagaoua M, Micol D, Cassar-Malek I, Hocquette JF and Terlow C 2014. Journal of Agricultural and Food Chemistry 62, 9808-9818.

463

Effects of supplementation during mid to late gestation on performance and carcass traits of progeny fed diets with or without high inclusion of rumen-protected fat

Germán Zamudio[1], Marcio Ladeira[1], Wendell Cruz[1], Liziana Rodrigues[1], Flavio Resende[2], Gustavo Siqueira[2], Ana Carolina Santos[1], Tiago Cuissi[1]

[1]Federal University of Lavras, Lavras, Brazil, [2]APTA, Colina, Brazil

E-mail: mladeira@dzo.ufla.br

Take home message High inclusion of calcium-salts reduces intake and gain of steers without effect on feed efficiency

Introduction Maternal nutrition affects muscle and adipose tissue development, which may influence performance and carcass traits of the progeny. Therefore, the objective of this work was to evaluate the effects of grazing Nellore cows supplementation on performance and carcass traits of progeny fed diets with or without high inclusion of rumen-protected fat.

Materials & methods Forty Nellore steers with initial average BW of 340 ± 9.38 kg were allotted in a completely randomized design using a 2 x 2 factorial arrangement, with 10 replicates per treatment. Factors were maternal nutrition and progeny feedlot diets. Therefore, the following treatments were assessed: nutritional management of the dams during gestation without supplementation (NSUPP) or with supplementation (SUPP); and progeny feedlot diets, without rumen-protected fat (NRPFAT), or with rumen-protected fat (RPFAT, 6% calcium salts). Cows supplementation (330 g of CP and 2.11 Mcal/day) started after 124 ± 21 days of gestation. Steers were housed in individual pens for 131 days, and half of the progeny from NSUPP or SUPP cows fed NRPFAT diet (CP = 147 and EE = 33 g/kg DM; ME = 2.82 Mcal/kg) and RPFAT diet (CP = 147 and EE = 81.1 g/kg DM; ME = 3.07 Mcal/kg). To measure average daily gain (ADG), bulls were individually weighed at the beginning and at the end of the experimental period, as well as meals and food leftovers were weighed daily to calculations of dry matter intake (DMI), and feed efficiency (G:F). The steers were slaughtered by captive bolt and exsanguination, followed by hide removal and evisceration to evaluate the following carcasses traits: hot carcass weight (HCW), hot carcass yield, *longissimus* muscle area (LM area), subcutaneous fat and pH_{24h}. All evaluated variables were analysed using Proc GLM of statistical software SAS, adopting $p < 0.05$ for statistically significant difference.

Results & discussion There were no differences in performances and most of carcass traits of the progenies of supplemented cows compared to those not supplemented ($p > 0.05$; Table 1). However, the carcass pH_{24h} was greater in muscle of the progeny from supplemented cows. The pH_{24h} difference may be attributed to an increase in the ratio of glycolytic:oxidative fibers in *longissimus muscle*, which, according to Daniel *et al.* (2007), progenies from undernutrition ewes had greater proportion of this type of fiber. Steers fed rumen-protected fat had lesser DMI, ADG and LM area. In addition, there was a interaction of rumen-protected fat and fetal programming for final BW. Steers fed RPFAT from NSUPP had lesser final BW, while lipid supplementation did not affect final BW the progeny from SUPP cows.

Table 1. Performances and carcass traits of progeny from not supplemented (NSUPP) or supplemented (SUPP) cows and fed diets without (NRPFAT) or with (RPFAT) rumen-protected fat

	NSUPP		SUPP			*p*-value		
Item	NRPFAT	RPFAT	NRPFAT	RPFAT	SEM	SUPP (F)	RPFAT (D)	F*D
Initial BW, kg	340	330	339	350	9.383	0.28	0.99	0.24
Final BW, kg	470	419	459	461	12.63	0.20	0.05	0.04
DMI, kg/d	8.35	6.28	8.13	7.09	0.311	0.33	<0.01	0.09
DMI, %BW/d	2.1	1.7	2.0	1.8	0.057	0.82	<0.01	0.34
ADG, kg/d	0.959	0.662	0.888	0.816	0.059	0.47	<0.01	0.06
G:F, kg/kg	0.116	0.104	0.109	0.113	0.006	0.79	0.51	0.16
HCW, kg	270	243	267	267	7.540	0.17	0.08	0.07
Hot carcass yield, %	57.6	58.2	58.1	58.0	0.497	0.70	0.60	0.51
LM area, cm^2	69.6	61.8	70.3	64.7	2.518	0.47	0.01	0.65
Subcutaneous fat, mm	4.7	4.4	4.9	4.4	0.489	0.78	0.43	0.80
pH_{24h}	5.60	5.55	5.69	5.68	0.042	0.01	0.42	0.57

Conclusion Maternal supplementation of grazing Nellore cows during mid to late gestation had no effect on ADG, feed efficiency and carcass traits of the progeny. However, high inclusion of rumen-protected fat in feedlot diets decreases steers performance.

Acknowledgements We thank for funding support Trow Nutrition, APTA/Colina, Fapemig, CNPq and Capes.

References

Daniel ZCTR, Brameld JM, Craigon J, Scollan ND and Buttery PJ 2007. Journal of Animal Science. 85, 1565-1576.

Field pea can be included up to 30% in the fattening concentrate of lambs, as it had no effect on meat colour and only minor effects on meat tenderness

Mireia Blanco, Guillermo Ripoll, Isabel Casasús, Rebeca Oliver-De la Esperanza, Margalida Joy

Centro de Investigación y Tecnología Agroalimentaria de Aragón, Zaragoza, Spain

E-mail: mblanco@aragon.es

Take home message Field pea can be included in the fattening concentrate of lambs as it only had a minor effect on meat tenderness.

Introduction There is interest to include pea in lamb's diets as a source of local protein to increase the self-sufficiency of Europe, which is extremely dependent on soya imports of very volatile prices (Peyraud *et al*., 2014). But the effect of pea on meat traits as colour and tenderness has not been studied yet. Thus, the aim of the study was to evaluate if different proportions of field pea in the concentrate of fattening lambs affected meat colour, lipid oxidation and tenderness.

Materials & methods 54 weaned Rasa Aragonesa lambs (13.4 kg and 31 days of age) were randomly assigned to four treatments that differed in the inclusion of field pea [0% (n=13), 10% (n=13), 20% (n=14) and 30% (n=14)] in the fattening concentrate. The concentrates were formulated to be iso-energetic (1.18 MJ/kg FM) and iso-proteic (175 g CP/kg FM). The concentrates were fed from weaning until the slaughtering (22-24 kg LW). Carcasses were chilled for 24 h and *Longissimus thoracis* (LT) muscle was excised, sliced and stored (4 ºC in darkness) in trays for 0, 2, 5, 7 and 9 days to measure the instrumental colour (CIEL*a*b*) (Lobón *et al*., 2017). For meat tenderness measurements, the *Semitendinosus* muscle was extracted to determine the Warner-Bratzler shear force registering maximum stress (N/cm^2) in cooked meat and the *Semimembranosus* muscle was extracted to study the force of compression at 20% and 80% (N/cm^2) in raw meat (Sañudo *et al*., 2004). The statistical analyses were performed with SAS. Colour parameters were evaluated with a mixed model with the inclusion of pea, the time and its interaction as fixed effect and the animal as random effect. The models for tenderness included the pea as fixed effect. Means were analysed with linear, quadratic and cubic contrasts.

Results & discussion Meat colour parameters were not affected by the inclusion of pea in the fattening concentrate of the lambs ($P>0.05$) but they were affected by the exposure time ($P<0.001$) (Figure 1). The inclusion of pea did not affect maximum stress and compression at 80% ($P<0.05$) but affected compression at 20% ($P<0.05$) (Figure 2). The compression at 20% was greater in 10% than 0% pea, whereas 20% and 30% pea presented intermediate values ($P<0.05$).

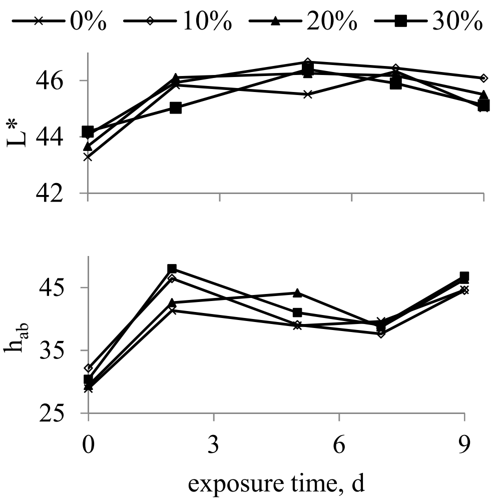


Figure 1 Effect of the inclusion of field pea on *LT* muscle lightness (L*) and Hue angle (h_{ab})

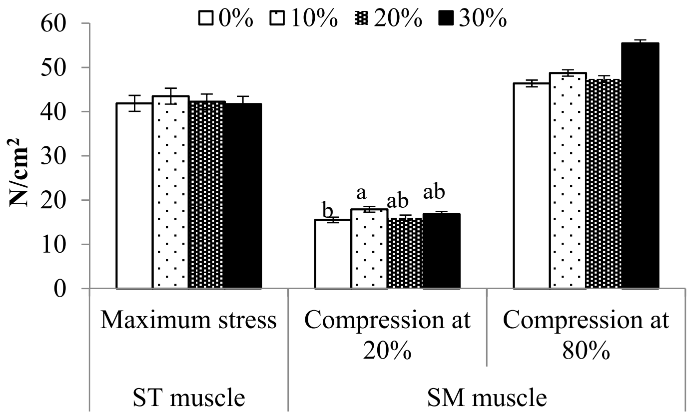


Figure 2 Effect of the inclusion of field pea on tenderness measured in *Semitendinosus* (ST) and *Semimembranosus* (SM) muscles. Means with different letters differ at $P<0.05$

Conclusion The inclusion of field pea did not affect meat colour and only affected compression at 20%.

Acknowledgements Project RTA2014-00032-c02-01; INIA and ESF funded M. Blanco contract.

References

Lobón S, Blanco M, Sanz A, Ripoll G, Bertolín JR and Joy M 2017. Journal of Animal Science 95, 4998-5011.
Peyraud J-L, Taboada M and Delaby L 2014. European Journal of Agronomy 57, 31-42.
Sañudo C, Macie ES, Olleta JL, Villarroel M, Panea B and Alberti P 2004. Meat Science.66, 925-932.

Effect of rearing practices on the abundance of protein biomarkers of tenderness and intramuscular fat content in the French Rouge des Prés cows

Brigitte Picard, Mohammed Gagaoua, Muriel Bonnet

INRA, Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès-Champanelle, France

E-mail: brigitte.picard@inra.fr

Take home message The abundance of some protein biomarkers of tenderness or intramuscular fat content are modified by rearing practices during the finishing period with differences depending on the muscle type.

Introduction Tenderness and intramuscular fat content (IMF) constitute internationally major quality traits to be controlled in beef. Recently some proteins considered as biomarkers of these quality traits have been identified (for review: Picard and Gagaoua, 2017; Ceciliani *et al.,* 2018). The present study used multivariate analyses to assess the effect of rearing practices applied during the finishing period on the relative abundance of 20 of these proteins quantified in 86 Rouge des Prés cows.

Materials & methods The relative abundance of 20 biomarkers of tenderness and/or IMF content were quantified by Reverse Phase Protein Array technique (RPPA) in 5 muscles: *Longissimus thoracis* (LT), *Semimembranosus* (SM), *Rectus abdominis* (RA), *Triceps brachii* (TB) and *Semitendinosus* (ST) (Picard *et al.,* 2017). The finishing period data [part of hay, haylage and/or grass in the finishing diet (% w/w)]; amount of concentrate (kg); duration (days) and physical activity (% days out) were used to identify rearing practise classes. For that we used the procedure based on principal component analysis (PCA) and *k*-means, recently reported by Gagaoua *et al.* (2017). The clusters were then compared for muscle biomarkers relative abundance by using the PROC GLM of SAS.

Results Rearing practise analysis allowed to distinguish 3 clusters mainly characterized by "Grass", "Haylage" or "Hay" diet during the finishing period (Figure 1, only biomarkers with eigenvectors >0.5 were considered). Grass finishing diet had an impact mainly on the properties of the SM and ST muscles known as fast glycolytic muscles. This cluster is characterized by higher relative abundance of MLC1F and PRDX6 (an antioxidant enzyme). Hay finishing practice impacted the properties of RA muscle known as slow oxidative muscle. This cluster is characterised by high abundance of small Heat Shock Proteins (HSP) (HSP20, 27, CRYAB) and HSP70-1A, TNNT1 (slow isoform) and ALDH1A, and by a low abundance of MyHC IIX (fast glycolytic). The results revealed also that LT and TB muscles, known as mixed oxido-glycolytic muscles, were less impacted by finishing diet than the 3 other muscles. Interestingly, the abundances of 3 proteins: FHL1, MDH1 and PYGB were not modified by rearing practices whatever the muscle.

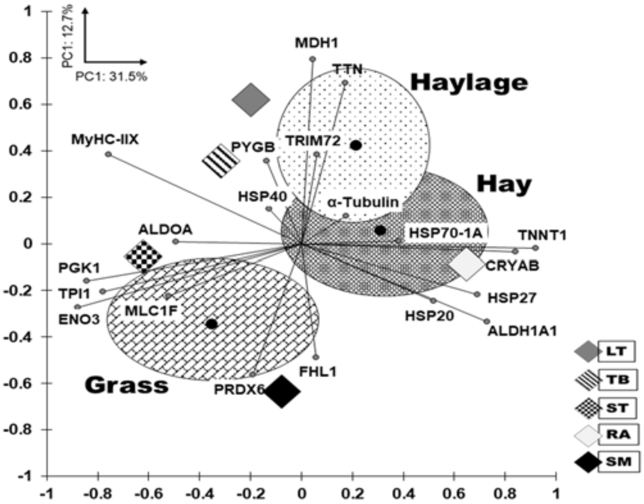


Figure 1: PCA highlighting both the projection of the variables in the first two axes and the relationships between rearing practices with the relative abundance of the 20 biomarkers by muscle type.

Conclusion These data highlighted at which extent the abundance of some proteins biomarkers of tenderness and IMF traits could be affected by the rearing practices applied during the finishing period in Rouge des Prés cows. This knowledge is important for the management of beef quality as well as to establish a molecular phenotyping strategy.

Acknowledgements The authors thank SICA Rouge des Prés and Région Pays de la Loire for the financial support and Institut Curie centre de recherche, Plateforme RPPA, Paris France, for the quantification of the biomarkers.

References

Ceciliani F, Lecchi C, Bazile J and Bonnet B 2018. In: Proteomics in Domestic Animals: from Farm to Systems Biology. 233-255. Springer

Gagaoua M, Monteils V, Couvreur S and Picard B 2017. Journal of Agricultural and Food Chemistry 65, 8264-78.

Picard B, Al Jammas M., Couvreur S, Pécot M, Aminot G, Leger S, De Koning L, Valais A and Bonnet M 2017. In: Proceedings of the 63rd International Congress of Meat Science and Technology. 819-820, Cork, Ireland.

Picard B and Gagaoua M 2017. Chapter 11 - In: Proteomics in Food Science: from farm to fork. 177-197. Academic Press.

Field pea can be included up to 30% in the fattening concentrate of lambs with minor effects on meat chemical composition

Mireia Blanco, Juan Ramón Bertolín, Isabel Casasús, Margalida Joy

Centro de Investigación y Tecnología Agroalimentaria de Aragón, Zaragoza, Spain

E-mail: mblanco@aragon.es

Take home message The replacement of soya by field pea is advisable due to the minor changes produced in meat chemical composition

Introduction There is a growing interest to increase the use of field pea (*Pisum sativum*), which has a high content of starch (48%, Petit *et al*., 1997) and protein (19-26%, Soto-Navarro *et al*., 2012) in the diet of ruminants. It can be incorporated in lambs diets, the effects on meat quality being dependent on the breed (Colonna *et al*., 2014) or the rate of inclusion of field pea (Lestingi *et al*., 2016). The aim of the study was to evaluate if different proportions of field pea in the concentrate of fattening lambs affected meat chemical composition.

Materials & methods 54 weaned Rasa Aragonesa lambs (13.4 kg and 31 days of age) were randomly assigned to four treatments that differed in the inclusion of pea [0 (control), 10, 20 and 30%] in the fattening concentrate fed from weaning to slaughter (22-24 kg). The concentrates were formulated to be iso-energetic (1.18 MJ/kg FM) and iso-proteic (175 g CP/kg FM). After 24 h cooling, the *Longissimus thoracis et lumborum* muscle was excised and sliced to determine the chemical composition, cholesterol, lipid oxidation during oxygen exposure (0 to 9 days) and fatty acid profile (FA). The statistical analyses were performed using SAS v9.3. The chemical composition, FA and cholesterol were analysed with the GLM procedure with the inclusion of pea as the fixed effect. Lipid oxidation was analysed with a mixed model with pea, time and its interaction as fixed effects and the animal as random effect.

Results & discussion The inclusion of pea did not affect the chemical composition (Table 1), as reported with 24% inclusion (Scerra *et al*., 2011), but affected cholesterol content (Table 1), having the inclusion of 20% pea greater cholesterol content than the inclusion of 30% pea (P<0.05). The inclusion of pea affected only some individual saturated FA (C13:0, C15:0 and C17:0; data not shown) and consequently total saturated FA, the inclusion of 20% pea presenting greatest content (P<0.05). An inclusion of 24% pea modified saturated, monounsaturated and polyunsaturated FA (Scerra *et al*., 2011). Lipid oxidation was affected by the exposure time but not by the inclusion of pea (Figure 1).

Table 1 Effect of the inclusion of field pea on the chemical composition, cholesterol and FA profile.

	0%	10%	20%	30%	P-value
Dry matter, %	22.0	21.8	22.2	21.5	0.15
Crude protein, %FM	20.3	20.3	20.4	19.9	0.26
Ether extract, %FM	1.7	1.7	1.9	1.7	0.25
Cholesterol, mg/g	0.51^{ab}	0.51^{ab}	0.53^{a}	0.49^{b}	0.01
Saturated FA, %	44.98^{b}	44.97^{b}	46.67^{a}	45.16^{b}	0.01
Monounsaturated FA, %	40.19	40.15	40.11	40.47	0.98
Polyunsaturated FA, %	14.16	14.25	12.62	13.72	0.20
n-6, %	8.75	8.59	7.69	8.26	0.61
n-3, %	1.57	1.60	1.44	1.53	0.22

Means with different superscript differ at P<0.05

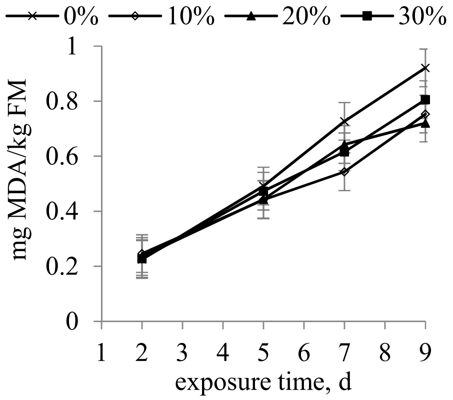


Figure 1 Effect of field pea inclusion on lipid oxidation during exposure.

Conclusion The inclusion of field pea had minor effects on the chemical composition and fatty acid profile. However, the inclusion of 20% of field pea increased the cholesterol and total SFA compared with the other concentrates.

Acknowledgements Project RTA2014-00032-c02-01; INIA and ESF funded M. Blanco contract; CITA staff

References

Colonna MA, Giannico F, Marsico G, Vonghia G, Ragni M and Jambrenghi AC 2014. Progress in Nutrition 16, 39-51.
Lestingi A, Facciolongo AM, Jambrenghi AC, Ragni M and Toteda F 2016. Small Ruminant Research 143, 15-23.
Petit HV, Rioux R and Ouellet DR 1997. Journal of Dairy Science 80, 3377-3385.
Soto-Navarro SA, Encinias AM, Bauer ML, Lardy GP and Caton JS 2012. Journal of Animal Science 90, 585-591.
Scerra M, Caparra P, Foti F, Cilione C, Zappia G, Motta C and Scerra V 2011. Meat Science 87, 229-233.

Meta-analysis of public transcriptomics data to understand and phenotype bovine body composition

Jeanne Bazile[1], Florence Jaffrezic[2], Denis Laloe[2], Muriel Bonnet[1]

[1]*Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, 63122 Saint-Genès-Champanelle, France,* [2]*INRA, UMR1313 Génétique Animale et Biologie Intégrative, Jouy-en-Josas, France*

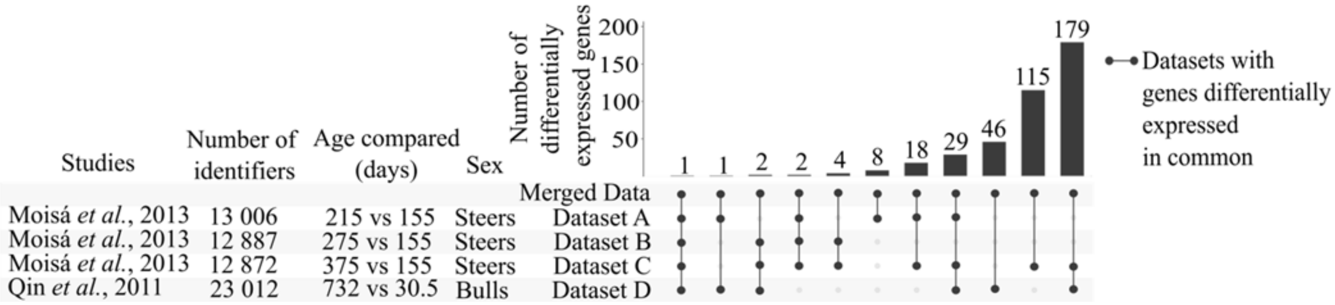
E-mail: jeanne.bazile@inra.fr

Take home message From a compilation of public genomics data, *HOXA5* was highlighted as a candidate driver or biomarker of bovine muscle mass accretion with age, in two bovine breeds, and for either steers or bulls.

Introduction Producing ruminant with adequate muscular and adipose tissues masses, *i.e.* lean-to-fat ratio, is an economic challenge for the bovine sector. The lean-to-fat ratio contribute to the animal adaptability, food efficiency and meat / carcass qualities. Despite numerous genomics studies that assayed how rearing factors affect the lean-to-fat ratio *(*Ceciliani *et al.*, 2018) there is no consensus on molecular biomarkers of the lean-to-fat ratio, perhaps due to the difficulty to compare large datasets produced with different quantitative protocols. One of the rare attempt to compile large datasets had merged micro-arrays data from a same quantitative protocol (Baron *et al*., 2011). Our challenge is to compile public data and to implement statistical tools such as meta-analysis methods, to identify genes that could be robust biomarkers of the lean-to-fat ratio.

Materials & methods For datasets comparisons, we used R software, first to merge identifiers over datasets and to perform descriptive statistics with the ade4 package. We then used the metaMA package to look for differentially abundant genes in each study and whatever the studies (named Merged Data), by performing Benjamini-Hochberg corrections at a significant level of 5%. This pipeline was bench tested with 4 datasets from 2 transcriptomics studies of bovine *Longissimus* muscle *(*Qin *et al.*, 2011; Moisá *et al*., 2013). These studies used different microarrays (UIUC Bos taurus 13.2K 70-mer condensed oligoarray and Affymetrix Bovine Genome Array), and cattle differing by the breed (Chinese Red Steppe vs Angus X Simmental), diet (compensatory growth plane of nutrition vs standard diet) and sex (steers vs bulls).

Results & discussion Only 855 GenBank accessions over thousands were shared by the 4 datasets. One gene (Homeobox protein Hox-A5, *HOXA5*), was overexpressed during muscle growth in each study and in the Merged Data (Figure 1), highlighting *HOXA5* as a potential marker of muscle accretion whatever the breed, diet and castration. The embryonic factor *HOXA5* has been found at the adulthood involved in fat depot in adipose tissue (Gesta *et al.*, 2006), but was never related to post-natal muscle biology. In dataset C, D and Merged Data, 179 genes were differentially abundant and may be drivers of muscle (including marbling) accretion with age. Of these, genes involved in *SMAD*, *TGF*-β or *activin* signalling already known to modulate muscle hypertrophy underscored the meta-analysis method.



Studies	Number of identifiers	Age compared (days)	Sex	
Moisá *et al.*, 2013	13 006	215 vs 155	Steers	Dataset A
Moisá *et al.*, 2013	12 887	275 vs 155	Steers	Dataset B
Moisá *et al.*, 2013	12 872	375 vs 155	Steers	Dataset C
Qin *et al.*, 2011	23 012	732 vs 30.5	Bulls	Dataset D

Figure 1 Descriptive data and number of differentially abundant genes in datasets used to bench test the meta-analysis.

Conclusion We provide methods to compile transcriptomics data that should foster the discovery of keys pathways or biomarkers of the lean-to-fat ratio, such as *HOXA5*. The next step is to increase the number of gene under compilation by a reannotation of transcriptomics data on the current bovine genome.

Acknowledgements The regional council of Auvergne-Rhône-Alpes and the Inra-Phase Division for funding J. Bazile.

References

Baron D, Dubois E, Bihouée A, Teusan R, Steenman M, Jourdon P, Magot A, Péréon Y, Veitia R, Savagner F, Ramstein G and Houlgatte R 2011. BMC Genomics 12, 113.

Ceciliani F, Lecchi C, Bazile J and Bonnet M 2018. In Proteomics in Domestic Animals: from Farm to Systems Biology, pp. 233–254. Springer International Publishing.

Gesta S, Blüher M, Yamamoto Y, Norris AW, Berndt J, Kralisch S, Boucher J, Lewis C and Kahn CR 2006. Proceedings of the National Academy of Sciences of the United States of America 103, 6676–6681.

Moisá SJ, Shike DW, Graugnard DE, Rodriguez-Zas SL, Everts RE, Lewin HA, Faulkner DB, Berger LL and Loor JJ 2013. Bioinformatics and Biology Insights 7, 253–270.

Qin L, Zhang G, Cao Y, Zhang J, Zhao Y and Zhao Z 2011. Animal and Veterinary Advances 10, 428–436.

Traceability of the ewe's diet using the fatty acids of the milk and the meat of the suckling lamb

Sandra Lobón[1], Margalida Joy[1], Albina Sanz[1], Javier Álvarez-Rodríguez[2], Mireia Blanco[1]

[1]Centro de Investigación y Tecnología Agroalimentaria de Aragón, Zaragoza, Spain, [2]Universitat de Lleida, Lleida, Spain

E-mail: mjoy@cita-aragon.es

Take home message Milk fatty acids are useful to trace accurately ewe's diet.

Introduction There is growing interest in identifying strategies to enhance the concentration of healthy fatty acids (FA) in food (Chikwaha *et al.* 2017). Grazing is an advisable system to increase healthy FA in milk, and consequently in suckling lamb meat (Joy *et al.* 2012). Condensed tannins (CT) have also been used to modify the FA profile of ewe´s milk and meat of the lambs (Carreño *et al.* 2015). Quebracho (*Schinopsis balanseae*), which is rich in CT, can be included in the ewe´s concentrate. Moreover, consumers demand guarantees of the feeding given to the animals. The aim of this study was to evaluate if the FA profile discriminated the type of forage and the inclusion of quebracho in the diet fed to ewes.

Materials & methods Thirty-nine ewe-lamb pairs were separated into 2 groups at lambing. One group grazed in mountain pastures (Pasture) and the other group was housed indoors and fed pasture hay (Hay). Within each type of forage, half of the ewes received 300 g/d of a commercial concentrate (Control), and the other half received 300 g/d of a concentrate containing 10% quebracho (QUE). Ewes were milked weekly and suckling lambs were slaughtered when they reached 10-12 kg of live weight. The FA profiles of milk and of the *Longissimus thoracis* muscle of the suckling lambs were analysed (Lobón *et al.* 2015). Fatty acid composition was used to classify individuals into the four feeding strategies (Pasture + QUE, Pasture + Control, Hay + QUE, Hay + Control) through canonical discriminant analyses using SAS V.9.3. Canonical correlations with a P-value lower than 0.05 were considered significant and thereby shown in the text.

Results & discussion The discriminant analysis of milk FA (Figure 1) accounted for 98.5% of the total variation between treatments and it was mainly determined by Can 1 including C18:2 n-6tt (r=-0.86), C6:0 (r=-0.86) and C18:1 n-9 (r=0.84) and discriminated by the type of forage. Can 2 included ATT (r=-0.28) and C17:0 (r=0.26) and discriminated by the inclusion of quebracho in the concentrate. Meat FA discriminant analysis (Figure 2) accounted for 81% of the total variation. Can 1 included ATT (r=0.43), ratio n-6:n-3 (r=0.43) and C20:5 n-3 (r=-0.34) as the main discriminator variables. Whereas, Can 2 included as main variables C20:3 n-6 (r=0.36), C20:4 n-6 (r=-0.27) and C18:1 n-7 (r=-0.26). All ewes were correctly classified using their milk FA profile, while some lambs were misclassified using the FA profile of the meat (10-18%).

Figure 1 Canonical discriminant analysis between animal feeding based on fatty acid composition of milk.

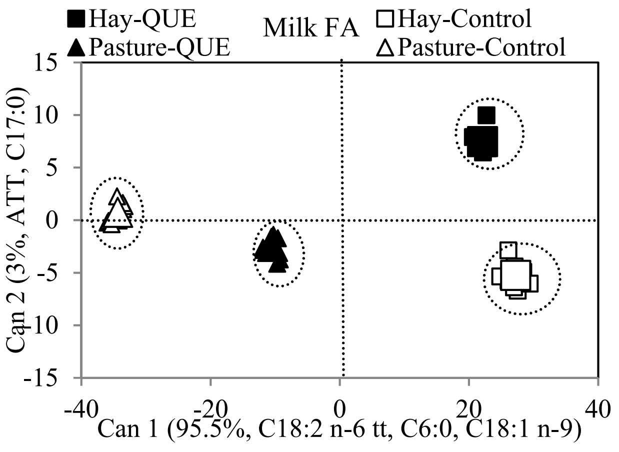
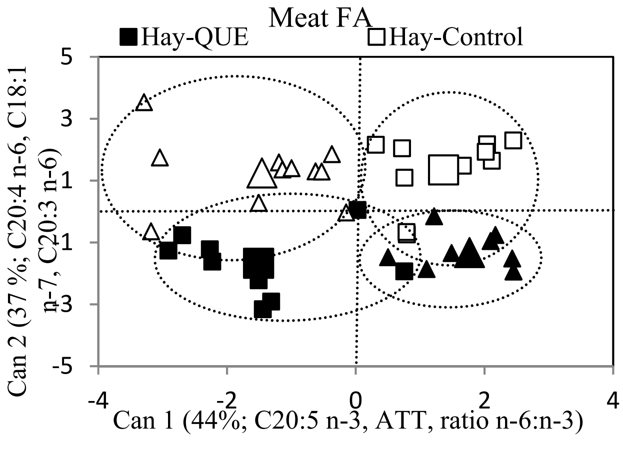


Figure 2 Canonical discriminant analysis between animal feeding based on fatty acid composition of lamb meat.



Conclusion Discriminant analyses were able to classify the different treatments studied according to their FA profiles. It was accurate with milk FA profile of the dam rather than with meat FA profile of the suckling lamb.

Acknowledgements Project RTA2012-080-00, INIA, RZP2009-00005, RZP2013-00001-00, CITA-staff.

References

Carreño D, Hervás G, Toral PG, Belenguer A and Frutos P 2015. Animal Feed Science and Technology 202, 42-51.
Chikwanha OC, Vahmani P, Muchenje V, Dugan MER and Mapiye C 2017. Food Research International 104, 25-38
Joy M, Ripoll G, Molino F, Dervishi E and Álvarez-Rodríguez J 2012. Meat Science 90, 775-782.
Lobón S, Sanz A, Ripoll G, Blanco M and Joy M 2015. XVI Jornadas sobre Producción Animal, AIDA 164-166

469

Expression of myogenic and adipogenic genes in muscle of progeny from Nellore cows supplemented during gestation

Liziana Rodrigues[1], Marcio Ladeira[1], Jon Schoonmaker[3], Tathyane Gionbelli[1], Flavio Resende[2], Gustavo Siqueira[2], Otavio Machado Neto[4], David Soares[1]

[1]Federal University of Lavras, Lavras, Brazil, [2]APTA, Colina, Brazil, [3]Purdue University, West Lafayette, USA, [4]State University Paulista / Unesp, Botucatu, Brazil

E-mail: mladeira@dzo.ufla.br

Take home message: Protein supplementation during gestation affect myogenesis and adipogenesis.

Introduction Little is known about developmental programming in *Bos indicus*, particularly in Nellore, which is the predominant beef breed in Brazil. The reason why nutrition of the dam can change cell tissues proliferation is that mesenchymal stem cells originate muscle and adipose tissues by the action of transcription factors (Du *et al*., 2010). Therefore, the objective of this study was to evaluate the effect of maternal nutrition on expression of genes involved in myogenesis and adipogenesis in muscle of progeny from birth until weaning.

Materials & methods Ninety-two multiparous Nellore cows (initial BW 387 ±9.0 kg) were allotted in a completely randomized design with two treatments. Cows were inseminated with semen from the same bull and were male pregnant. Cows were grazed on *Brachiaria brizantha* cv Marandu pastures (8.2% crude protein, CP, and 68.5% neutral detergent fiber). Forty-six cows were fed only mineral premix (NSUPP) and the other 46 (SUPP) were fed a protein supplement (36% CP, 0.2%BW) from day 125 of gestation until calving. At calving, six calves from each treatment were slaughtered and collected samples of *longissimus thoracis* (LT) muscle for histological and gene expression analyzes. In addition, 10 calves from each treatment were chosen randomized to collect LT muscle using a biopsy procedure at 11 ±4 days after birth and at weaning for gene expression analysis. It was evaluated myogenic markers (*WNT10B, β-catenin, mTOR*), adipogenic markers (*PPARγ, C/EBPα, ZFP423, CD36, FABP4*) and fibrogenic markers (*TGFβ1, COL3A1, FN1*) through RT-qPCR. Gene expression was analyzed using an Eppendorf Realplex system and a SYBR Green detection system. Relative expression levels were calculated according to the method described by Pfaffl (2001). Histological samples were staining with hematoxylin and eosin and analyzed under a light microscope with 20X objective, and five images per sample were examined in the ImageJ® analyzer software. To measure the number of cells per field, cell density in 15,000 μm^2, muscle cell diameter (µm), and then cell area (μm^2) were calculated. A Shapiro-Wilk test was performed to assess the normality of all collected data. When data were not normally distributed, they were transformed using PROC RANK from SAS 9.4. Least square means of all data were analyzed using the GLM procedure of SAS.

Results & discussion Muscle of calves from SUPP group had a tendency of greater number of muscle cells per field ($p = 0.07$), while calves from NSUPP compensated the smaller number of cells with a trend of largest cells area ($p = 0.06$) and ribeye area ($p = 0.07$). This is in agreement with Du *et al.* (2010) where animal restricted during fetal development impairs muscle hyperplasia and compensate the growth with hypertrophy. Twenty-four hours after birth muscle of calves from SUPP group had greater expression of *WNT10B* ($p = 0.01$), *PPARG* ($p = 0.03$), *CD36* ($p = 0.04$) and *TGFβ1* ($p = 0.01$) than muscle of calves from NSUPP cows without differences in the other genes. This greater expression of *WNT10B*, *PPARG*, *CD36* and *TGFβ1* indicates that 24 hours after birth, calves from SUPP group still had multipotent cells performing the differentiation for myogenesis, adipogenesis and fibrogenesis. Muscle of SUPP calves had lesser expression of *C/EBPα* ($p = 0.01$) and *FABP4* ($p = 0.07$) than calves from NSUPP cows 11 days after birth, suggesting greater amount of adipocytes resulted of an early adipogenesis in the non-supplemented calves as a metabolic adaptation to increase the body reserves. At weaning, muscle of SUPP calves had a tendency to have greater expression of *PPARG* ($p = 0.08$), and greater *ZFP423* ($p = 0.04$) and *TGFβ1* ($p = 0.02$) than muscle of calves from NSUPP cows. The increase in expression of adipogenic genes in SUPP calves supports the hypothesis that these animals had a late adipogenesis. *β-catenin, mTOR COL3A1* and *FN1*genes were not affected by maternal diet ($p > 0.05$).

Conclusion Fetal programming affected the histology of the *longissimus* muscle, increasing the potential for growth of calves whose dams were supplemented during mid to late gestation. In addition, expression of genes involved in myogenesis, adipogenesis, and fibrogenesis of the progeny was affected by the dam nutrition during gestation, suggesting greater myogenesis in fetal phase and a delay in the adipogenesis of calves whose mothers were supplemented.

Acknowledgements Authors would like to thank CNPq, CAPES, FAPEMIG, APTA, and Bellman – Trouw Nutrition Company for the financial support of this research.

References

Du M, Tong J, Zhao J, Underwood KR, Zhu M, Ford SP and Nathanielsz PW 2010. Journal of Animal Science 88, E51-E60.
Pfaffl MW 2001. Nucleic Acids Research 29:e45.

Effects of saffron petals ethanolic extract and vitamin E on plasma antioxidant status in Baluchi male lambs

Fereshteh Alipour, Alireza Vakili, Mohsen Danesh Mesgaran, Hadi Ebrahimi

ferdowsi mashhad university, Mashhad, Iran

E-mail: savakili@um.ac.ir

Take home message Saffron petal extract like vitamin E has improved the antioxidant status in plasma of Baluchi male lambs.

Introduction Lately consumers have rejected synthetic antioxidants because of their carcinogenicity. According to the literature, inclusion foods containing phenolic combinations in the diet of animals, contribute to healthful animal yields, moreover the antioxidant properties of these compounds inhibited of cholesterol oxidation in blood and meat. Saffron petal as the important by-product of saffron is with a production of more than 10,000 tons in year that is discarded as a waste. The phenolic combinations among, flavonoids and anthocyanins are presumably the biologically active components of the saffron petal (Kubo and Kinst-Hori, 1999). There is little information about the use of saffron petal as a rich phenolic source on the antioxidant status of the livestock Therefore, this study can be done to investigate the effects of saffron petal extract on the antioxidant status of Baluchi male lambs.

Materials & methods Sixteen Baluchi male lambs (initial body weight 35.22 ± 5.75 kg) were used in a 42-day trial during to determine the effects of ethanolic saffron petals extract (SPE) and vitamin E on antioxidant status and lipid oxidation. The lambs were randomly assigned into four groups ($n = 4$). The first group weekly subcutaneous injection (5 ml) of normal saline (control). The second group, weekly subcutaneous injection of the ethanolic SPE (25 mg/kg BW). The third group, subcutaneous injection of DL-α-tocopheryl acetate a weekly (Total dose was 1350 IU). The fourth group, administered as a weekly oral dose of ethanolic SPE (500 mg/kg BW). The lambs were fed with a basal diet (40:60 forage:concentrate). Blood samples were taken from lambs in days 1, 14, 28 and 42 to measure the enzymatic activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), the total antioxidant capacity (TAC) and lipids oxidation by forming Malondialdehyde (MDA), using commercial kits (Cayman Chemical Company, Ann Arbor, MI, USA). All data were analysed using repeated measures and the Proc Mixed model (SAS 9.1). Treatments, lambs within treatment, week of measurement and the interaction of treatment × measurement week as the main effect. Lambs within treatment was a random variable. Data are presented as the least square mean ± SE, and the means evaluation was done using Tukey's test. Differences were considered significant at $P < 0.05$.

Results The effects of treatments on plasma antioxidant status and lipids oxidation are presented in Table 1. There were significant differences between treatments for the activities of GPx, SOD and MDA ($P < 0.05$). However, there were no significant differences for TAC ($P > 0.05$). The GPx activity significantly increased just with the oral SPE, whereas all treatments significantly increased SOD activity compared to control. There was treatment × week interaction for GPx ($P < 0.05$). The reason for the increase of antioxidant enzymes by saffron petals can be attributed to its rich phenolic compounds (3.4 mg GA/g dry weight) including flavonoids and anthocyanins. The antioxidant capacity of saffron petals is related to the presence of flavonoids (Termentzi and Kokkalou. 2008). Vitamin E acts as a free radical sweeper in the prevention of diseases and thereby inhibits lipid peroxidation (EL-Demerdash, 2004). Also, the reports were of protected lipid oxidation with injective vitamin E in lambs and pork (Maiorano *et al.*, 2016).

Table 1 Effects of saffron petals ethanolic extract and vitamin E on plasma antioxidant status in Baluchi male lambs.

Item	Treatment					P-value		
	Control	Injective SPE	Injective Vit E	Oral SPE	SEM	Treat	Week	Treat×Week
GPX (u/l)	33.26	39.35	40.85	53.49	1.930	0.0001	0.137	0.0001
SOD (u/l)	16.15	27.16	38.27	21.87	2.240	0.0004	0.016	0.415
MDA (nmol/dl)	1.51	1.42	1.62	1.82	0.088	0.040	0.018	0.107
TAC (mmol/l)	0.28	0.27	0.31	0.30	0.017	0.57	0.000	0.14

Conclusion These results indicate that petal extract of saffron, such as vitamin E improved the plasma antioxidant status in lambs.

Acknowledgements Researchers gratefully acknowledge funding from department of animal science, Ferdowsi University of Mashhad, Iran.

References

EL-Demerdash, FM 2004. Journal of Trace Elements in Medicine and Biology 18, 113–21.
Kubo I and Kinst-Hori I 1999. Agricultural and Food Chemistry 47, 4121-4125.
Maiorano G, Angwech H, Di Memmo D, Wilkanowska A, Mucci R, Abiuso C and Tavaniello S 2016. Small Ruminant Research 139, 52-59.
Termentzi A and Kokkalou E 2008. Planta Medica 74: 573–581.

Do the coagulation properties of milk produced from cows fed grass or maize silage differ from those obtained from cows receiving no silage?

Elisa Manzocchi[1], Werner Hengartner[2], Michael Kreuzer[1], Katrin Giller[1]

[1]*ETH Zurich Animal Nutrition, Zurich, Switzerland,* [2]*Bildungszentrum Strickhof, Lindau, Switzerland*

E-mail: elisa.manzocchi@usys.ethz.ch

Take home message Feeding ensiled forages did not affect the coagulation properties of the milk of individual cows.

Introduction Milk from cows fed ensiled forages has a poor reputation among a distinct consumer segment and producers of hard cheese when compared with milk from cows fed hay. However, no sensory difference (P>0.10) was detected in a triangle test comparing hay- and grass-silage based milk conducted with 235 untrained participants (unpublished results).

Materials & methods Twenty-four cows of the breeds Brown Swiss, Holstein and Red Holstein (2±1.5 lactations, 209±68 DIM) housed in a single pen within a free-stall barn with access to individual feeding troughs were allocated to four treatment groups. In the experiment, 12 days of adaptation were followed by 10 days of sampling period. The first group (HAY) was fed hay (NDF 447, CP 92 g/kg DM) supplemented with grass pellets (NDF 418, CP 157 g/kg DM). The second group (GRASS) was fed grass silage (NDF 455, CP 143 g/kg DM) and grass pellets. The third group (C-MAIZE) was fed conventional maize silage (NDF 331, CP 82 g/kg DM) and hay (4:1 in DM), whereas the fourth group (S-MAIZE) received long-chopped maize silage (shredlage) (NDF 366, CP 77 g/kg DM) and hay (4:1 in DM). To reach an equivalent milk production potential (26 kg/d ECM), the four diets were adjusted with wheat flakes or soybean meal or both. The cows received NaCl (50 g/d) and a vitaminised mineral mix (120 g/d). Milk samples were collected at each milking and pooled according to milk yield. Milk composition was analysed by infrared analysis (MilkoScan FT1, Foss, Hilleroed, Denmark). Milk coagulation properties were determined twice during the sampling period with a Lattodinamografo (Foss, Padua, Italy) in triplicates of 10 ml milk incubated with 100 µl of 3%v/v rennet solution (Pacovis Premium, 215 IMCU/kg, Pacovis Amrein AG, Belp). Data were analysed with the Mixed procedure of SAS with treatment, breed, parity and their interactions as fixed effects in the model, DIM as a covariate and the individual animals as random factor. Multiple comparisons among treatment means were performed with Tukey's procedure. The data of two cows were excluded due to several outliers.

Results Cows of the four treatments did not differ significantly in milk yield and composition, except for the high milk urea content in the GRASS group (Table 1). Although there was a tendency (P<0.10) towards a difference in rennet coagulation time, along with a tendency towards different acidity, the coagulation properties were not affected by the treatments.

Table 1 Treatment means of milk yield and composition as well as coagulation properties (rennet coagulation time (RCT), rate of firming (k_{20}), curd firmness after 30 min (A_{30}) and after twice the RCT (A_{2R})).

Treatment group	HAY (n=6)	GRASS (n=6)	C-MAIZE (n=5)	S-MAIZE (n=5)	SEM	P-values[1]
Milk yield, kg/d	25.3	24.0	25.3	21.1	1.7	0.23
Milk main constituents						
Fat, g/100 g	4.26	4.52	3.94	4.09	0.28	0.40
Protein, g/100 g	3.81	4.97	3.78	3.68	0.14	0.55
Casein, g/100 g	2.83	2.97	2.81	2.72	0.11	0.47
Lactose, g/100 g	4.77	4.60	4.77	4.76	0.09	0.39
Urea, mg/dl	16.9^b	26.6^a	19.7^b	19.3^b	0.96	<0.001
Acidity, °SH	7.16	7.73	6.99	6.92	0.23	0.08
Milk coagulation properties						
RCT, min	10.3	8.4	10.7	11.3	0.74	0.08
k_{20}, min	1.37	1.36	1.45	1.86	0.38	0.76
A_{30}, mm	56.3	59.8	53.7	55.8	3.98	0.66
A_{2R}, mm	54.5	54.7	49.8	50.4	4.03	0.65

$^{a\text{-}b}$ Means within row carrying no common superscript letters are significantly different (P<0.05) between the means.

[1] Probability values of the effect of treatment. No other effects were significant for any variable.

Conclusion The principal coagulation properties of milk from ensiled forages do not differ from milk of cows fed no silage. Further parameters like volatile compounds in milk that might affect the sensory properties are under investigation.

Acknowledgments We thank the staff of AgroVet-Strickhof and the H. Wilhelm Schaumann Stiftung for funding the study.

Fatty acid composition of Fontina-type cheeses produced from mountain pasture of South Korea

Seungyong Park[1], Mauro Coppa[2], Andrea Barmaz[3], Kyeongil Sung[4]

[1]Yonam College, Cheonan, Chungnam, Republic of Korea, [2]University of Turin, Torino, Piemonte, Italy, [3]Institute of Regioan Agriculture, Aosta, Valle d'Aosta, Italy, [4]Ganwon National University, Chuncheon, Gangwon, Republic of Korea

E-mail: psyonam@daum.net

Take home message Because of topological reason, South Korea is deficient in pasture in the lowland. Introducing grazing in mountain areas will allow to obtain a healthier milk.

Introduction The ratio of ω-6 to ω-3 fatty acids (FA) may be one indicator for diet with positive effect on human health. Although the optimal ratio is near to 1:1 in the milk, when its value is estimated 5:1, it represents imbalance diet. This ratio in milk is improved by herbage feeding (Coppa *et al.*, 2012). However, a total of 404,000 heads of dairy cows are mostly fed diet with 52% of concentrate and 48% of forage in South Korea. In 2016, 35% of a total 5.4 million tonnes of forages consumed annually were supplied with the dried rice straw, and 45% were mostly Italian Rye grass from the land of 107,793 hectares, and a deficiency of 20% were supplied by the imported dry grass (mainly Tall Fescue). The aim of this work was to improve the FA composition of the cheese produced in South Korea by introducing a grazing system in the mountain area of the country.

Materials & methods A group of 10 Holstein cows were fed at pasture (expected daily intake 15 kg DM/cow), composed of a timothy and reed canary grass mixture (70%:30%), with a supplementation of 4.6 kg (on DM base) of concentrate (crude protein 19.5, crude fat 2.0, crude fibre 15, ash 12, total digestible nitrogen 75%, Table 1). The cheese obtained from the pasture group was compare to those obtained from a control group fed indoor a concentrate-based TMR (total intake 24.8kg DM/cow*day). The milk of the two groups was used separately to obtain 7 wheels of Fontina-type cheese. The FA composition was analysed using a gas chromatograph (Shimadzu) and a cyanopropyl capillary column (SP-2560, 100 m × 0.25 mm, SUPELCO). The analysis conditions were as follows; carrier gas was helium at flow rate of 1.80 mL/min. Injector temperature was 250℃ and the oven temperature was programed 60℃ for 5 min, 175℃ at the rate of 10℃/min, 230℃ at the rate of 2℃/min, and hold it for 10 min. FID detector temperature set at 270℃.

Results & discussion The effective herbage intake (7.6 kg of DM7cow*day) at pasture was lower than expected. Even though it was sufficient to increase the ω-3 FA concentration in Fontina–type cheese (0.71g/100g FA) when compare to the control cheese (Table 2). Consequently, the ω-6 FA to ω-3 FA ration decreased from 6.54 to 3.37, being closer to the recommended values for human nutrition. Similarly, also the SFA content of pasture-derived cheese was 74.87g/100 g FA lower than that of the control cheese. This is in coherence with similar finding in Europe (Coppa *et al.*, 2015; Bovolenta *et al.*, 2014).

Table 1 Diet compositions and milk yield.

Feeding Regime	Pasture	Control
Grazing time (hr.)	24	0
Concentrates (kg DM/day)	4.6	9.9
TMR* (kg DM/d)	0	14.2
Herbage intake (kg DM/d)	7.6	0
Milk per cow (kg/cow*day)	27.6	41.8

*TMR was composed of a mixture of hays (timothy 2.5, alfalfa 3, tall fescue 1, oat grass 3, and reed canary grass 1.5), with ground corn (2.5), TMR base (7.5), health corn (2.5) and protected fat (0.2) for indoor feeding (average 24.8kg/day).

Conclusion Fontina-type cheeses made with milk from grazing cows on mountain decreased the ω-6 FA to ω-3 FA ratio to a more favourable value for heath of human.

Acknowledgements This study was supported by project No. PJ01020902 of RDA, 2017, Korea.

Table 2. Fatty acid composition of Fontina-type cheese.

Constituents [1]		Season Off	Control[2]	Pasture[3]
Total fat (g/100g cheese)		19.96±1.68	21.97±1.17	20.38±0.89
Saturated fatty acids (g/100g FA)		87.59±3.93	82.39±1.00	74.87±4.56
Unsaturated fatty acids	Mono unsaturated fatty acids	8.46±3.91	13.70±10.31	21.49±4.35
	Poly unsaturated fatty acids	3.95±0.02	3.91±0.31	0.81±0.22
Total unsaturated fatty acids (g/100g FA)		12.41±3.93	17.61±10.00	25.13±4.56
ω-6 Fatty acids	Linoleic acid *	2.93±0.03	2.82±0.25	2.29±0.13
	γ-Linolenic acid	0.06±0.03	0.08±0.01	0.06±0.02
	Arachidonic acid	0.05±0.07	0.15±0.05	0.22±-.21
Total ω-6 fatty acids (g/100g FA) **		3.04±0.02	3.05±0.19	2.58±0.06
ω-3 Fatty acids	α-Linolenic acid **	0.45±0.00	0.48±0.04	0.62±0.04
	Eicosapentaenoic acid	0.00±0.00	0.00±0.00	0.08±0.08
	Docosahexaenoic acid	0.00±0.00	0.00±0.00	0.00±0.00
Total ω-3 fatty acids (g/100g FA) *		0.45±0.00	0.48±0.04	0.71±0.13
Ratio of ω-6 to ω-3		6.82	6.54	3.37

[1] Values presented as Mean±SD; [2] TMR fed during gazing season; [3] Grazing on pasture; * P<0.1, ** P<0.05

References

Coppa M, Chassaing C, Ferlay A, Agabriel C, Laurent C, Borreani G, Barcarolo R, Baars T, Kusche D, Harstad OM, Verbič J, Golecký J, Delavaud C, Chilliard Y, and Martin B. 2015. Journal of Dairy Science 98, 1539–1551.

Bovolenta S, Romanzin A, Corazzin M, Spanghero M, Aprea E, Gasperi F and Piasentier E. 2014. Journal of Dairy Science 97, 7373-7385.

Growth performance and slaughter traits of Baladi and Shami-Baladi kids raised during summer in Jordan

Mohammad Diya Obeidat, Belal Obeidat

Jordan University of Science and Technology, Irbid, Jordan

E-mail: mdobeidat@just.edu.jo

Take home message Crossbreeding is a mating system that has been widely used by animal producers to improve the productivity of their animals. However, a crossbred animal not mean necessarily better animal. This study showed that crossing Baladi and Shami goat breeds did not have a great positive effect on male kid's growth and slaughter traits during the period of hot summer in Jordan.

Introduction In developing countries such as Jordan, sheep and goats play a major role in household economic through meat and milk production (Steinfeld *et al.* 2006). Due to some health considerations and consumer preferences, the demand for goat meat has increased during the past several years. Lee *et al*., (2008) reported that goat meat had lower levels of hypercholesteremic fatty acids and higher levels of unsaturated fatty acids which make it healthier compared to lamb meat. However, meat production of local goat breeds is not adequate to meet the high demand. As a result, some exotic breeds with noticeable meat and milk production have been imported to the country and raised as pure breeds or crossed with local goats. During the last decade, the most famous exotic breed that has been widely used in Jordan is Shami goat.

Materials & methods A total of thirty newly weaned kids (15 Baladi (BB) kids and 15 Shami-Baladi (SB) kids) were evaluated for growth performance and slaughter traits. The trial lasted for 77 days (7 days for adaptation and 70 days for data collection). Feed intakes were measured on daily basis. Body weight of kids was measured at the beginning of the study and biweekly thereafter. At the end of the trial kids were slaughtered to examine carcass traits. Data were analysed using the MIXED procedures of SAS (SAS Inst., Inc., Cary, NC). Fixed effect of growth performance traits included only genotype, while for carcass traits the model included cold carcass weight as a covariate. Kids were modelled as a random effect.

Results & discussion Initial weight, final weight, and ADG were not affected ($p > 0.05$) by kid's genotype. In addition, genotype of kid showed no significant effect on feed to gain ratio. Cold carcass weight, dressing percentage were also not affected by kid's genotype ($p > .05$). Offal formed about 13% of the carcass, with no difference between both genotypes. Kid genotype had no significant effect on shoulder, rack and legs percentages. However, SB kids had higher loin cut percentage compared to BB kids ($p < 0.05$).

Table 1 Growth performance an carcass traits of Baladi (BB) and Shami Baladi (SB) kids rasied in the summer.

	Genotype	
Trait	BB	SB
Initial weight (kg)	18.9 ± 0.32	18.3 ± 0.32
Final Weight (kg)	29.2 ± 0.52	29.6 ± 0.52
ADG (g)	155 ± 8.9	156 ± 8.9
Feed to Gain	8.2 ± 0.31	7.6 ± 0.31
CCW (kg)	13.2 ± 0.23	13.3 ± 0.23
DP %	44.7 ± 0.01	45.2 ± 0.01
Offal %	13.3 ± 0.5	13.4 ± 0.5
Shoulders %	44.7 ± 0.01	45.2 ± 0.01
Loin %	6.9 ± 0.3^b	7.6 ± 0.3^a
Rack %	9.8 ± 0.13	10.1 ± 0.13
Legs %	33.3 ± 0.64	32.5 ± 0.64

^{a,b} means with different superscripts with the same row differ ($P < 0.05$)

Conclusion Results of this study indicate that crossing Shami and Baladi goat breeds did not have a significant effect on either growth performance or slaughter traits. This could be due to the high temperatures during summer season. Future studies on different seasons may merit further investigation.

Acknowledgements The authors wish to thank the Deanship of Scientific Research at Jordan University of Science and Technology for the financial support of this project (192/2016).

References

Lee JH, Kannan G, Eega KR, Kouakou B and Getz WR 2008. Small Ruminant Research 74, 255–259.

Steinfeld H, Wassenaar T and Jutzi S 2006. Revue Scientifique et Technique 25, 505-516.

Expression of chemerin and chemerin receptor in plasma, subcutaneous adipose tissue and granulosa cells during lactation in dairy cows: potential effect of diet energy content

Namya Mellouk, Christelle Ramé, Mélodie Diot, Eric Briant, Jean-Luc Touzé, Joëlle Dupont

INRA-CNSR-Université François Rabelais Tours-IFCE, Nouzilly, France

E-mail: namya.mellouk@inra.fr

Take home message Chemerin is a potential key actor for the regulation of metabolism and reproduction in lactating cows.

Introduction In dairy cows, the energetic cost of milk production during early lactation is greater than energy consumed resulting in a prolonged period of negative energy balance (NEB) and consequent mobilization of body tissue reserves. The energy balance of the animals influences the amount of adipose tissue mobilized that negatively affects reproductive performance. Emerging evidence indicates that bioactive molecules produced by adipose tissue named adipokines are not only involved in adipose tissue metabolism but also in the regulation of the physiology of the reproductive tract and more precisely in ovary. Chemerin is a new adipokine highly expressed in the adipocytes where it plays a role in adipogenesis (Song *et al*., 2010). To exert its physiological functions chemerin is able to bind predominantly a G protein-coupled receptor, the chemokine-like receptor 1 (CMKLR1). We have previously shown that chemerin can regulate ovarian functions including steroidogenesis (Reverchon *et al*., 2014). In this study we have investigated the expression of chemerin and its receptor in both subcutaneous adipose tissue and granulosa cells at different times of body fat mobilization (4, 8, 20 and 44 weeks after calving). Then, we explored the effect of a high and a low energy diet on their expression in subcutaneous adipose tissue during lactation in dairy cows.

Materials & methods Our study included two independent experiments that took place at the experimental unit UEPAO (Institut National de la Recherche Agronomique, Nouzilly, France). During the first experiment, multiparous Holstein dairy cows were fed with a high-energy diet (HE) from calving to 44 weeks postpartum (pp, n = 9). During the second experiment primiparous Holstein dairy cows were fed either with a HE diet (n = 8) or with a low energy diet (LE, n = 8) from -4 to 16 weeks pp during the first lactation and from -4 to 8 weeks pp during the second lactation. HE diet was calculated to produce 35 kg milk/day whereas LE was calculated to produce 25 kg milk/day. Biopsies of subcutaneous adipose tissue were performed at the dewlap at 4, 8, 20 and 44 weeks pp for the first experiment and at -4, 1 and 16 weeks pp (or 8 weeks pp for the second lactation) during the second experiment. Granulosa cells from small (SF, 3–5 mm), medium (MF, >5 and ≤7 mm), and large (LF, >7 mm) follicles were obtained only during the first experiment after ovum pick up at 8, 20 and 44 weeks pp. Plasma concentration and mRNA expression of chemerin and CMKLR1 were obtained by ELISA and RT-qPCR respectively.

Results & discussion We have shown that plasma chemerin levels significantly decreased from 8 weeks pp to the dry period (44 weeks pp). In adipose tissue, the mRNA expression of chemerin and CMKLR1 increased between 4 and 8 weeks pp then decreased until the dry period. In addition, chemerin mRNA expression was decreased at 1 week pp compared to -4 and 16 weeks pp in cows fed with HE diet during the first and the second lactation while opposite results were obtained for CMKLR1. Similar results were observed for mRNA expression of CMKLR1 in cows fed with LE diet while there was no difference of chemerin mRNA levels. We have also found that the LE diet decreased the expression of chemerin in adipose tissue at 1 week pp of the first and the second lactation compared to HE diet while no difference was observed at -4 and 16 weeks pp between the two groups. Interestingly, we have shown that mRNA expression of chemerin system was higher in granulosa from SF than in granulosa from MF and LF. In granulosa from SF, mRNA expression of chemerin and CMKLR1 was higher at 8 weeks pp and then decreased from 20 weeks to the dry period.

Conclusion Regarding to these results, we showed that the energetic state and the diet of Holstein dairy cows influence chemerin system expression in adipose tissue and similarly in ovarian cells such as granulosa cells in Holstein dairy cows. The regulation of chemerin tissue expression is similar to those observed in plasma that makes chemerin a good marker for evaluating metabolism and reproductive efficiency in Holstein dairy cow.

Acknowledgements The research leading to these results has received funding from the European Union Seventh Framework Program (FP7/2007-2013) under the grant agreement n°311776 and from the Région Centre under the grant agreement n°32000407.

References

Song SH 2010. Domestic Animal Endocrinology 39, 97-105.
Reverchon M 2012. Biology of Reproduction 90, 102

Azgp1 knockout changes mammary gland, adipose tissue and liver gene expression in lactating mice

Yannick Faulconnier[1], Céline Body[1], José Pires[1], Cyril Labonne[1], Sébastien Bes[1], Emilie Tixier[1], Christine Leroux[1,2]

[1]Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès-Champanelle, France, [2]Department of Food Science and Technology, University of California Davis, Davis, CA, 95616, USA

E-mail: yannick.faulconnier@inra.fr

Take home message *Azgp1* influences mammary gene expression that could regulate milk fat composition.

Introduction The alpha-2-glycoprotein 1, zinc-binding (ZAG encoded by *AZGP1* gene) is an adipokine involved in the regulation of mobilization of body reserves in adipose tissue (AT, Bing *et al.*, 2010). *AZGP1* gene expression was observed in the mammary gland (MG) of ruminants. Modifications of its expression in response to different dietary conditions suggest that *AZGP1* may be involved in the regulation of milk fat composition (Ollier *et al.*, 2007; Faulconnier *et al.*, 2011). Our objective was to study *AZGP1* function in MG and assess its role in overall lipid metabolism of lactating animals.

Materials & methods Both alleles of *Azgp1* were inactivated by gene targeting in a knockout (KO) mice model. The effects of *Azgp1* KO (or $^{-/-}$) on the lactation function were studied on Azgp1$^{-/-}$ *vs Azgp1* $^{+/+}$ mice by measuring the offspring growth *via* litter weight and the milk fatty acid composition by gas chromatography. The expression of 40 genes mostly involved in lipid metabolism was analyzed in MG, AT and liver (n = 10) by RT-qPCR using TaqMan Low Density Arrays (Applied Biosystems, Foster City, CA, USA). Results were expressed as fold change of threshold cycle (Ct) values relative to the control. Animal and gene expression data were analyzed using SAS version 9.4 and XLSTAT Software, respectively.

Results & discussion The body weight of lactating *Azgp1*$^{-/-}$ mothers was greater after parturition ($p < 0.01$) and at 10 days of lactation ($p < 0.04$) than *Azgp1*$^{+/+}$. The milk polyunsaturated fatty acid content increased ($p < 0.01$) in *Azgp1* KO compared to the wild type, although they were fed with the same standard diet.. A difference in milk secretion and composition could explain in part the higher growth rate of *Azgp1*$^{+/+}$ pups nursed by *Azgp1*$^{-/-}$ mothers. In addition, among the 40 studied genes, 9, 10 and 3 genes were ($p < 0.05$) or tended ($p < 0.1$) to be downregulated in MG, AT and liver of *Azgp1* KO mice, respectively. Most of these genes are involved in fatty acid synthesis and transport, or in triglyceride synthesis (Figure 1).

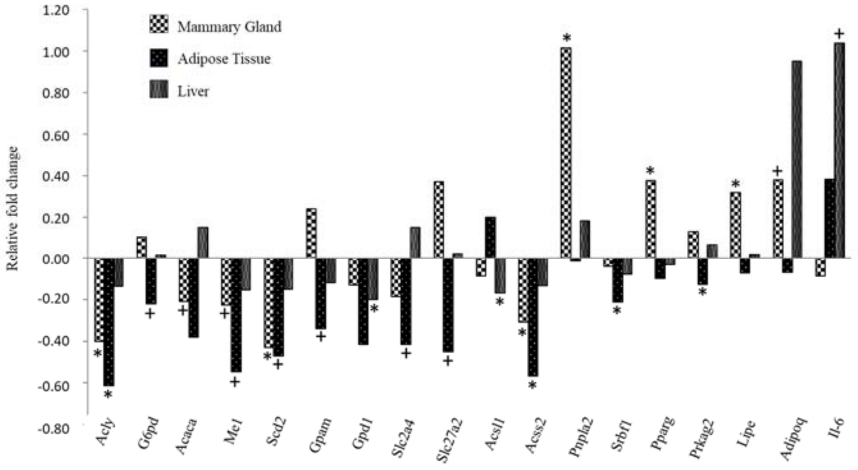


Figure 1 Effect of *Azgp1* KO on the expression of genes involved in metabolism in mice MG, AT and liver (comparison of *Azgp1*$^{-/-}$ (n = 10) to *Azgp1*$^{+/+}$ (n = 10) lactating mice). Significance: +: $P < 0.1$; *: $P \leq 0.05$

Conclusion In lactating mice, *Azgp1* KO modified the expression of genes involved in MG, AT and liver lipid metabolism. The changes in MG gene expression may explain the modifications observed on fatty acid composition in milk. This study supports a role of *Azgp1* on the lactation function and regulation of nutrient partitioning, by modifications of gene expression in MG, AT and liver of *Azgp1*$^{-/-}$ mice.

Acknowledgements We thank the UMS344/US8 SFR Biosciences (Lyon, France) for transgenic mouse production, C. Del'Homme, A. Teynie and A. Teurrisse ofthe Nutrition Experimental Installation (INRA, St Genes Champanelle, France) for mice management, F. Le Provost (INRA, Jouy-en-Josas, France) for helpful insights on milk collection technique.

References

Bing C, Mracek T, Gao D and Trayhurn P 2010. International Journal of Obesity 34, 1559-1565.
Faulconnier Y, Bernard L, Boby C, Domagalski J, Chilliard Y and Leroux C 2017. Animal 10, 1-12.
Ollier S, Robert-Granié C, Bernard L, Chilliard Y and Leroux C 2007. Journal of Nutrition 137, 560-567.

Quest for plasma biomarkers for beef tenderness

Sabrina Boudon[1], Joelle Henry-Berger[2], Isabelle Cassar-Malek[1]

[1]Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès- Champanelle, France, [2]Université Clermont Auvergne, CNRS, GReD, UMR6293 U1103, F-63000 Clermont-Ferrand, France

E-mail: isabelle.cassar-malek@inra.fr

Take home message We have made an *in-silico* identification of 63 candidate plasma biomarkers for beef tenderness by a meta-analysis of published data.

Introduction A challenge for the ruminant sector is to predict and manage the complex phenotypic traits related to meat production and quality especially tenderness a top priority for the beef industry. Meat quality is a complex phenotype that can be evaluated only after slaughtering and ageing. Previous research efforts have investigated the potential of muscle-derived markers to assess meat quality (Picard *et al.*, 2015; Cassar-Malek and Picard, 2016). However, their evaluation requires post-mortem muscle sampling or biopsies in the living animals. Thus, the identification of low invasive biomarkers is an issue for the beef sector. In this study, we hypothesized that available published data may help identifying candidate plasma biomarkers for beef quality.

Materials & methods Proteomic, transcriptomic and genetic muscle data related to beef tenderness (with significant correlation, differential abundance in extreme groups, or gene polymorphism) were collected from 54 publications including 1 GEO dataset (GSE9256) and computed according to Bonnet *et al.* (2016). Aggregation of the Gene Names (GN) from 3 datasets (proteomics, transcriptomics and genetics) were analysed using ProteINSIDE (http://www.proteinside.org/) to find biological information and predict secretion through a signal P sequence (SignalP score ≥ 0.5; TargetP score ≤ 2) or through a non-classical pathway without signal P (TargetP score ≤ 2). The lists of secreted proteins (named SignalP, other secreted) and cytoplasmic proteins were compared to a list of bovine plasma proteins from publications and experimental data (n=1106).

Results & discussion. The numbers of GN included in each dataset are shown in Table 1. Among the total 681 unique GN, 145 corresponded to secreted proteins, and 543 to cytoplasmic proteins. The comparison of the GO annotations revealed 73 common GO Biological Pathways (BP) between the datasets (oxidation-reduction process, response to oxidative stress, apoptotic process, proteolysis, lipid metabolic process, response to hypoxia, transport) consistent with tenderness mechanisms (Cassar-Malek and Picard, 2016). Twenty-four of the secreted proteins and 39 of the cytoplasmic proteins were hypothetically released in the bovine plasma. In both repertoires the GO BP were mainly related to oxidation-reduction process and neutrophil degranulation. Moreover, glycolytic process, ATP metabolic and apoptotic process, response to oxidative stress and extracellular matrix were specific to cytoplasmic plasma proteins whereas transport, lipid metabolic process and response to hypoxia were more specifically associated with secreted plasma proteins.

Table 1 Candidate plasma proteins secreted by muscle as computed from proteomic, transcriptomic or genetic data.

Dataset	Nb of unique GN	Signal P	Other secreted	Cytoplasmic	Secreted + Plasma	Cytoplasmic + Plasma
[Proteomics] n= 25 publications	156	10	31	115	11	20
[Transcriptomics] n= 9 publications	226	28	13	185	4	10
[Genetics] n= 20 publications	328	47	20	261	9	13
[General bilan] (unique Gene Names)	710 (681)	85 (83)	64 (62)	561 (543)	24	39

Conclusion Meta-analysis and bioinformatic analysis of published data enabled to identify putative plasma biomarkers for beef tenderness. Their detection in the plasma proteome of cattle from extreme groups of tenderness is on-going.

Acknowledgements This research was supported by grants from Auvergne-Rhône-Alpes in Europe division (FEDER).

References

Bonnet M, Tournayre J and Cassar-Malek I 2016. Molecular BioSystems. 12, 2722–2734.
Cassar-Malek I and Picard B 2016. Scientific World Journal 2016, 1–11.
Picard B, Lebret B, Cassar-Malek I, Liaubet L, Berri C, Le Bihan-Duval E, Hocquette JF and Renand G 2015. Meat Science 109, 18–26.

The effects of monensin and physical form of concentrate on carcass quality of male Holstein calves

Hamid Mohammadzadeh, Reza Rastkari, Ali Hosseinkhani

University of Tabriz, Tabriz, Iran

E-mail: hamidmhz@tabrizu.ac.ir

Take home message In contrast with pelleting the diet, application of monensin in diets of male Holstein calves improved carcass quality with increasing rib muscle area and decreasing the back fat thickness.

Introduction Antimicrobial compounds such as ionophore routinely incorporated into beef cattle diets improve production efficiency (Callaway and Martin, 1996). Monensin inhibits growth of gram-positive organisms, reduces lactic acid production and methanogenesis in the rumen, increases molar proportion of propionate and improves nitrogen retention (Goodrich *et al.,* 1984; Van Nevel and Demeyer, 1988). These situations may lead to improvement in animal growth rate, milk yield, feed intake and feed conversion (Chaucheyras-Durand and Fonty, 2002; Stella *et al.,* 2007). However there is very little information about the effects of ionophore and physical form of diet or concentrate on beef cattle carcass quality. Therefore, our hypothesis was to investigate the main and interaction effects of monensin application and physical form of diet (mesh or pellet) which may affect digestion rate in rumen, on carcass quality of Holstein male calves.

Materials & methods Sixteen Holstein male calves with average body weight of 258 ± 12 kg and average 9 ± 1 months old were used in an experiment with four treatments as a completely randomized design. Treatments were: Mesh concentrate without monensin (MWM), Mesh concentrate with monensin (300mg/d; MM), pelleted concentrate without monensin (PWM) and pelleted concentrate with monensin (300mg/d; PM). The experimental period was 6 month with an initial adaptation to diets for 14 d. Diets were similar in terms of energy concentration, protein concentration, forage source and forage to concentrate ratio. Diets were offered as totally mixed rations, three times per day as *ad libitum*. Feed intake was measured daily, and animals were weighted every two weeks. Data were analyzed with proc mixed of SAS (9.1) using treatment as fixed effect and animal within treatment as random effect.

Results According to the results in Table 1, application of monensin resulted in lower back fat in carcass ($P<0.05$). However, application of monensin did not affect kidney, pelvic and hurt fats but increased rib muscle area ($P<0.05$). In contrast, pelleting the concentrate resulted in higher back fat, lower pelvic and hurt fats and lower rib muscle area ($P<0.05$).

Table 1 The effects of monensin and physical form of concentrate on carcass quality.

	Treatments[1]				main and interaction effects			
	MWM	MM	PWM	PM	monensin	concentrate form	monensin × concentrate form	SEM
Back fat thickness (cm)	0.99	0.90	1.28	1.24	0.0042	0.0001	0.2820	0.035
Kidney fat (kg)	4.74	5.17	4.72	4.90	0.1209	0.6803	0.5155	0.152
Pelvic fat (kg)	4.00	4.40	3.91	3.94	0.0896	0.0429	0.2993	0.107
Hurt fat (kg)	0.91	0.92	0.77	0.73	0.6518	0.0001	0.5583	0.034
Rib muscle area (cm^2)	80.18	90.20	70.89	79.55	0.0464	0.0392	0.8833	4.027

[1] MWM: Mesh concentrate without monensin, MM: Mesh concentrate with monensin, PWM: pelleted concentrate without monensin, PM: pelleted concentrate with monensin

Conclusion In general, in contrast with pelleting the diet, application of monensin in diets of male Holstein calves improved carcass quality with increasing rib muscle area and decreasing the back-fat thickness. However, monensin increased hurt, pelvic and kidney fat.

Acknowledgements This research was supported by University of Tabriz (Iran).

References

Callaway TR and Martin SA 1996. Journal of Animal Science 74, 1982-1989.
Chaucheyras-Durand F, G and Fonty G. 2002. Microbial Ecology Health Diseases 14, 30-36.
Goodrich RD, Garrett JE, Gast DR, Kirick MA, Larson DA and Meiske JC 1984. Journal of Animal Science 58, 1484-1498.
Stella AV, Paratte R, Valnegri L, Cigalino G, Soncini G, Chevaux E, Dell′ Orto V, Savoini G 2007. Small Ruminant Research 67, 7-13.
Van Nevel CJ, Demeyer DI 1988. Elsevier Applied Science, London, pp. 387-444.

Consumer acceptance of milk of cows fed cold pressed sunflower cake

Idoia Goiri, Raquel Atxaerandio, Izaro Zubiria, Roberto Ruiz, Aser Garcia-Rodriguez

Neiker, Vitoria-Gasteiz, Spain

E-mail: aserg@neiker.eus

Take home message Feeding cold pressed sunflower cake did not negatively affect consumer acceptance of milk.

Introduction. The use of polyunsaturated (PUFA)-rich cold pressed sunflower (SUN) cake in ruminant nutrition could modify milk fatty acid (FA) profile partially replacing the saturated FA (SFA) content of milk with unsaturated FA (Amores *et al*, 2014). Although these changes in milk FA profile could have a beneficial effect in consumer's health, they could also affect milk sensory characteristics (Timmons *et al* 2001). Since consumers have been recognized as the key driver for product success (Moskowitz *et al*., 2012), it should be ensured that no detrimental effect in milk sensory traits appeared. Therefore, the objective of the present study was to study the effect of feeding SUN on consumer acceptance of milk.

Materials & methods 6 Brown Swiss and 4 Holstein cows were used in a crossover design with two treatments (CTR, SUN) and two 63-day experimental periods. Concentrates were CTR and SUN (230 g kg^{-1} sunflower cold pressed cake) and were individually fed. Concentrates were formulated to provide similar amounts of energy, protein and fat. Forage was group fed *ad libitum*. On day 60 of each experimental period, a composite milk sample (9 L) from each treatment and breed was collected into stainless steel milk cans, pasteurized, cooled and aseptically packaged into cartons and stored at 4ºC. A duotrio test was performed to analyse differences between treatments within the two breeds. The acceptance test was carried out using a non-trained sensory panel (n=58). A 9-point line scale was used, with 1 being the lowest and 9 representing the highest intensity, for the attributes of appearance, flavour, odour, texture and overall acceptability. A balanced incomplete block design was used to assign 4 tempered milk samples to each panellist. Data were analysed using the MIXED procedure of SAS, with fixed effects of concentrate, breed, and their interaction, and the random effect of panellist. Treatment means were separated using Tukey test

Results & discussion Consumers discriminated milk of the cows fed SUN of that of cows fed CTR within the samples of Holstein cows ($P<0.05$), but not in the Brown Swiss ones (Table 1). An interaction between concentrate and breed was observed for milk overall acceptability (Table 1), in this sense the milk of cows fed SUN presented a higher overall acceptability only with the Holstein cows. This can be a consequence of the improved flavour observed in this milk. No significant differences were observed in terms of appearance, odour or texture.

Table 1 Mean ratings (n=58) of overall acceptability, liking of appearance, odour, flavour and texture for milk samples of two breed of cows fed cold pressed sunflower cake.

Item	Holstein-Friesian		Brown Swiss			P-value		
	CTR	SUN	CTR	SUN	SEM	CON	BR	CON*BR
Overall acceptability	5.6	6.5	6.3	6.4	0.19	0.003	0.111	0.022
Appearance	6.6	6.8	6.8	6.5	0.17	1.000	0.921	0.166
Odour	5.6	6.0	6.1	5.8	0.18	0.702	0.444	0.070
Texture	5.9	6.4	7.4	6.4	0.55	0.629	0.148	0.177
Flavour	5.6	6.6	6.5	6.6	0.29	0.008	0.048	0.058

CTR: control; SUN: cold pressed sunflower cake; SEM: standard error of the mean; CON: concentrate; BR: breed

Conclusion Inclusion of SUN in cows' ration did not negatively affect consumer's acceptance of the produced milk. However, the effect on acceptance traits depends on the breed of the cow. In this sense, the inclusion of SUN in the concentrate fed to Brown Swiss cows did not modify milk acceptance traits, but improved overall acceptability of milk in Holstein cows.

Acknowledgements This research was supported by RTA2015-00022-C03-02.

References

Amores G, Virto M, Nájera AI, Mandaluniz N, Arranz J, Bustamante MA, Valdivielso I, Ruiz de Gordoa JC, García-Rodríguez A, Barron LJ and de Renobales M 2014. Journal of Dairy Research 81, 410-416

Moskowitz HR, Beckley JH and Resurreccion AV 2012. Sensory and Consumer Research in Food Product Design and Development, ed. by IFT press, Wiley-Blackwell, New Jersey, 1–16.

Timmons JS, Weiss WP, Palmquist DL and Harper WJ 2001. Journal of Dairy Science 84, 2440-2449

Addition of mineral premix enriched with 'BAV' blend of essential oils in the ration of lactating dairy cattle on the feed intake, body weight and milk production

Ali Agus[1], Muhsin Al Anas[1], Chusnul Hanim[1], Haig Babikian[2], Yosef Babikian[2], Benjamin Jiaravanon[2]

[1]*Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia,* [2]*PT Central Proteina Prima, Jakarta, Indonesia*

E-mail: muhsin_alanas@mail.ugm.ac.id

Take home message Mineral premix combined with essential oils have a potential feed supplement to be developed to improve feed intake, health and productivity of lactating dairy cattle.

Introduction During lactating period, imbalance nutrients including mineral will affect body weight loss and milk production of dairy cattle. Minerals under-nutrition especially during early lactation risk to excessive bone mineral mobilization in favour to milk production. This condition may also impair to the animal health and reproductive performances. Utilization of antibiotic and growth promoters (AGP) to maintain the animal health and increasing livestock productive performances was banned in many countries, including Indonesia. Essential oils as an alternative use of AGP replacer has been documented and gained much interest in recent years. It was reported that essential oil has an antimicrobial properties, immunostimulators and thus improving health status of the animals (Soanghero *et al*., 2009). This research was done to evaluate the addition of mineral premix enriched with 'BAV' blend of essential oils (MP-BAV) in the ration of lactating dairy cattle on the body weight and milk production.

Materials & methods The 'BAV' is a product from the blend of nine different of essential oils produced by Central Proteina Prima Tbk Jakarta. BAV consist of *Pine oil, Gardenia jasminoides, Cocos nucifera, Eucalyptus globules, Herba Origani, Gummi Myrrha, Gummi Boswellii, Cymbopogon citrates* and Carrot seed oil. Total of 20 multiparous Holstein Friesian dairy cattle, after peak lactation (2-3 months post calving), were divided into two groups (control and treatment) of 10 cows per group. The trial was conducted during 7 weeks. The treatment group received ration as control group (grass + concentrate; 60:40) but added with 50 g of MP-BAV per head/day. Daily dry mater intake (DMI), body weight gain (ADG) and milk production (FCM 4%) were controlled and data collected was statistically analysed by Student's t-test.

Results & discussion Addition of MP-BAV has no effect on DMI, ADG and FCM 4%. However, cattle in treatment group tend to be higher in DMI (0.51 kg; p=0.12) and higher ADG (0.19 kg; p=0.11). After peak lactation, priority of nutrients utilization is for body weight reconstution rather than for milk secretion, explaining the indiference in milk production. Further trial with higher dose and number of replication would be interesting to be done.

Table 1. Effect of mineral premix enrich with 'BAV' blend essential oils in the ration of lactation dairy cattle on dry matter intake, body weight and milk production.

Item	Treatment		St.dev	p-value
	Control	MP-BAV		
DMI, kg/head/day	18.18	18.69	0.92	0.12
Body Weight (kg)				
Initial BW, kg	514.20	514.55	62.46	0.50
Final BW, kg	547.30	557.30	60.34	0.36
ADG, kg/d	0.68	0.87	0.350	0.11
Milk production (4% FCM)				
Initial (kg/head/day)	12.11	13.00	2.93	0.26
Day 1-21 (kg/head/day)	10.91	11.77	2.74	0.26
Day 22-42 (kg/head/day)	12.37	12.07	3.63	0.43

Conclusion The addition of 50 g MP-BAV per head/day has no effectsignificantly on DMI, BW gain and milk production.

Acknowledgements The authors wish to express their gratitude to Balai Besar Pembibitan Sapi Perah Baturaden, Central Java for supporting the research by providing dairy cattle for the trial, and also to PT. Central Proteina Prima Tbk Jakarta for providing BAV essential oils.

References

Soanghero M, Robinson PH, Zanfia C and Fabbro E 2009. Animal Feed Science and Technology 153, 153–157.

Supplementing goats with sainfoin pellets (*vs* alfalfa) modifies cheese sensory properties

Bruno Martin[1], Steffen Werne[3], Cécile Bord[2], Anne Ferlay[1], Amélie Lèbre[4], Matthias Klaiss[3], Annick Lebecque[2], Félix Heckendorn[3]

[1]Université Clermont Auvergne, INRA, VetAgro Sup, UMR1213 Herbivores, Saint Genès Champanelle, France, [2]Université Clermont Auvergne, VetAgro Sup, UMR545 Fromage, Lempdes, France, [3]Research Institute of Organic Agriculture, Frick, Switzerland, [4]Research Institute of Organic Agriculture, French Branch, Divajeu, France

E-mail: bruno.martin@inra.fr

Take home message Supplementing goats with sainfoin pellets instead of alfalfa modifies cheese texture and increases favour intensity without off-flavours.

Introduction Sainfoin is a legume fodder rich in condensed tannins particularly interesting for small ruminants because of its anthelmintic properties (Hoste *et al*., 2006) and interest for milk lipid composition (Girard *et al*., 2016). It is important to verify that the supplementation of goats with sainfoin does not adversely affects the cheese sensory properties. This feeding strategy was evaluated in Gruyère production (Girard *et al*., 2016) but has never been tested in farmhouse goat cheesemaking.

Materials & methods Our experiment was carried out in two commercial farms producing goat milk cheeses (Mutschli in Switzerland and Picodon in France). In each farm, the Alpine goats were divided in two groups of goats (10 each in the Mutschli farm and 14 and 18 in the Picodon farm). Each group was fed with either 700g sainfoin or 700g alfalfa pellets daily and all animals received an additional 100g/day of corn-grit (Mutschli) or 50g/day of whole barley grain (Picodon). The experimental feed was given twice daily in the milking parlour. Except for milking and feeding, all animals were kept in one group and had access to pasture for approx. 5 hrs daily and had a*d libitum* access to non-tanniferous hay when stabled. In both farms, the milk from the goats of the two groups was collected separately and used for parallel cheesemaking with identical process. During weeks 5 and 6 of the sainfoin/alfalfa feeding period, cheesemaking was repeated 4 and 6 times in the Mutschli and Picodon farms respectively. Once ripened (8 and 2 weeks respectively), the cheeses were analysed for gross and fatty acid composition. Two types of sensory tests were performed: a triangular test with an untrained panel (n=38) and a descriptive analysis by a trained panel (n = 10). A specific list of sensory attributes was developed for the description of each cheese variety (32 and 33 for Mutschli and Picodon) by the panellists who were trained to recognise them during 7 training sessions. Data from the descriptive analysis evaluation were analysed using the Mixed procedure of SAS, with feeding considered as fixed effect, the panellist as random effect and the day as the repeated statement.

Results In the triangular test, both Mutschli and Picodon cheeses made with milk from goats supplemented with sainfoin were found significantly different from those made with milk from goats supplemented with alfalfa. The differences seemed more important for the Picodon (79% of correct answers, $p<0.0001$) than for the Mütschli cheese (61% of correct answers, $p<0.001$). The descriptive test showed that Picodon and Mutschli sainfoin cheeses developed stronger flavours characterised by animal notes in comparison to alfalfa cheeses. The differences were more important for Picodon than for Mutschli: sainfoin Picodon cheeses had a significantly stronger odour, characterised as more “animal”, and stronger aromas like “goat aroma” and “animal aroma” (Table 1). These cheeses were also less “fruity” and had a more bitter taste. Similarly, sainfoin Mutschli cheeses tended to have higher “goat” and “hay” aromas (Table 2). These common trends can be linked to the higher secondary proteolysis of Sainfoin cheese (+13%) and/or to their higher PUFA content (including C18:3n-3, 1.6-fold higher in sainfoin compared to alfalfa) in both cheese varieties. In comparison to alfalfa Picodon cheese, the texture of the sainfoin Picodon cheese was less firm, dry and crumbly and more creamy, possibly because of their lower dry matter (-3.7%). In contrast, the texture of the sainfoin Mutschli cheese was drier and less melting and sticky than the texture of the alfalfa Mutschli cheese.

Table 1 Sensory properties of Picodon cheeses (score 0-10).

Feeding	Alfalfa	Sainfoin	SEM	P Value
Odour intensity	5.5	5.8	0.09	0.015
Animal Odour	2.2	2.6	0.01	0.010
Goat Aroma	4.7	5.2	0.08	0.048
Creamy texture	1.1	1.5	0.09	0.006
Firm texture	5.8	4.9	0.13	0.001

Table 2 Sensory properties of Mutschli cheeses (score 0-10).

Feeding	Alfalfa	Sainfoin	SEM	P Value
Odour intensity	6.3	6.3	0.12	0.940
Hay Aroma	1.4	1.7	0.14	0.065
Goat Aroma	5.5	5.9	0.16	0.074
Melting texture	2.1	1.5	0.17	0.025
Sticky texture	2.0	1.5	0.16	0.022

Conclusion Using sainfoin as a supplement for goats modifies the texture and flavour of cheeses but is not responsible for the development of any off-flavours.

Acknowledgements We thank famille Gilles and the agricultural center in Visp. This research was funded by the Auvergne Rhône-Alpes Region (PSADER project), the Federal Office for Agriculture in Switzerland and the Canton du Valais.

References

Girard M, Dohme-Meier F, Wechsler D, Goy D, Kreuzer M and Bee G 2016. Journal of Dairy Science 99, 205–220.
Hoste H, Jackson F, Athanasiadou S, Thamsborg SM and Hoskin SO 2006. Trends in Parasitology 22, 253-261.

RNA-Seq showed that lactation modified gene expression profile in goat adipose tissue

Céline Boby[1], Yannick Faulconnier[1], Sébastien Bes[1], Yannick Gestin[1], Patrice Martin[3], Christine Leroux[1,2]

[1]*Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès-Champanelle, France,* [2]*Department of Food Science and Technology, University of California Davis, Davis, CA, 95616, USA,* [3]*GABI, INRA, AgroParisTech, Université Paris Saclay, 78350, Jouy-en-Josas, France*

E-mail: celine.boby@inra.fr

Take home message The lactation status of goats induces changes in adipose tissue gene expression profiles, especially those of lipid metabolism.

Introduction A major function of ruminant adipose tissue (AT) is to store lipids for use in productive functions. Body fat mobilization is required during periods of negative energy balance such as early lactation period. Changes in AT transcriptome are known in dairy cattle peripartum (Khan *et al.*, 2013; McNamara *et al.*, 2016). In contrast in goat, few information is available on AT gene expression profiles except modifications of AT transcriptome in response to food deprivation during lactation (Faulconnier *et al.*, 2011). The objective of this study was to compare adipose tissue gene expression in AT of goats at two different lactation stage, lactation and post-lactation by analyzing RNA-Seq data sets.

Materials & methods Four lactating (74 days in milk) and four dry (120 days post-lactation) Alpine goats were fed a standard diet of mixed hay grass *ad libitum* with concentrate. Samples of omental AT were collected immediately post mortem. Total RNAs were extracted and RNA-Seq analyses performed from AT using a HiSeq. 1500 (Illumina, USA). After normalization and cleaning, sequences were mapped to the reference *Bos taurus* genome. The statistical analyses were performed using R software with DEseq1.8 including a Benjamini-Hochberg (BH) multitesting correction at 5%. Bio-informatic analyses were performed using Panther tools for Gene Ontology and KEGG for pathways identifications.

Results & discussion RNA-Seq analysis allowed to detect the expression of 19 271 genes in AT of lactating and post-lactating goats. Among them, 252 genes were differentially expressed ($p_{adj} \leq 0.05$), 118 upregulated and 134 downregulated, and 11 genes were validated by RT-qPCR. Data mining using bio-informatics tools revealed that among genes involved in metabolic processes (representing 31% of the differentially expressed genes), 38% were involved in lipid metabolism (29 genes differentially expressed). Most of genes involved in lipid synthesis were increased and most of those involved in lipid transport and storage were decreased in AT of lactating goats compared to post-lactating goats (Table 1), that could correspond to the recovery of lipid reserves after lactation peak.

Table 1 Genes involved in lipogenesis, lipid transport and lipid storage differentially expressed during lactation compared to the dry period.

Functional class	Gene Name	Description	Fold Change	P_{adj}	Mean reads Mid-lactation	Mean reads Post-lactation
Lipogenesis	PC	Pyruvate carboxylase	1.96	0.02	6023	3068
	DGAT1	Diacylglycerol O-acyltransferase 1	2.38	0.00	3484	1463
	LPL	Lipoprotein lipase	2.48	0.01	153062	61735
	ACSL1	Acyl-CoA synthetase long-chain family member 1	2.57	0.02	103471	40194
	ACOT2	Acyl-CoA thioesterase 2	3.10	0.00	565	182
	GPAT3	Glycerol-3-phosphate acyltransferase 3	4.19	0.00	7951	1899
Lipid transport	CRABP2	Cellular retinoic acid binding protein 2	0.17	0.00	440	2635
	VLDLR	Very low-density lipoprotein receptor precursor	0.27	0.02	1476	5546
	GM2A	GM2 ganglioside activator	0.55	0.05	1030	1866
Lipid storage	PLIN4	Perilipin 4	0.47	0.05	33988	73035

Conclusion To our knowledge this is the first report on AT transcriptome data using RNA-Seq in goat. It revealed different gene expression profiles of AT during and after lactation, specifically those involved in lipid metabolism. This exhaustive repertoire of genes expressed improve our knowledge in a species with few genomic data. These RNAseq information will serve as valuable data to better understand the molecular mechanisms of lipid metabolism regulation in this dairy species.

Acknowledgements This research was supported by grants from Agence National de la Recherche and APIS-GENE. We thank the “Ecole Normale Supérieure” France Genomic Platform for the sequencing analysis.

References

Khan MJ, Hosseini A, Burell S, Rocco SM, McNamara JP and Loor JJ 2013. Journal of Dairy Science 96, 2171-2182.

McNamara JP, Huber K and Kenéz A 2016. Journal of Dairy Science 99, 5649-5661.

Faulconnier Y, Chilliard Y, Torbati MB and Leroux C 2011. Comparative Biochemistry and Physiology Part D Genomics Proteomics 6, 139-149.

Pasture and extruded linseed influence on ewes milk qualities and ricotta sensory characteristics

Isa Fusaro[1], Melania Gianmarco[1], Michael Odintsov Vaintrub[1], Matteo Chinacarini[1], GianPiero Mazzone[1], Andrea Formigoni[2], Giorgio Vignola[1]

[1]University of Terano, Teramo, Abruzzo, Italy, [2]University of Bologna, Emiglia-Romagna, Italy

E-mail: modintsovvaintrub@unite.it

Take home message Adding extruded linseed to dairy ewes' diet when pasture is lacking improves milk parameters. Ricotta made from such milk results in having better sensorial qualities in comparison to standard product.

Introduction In central Italy (Lazio and Abruzzo regions) the diet of dairy ewes is almost exclusively based on pasture during summer, spring and autumn, while during winter they kept indoors and given a standard feeding (hay and grains). Ricotta is a milk product highly associated with freshness and low-fat and thus considered as healthy food. Seasonal feeding alteration can impact the quality of the product. The aim of this study was to evaluate the possibility of using extruded linseed with the purpose of maintain the quality of milk and ricotta when pasture is scarce.

Materials & methods 54 ewes of Comissana breed (farm located in Lazio, Central Italy) were randomly divided into three groups of equal size. Each group was fed one of three experimental diets: 1) Pasture (P)- typical pasture grazing; 2) Enriched diet (L)- supplemented with 0.190 kg of extruded linseed; 3) Farm diet (F)- typical winter ration (control group). The experimental diets were administered for 80 days in total. The first month after delivery, the ewes milk was used for lamb production, as traditionally common in these parts. After this first period they were milked twice a day and weekly bulk milk was sampled and analysed for chemical composition and lipid profile analysis. Ricotta was obtained from this milk using traditional methods (by whey protein coagulation) and sensory panel was then performed using a modified protocol from Pizzillo *et al.* (2005).

Before milk analysis, the data on fatty acid composition were processed to compute the Δ9-desaturase activity index as the C14:0/C14:1 ratio (Addis *et al.*, 2005), and the atherogenic index was calculated according to Chilliard *et al.* (2000). The data on milk fatty acids and structure was analysed using ANOVA. For models used for milk sampling time was not included because it was found not to be significant ($P>0.05$). The final model included the fixed effect of dietary treatment and the residual error expressed as $Yij = \mu+Di +\varepsilon ij$. The sensory test data were normalised, then subjected to analysis of variance for repeated measures, with diet as sole factor. Duncan's test was used to determine the groups significantly different from each other.

Results & discussion Milk from the L group had higher fat content (44.75 %DM, 5.21% total) while P group had higher content of milk proteins (L 4.25%; F 4.76%). Fatty acid analysis of milk resulted with distinct differences. L group had higher n-3, n-6 FA and P group was higher on 9-desaturas index and IA (Table 1). In ricotta sensory panel Greasiness and Spreadability were highest in L group, while in parameter of Whiteness group P scored the lowest (Table 2).

Table 1 Key fatty acid profile (g/100 g of total fatty acid) of bulk milk.

Fatty acid	F Group	L Group	P Group	*p*
N-3	0.7^{c}	2.15^{A}	0.99^{B}	0.000
N-6	2.26^{a}	1.81^{b}	1.78^{b}	0.013
N-6/N-3	3.22^{A}	0.84^{C}	1.63^{B}	0.000
Δ9-D index	0.039^{a}	0.048^{b}	0.057^{c}	0.015
IA	3.54^{c}	1.75^{a}	1.98^{b}	0.01

Table 2 Differences of organoleptic qualities in various ricotta (attributed points).

Attribute	F Group	L Group	P Group	*p*
Greasiness	4.6^{b}	5.7^{a}	4.9^{b}	0.31
Spreadability	4.9^{C}	7.1^{a}	6.3^{b}	0.44
Whiteness	7.2^{A}	6.9^{A}	3.5^{B}	0.001

Mean values in the same row with different letter present significant differences: a,b,c= $p< 0.05$; A,B,C= $p< 0.01$

Conclusion On the basis of our results, addition of extruded linseed to the farm-based diet is effective in obtaining milk of high quality fatty acid composition. Although having different properties than pastured animals, ricotta cheese produced by linseed fed ewes has greasier and softer texture as a product, which adds to its organoleptic value.

Acknowledgements We thank "Azienda Agricola Gasbarri" for farm support and herd management.

References

Pizzillo M, Claps S, Cifuni GF, Fedele V and Rubino R 2005. Livestock Production Science 94, 33–40.
Addis M, Cabiddu A, Pinna G, Decandia M, Piredda G, Pirisi A and Molle GJ 2005. Journal of Dairy Science 88, 3443-3454
Chilliard Y, Ferlay A, Mansbridge MR and Doreau M 2000. Annales de Zootechnie 49, 181-205.

Association of leptin gene polymorphism with beef quality traits in two Sudanese baggara cattle (*Bos indicus*) subtypes

Khaleel Jawasreh[1], Romaz Omer[2], Mai Masri[2], Ikhlas Nour[2], Mohamed-Khair Ahmed[2]

[1]*Jordan University of Science and Technology, Irbid, Jordan,* [2]*Faculty of Animal Production, Department of Genetics and Animal Breeding, University of Khartoum, Khartoum, North Sudan*

E-mail: kijawasreh@just.edu.jo

Take home message: Baggara cattle strains (Nyalawi and Mesairi) differed in their Growth performance meat quality and their genetic structure of the leptin gene.

Introduction The leptin gene encodes a 16 kDa peptide hormone, leptin, which is synthesized and released from adipocytes in adipose tissue. Therefore, the leptin gene could be an excellent candidate gene controlling fat deposition, carcass traits and meat quality in beef cattle. The aims of this study were to investigate the difference between two major Sudanese Baggara cattle strains (Nyalawi and Mesairi) and also to study the association between the possible mutations detected in *Leptin* gene and meat quality traits.

Materials & methods The present investigation was carried out on 112 Baggara bulls (4-5 years of age) that belong to two subtypes (56 Nyalawi and 56 Mesairi) were commercially finished under aseptic conditions, 112 meat samples were cut from the *Longissmus thoracis* muscles (left half of the carcass) between the 11th and the 12th ribs within 1 hour post-slaughter. before slaughtering, blood samples were taken from the jugular vein of each bull using vacutainers containing EDTA as an anticoagulant and held at 4◦C. Samples were transferred to Animal biotechnology Lab, at department of Animal production/ Jordan University of Science and Technology for genotyping and sequencing analysis. A single nucleotide polymorphism (C/T) in exon 2 of the bovine *leptin* gene that causes an arginine to cysteine amino acid substitution (Arg25Cys) was investigated. The (C/T) SNP associations with meat quality attributes in two strains of Sudanese Baggara cattle (Nyalawi and Mesairi) was studied via PCR - RFLP techniques. Slaughter and carcasses weights, dressing percentage were determined. Chemical composition, Water Holding Capacity (WHC), cooking loss and myofibril fragmentation index (MFI) were determined using standard methods. Statistical analysis was done using ANOVA and Chi-square test.

Results & discussion A total number of 112 blood samples (56 Nyalawi and 56 Mesairi,) commercially finished for 3 months. The digestion of the PCR product (94bp band) with Kpn21 enzyme showed the existence of the three genotypes (CC) (75-19bp),TT (94bp) and (CT) (94-75-19bp) in the two Baggara strains. However the genotype frequencies were; 37.5% (CC) , 39.3% (CT) and 23.2% (TT) in Nyalawi compared to 46.4%, 28.6% and 25% in Mesairi, respectively. The Chi-square (X^2) test indicated that the Mesairi strain was not in HWE (X2 = 5.862), while the Nyalawi subtype was in HWE ($X^2 = 2.388$) with regard to this locus there was no significant associations in SNP C>T of the exon 2 of leptin gene with, carcass weight, dressing and fat percentages of the strains. MFI was close to significance (P=0.072). ANOVA showed that that differences between strains in hot carcass weight, dressing percentage MFI, WHC, cooking loss, moisture and fat were highly significant (P<0.01). The effects of exon 2 of the Leptin genotypes on WHC was significant (P= 0.032). Leptin genotypes interactions with breeds were not significant (P>0.05). Previously Woronuk *et al*. (2012) who studied Western Canadian feedlot cattle reported that the T allele was associated with progressive increases in backfat thickness.

Conclusion Differences between Baggara cattle strains in hot carcass weight, dressing percentage, MFI, WHC, cooking loss, moisture and fat were highly significant. The effects of exon 2 of the Leptin genotypes on WHC was significant (P= 0.032) and MFI was close to be significance (P=0.072).

Acknowledgements we would like to thank the Deanship of research of Alkhartoum University for funding the project.

References

Woronuk GN, Marquess FL, James ST, Palmer J, Berryere H, Deobald T, Howie S and Kononoff P 2012. Animal Genetics 43, 608-10.

Impact of rearing practices applied during the lifetime of heifers on 20 protein biomarkers of tenderness or adiposity in *rectus abdominis* muscle

Julien Soulat, Valérie Monteils, Muriel Bonnet, Brigitte Picard

Université de Clermont Auvergne, INRA, VetAgro-Sup, UMR Herbivores, 63122 Saint-Genès-Champanelle, France

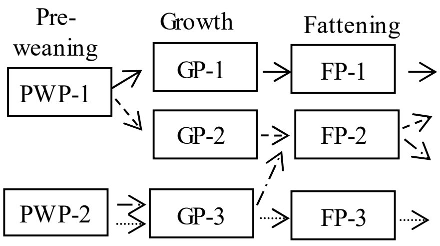
E-mail: julien.soulat@inra.fr

Take home message Among 20 protein biomarkers of tenderness or adiposity in *rectus abdominis* muscle, only the relative abundance of α-tubulin, a negative biomarker of adiposity, was modified by rearing practices.

Introduction In cattle skeletal muscles, proteins represent 20% of the chemical composition. Several studies had identified some of these proteins as biomarkers of tenderness or adiposity of beef meat (Picard *et al.*, 2015). Muscle, breed and animal type are known to influence the abundance of proteins. However, few studies had analyzed the effect of the rearing practices on these abundances. The aim of the present work was to study the effect of rearing practices applied during the lifetime of heifers (Life-Pract) on the relative abundance of biomarkers in *rectus abdominis* (RA).

Material & methods The full RA muscle was collected 24h *post-mortem* from Charolais x Aubrac heifers (n = 48). After 14 days of ageing at 4°C and cooking at an internal temperature of 55°C, a trained tasting panel evaluated their overall tenderness and fat presence. The relative abundance of 20 biomarkers was measured on samples frozen 24h *post-mortem*, using the Reverse Phase Protein Array method (Gagaoua *et al.*, 2018). The Life-Pract applied in farm, were characterized by different rearing practices applied at each life period of the heifers: pre-weaning (PWP, *i.e.* from birth to weaning), growth (GP, *i.e.* from weaning to beginning of fattening) and fattening (FP, *i.e.* from beginning of fattening to slaughter). Forty-six factors (*e.g.* weight, pasture duration, diets, etc.) collected thanks to surveys (Soulat, 2017), were used to define the different rearing practices using a factor analyse for mixed data followed by a hierarchical clustering on principal components with R software. The Life-Pract were determined thanks to the combination of the rearing practices applied during PWP, GP and FP. For each meat sensory traits and biomarkers' relative abundance, ANOVAs were realized to evaluate their dependence on the Life-Pract.

Results & discussion The combinations of the different rearing practices defined allowed characterizing 4 Life-Pract (Figure 1). Our results displayed that the tenderness, the fat presence in meat and the relative abundance of 19/20 biomarkers were not impacted by the Life-Pract. Only one protein: α-tubulin showed differences in relative abundance among the 4 Life-Pract. The α-tubulin was significantly most abundant in the Life-Pract-A and less abundant in the Life-Pract-B. In Life-Pract-A, the heifers had a longer pasture duration during their lifetime and intake a lower concentrate quantity during GP and FP than in Life-Pract -B. An increase of the relative abundance of α-tubulin was linked with a decrease in the animal adiposity (Taga *et al.*, 2012). These data are coherent, as cattle at pasture are known to produce lower fatness carcasses.



		Main characteristic	α-tubulin abundance
Life-Pract-A	(n = 8)	Long pasture duration during whole life	0.11^{a} ± 0.28
Life-Pract-B	(n = 8)	High concentrate quantity intake (GP and FP)	-0.26^{b} ± 0.24
Life-Pract-C	(n = 12)	Main forage in diet: Hay (GP and FP)	-0.07^{ab} ± 0.32
Life-Pract-D	(n = 10)	Short pasture duration during whole life	-0.09^{ab} ± 0.30

Figure 1 Description of the 4 Life-Pract characterized by different rearing practices and their effect on the α-tubulin abundance, with n: the number of heifers per system.

Conclusion These results showed that the establishment of different Life-Pract could result in the same tenderness and adiposity of RA muscle. Among protein biomarkers of these traits, only α-tubulin abundance was modified.

Acknowledgements Authors thanks the protected geographical indication "Fleur d'Aubrac", Bordeaux Sciences Agro for sensory analysis and Curie institute research center for Reverse Phase Protein Array.

References

Gagaoua M, Bonnet M, Ellies-Oury MP, De Koning L and Picard B 2018. Food Chemistry 250, 245-252.
Picard B, Lebret B, Cassar-Malek I, Liaubet L, Berri C, Le Bihan-Duval E, Hocquette JF and Renand G 2015. Meat Science 109, 18-26.
Taga H, Chilliard Y, Meunier B, Chambon C, Picard B, Zingaretti MC, Cinti S and Bonnet M 2012. Journal of Cellular Physiology 227, 1688-1700.
Soulat J 2017 PHD Université Clermont Auvergne, Clermont-Ferrand, 419.

Undernutrition combined with dietary mineral oil: a nutritional strategy enhancing removal of dioxins and polychlorinated biphenyls in contaminated ewes

Sylvain Lerch[1], Lucille Rey-Cadilhac[1,2], Ronan Cariou[3], Catherine Jondreville[1], Yannick Faulconnier[2], Denis Roux[4], Gaud Dervilly-Pinel[3], Bruno Le Bizec[3], Stefan Jurjanz[1], Anne Ferlay[1]

[1]Université de Lorraine / INRA, Vandoeuvre-lès-Nancy, Lorraine, France, [2]INRA / Université Clermont Auvergne / VetAgro Sup, Saint-Genès-Champanelle, Auvergne, France, [3]LUNAM Université / INRA, Nantes, Pays de la Loire, France, [4]INRA, Laqueuille, Auvergne, France

E-mail: sylvain.lerch@univ-lorraine.fr

Take home message Undernutrition combined with dietary mineral oil hastens the removal of dioxins and polychlorinated biphenyls in ewes' empty body by increasing their faecal excretions by 2.0 to 2.5-fold, when compared to a control treatment.

Introduction Last decades, livestock production has faced several sanitary crises involving contamination of products by persistent organic pollutants (POP, Lake *et al.,* 2005). In order to avoid the disposal of products originating from contaminated herds, strategies hastening POP removal should be implemented. This study aimed at assessing the efficiency of undernutrition (release of lipophilic POP from adipose tissue to blood, Lerch *et al.,* 2016) combined with dietary non-absorbable lipid supplementation (enhancement of POP transfer from blood to faeces by passive diffusion across the intestinal tract wall, Rozman *et al.,* 1984) as a nutritional strategy to hasten dioxins (TCDD) and polychlorinated biphenyls (PCB) removal in contaminated ewes.

Material & methods The experiment was divided into three consecutive periods: i) a 27-day exposure period during which nine non-lactating ewes were daily orally exposed to POP through a spiked concentrate [280±35 pg TCDD, 285±35 pg PCB 126 and 281±35 pg PCB 153 / kg body weight / day]; ii) a 8-day buffering period with a non-spiked diet; and iii) a 58-day depletion period according to two treatments: control well-fed (96% of maintenance energy requirement (MER), CTL, $n = 4$) or underfed (37% of MER) and supplemented with mineral oil (10% of dry matter intake, Codex 68, IGOL, Amiens, France, UFMO, $n = 5$). Faeces were individually pooled over the depletion period. At the end of the depletion period, ewes were slaughtered by stunning followed by exsanguination, before empty body (whole body minus gut contents and wool) was weighed, minced, mixed and homogenized using an industrial mixer-grinder. Total lipids, TCDD and PCB concentrations were determined in faeces and empty body. Faecal flows of POP were estimated by means of acid-insoluble ash analysed in both feedstuffs and faeces. Data were analysed using the MIXED procedure of SAS (2003, Cary, USA) with a model including the depletion treatment (CTL or UFMO) as a fixed effect and the ewe as a random effect.

Results & discussion Faecal POP concentrations (DM basis) in UFMO ewes were 2.1 to 2.5-fold higher than in CTL ewes ($p < 0.01$). In accordance with our results, supplementing a well-fed diet with mineral oil enhanced mirex (a lipophilic organochlorine pesticide listed as POP) faecal concentration by 2-fold in dairy goats (Rozman *et al.,* 1984). Thus, decreases in empty body POP burdens after depletion period were 2 to 3-fold higher in UFMO than in CTL ewes, but these decreases in UFMO ewes accounted for only 7%, 2% and 6% of initial burdens of TCDD, PCB 126 and 153, respectively (Table 1). Concomitantly, estimated elimination half-lives of TCDD were reduced from 1,200 in CTL to 523 days in UFMO ewes. We suspect that the rate of POP transfer from blood to intestine lumen was the limiting step of faecal excretion enhancement due to UFMO. Indeed, the high lipophilicity of some POP (logarithm of octanol/water partition coefficient, log $K_{ow} > 6.8$) was reported to limit their ability to cross the intestinal tract wall by passive diffusion (Moser and McLachlan, 1999).

Table 1 Body weight, fatness, TCDD and PCB burdens at days 0 and 57 of depletion period.

	Day 0			Day 57			Difference (d 57 – d 0)			
	CTL	UFMO	SEM	CTL	UFMO	SEM	CTL		UFMO	SEM
Body weight (kg)	62	64	2.8	59	51	5.6	-3	*	-14	1.4
Empty body lipids (kg)	12.6	14.7	1.19	11.8	9.8	1.60	-1.0	*	-5.2	0.51
Empty body burden[1]										
TCDD (ng)	247	233	18.7	239	216	19.2	-8	*	-17	1.8
PCB 126 (ng)	220	241	42.0	227	236	40.9	+7	*	-6	2.2
PCB 153 (µg)	258	256	14.7	253	241	15.1	-5	*	-15	1.9

[1] Estimated burden at day 0 = burden measured at day 57 + amount of pollutant excreted over the depletion period through faeces and wool – amount of pollutant ingested over the depletion period. * CTL and UFMO means differs at $p < 0.05$.

Conclusion The current results combining a fine description at once of POP toxicokinetics and body lipid dynamics will be useful to calibrate mechanistic models aiming to predict the transfer of POP in ruminants and fine tune relevant strategies of decontamination.

Acknowledgements Research co-funded by "Conseil Régional Lorraine" and the French dairy professional organization "CNIEL".

References

Lake IR, Foxall CD, Lovett AA, Fernandes A, Dowding A, White S and Rose M 2005. Environmental Science and Technology 39, 9033-9038.
Lerch S, Guidou C, Thomé JP and Jurjanz S 2016. Journal of Agricultural and Food Chemistry 64, 1212-1220.
Moser GA and McLachlan MS 1999. Chemosphere 39, 1513-1521.
Rozman K, Rozman T and Smith GS 1984. Bulletin of Environmental Contamination and Toxicology 32, 27-36.

Comparison of miRNome from cow milk fat fraction and mammary gland tissue

Karol Pawlowski[1,3], Pierre-Alexis Billa[1], Yannick Faulconnier[1], Sébastien Bes[1], José Pires[1], Christine Leroux[1,2]

[1]*INRA, Saint-Genès-Champanelle, France,* [2]*University of California Davis, Davis, CA, USA,* [3]*Warsaw University of Life Sciences, Warsaw, Poland*

E-mail: christine.leroux@inra.fr

Take home message Majority, but not all, of miRNAs found in milk fat fraction are the same as those found in mammary gland.

Introduction MicroRNAs (miRNA, small non-coding RNAs) regulate the expression of genes involved in many cellular processes, including mammary gland (MG) development and lactation (Le Guillou *et al.*, 2014). MG gene expression is usually studied *via* invasive methods (biopsy or post-mortem), or *via* non-invasive methods using milk cells (Canovas *et al.*, 2014), but the latter requires a relatively large volume of milk, which limits high through put applications. Milk fat (MF) globules were used as a source of mammary mRNA (Canovas *et al.*, 2014). More recently, MF was also shown to be a source of 7 miRNAs in the bovine (Lago-Novais *et al.*, 2016). The dual objectives of our study were to deepen the description of miRNA from MF and to ensure that the latter are representative of MG miRNA expression during mammary inflammation. To achieve these objectives, we employed microarray technology to compare the expression of miRNA in MG and MF, in an experimental model of inflammation in early lactation cows (Pires *et al.*, 2016).

Materials & methods MG tissue and MF were collected from six early lactation multiparous Holstein cows (28 ± 3 DIM), 24 h after injection of 50 µg of lipopolysaccharide (LPS, E. coli 0111:B4) to induce an inflammation (Pires *et al.,* 2016). Total RNAs were extracted from MF and MG using TRIzol (ThermoFisher, Inc, USA). Customized 8 x 60K microarrays containing 786, 276 and 105 miRNAs from bovine, caprine and ovine sequences, spotted at least twice, were hybridized using a single colour method, following the manufacturer recommendations (Agilent Technologies, Foster City, CA, USA). Statistical analyses were performed using paired t-test and P-values were adjusted using Benjamini-Hochberg correction (GeneSpring Software, Agilent, USA). Differences were considered significant at adjusted value $p_{adj} \leq 0.10$. Bioinformatics analyses were performed using KEGG software.

Results & discussion Preliminary analyses of MG and MF miRNA profiles (miRNomes) showed that 97% of the 2,583 detected sequences did not presented statistical difference in their abundance in MG and MF. This result suggests that MF miRNomes seem mostly representative to MG miRNomes, in our inflammatory model. Among the 47 miRNAs differentially present in MG and MF, 29 had a fold change (FC) ≥1.5. Bioinformatics analyses of coding genes potentially targeted by these miRNAs revealed that they are involved in different cellular pathways, such as calcium signalling, adherents, tight and gap junction and endocytosis pathways. In addition, among those presenting higher FC, were let-7c, miR-126-3p and the miR-200 family which were more abundant in MG. These latter could target genes involved in cell adhesion, migration or vascular integrity, as in the epithelial-mesenchymal transition, therefore determining the epithelial cell phenotype. These results could suggest a sorting of miRNAs from mammary epithelial cells (MEC) to MF globules, and/or MG cell type changes in response to inflammation.

Conclusion These results suggest that miRNomes in milk fat fraction are mostly representative of MG miRNome. However, weak differences were detected. Our results could suggest a sorting of miRNAs from MEC to MF globules. We cannot exclude that these differences could be due to the specific inflammation challenge inducing cell type changes in MG. These hypotheses need to be confirmed by further investigations.

Acknowledgements Authors are grateful to C. Soulard, C. Boby and A. de la Foye for microarray analyses performed at the PlateForme d'Exploration du Métabolisme (PFEM, Theix, France), the staff at Herbipôle (INRA, UE1414, Theix, France) for animal care and sample collection. This research was funded in part by GISA program of INRA (Ruminflame and LongHealth projects).

References

Lago-Novais D, Pawslowski K, Pires JA, Mobuchon L, Bes S, Martin P and Leroux C 2016. In: 2016 ADSA/ASAS Joint Annual meeting, p. 401, Salt Lake City, USA

Le Guillou S, Marthey S, Laloë D, Laubier J, Mobuchon L, Leroux C and Le Provost F 2014. PLoS One 21, e91938.

Canovas A., Rincon G, Bevilacqua C, Islas-Trejo A, Brenaut P, Hovey RC, Boutinaud M, Morgenthaler C, Van Klompenberg, MK, Martin P and Medrano JF 2014. Scientific Reports 4, 5297

Pires J, Pawlowski K, Rouel J, Delavaud C, Foucras G, Rainard P, Germon P and Leroux C 2016. In: 2016 ADSA/ASAS Joint Annual Meeting (p. 64-65), Salt Lake City, USA.

487

Comparing palm oil-derived and rapeseed-derived dietary fat additives in the ration of Norwegian dairy goats: effects on milk fat content and fatty acid composition

Margrete Eknæs[1], Siv Skeie[1], Laurence Bernard[2], Carole Delavaud[2], Knut Hove[1], Harald Volden[1]

[1]Norwegian University of Life Sciences, Ås, Norway, [2]UMR1213 Herbivores, Institut National de la Recherche Agronomique, Saint-Genès-Champanelle, France

E-mail: margrete.eknas@nmbu.no

Take home message The amount of unsaturated fatty acids in goat milk is increased by substituting palm oil with rapeseeds, without negative effects on milk yield, milk fat content or milk fat yield.

Introduction Dietary lipid supplements are commonly used in ruminants diets to increase energy intake, and improve milk yield and milk composition. Among them, palm oil-derived fatty acids are highly used as energy supplements for dairy goats due to the high availability on the global market. However, this resource is heavily criticized mainly from a sustainability point of view, and also from a consumer health perspective, as its high content of palmitic acid (PA) led to increased milk PA. Hence, the search for substitutes to palm oil in animal feeds by locally produced resources to increase sustainability is an ongoing issue.

The aim of the study was to evaluate if rapeseed locally produced in Norway and characterized by its richness in long chain unsaturated fatty acids, can substitute palm oil in concentrates to dairy goats without influencing milk fat content and fatty acid composition in undesirable ways.

Materials & methods The experiment included 48 multiparous Norwegian dairy goats, lasting 225 days from February to October 2016, comprising of indoor feeding days 1-115 of lactation (DIM), mountain grazing 115-185 DIM and indoor feeding 185-225 DIM. Grass silage was fed according to appetite during indoor feeding periods. The goats were assigned to six experimental groups (8 animals/group). Treatments were: concentrates with inclusion of lipids from hydrogenated palm oil at 2% and 8% level (POFA_2 and POFA_8), and from rapeseeds at 2, 4, 6 or 8% fat (RS_2, RS_4, RS_6 and RS_8), corresponding to a fat intake ranging from 22 to 43 g/kg total DMI. Individual milk samples taken at 30, 55, 85, 115, 185 and 225 DIM were analysed for milk fat content and fatty acid composition. Statistical differences were assessed using the MIXED procedure of SAS with lipid source, lipid level and DIM as fixed effects and goat as the random effect.

Results & discussion Increased rates of lipid supplementation increased milk fat content (Table 1). There was no difference between the two sources of lipid supplements on milk yield, milk fat content or yield. Goats receiving POFA_8 diet had a higher content of C16-fatty acids in milk, compared to the other groups. The content of C18:0 and C18:1 fatty acids were higher in milk from the goats receiving rapeseeds as lipid supplement than that from goats receiving palm oil. Increased amounts of lipid supplement from rapeseeds increased the total content of monounsaturated fatty acids in milk. RS_8 compared to POFA_8 resulted in a higher milk content of CLA (C18:2 cis-9,trans-11). Even though numerically RS_8 diet resulted in the highest concentration of CLA, no dose-response effect of RS was observed for this fatty acid.

Table 1 Milk yield, fat content, fat yield and fatty acid composition in milk from goats fed either palm (POFA) or rapeseeds (RS) at different levels of inclusion.

	POFA_2	POFA_8	RS_2	RS_4	RS_6	RS_8	SEM	POFA_2 vs RS_2	POFA_8 vs RS_8
Milk yield, kg/d	3.07	2.76	2.78	2.99	3.05	2.76	0.12	0.096	0.997
Fat, g/kg	40.9^{c}	49.0^{ab}	42.7^{abc}	42.0^{bc}	45.4^{abc}	49.8^{a}	2.50	0.621	0.826
Fat yield, g/d	125.9^{ab}	134.5^{ab}	118.0^{b}	124.8^{ab}	139.6^{a}	137.1^{a}	6.53	0.393	0.780
Fatty acids, g/kg milk									
C16:0	12.56^{b}	17.35^{a}	11.03^{bc}	10.24^{c}	10.42^{c}	10.85^{bc}	0.63	0.092	<0.001
C18:0	3.91^{c}	4.70^{c}	4.80^{c}	5.89^{b}	6.66^{b}	7.95^{a}	0.37	0.093	<0.001
C18:1	8.34^{e}	9.94^{cd}	9.46^{de}	10.82^{bc}	11.52^{b}	13.37^{a}	0.45	0.084	<0.001
C18:2	1.07	1.10	1.21	1.19	1.23	1.25	0.06	0.113	0.077
C18:2 cis-9,trans-11	0.18^{b}	0.18^{b}	0.22^{ab}	0.20^{ab}	0.21^{ab}	0.25^{a}	0.01	0.086	<0.001

$^{a-e}$ LSM within a row with different superscripts are significantly different ($P<0.05$) from each other.

Conclusion Milk fat yield of goats increases with supplementation of either palm oil fatty acids or lipid-rich rapeseeds. The amounts of unsaturated fatty acids secreted in milk is enhanced when substituting palm oil fatty acids by rapeseed, a locally grown fat resource, without negative effects either on milk yield, fat content or fat yield.

Acknowledgements This study was financed by the Norwegian Research Council, TINE BA (Oslo, Norway), Fiskå Mølle AS (Sandane, Norway) and Norgesfôr AS (Oslo, Norway).

Black Solder Fly Larvae: novel feed source for ruminants and effective processing methods to reduce the microbial load

Alexandros Stratakos[2], Tara Elliott[1], Mairead Campbell[1], Mark Linton[2], Nicolae Corcionivoschi[2], Katerina Theodoridou[1]

[1]Queen's University, Institute for Global Food Security, Belfast, UK, [2]Agri-Food and Biosciences Institute, Veterinary Sciences Division, Bacteriology Branch, Belfast, UK

E-mail: k.theodoridou@qub.ac.uk

Take home message Black solder fly larvae (BSFL) could be an alternative protein source for animal diets

Introduction The EU is moving towards considering insects as alternative protein source for animal feed. However, scientific knowledge on the suitability of insects as a feed ingredient in ruminant diets is scarce (Makkar *et al*., 2014). It is necessary to explore effective processing methods to certify insect feed as a safe ingredient. We investigated the nutritive value of BSFL and the effect of two processing techniques, High-Pressure Processing (HPP) and boiling, on microbial contamination and *in vitro* true dry matter digestibility (IVDMD).

Materials & methods Representative samples (n=9) of BSFL were obtained from Hexafly (Co.Meath, Ireland). On arrival, the larvae were fasted for 24h before being frozen at -20°C. Then subjected to the following treatments: control (untreated), HPP (400Mpa for 90s/10min; 600Mpa for 90s/10min) and boiling (90°C for 10/15min). Microbiological analyses considered total Viable Counts (TVC), enterobacteria, lactic acid bacteria and yeast and moulds. IVDMD was determined by incubating the larvae in buffered rumen fluid for 48h according to the Daisy^{II} incubation method, followed by NDF digestion in an ANKOM 200 Fibre Analyser. Prior to chemical analyses of untreated samples, larvae was lyophilized and ground to 1mm. Samples were analysed for ether extract (EE), gross energy and crude protein (CP), according to AOAC (1990) and NDF (Van Soest and Mason, 1991). Polyunsaturated (PUFAs) and saturated (SFAs) fatty acids were analysed by Gas Liquid Chromatography (Table 1). All samples were analysed in triplicate. Statistical analyses were performed using the MIXED procedure of SAS (version 9.4) (SAS Institute, 2016).

Results & discussion CP content was high and similar to soymeal in the literature. The level of PUFAs was low but this could be changed, as use of different growing substrates, influence the chemical composition of BSFL. HPP treatment increased IVDMD whereas microbial load was reduced with boiling heat treatment which is an easy method to certify insect feed as a safe ingredient (Table 2).

Table 1 Chemical composition of BSFL (mean of triplicates ± standard deviation).

(g/kg DM)	NDF	CP	EE	PUFAs (%)	SFAs (%)	Gross energy (MJ/kg)
BSFL	376.7 ± 1.1	441.5 ± 0.5	258.3 ± 0.8	19.62	64.51	23.7 ± 0.1

Table 2 Effect of high pressure and thermal treatments of BSFL on microbial load and on IVDMD (n=9).

Item	Control	HPP[1]	Boiled[2]	SEM	P value
Microbial load					
TVC	7.97^{a}	6.91^{b}	5.62^{c}	0.158	<0.0001
Enterobacteria	7.65^{a}	3.51^{b}	1.22^{c}	0.323	<0.0001
Lactic acid bacteria	6.50^{a}	4.21^{b}	1.47^{c}	0.415	<0.0001
Yeast and Moulds	5.07^{a}	3.96^{b}	2.00^{c}	0.130	<0.0001
Digestibility					
IVDMD (%)	87.81^{b}	93.56^{a}	89.44^{b}	0.572	<.0001

SEM= Standard error of mean; $^{a, b, c}$ Means with different letters within the same row differ ($P < 0.05$); [1]Average over all pressure and duration treatments; [2] average over all duration treatments.

Conclusion Boiling was the most effective processing method at reducing microbial load whilst IVDMD improved after HHP treatment. The results of this study highlight BSFL as a potentially novel protein source for inclusion in animal diets, including ruminants that can be sustainably and locally sourced thus reducing dependence on imported protein crops.

Acknowledgements 'HEXAFLY' for providing the insect larvae for this research work.

References

Association of Official Analytical Chemists (AOAC), Official Methods of Analysis. AOAC Washington, DC 1990.
Makkar HPS, Tran G, Heuzé V and Ankers P 2014. Animal Feed Science and Technology 197, 1-33
Van Soest PJ and Mason VC 1991. Animal Feed Science and Technology 32, 45-53.

Quantitative analysis of volatile fatty acids, hydrogen and methane production in cows fed highly digestible rice silage

Kouji Higuchi[1], Itoko Nonaka[1], Fumihiro Ohtani[1], Atsuko Fukuda[2]

[1]National Agriculture and Food Research Organization (NARO), Institute of Livestock and Grassland Science, Tsukuba, Ibaraki, Japan, [2]Kumamoto Prefectural Research Center, Koushi, Kumamoto, Japan

E-mail: higuchik@affrc.go.jp

Take home message Greater escape of hydrogen from rumen decreased methane emission in cows.

Introduction Ruminal fermentation is the process that converts dietary carbohydrates to volatile fatty acids (VFA) producing metabolic hydrogen. Metabolic hydrogen is an essential substrate for methanogenesis in rumen. Therefore, management of metabolic hydrogen in the rumen has been identified as an important consideration especially for reducing ruminant methane emission. However, few studies have reported on quantitative evaluation of VFA, hydrogen and methane production *in vivo*. On the other hand, 'Tachisuzuka' is a rice (*Oryza sativa* L.) cultivar for whole-crop silage use and has a highly digestibility as compared to conventional strains of rice for feed (Matsushita *et al*., 2011). Usually rice receives chopping process in which the chop length depends on the system during harvesting or before ensiling. Chop length of roughage strongly influences the particle size, duration of eating and rumination time, and ruminal fermentation (Mertens 1997). Therefore, the difference in chopping process of rice may affect ruminal fermentation. Thus, in this study, the effect of the rice harvested differently on ruminal fermentation especially on VFA, hydrogen and methane production were determined.

Materials & methods This study was conducted in accordance with the animal care and use guidelines of the NARO Institute of Livestock and Grassland Science of Japan. Four non-lactating cannulated cows were housed in a temperature and humidity controlled room and given two diets (S, L) containing one of ensiled whole crop rice 'Tachisuzuka', average particle length of rice for S and L were 5.3 and 9.0 cm, respectively, with supplementation of soybean meal and minerals for 21-days experimental period in a cross over design. At day 13, each cow was introduced into respiration chamber, and hydrogen and methane emissions were determined during a day. The next day, at the outside of the chambers, cows were received intra-ruminal administration of Cobalt-EDTA solution just before morning meal to estimate ruminal volume and liquid phase passage rate. Ruminal fluid samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 5 and 6 hours after feeding and pH, VFA, hydrogen and cobalt were determined. Hydrogen concentration in ruminal fluid was determined by electrode (Guyader *et al*., 2014). The production of each VFA was calculated by considering the amount in rumen, absorbed through rumen wall, and of flow to the omasum. The amount through rumen wall was estimated from equation showing fractional absorption rate of each VFA shown by Dijkstra *et al*., (1993). The amount emitted from rumen was additionally considered for the calculation of hydrogen production. From day 17 to 21, digestibility of diets was determined by collecting whole faeces. Data were analyzed by SAS. The model used in GLM procedure included the fixed effect of experimental feed, term and cow, while the fixed effect of time was added and treated as a repeated measure in MIXED procedure.

Results & discussion Molar proportion of acetate was significantly higher in L diet (68 vs 65), while propionate was higher in S (19 *vs* 17). Hydrogen concentration in rumen fluid was higher in S diet. There were no significant differences between L and S diet on acetate, propionate, and total VFA production, methane and hydrogen emission during 6 hours after feeding. However, hourly emitted hydrogen was significantly increased at 3 hours after feeding, while methane emission was significantly decreased at 4 hours after feeding in S diet (Figure 1 and 2). Whole day emission of methane was significantly increased in L diet, while hydrogen emission was greater in S (Figure 3). Total digestible nutrients content was greater in S diet.

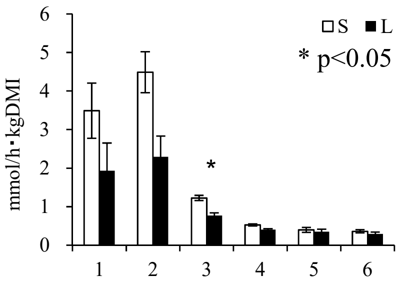


Figure 1 H_2 emission after feeding.

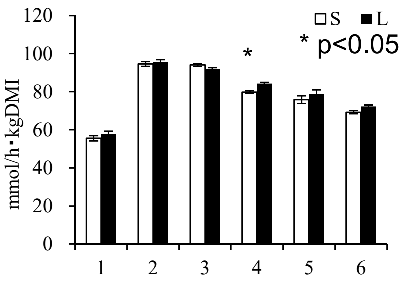


Figure 2 CH_4 emission after feeding.

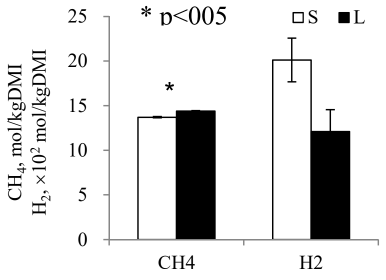


Figure 3 Whole day CH_4 and H_2 emission.

Conclusion Rice silage chopped in shorter length might have increased the fermentation in rumen resulting in increased hydrogen release and decreased methane emission.

Acknowledgements This research was supported by a grant from Ministry of Agriculture, Forestry and Fisheries, Japan.

References

Dijkstra J, Boer H, Van Bruchem J, Bruining M and Tamminga S 1993. British Journal of Nutrition 69, 385-396.
Guyader J, Silberberg M, Morgavi DP and Martin C 2014. Joint ISNH/ISRP International Conference.
Matsushita K, Iida S, Ideta O, Sunohara Y, Maeda H, Tamura Y, Kouno S and Takakuwa M 2011. Breeding Science 61, 86-92.
Mertens DR 1997. Journal of Dairy Science 80, 1463-1481.

Palatability of plants rich in phenols and their effect on milk yield and composition in cows

Melissa Terranova[1], Shaopu Wang[1], Michael Kreuzer[1], Lukas Eggerschwiler[2], Angela Schwarm[1]

[1]ETH Zurich, Institute of Agricultural Science, Zurich, Switzerland, [2]Agroscope, Posieux, Switzerland

E-mail: melissa.terranova@usys.ethz.ch

Take home message Palatability of phenol-rich plants to cows depends on phenol content but also on lignin content.

Introduction Plant secondary compounds (PSC) such as phenols play an important role in the development of feeding strategies for ruminants with the aims to reduce methane emissions and to influence digestion. However, high levels of PSC can reduce the palatability of the feed and, concomitantly, feed intake and productivity of the animals. This explains why the mitigation of methane emission from ruminal fermentation with promising plant species is often less successful *in vivo* than expected from *in vitro* studies. In addition, PSC may affect also milk yield and composition. This cannot be simulated *in vitro.* The goal of the present study was, therefore, to quantify the palatability and the effects on milk yield and milk composition of six PSC-rich plant materials when provided in the short-term as supplements to the diet of dairy cows. The plants selected reduced methane and ammonia formation at unchanged *in vitro* digestibility as determined in a preceding *in vitro* study (Terranova *et al*., 2017).

Materials & methods The following plant materials were tested in a cross-over design using six late-lactating Brown Swiss cows (609 ± 37 kg body weight) in their first to fifth lactation: leaves from silver birch (*Betula pendula*), hazel *(Corylus avellana*), wood avens (*Geum urbanum*)*,* blackcurrant (*Ribes nigrum*) and green grape vine (*Vitis vinifera*) as well as the herb from rosebay willow (*Epilobium angustifolium*)*.* Pellets were produced from mixtures of lucerne and ground test plant material in different proportions (54 to 98%) to achieve the same total phenol (TP) content of 41 g/kg in dry matter (DM). These pellets were provided separately from a mixed basal ration (MBR, grass silage, maize silage, hay and concentrate) in a ratio of 0.4:0.6 three times daily. First, during 7 days of adaptation period pellets consisting only of lucerne were fed besides the mixed ration (control diet). Thereafter, the cows were offered the pellets including the different plants. All six types of plant pellet were fed in randomized order to each cow for a duration of 3 days each. The cows were milked twice per day. Milk yield was recorded and on day 3 of each 3-day period milk samples from morning and evening milking were taken and analysed for fat, protein, lactose, urea and cell count. The intakes of the MBR and the pellets were recorded separately once per day. The lucerne control pellets were considered well palatable and their intake during the adaptation period served as a reference for calculating the short-term Palatability Index (sPAL). The following equation was used: sPAL (%) = (test pellet intake (kg)/test pellet offered (kg))/(lucerne pellet intake (kg)/lucerne pellet offered (kg))×100 (Ben Salem *et al*., 1994). The data were analysed with the mixed procedure of SAS with a Tukey-Kramer adjustment.

Results & discussion The crude protein and neutral detergent fibre content of the pellets varied from 149 (rosebay willow) to 198 g/kg DM (lucerne-only) and from 296 (blackcurrant) to 432 g/kg DM (lucerne-only), respectively. The lignin content of the pellets supplemented with birch and blackcurrant was almost twice as high as that containing rosebay willow (13 vs. 6.8 g/kg DM). The TP content ranged from 48 (hazel) to 95 g/kg DM (rosebay willow). The offered MBR (11 kg DM/day) was completely consumed by all animals independent of the pellet supplemented. The intake of the test pellets compared to lucerne control pellets (6.6 kg DM/day) and therefore their sPAL (%) was higher ($p < 0.05$) with hazel (110), wood avens (107), rosebay willow (103) and grape vine (102) than those with blackcurrant (67) and birch (54). This means that hazel pellets were consumed 1.1-fold more than the lucerne-only pellets. Test feeds accepted over the duration of palatability measurements of a few days are likely to be accepted over longer periods as well (Ben Salem *et al*., 1994). Only feeding pellets with birch leaves reduced ($p < 0.05$) daily milk yield. Contents of milk fat, protein and lactose as well as cell counts did not differ between treatments, but milk urea content was reduced ($p < 0.05$) by all test pellets, except by birch.

Conclusion The hazel pellets with the lowest TP content were the most palatable test pellets in the present study. Unexpectedly, the rosebay willow pellets with the highest TP content were well palatable, too. The results suggest that a high lignin content, as found in blackcurrant and birch, was also very adverse to palatability. The reduced milk yield found with birch leaves pellets was a direct consequence of their poorest palatability. The reduced milk urea levels show that the phenols of five of the six test materials seem to have the ability to bind part of the dietary protein and prevent its ruminal digestion to ammonia. This can be favourable from an environmental perspective when diets contain excessive nitrogen.

Acknowledgements This study was supported by the ETH Zurich Research Grant and by the China Scholarship Council.

References

Ben Salem H, Nefzaoui A and Abdouli H 1994. Animal Feed Science and Technology 46, 143-153.
Terranova M, Wolter M, Kreuzer M, Braun U and Schwarm A 2017. Proceedings of the Society of Nutrition Physiology 26, 130 (Abstr.).

Tagasaste (*Chamaecytisus palmensis*) as a source of high quality fodder supplement in smallholder mixed crop-livestock systems

Melkamu Bezabih[1], Kindu Mekonnen[1], Alan Duncan[1], Adugna Tolera[2], Meron Mengesha[2], Peter Thorne[1]

[1]*ILRI, Addis Ababa, Ethiopia,* [2]*Hawassa University, Hawassa, Ethiopia*

E-mail: derseh7@yahoo.com

Take home message Tagasaste grows well in cooler highlands and provides good quality forage year-round with a considerable potential to increase livestock productivity.

Introduction Feed shortage and poor quality of available feeds have become major constraints for livestock production in tropical Africa. Due to shrinking grazing lands, crop residues have become a major component of the diet of ruminant livestock (Duncan *et al*. 2016). Supplementation of crop residues with readily fermentable energy and protein concentrate supplements improves their utilization. A trial was conducted to evaluate the potentials of tagasaste fodder to serve as a source of year-round protein and energy supplement and determine optimal levels of supplementation for small ruminants consuming barley straw basal diets in the Ethiopian highlands.

Materials & methods The study had two components: the first involved establishing tagasaste seedling on-farm and monitoring its yield performance over 1-2 years, and the second involved an on-station feeding and digestibility trial with sheep. For the on-farm fodder performance evaluation, a total of 250 purposely selected farm households across four districts participated. Each participant planted a minimum of 50 tagasaste seedling. After 9 months of growth, tagasaste trees were subjected to two cutting height (1m and 1.5 m) and 3 cutting intervals (3, 4 or 6 months). For the feeding trial a total of 24 yearling intact Menz sheep (weight 17±0.83 kg) were used. This trial had four treatments, which involved supplementation of dried tagasaste leaf at 100, 200, 300 and 400 g/d/head to a basal diet of barley straw. The sheep were assigned to one of the four treatments in a randomized complete block design. The feeding trial lasted for 90 days, followed by a digestibility trial and carcass evaluation using all animals. Samples of the feed consumed, refused and faeces were analysed for nutrients.

Results & discussion The leaf biomass yield ranged from 4-8 tons DM/ha, with 6 months cutting interval and 1.5 m cutting height producing the maximum yield ($P<0.01$) (Figure 1). The leaf had on average 21.5% crude protein (CP) and 43% neutral detergent fibre (NDF). Intake and digestibility of dry matter and nutrients increased linearly ($P<0.001$) as tagasaste supplementation increased from 100 to 400 g/d (Table 1). Similarly, average daily body weight gain, feed conversion efficiency and dressing percentage increased linearly ($P<0.001$) with the supplementations. Proportion of tagasaste leaf in the diet increased from 20 % to 50 %, while the ratio of NDF to CP decreased from 7.9 to 5.3, as the level of supplementation increased.

Table 1 Effect of supplementing dried tagasaste leaf to Menz sheep fed on barely straw basal diet in the Ethiopian higlands.

Variables	Supplementation levels (g DM/d/head)				P
	100	200	300	400	
DMI (g)	477^{d}	559^{c}	635^{b}	717^{a}	<0.001
DMD (%)	57.6^{c}	60.3^{c}	66.1^{b}	72.1^{a}	<0.001
DWG (g)	19.8^{d}	40.3^{b}	55.7^{ab}	72.5^{a}	<0.001
FCE	0.04^{c}	0.07^{b}	0.09^{ab}	0.10^{a}	<0.001
DP (%)	40.5^{c}	45.0^{b}	47.0^{ab}	48.2^{a}	<0.001

DMI=dry matter intake; DMD=dry matter digestibility, DWG=daily weight gain, FCE=feed conversion efficiency (g DWG/g DMI), DP=dressing percentage.

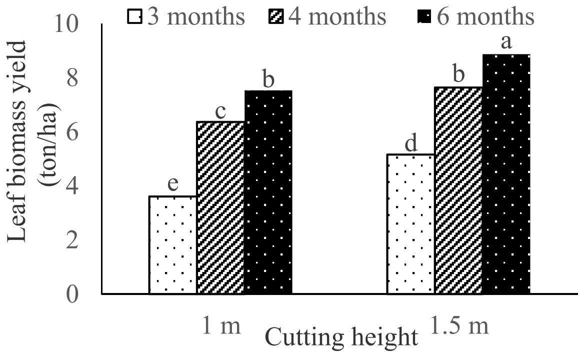


Figure 1 On-farm leaf biomass yield of 1-2 years old tagasaste tree as affected by cutting height and cutting interval in the highlands of Ethiopia.

Conclusion Tagasaste tree performed very well under smallholders' management to provide year-round fodder supplement, in addition to other environmental functions. Supplementation considerably enhanced nutrient digestibility, intake, live weight gain, and carcass yield, while simultaneously improving feed conversion and nitrogen use efficiency. Inclusion of dried tagasaste leaf up to 50% of the diet DM, which results in a NDF:CP ratio of 5.3, showed no deleterious effects on the sheep, and resulted in superior growth performance and carcass yield.

Acknowledgements This research was undertaken with supported from Africa RISING, a program financed by USAID.

References

Duncan AJ, Bachewe F, Mekonnen K, Valbuena D, Rachier G, Lule D, Bahta M. and Erenstein O 2016. Agriculture, Ecosystems and Environment 228, 101-10.

Including bioactive legumes in grass-based silage to improve performances and reduce methane emissions in sheep

Vincent Niderkorn, Giuseppe Copani, Cécile Ginane

INRA, Saint Genes Champanelle, France

E-mail: vincent.niderkorn@inra.fr

Take home message Red clover containing polyphenol oxidase can improve lamb growth while sainfoin containing condensed tannins can reduce methane yield when these legumes are incorporated in grass silage.

Introduction Some forage legumes may have a particular interest as they contain bioactive compounds that can positively affect silage quality (Copani *et al*., 2014) and reduce nematode infection (Hoste *et al*., 2006). The aim of this study was to quantify the benefits of including in grass-based silages two bioactive legume species, namely sainfoin (SF) and red clover (RC) containing condensed tannins (CTs) or polyphenol oxidase (PPO) respectively, on digestive efficiency, performances, and methane (CH_4) emissions in sheep.

Materials & methods Five large-scale silos were prepared as followed: pure timothy (T, control), 50-50% binary mixtures (T-SF, T-RC, SF-RC) and a ternary mixture (50% T, 25% SF, 25% RC). Two experiments were simultaneously conducted to measure i) performances in growing lambs ($n = 8$ per silage) fed *ad libitum* with the experimental silages and a restricted barley supplement, through measurements of voluntary intake and weight gain, and ii) OM digestibility and CH_4 emissions (SF_6 technique) using 10 young sheep fed with the experimental silages according to a repeated latin square design. Data were analyzed with the mixed procedure of SAS using the repetition of silage sampling or animals as random factors.

Results & discussion In addition to silage characteristics shown in Table 1, we observed that the CT content decreased from 1.0-1.2 g/kg DM in the binary mixtures to 0.6 g/kg DM in the ternary mixture. The PPO activity in RC at harvest was 7.02 dOD_{420}/min/g using 4-methylcatechol as phenolic substrate. We showed that the presence of RC within silage can lead to greater DM intake ($P < 0.001$) and weight gain ($P < 0.001$) than for T or T-SF. This could be due to differences in fibre profiles between SF and RC and the presence of CT which led to lower OM digestibility of SF- than RC-containing silages ($P = 0.002$). When animals were fed with SF-containing mixtures, CH_4 yield was lower compared to pure grass ($P = 0.003$).

Table 1 Silage characteristics, animal performance data of lambs and digestion parameters of young sheep fed with experimental silages containing timothy (T), sainfoin (SF) and red clover (RC).

Item	Silages					s.e.m	*P*-value
	T	T-SF	T-SF-RC	T-RC	RC-SF		
Silage characteristics							
pH	4.6	4.3	4.4	4.5	4.3	0.08	0.200
NDF, g/kg DM	594^a	504^b	501^b	487^c	431^d	5.4	< 0.001
CP, g/kg DM	152^d	167^c	181^b	179^b	197^a	1.5	< 0.001
NH_3, % total N	11.5	7.8	6.7	7.6	7.2	0.59	0.001
Performance experiment							
DM intake, kg/d	1.06^b	1.02^b	1.19^a	1.21^a	1.25^a	0.038	< 0.001
ADG, kg LWG/d	0.18^{bc}	0.15^c	0.22^a	0.24^a	0.21^{ab}	0.013	< 0.001
Digestion experiment							
OM digestibility, %	74.4^a	69.9^{bc}	70.9^{bc}	73.5^{ab}	70.0^c	1.06	0.002
CH_4 emissions, g/kg DM intake	35.7^a	29.7^b	29.3^b	30.5^{ab}	27.2^b	1.06	0.003

ADG: average daily gain; LWG: live weight gain; s.e.m: standard error of the means. Within a row, means followed by different letters are significantly different ($P < 0.05$).

Conclusion Under our experimental conditions, the inclusion of RC in grass silage appeared to provide greater benefit for DM intake and weight gain, while SF led to lowered CH_4 emissions.

Acknowledgements These investigations were supported by the European Comission through a Marie Curie Initial Training Network (PITN-GA-2011–289377, 'LegumePlus' Project).

References

Copani G, Ginane C, Le Morvan A and Niderkorn V 2014. Animal Production Science 54, 1826-1829.

Hoste H, Jackson F, Athanasiadou S, Thamsborg SM and Hoskin SO 2006. Trends in Parasitology 22, 253-261.

pH of wheat straw during fungal treatment and storage at different temperatures

Lei Mao, Anton S.M. Sonnenberg, Wouter H. Hendriks, John W. Cone

Wageningen University & Research, Wageningen, The Netherlands

E-mail: lei.mao@wur.nl

Take home Message: Acidification of lignocellulose by white rot fungi has a preservative effect and continues somewhat during anaerobic storage.

Introduction Agricultural by-products, such as wheat straw, are usually difficult to be utilized by microbiota in the rumen, because of high contents of lignin. Many studies have shown that fungi, such as *Ceriporiopsis subvermispora* and *Lentinula edodes*, have a great lignin degradation ability with low utilization of cellulose resulting in an increasing *in vitro* degradability in rumen fluid. To test the stability of fungal-treated wheat straw, it was stored anaerobically for ten weeks at different temperatures, mimicking prolonged storage conditions.

Materials & methods The fungi *C. subvermispora* (CS) and *L. edodes* (LE) were selected for the present study. Spawn was made as described by Van Kuijk *et al.*, (2015). Organic wheat straw was chopped and soaked in tap water for 3 days and then drained for 5-h. The fungal treatment was carried out in 4L plastic containers, with a filter on the lid allowing gas exchange. Containers were filled with wet wheat straw and sterilized at 121°C for 1h and then cooled down to room temperature. 16g spawn of each fungus was added to the autoclaved wheat straw and mixed. The cultures were then incubated aerobically at 24°C for 7 weeks. The fungal treated wheat straw were then transferred into 0.5L glass jars and stored at 24.7, 35.0, 45.9 or 52.4°C. Three glass jars of each temperature were opened at 2, 4, 6, 8 and 10 weeks of incubation for pH and chemical composition analysis (Table 1). In the case of week 0, the fresh samples were directly collected after fungal treatment without putting into grass jars. Within one fungi, the difference between week 0 and 7 were statistically analysed with the GLM procedure in SAS 9.3. Significance was declared at p<0.05.

Results & discussion Treating wheat straw with *C. subvermispora* and *L. edodes* caused an increase ash, crude protein and cellulose and a decrease in hemicellulose, ADL and pH. The pH decreased significantly during the fungal incubation showing that both fungi produce organic acids during the aerobic incubation on wheat straw. During the storage afterwards, no obvious trend was observed in pH of the *C. subvermispora* treated straw, whereas a slight decrease on pH was shown in *L. edodes* treated straw at all temperatures (Figure 1).

Treatment	Ash	Crude protein	Cellulose	Hemicellulose	ADL	pH
C. subvermispora						
Week 0	36.4b	17.3b	457.0b	278.9a	67.9a	4.78a
Week 7	43.3a	22.4a	497.6a	141.1b	26.9b	3.47b
L. edodes						
Week 0	36.5b	18.7b	448.8b	277.7a	69.9a	4.88a
Week 7	42.3a	22.3a	507.5a	164.9b	45.3b	4.18b

Table 1 pH and chemical composition (g/kg DM) of *C. subvermispora* and *L. edodes* treated wheat straw at 0 and 7 weeks of fungal incubation.

*a and b shows significance (P<0.05) between 0 and 7 weeks within a fungi.

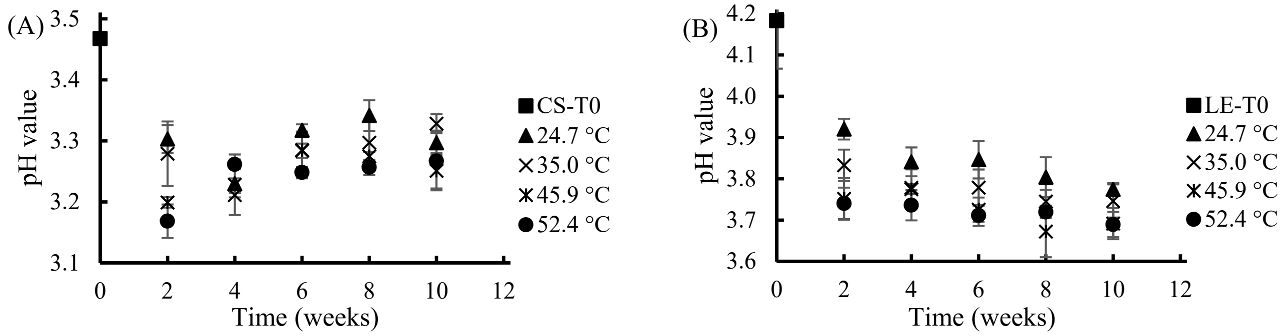


Figure 1. pH of (A) *C. subvermispora* and (B) *L. edodes* treated wheat straw stored at different temperatures.

Conclusions Both *C. subvermispora* and *L. edodes* acidifies wheat straw during the aerobic incubation and acidification continues slightly after anaerobic storage. It shows that fungal treatment is a good preservation method for lignocellulose during anaerobic storage.

Acknowledgements This project was financially supported by Victam, Deka, and For Farmer through the Wageningen University Fund and the Chinese Scholarship Council.

References

Van Kuijk SJA, Sonnenberg ASM, Baars JJ, Hendriks WH and Cone JW 2015. Animal Feed Science and Technology 209, 40-50.

In vitro digestibility and ruminal fermentation characteristic of fermented rice straw supplemented with tapioca by-product, palm kernel cake, and copra meal

Cuk Tri Noviandi, Andriyani Astuti, Ali Agus, Ristianto Utomo

Universitas Gadjah Mada, Yogyakarta, Indonesia

E-mail: c.t.noviandi@ugm.ac.id

Take home message Supplementing the right combination of protein and energy feeds is important in increasing digestibility of fermented rice straw basal diet.

Introduction A large range of agricultural by-products, such as *onggok* (tapioca by-product), palm kernel cake (PKC), and copra meal (CM), as well as forages and crop resources (legumes and cereal straws) are available for cattle diets in Indonesia and other Asian countries. However, most farmers formulate their rations based on the cheaper rather than nutritional principles, resulting in poor performance of the cattle. A good balance between energy and protein feeds in the ration is the key factor in achieving optimum performance. This *in vitro* experiment was done to determine the best combination ratio of two protein supplements in fermented rice straw based diets on *in vitro* digestibility and ruminal fermentation characteristics.

Material & methods The first stage of 2-stage *in vitro* technique (Tilley and Terry, 1963) was used in this experiment. Fermented rice straw and 150 g/kg of tapioca by-product were used as basal diet while palm kernel cake (PKC) and copra meal (CM) were added as protein sources in various combinations. Sixteen dietary treatments were tested in a 4 (0, 100, 200, or 300 g PKC/kg basal diet) × 4 (0, 100, 200, or 300 g CM/kg basal diet) factorial design. The crude protein (CP) contents of dietary treatments were ranging from 59.0 to 144.4 g/kg. Rumen fluid used for *in vitro* digestibility test was collected from 2 cannulated Bali cattle fed with king grass (*Paspalum purpurhoides*) and commercial concentrate in 80:20 ratio of ration. All data were analysed using the General Linier Model Multivariate procedure of SPSS ver. 22 (IBM, USA) and continued with Duncan's new multiple range test for any significant differences.

Results & discussion Results showed that both treatments had no significant effect on culture pH ranging from 7.22 to 7.26, which is in the normal pH range for rumen fluid of Bali cattle. PKC supplementation increased organic matter digestibility (OMD), volatile fatty acids (VFA), and ammonia-N (NH_3-N) concentrations when 100 g/kg of that feedstuff was included in the mixture (467 g/kg, 60.6 m*M*, and 127 mg N/L, respectively; $P<0.05$; Table 1 and 2). The OMD and NH_3-N concentrations were greater when 300 g/kg CM supplement was added into the diet (514 g/kg and 130 mg N/L, respectively; $P<0.05$; Table 1 and 2), while the greatest VFA concentration was noticed at 200 g/kg CM supplementation (76.0 m*M*; $P<0.05$). The greatest OMD, VFA, and NH_3-N concentrations were achieved when 100 g/kg PKC and 300 g/kg CM were combined as supplement (518 g/kg, 56.7 mM, and 150 mg N/L, respectively; $P<0.05$).

Table 1 *In vitro* organic matter digestibility (g/kg) of fermented rice straw supplemented with tapioca by-product, copra meal, and palm kernel cake.

Copra meal, g/kg	Palm kernel cake, g/kg 0	100	200	300	Mean
0	359^{p}	379^{pq}	389^{pq}	397^{pq}	381^{a}
100	420^{qr}	477^{st}	448^{r}	469^{s}	453^{b}
200	489^{st}	494^{st}	475^{st}	482^{st}	485^{c}
300	519^{u}	518^{u}	514^{u}	504^{tu}	514^{d}
Mean	447^{x}	467^{y}	457^{xy}	463^{y}	

Table 2 *In vitro* ammonia-N (mg N/L) production of fermented rice straw supplemented with tapioca by-product, copra meal, and palm kernel cake.

Copra meal, g/kg	Palm kernel cake, g/kg 0	100	200	300	Mean
0	94.3^{pqr}	108^{rs}	126^{u}	90.6^{pq}	105^{a}
100	86.0^{p}	124^{stu}	105^{r}	105^{qr}	105^{a}
200	112^{rst}	127^{u}	132^{uv}	109^{rs}	120^{b}
300	113^{rst}	150^{v}	132^{u}	124^{tu}	130^{c}
Mean	101^{x}	127^{z}	124^{y}	107^{x}	

$^{a–d}$ Means in the same column with different superscripts differ at $P < 0.05$. $^{x–y}$ Means in the same row with different superscripts differ at $P < 0.05$. $^{p–u}$ Means in the same row and column with different superscripts differ at $P < 0.05$.

Conclusion It can be concluded that 100 g PKC/kg basal diet and 300 g CM/kg basal diet is the best combination of protein meal to be added to fermented rice straw with 150 g tapioca by-product/kg basal diet to increase the *in vitro* digestibility and to optimize ruminal fermentation. However, this result has to be confirmed in *in vivo* trial.

Acknowledgements This research was supported by grants from Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia.

References

Tilley JMA, and RA Terry 1963..Grass Forage Science 18,104-111.

Alternative feed sources for ruminants in Indonesia – a comparison of fruit tree waste with *Leucaena leucocephala* for methane production and fermentability *in vitro*

Amriana Hifizah[1,3], Philip Vercoe[1,2], Graeme Martin[1,2], Zoey Durmic[1,2], Joy Vadhanabuti[1,2]

[1]School of Agriculture And Environment, UWA, Perth, Western Australia, Australia, [2]Institute Of Agriculture, UWA, Perth, Western Australia, Australia, [3]Department Of Animal Science, Faculty Of Science and Technology, UIN Alauddin, Makassar, South Sulawesi, Indonesia

E-mail: ahifizah@gmail.com

Take home message The leaves of *A. heterophyllus* (jackfruit), *I. batatas* (sweet potato), *A. muricata* (soursop) and *M. paradisiaca* (banana) have fermentability comparable to leucaena, while *P. guajava* (guava) leaves are likely to reduce CH_4 emissions.

Introduction High fermentability plus low methane (CH_4) production is a target in the screening of alternative feed sources. In Indonesia, many alternative feed sources, including fruit tree wastes, are available, but little is known about how well they ferment in the rumen. Moreover, little is known about their effect on the production of CH_4, a normal end-product of rumen fermentation that is a major greenhouse gas and also a loss of up to 12% of the gross energy of the diet consumed. These issues are driving our investigation of alternative feed sources in Indonesia. We began by collecting a wide variety of information on alternative feed sources from Aceh, and selected candidates for further analysis. This process led us to focus on fruit tree waste.

Material & methods Fruit tree waste products were compared with *Leucaena leucocephala*, a common forage for small ruminants in Indonesia. Fresh leaves of *L. leucocephala*, *Artrocarpus heterophyllus*, *Ipomoea batatas L*, *Annona muricata*, *Musa paradisiaca*, and *Psidium guajava* were collected from Lembah Seulawah district, freeze-dried and ground to pass through a 1 mm screen. Triplicate samples were tested in an *in vitro* fermentability system for 24h (Durmic *et al*., 2010). In this system, total gas, volatile fatty acid (VFA) and CH_4 production were measured and data were analysed in one-way ANOVA using JMP® software. Least significant difference (LSD) was used to compare the treatments to the control forage (*L. leucocephala*) and significant differences were declared at $P<0.05$.

Results & discussion For all plants except *P. guajava*, fermentability (total gas and VFA production) were comparable or higher than for leucaena (Table 1), implying better degradation by the rumen bacteria. As VFA provide 70% of the energy for the animal (Gethacew *et al.,* 2004), these results suggest that fruit tree waste would be able to meet the energy demands of sheep in same manner as leucaena.

Table 1 Total gas, VFA concentration and CH_4 production following *in vitro* fermentation of alternative forages in ovine rumen fluid. a-e: different letters within columns signify significant differences ($P<0.05$).

Treatment	Total gas (mL gDM^{-1})	VFA (mmol L^{-1})	CH_4 (mL gDM^{-1})
L. leucocephala (Control)	324^c	$75^{b,c}$	30.7^b
A. heterophyllus	346^a	86^a	36.2^a
I. batatas L	341^b	86^a	35.7^a
A. muricata	331^c	$80^{a,b}$	28.0^c
M. paradisiaca	308^d	68^c	$29.0^{b,c}$
P. guajava	251^e	52^d	14.0^d
P value	< 0.05	< 0.05	< 0.05
SEM	5.7	2.2	1.3

Compared to leucaena, *A. muricata* showed a small reduction in CH_4 production, whereas *P. guajava* reduced it by more than 50% but was also the least fermentable. This effect was probably due to interactions between the various chemical compounds in the plant with the rumen microorganisms (Makkar, 2003), which implies that the plant has anti-methanogenic properties and also general anti-microbial properties. Further study is warranted to look at the mechanism of action of these two potential forages in mixed diets *in vitro* as well as their effects *in vivo*.

Conclusion The leaves of some fruit trees are comparable with *L. leucocephala* in terms of rumen fermentability *in vitro*, so should be investigated further as alternative forages for ruminants. *P. guajava* greatly reduced CH_4, but also reduced fermentability, whereas *A. muricata* had some effect on CH_4, but without affecting fermentability. Further studies are needed to investigate these potential forages in mixed diet.

Acknowledgements This research was supported by an LPDP (Lembaga Pengelola Dana Pendidikan) Scholarship from the Indonesian government, and The University of Western Australia.

References

Durmic Z, Moate PJ, Eckard R, Revell DK, Williams R and Vercoe, P. E. 2010. Animal Feed Science and Technology 160, 98-109.
Getachew G, Robinson PH, DePeters EJ and Taylor SJ 2004. Animal Feed Science and Technology 111, 57-71.
Makkar HPS 2003. Small Ruminant Research 49, 241-256.

Hybrid rye grain as a substitute for wheat grain in diets for midlactating dairy cows

Piotr Micek[1], Jolanta Oprzadek[2], Pawel Gorka[1], Tomasz Schwarz[3], Patrycja Rajtar[1]

[1]Department Of Animal Nutrition And Dietetics, University Of Agriculture In Kraków, 30-059 Krakow, Poland, [2]Institute Of Genetics aOAnd Animal Breeding, 05-552 Jastrzebiec, Poland, [3]Institute Of Animal Sciences, University Of Agriculture In Kraków, 30-059 Krakow, Poland

E-mail: rzmicek@cyf-kr.edu.pl

Take home message Combination of wheat grain and hybrid rye grain in the diet for lactating dairy cows is more effective than the use of wheat grain or rye grain only.

Introduction Grain from traditional varieties (open-pollinated) of rye is not willingly used in animal nutrition. Compared to traditional varieties, hybrid varieties of rye are yield stable, resistant to disease, frost and ergot, and less often contaminated with significant level of mycotoxins (Grajewski *et. al.*, 2012). The effects of wheat, barley or maize grain use in diets for dairy cows have been widely investigated, but information on the effect of hybrid rye grain on feed intake of cows, milk yield and milk composition is limited. The aim of this study was to determine the effect of partial or full replacement of wheat grain with hybrid rye grain in the diet for dairy cows on dry matter intake (DMI), milk yield and milk composition.

Material & methods Thirty multiparous and midlactating Holstein-Friesian dairy cows (118 ± 39 days in milk) were randomly allocated to 3 groups of 10 cows each and fed isoenergetic and isoprotein diets (INRA 2007) consisted of whole crop maize silage (32%), meadow haylage (19%), ensiled maize grain (11%), wheat straw (5%) and concentrate mixture (33% on DM basis; CM). The latter one consisted of cereal grain (47%), soybean meal (33%), canola cake (12%) and mineral-vitamin supplement (8% on fresh basis). The treatments were: 1) wheat as the source of cereal grain in the CM (W); 2) wheat and hybrid rye as sources of cereal grain in the CM (50/50, wt/wt on fresh basis; WR); and 3) hybrid rye as the source of cereal grain in the CM (R). Diets were fed as TMR for a period of 56 days and feed intake was controlled using the Roughage Intake Control feeding system (RIC, Fusion Electronics B.V., Netherlands) during the last 28 days (data collection period). TMR was fed free of choice assuring 10% of refusals. Chemical composition of milk was determined with MilkoScan FT2 (Foss Analytical). Data were analyzed by the MIXED procedure of SAS (version 9.2; SAS Inst. Inc., Cary, NC). Statistical model included effect of the diet as fixed effect and pre-planned contrasts were used for scientific hypothesis verification (W *vs* WR and W *vs* R).

Results & discussion DMI was lower ($P<0.01$) for W compared to WR but did not differ between W and R ($P=0.41$; Table 1). Milk yield tended ($P=0.08$) to be lower for W compared to WR but tended ($P=0.07$) to be higher for W compared to R; however, fat corrected milk yield did not differ between treatments ($P\geq0.59$).With the exception of the lactose content, that was lower for W compared to WR ($P=0.05$), the contents of dry matter, protein, fat and urea in milk were not affected by treatment. Feed efficiency (DMI/kg of milk) was not affected by treatment. Tendency to lower milk production when R fully replaced W in the diet suggests that hybrid rye grain should be offered to lactating cows in a combination with other cereal grains, for example wheat (Svihus *et al.* 2005).

Table 1 Dry matter intake (DMI), milk yield feed efficiency, and chemical composition of milk. [1]concentrate mixture with wheat (W), wheat and rye (WR) or rye (R) grain. [2]Fat corrected milk (3.5% FCM) = (0.432 + 0.1625 × percentage of milk fat) × kilograms of milk.

Item	Treatment[1]			Contrast (*P*-value)		SEM
	W	WR	R	W *vs* WR	W *vs* R	
DMI, kg/d	24.4	25.4	24.2	<0.01	0.41	0.11
Yield, kg/day per cow						
Milk	30.7	31.5	29.9	0.08	0.07	0.19
Milk 3.5% fat (FCM[2])	32.3	33.2	31.0	0.68	0.59	0.94
DMI/kg of milk	0.82	0.82	0.83	0.99	0.90	0.02
Milk composition						
Dry matter, %	12.9	13.0	12.9	0.61	0.68	0.07
Protein, %	3.5	3.5	3.5	0.39	0.79	0.02
Fat, %	3.9	3.9	3.7	0.74	0.21	0.05
Lactose, %	5.0	5.1	5.0	0.05	0.65	0.02
Urea, mg/L	267	257	256	0.49	0.42	5.84

Conclusion Results of this study indicate that hybrid rye grain can be used with success in diets for midlactating dairy cows when combined with other sources of grain, such as wheat.

Acknowledgements This study was supported by the project No BIOSTRATEG2/297910/12/NCBR/2016.

References

Grajewski J, Błajet-Kosicka A, Twarużek M 2012. Journal of Animal Physiology and Animal Nutrition 96, 870-877.
Svihus B, Uhlen AK., Harstad OM 2005. Animal Feed Science and Technology 122, 303–320.

Influence of extruded faba bean-linseed or lupin-linseed blends on nitrogen partitioning in dairy cows

Solveig Mendowski[1,3], Patrick Chapoutot[2], Anne Ferlay[1], Guillaume Chesneau[3], Francis Enjalbert[4], Vincent Largeau[1], Lucette Genestoux[1], Pierre Nozière[1]

[1]*Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, Saint-Genès-Champanelle, France,* [2]*UMR 791 MoSAR, INRA, AgroParisTech, Université Paris-Saclay, Paris, France,* [3]*Valorex, Combourtillé, France,* [4]*GenPhySE, Université De Toulouse, INRA, ENVT, Castanet Tolosan, France*

E-mail: solveig.mendowski@inra.fr

Take home message Extruded faba bean or extruded lupin can substitute soybean meal in dairy cows diets, without changes in nitrogen partitioning.

Introduction To enhance the protein autonomy in France, soybean meal (SBM) could be substituted in dairy cows diets by proteaginous seeds grown in France (Poncet *et al.*, 2003). Proteaginous seeds contain relatively high level of proteins, but these proteins are highly degradable in the rumen, leading to a low protein value in the PDI system (Rémond *et al.*, 2003). A heat treatment, such as extrusion, reduces their degradability (Poncet *et al.*, 2003). Our objective was to compare two blends containing French produced faba bean or lupin, extruded or not, to SBM in a nitrogen balance experiment in dairy cows.

Material & methods Eight rumen cannulated lactating Holstein cows were fed *ad libitum* iso-energy (1680 kcal NEL/kg DM) and iso-nitrogenous (146 g CP/kg DM) diets with either a 90% faba bean-10% linseed blend or a 90% lupin-10% linseed blend according to a replicate 4×4 Latin square design, one for each blend. These blends were either raw (R), extruded in specific conditions, at 140°C (E1) or at 160°C (E2), and were compared to SBM as control (C). Blends and the SBM provided 40% of total CP in diets. Each cow was supplemented with 50 g Metasmart® per day to avoid deficit in methionine. Measurements took place the last 6 days of each 28-days period: animals were fed individually at 95% of *ad libitum*, intake was measured, faeces and urine were totally and separately collected. Feeds, faeces and urines samples were pooled per animal and period before nitrogen (N) analysis. Milk samples were taken during the morning and the evening milkings for 3 days in the last week of each period, for infra-red milk protein determination. During two days in the last week of each period, rumen ammonia kinetics (NH_3) were also analysed (spectrometry, 340 nm) by sampling rumen fluid through the cannula just before the morning meal, and then 1, 2.5, 5 and 8 h after the morning meal. Statistical analyses were performed using the Mixed procedure of SAS with Latin square (LS), treatment (Trt), their interaction, and period as fixed effects, and cows as a random effect. The repeated statement was used for the NH_3 kinetics.

Results There was no significant change *($P>0.05$)* in N partitioning between milk, faeces and urine when SBM was substituted by either raw or extruded blends (Table 1). Rumen NH_3 was higher with R diets *($P<0.05$)* and decreased with extrusion at a similar level to C (Figure 1), suggesting an effective N protection against microbial degradation in the rumen when faba bean or lupin were specifically extruded.

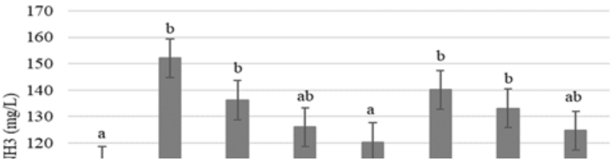


Figure 1 Effect of protein sources on rumen ammonia in dairy cows (means ± SEM)

Table 1 Effect of protein sources on nitrogen balance in dairy cows.

	Faba bean				Lupin					P-values		
	C	R	E1	E2	C	R	E1	E2	SEM	LS	Trt	LS× Trt
N intake, g/d	500	502	526	487	501	457	473	458	28.6	0.41	0.09	0.19
N milk, g/d	142	135	145	135	147	137	142	139	9.8	0.88	0.16	0.80
N urine, g/d	149	150	160	151	139	132	130	121	11.2	0.10	0.69	0.47
N faeces, g/d	185	179	180	181	171	148	152	164	12.4	0.18	0.14	0.51
N milk, %N intake	28.4	26.9	27.6	27.7	29.3	30.0	30.0	28.7	1.93	0.42	0.85	0.42

Conclusion In this experiment, soybean meal was substituted by faba bean-linseed (25% of the diet) or lupin-linseed (18% of the diet) blends without affecting N partitioning. Heat treatment by specific extrusion of these blends permitted a N protection against microbial degradation in the rumen, to a similar level to soybean meal.

Acknowledgements This experiment was conducted as part of the Proleval project, supported by Valorex and financed by Bpifrance.

References

Poncet C, Rémond D, Lepage E, Doreau M 2003. Fourrages 174, 205-229.
Rémond D, Le Guen MP, Poncet C 2003. Animal Feed and Technology 105, 55-70.

Effects of condensed and hydrolysable tannins on rumen metabolism with emphasis on the biohydrogenation of unsaturated fatty acids

Mónica Costa[1], Susana Alves[1], Alice Cappucci[2], Shaun Cook[3], Ana Duarte[1], Rui Caldeira[1], McAllister Tim[3], Bessa Rui[1]

[1]CIISA, Centro De Investigação Interdisciplinar Em Sanidade Animal, Faculdade De Medicina Veterinária, Universidade De Lisboa, Lisboa, Portugal, [2]Dipartimento Di Scienze Agrarie, Alimentari E Agro-ambientali, Università Di Pisa, Pisa, Italy, [3]Agriculture And Agri-Food Canada, Lethbridge Research Centre, Lethbridge, Alberta, Canada

E-mail: admonicacosta@fmv.ulisboa.pt

Take home message Ruminal metabolism is inhibited by mimosa condensed tannins compared to chestnut hydrolysable tannins.

Introduction The inclusion of tannins in ruminant diets has been reported to modulate ruminal biohydrogenation (Vasta & Bessa, 2012). Hydrolysable tannins can be depolymerised in the rumen and display a low affinity for lipoproteins of bacterial membranes but still causing disturbances in membrane fluidity, which might be lessened through adaptive responses of bacteria as the incorporation of *trans* fatty acids into microbial cells. Condensed tannins are unclearly prone to depolymerisation and they can bind to lipoproteins and decrease membrane permeability with an inhibition of growth of rumen microorganisms. The effects of both molecular types of tannins on ruminal metabolism were tested.

Material & methods Our experiment involved rumen fistulated ≈ 2-year old sheep (n = 4) allocated to 4 experimental periods and 3 treatments. Treatments consisted of common basal feed ingredients incorporated with: mimosa (*Acacia mearnsii*) condensed tannin extract (CT) (100 g/kg dry matter (DM)), chestnut (*Castanea sativa*) hydrolysable tannin extract (HT) (100 g/kg DM) or a mixture of CT and HT (MIX) (50 g/kg DM of each tannin extracts). Rumen contents were collected, before the morning meal, in the last week of each period over 2 days with 3 days between them, to analyse fatty acid and dimethyl acetal (microbial marker) composition of rumen contents and bacterial biomass (Alves *et al.*, 2013), and to extract DNA for selected rumen bacteria analysis by real time qPCR. Data were analysed using the MIXED procedure of SAS considering a change-over design. Data of bacterial 16S rRNA copy numbers were averaged for each experimental unit (i.e. animal within period) and analysed using Proc GLIMMIX with negative binomial distribution.

Results A reduction of volatile fatty acid ($p < 0.001$) and microbial biomass concentrations in the rumen was found with CT compared to HT (Figure 1A), which was associated with lower concentrations of *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, *Ruminococcus albus* and *Butyrivibrio proteoclasticus* ($p \leq 0.02$). The extension of ruminal biohydrogenation of 18:2n-6 and 18:3n-3 did not differ among treatments ($p > 0.05$), but was much more variable with CT and MIX than with HT (Figure 1B). The *trans-*/*cis-*18:1 ratio in bacterial biomass was higher with HT than CT ($p = 0.002$, Figure 1C).

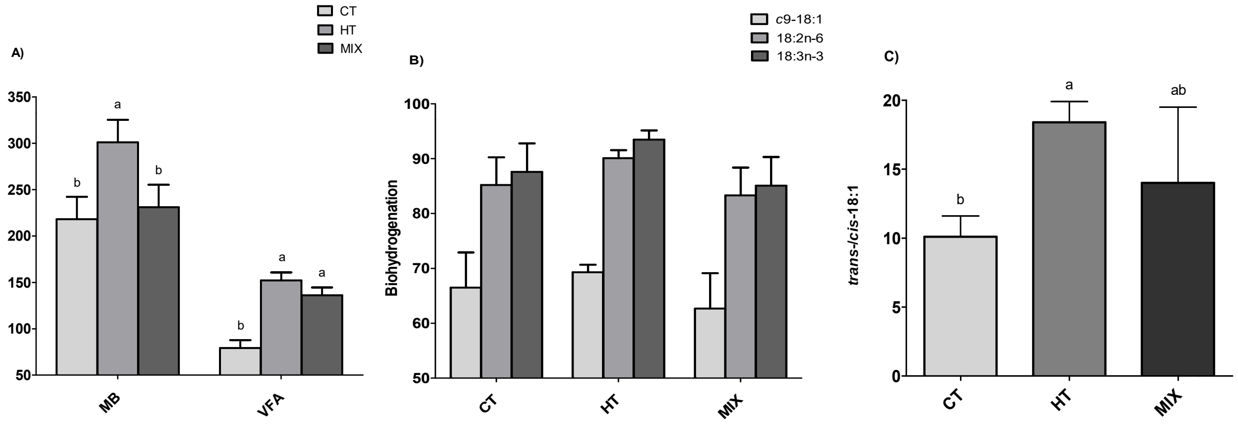


Figure 1 Effect of tannin extracts (mean ± s.e.) on: **A**) microbial biomass (MB) (g/kg DM) and total volatile fatty acid (VFA) (mmol/L) concentrations in the rumen; **B**) ruminal biohydrogenation estimates (%); **C**) *trans-*/*cis-*18:1 ratio (mg/g total C18) in rumen bacterial biomass. ^{a,b}Lowercase superscripts indicate differences among treatments.

Conclusion The CT treatment led to lower rumen fermentative activity and microbial biomass, as well as an occasional inhibition of biohydrogenation, compared to HT. Besides, a greater *trans-*/*cis-*18:1 ratio in rumen bacterial biomass was found with HT, as a possible homeoviscous adaptation to counteract the toxicity of tannins.

Acknowledgements This research was supported by grants from Fundação para a Ciência e Tecnologia (FCT).

References

Alves SP, Santos-Silva J, Cabrita ARJ, Fonseca AJM and Bessa RJB 2013. Plos One 8, e58386.
Vasta V, and Bessa RJB 2012. In Dietary phytochemistry and microbes (ed. AK Patra), pp. 263-284. Springer, Netherlands.

499

The effect of grass ploidy and white clover inclusion on dry-matter intake of grazing dairy cows

Brid McClearn[1,2], Trevor Gilliland[2,3], Norann Galvin[1], Clare Guy[1], Michael Dineen[1], Fergal Coughlan[1], Brian McCarthy[1]

[1]*Teagasc, Animal And Grassland Research And Innovation Centre, Moorepark, Fermoy, Cork, Ireland,* [2]*Institute Of Global Food Security, Queen's University Belfast, Belfast, UK,* [3]*Agri-food And Biosciences Institute, Large Park, Hillsborough, Down, UK*

E-mail: brid.mcclearn@teagasc.ie

Take home message White clover inclusion in grazing swards had a significant positive effect on dry-matter intake of grazing dairy cows, however grass ploidy had no effect.

Introduction Grazed perennial ryegrass (*Lolium perenne* L.; PRG) is considered the cheapest feed available for dairy cows in temperate regions and so to maximise profits, dairy farmers must utilise this high quality feed where possible (Dillon *et al.,* 2005). Recent research reported that including white clover (*Trifolium repens* L.; WC) in grass swards can have a positive effect on milk production. (Ribeiro Filho *et al.,* 2005). This positive effect is thought to be due to a higher dry matter intake (DMI) from cows grazing swards with WC.

Material & methods Over two years (2015 and 2016), 120 dairy cows were randomly assigned to one of four grazing treatments (tetraploid-only, diploid-only, tetraploid-WC or diploid-WC). Each treatment was stocked at 2.75 cows ha^{-1} and nitrogen fertiliser application was 250 kg ha^{-1} year^{-1}. There were 30 cows per treatment with equal numbers of three breeds (n = 10) used in each treatment. The breeds used were; Holstein-Friesian (HF), Jersey ^{x} HF and Norwegian Red ^{x} Jersey ^{x} HF. Within breed, cows were assigned to treatment based on calving date, parity and economic breeding index. Cows remained on their treatments for the entire grazing season in each year. Treatments were rotationally grazed from early-February to mid-November each year and target post-grazing sward height was 4 cm. Sward WC content was measured before grazing in each paddock during the DMI measurement periods by cutting 15 random grab samples to 4 cm with a Gardena hand shears, separating the sample into PRG and WC fractions and drying at 60°C for 48 hours. Milk yield was recorded daily and milk composition weekly by taking milk samples from a consecutive evening and morning milking. Individual animal DMI was estimated using the *n*-alkane technique (Mayes *et al.,* 1986) as modified by Dillon & Stakelum (1989) three times in 2015 (corresponding to early (March), mid (May) and late (August) lactation) and twice in 2016 (corresponding to early (April) and late (August) lactation). The ratio of herbage C33 to dosed C32 was used to estimate DMI. The animal production data from the DMI measurement periods was analysed using PROC MIXED of SAS (SAS, 2006). Terms included as fixed effects in the model were ploidy, white clover treatment, parity, year and their interactions, with cow included as a random effect.

Results & discussion Average pre-grazing herbage mass for all treatments was 1,753 kg DM^{-1} ha. Ploidy had a significant effect ($P < 0.05$) on pre-grazing herbage mass. Average post-grazing height of the four treatments was 4.14 cm with both PRG ploidy and white clover inclusion having a significant effect ($P < 0.05$) on post-grazing sward height. Perennial ryegrass ploidy had no effect on herbage DMI. There was a significant positive effect of WC inclusion on herbage DMI. On average, there was a 0.5 kg difference in herbage DMI between the grass-only (tetraploid-only and diploid-only) and grass-WC (tetraploid-WC and diploid-WC) treatments. However, the effect of WC on DMI was not consistent according to time of year as there was no difference ($P > 0.05$) in herbage DMI between grass-only and grass-WC treatments in early lactation (14.6 and 14.5 kg cow^{-1} day^{-1}) whereas there was a significant difference ($P < 0.05$) in DMI between grass-only and grass-WC treatments in mid (16.4 and 17.2 kg cow^{-1} day^{-1}) and late lactation (16.9 and 17.6 kg cow^{-1} day^{-1}), respectively. This may be due to the WC content of the sward, as sward WC content was low in early lactation (71 g kg^{-1} DM) but increased in mid and late lactation (183 g kg^{-1} DM and 285 g kg^{-1} DM, respectively).

Table 1 Herbage dry matter intake (HDMI) and total dry matter intake (TDMI) of the four grazing groups; tetraploid-only (TO), diploid-only (DO), tetraploid-white clover (TC) and diploid-white clover (DC) over two years.

	TO	DO	TC	DC	SE[1]	P[2]	WC[3]	P ^{x} WC
HDMI (kg cow^{-1} day$^{-1)}$	15.8	16.1	16.5	16.4	0.19	0.88	0.009	0.25
Total DMI (kg/cow^{-1} day$^{-1)}$	16.8	17.3	17.5	17.3	0.20	0.52	0.10	0.04

[1]SE = Standard error; [2]P = Ploidy; [3]WC = White clover

Conclusion This study shows that herbage DMI can be significantly increased by WC inclusion in a grazing sward. However, further research is warranted to investigate what level of WC is needed in the sward to have an effect on DMI.

Acknowledgements The authors acknowledge the Teagasc Walsh Fellowship and the Dairy Levy Research Fund for financial support.

References

Dillon P, Roche J, Shalloo L, and Horan B 2005. Proceedings of a satellite workshop of the XXth international grassland congress.
Dillon P, and Stakelum G.1989. Irish Journal of Agricultural Science and Research 28,104.
Mayes R, Lamb C, and Colgrove PM 1986. The Journal of Agricultural Science 107, 161-170.
Ribeiro Filho H,. Delagarde R, and Peyraud J 2005. Animal Feed Science and Technology 119, 13-27.
Statistical Institute of Analysis (SAS) 2006. Users guide: statistics. Version 9.4. SAS Institute, Cary, NC, USA.

Evaluation of sprouted barley production systems on organic dairy farms in temperate regions of the United States

Kathy Soder[1], Bradley Heins[2], Hugh Chester-Jones[2], Aimee Hafla[1], Melissa Rubano[1]

[1]USDA-ARS, University Park, PA, USA, [2]University Of Minnesota, Morris, MN, USA

E-mail: kathy.soder@ars.usda.gov

Take home message High-quality forage is more economical than producing sprouted barley for USA organic dairy farms.

Introduction Organic dairy farmers have shown increased interest in growing sprouted barley (SB) in temperate regions of the USA where producing high-quality forage has become more challenging due to changing weather patterns and a decline in availability of arable land (Griffin *et al*., 2014). Increasing costs of grain, a desire to eliminate grain supplements, and an interest in alternative forages have also been cited by dairy farmers considering using sprouting systems. High-profile claims by manufacturers marketing sprouted grain systems have also fuelled interest. However, little scientific evidence is currently available to support these claims. Previous research indicates that benefits of sprouting may be negated by net DM loss from sprouting coupled with no significant improvement in nutrient concentrations or digestibility (Hafla *et al.,* 2014). Currently, no information is available regarding the feeding value of SB with high-quality forages such as the conserved forages and pastures found on well-managed organic dairy farms in temperate regions of the United States. The objective of this study was to evaluate the feasibility and challenges of implementing SB systems on organic dairy farms in the USA.

Material & methods Three experiments were conducted: 1) 5 grains [barley (*Hordeum vulgare* L.), oats (*Avena sativa)*, wheat (*Triticum* spp.), rye (*Secale cereal)*, and triticale (× *Triticosecale)*] were sprouted for 7 d to evaluate biomass yield, nutritional quality and mould growth; 2) lactating dairy cows fed a TMR containing corn silage, alfalfa silage and corn/grain mix (65% forage, 35% concentrate) were supplemented with either no SB or 1.4 kg DM of SB (which substituted for 2.4 kg of corn DM) to evaluate DMI, milk production, milk composition and fatty acid profile, and net income over feed costs (IOFC); and 3) in a case study, monthly farm visits were made to three certified organic dairy farms in Pennsylvania for 1 yr to evaluate feasibility and challenges of implementing a SB system. The sprouting and lactating cow studies were analysed using the MIXED procedure of SAS. Due to the nature of the case study and the inconsistent data collection due to farmer input and discontinuation of SB feeding on 2 of the farms during the study, statistical analysis was not feasible. Rather, case study data were transferred to Microsoft Excel files, summarized and included as supporting information regarding practical information from farmers in making decisions relative to feeding SB.

Results & discussion In the sprouting study, barley showed the greatest ($P < 0.05$) fresh biomass yield (9.3 kg) and least ($P < 0.05$) mould score [0.04 on a scale of 0 (least) to 5 (greatest)] while triticale had the least ($P < 0.05$) fresh biomass yield (6.3 kg) and greatest ($P < 0.05$) mould score (4.8). This information, combined with accessibility of barley seed in the USA, makes barley one of the most popular seeds for sprouting. In the lactating cow study, cows ($n = 36$) fed SB had reduced ($P < 0.05$) total DMI compared to cows not fed SB. SB did not affect ($P > 0.05$) milk yield or fatty acid profile. Cows fed SB had greater ($P < 0.05$) milk urea N which was probably a result of greater CP concentration and digestibility of SB compared with the corn for which the SB was substituted. The IOFC favoured not feeding SB by $0.22 USD/cow/d. In a sensitivity analysis, IOFC did not favour SB feeding until grain prices were increased by 50% over current organic corn prices. However, IOFC does not entirely capture the total economics of the SB systems because labour, cost of production, and the initial investment in the SB system are not included in the analysis. In the case study, two of the case study farms ceased feeding SB during data collection, citing mould issues, inability to source high-quality certified organic barley seed (free of mould and impurities), high maintenance costs of sprouting systems, greater than expected labour requirements, and lack of milk response, as reasons for discontinuing sprouting. The third farm continued to feed SB, but had a very low-cost homemade system, a small herd (20 cows), family-sourced labour (whereas the other farms had part-time hired help to assist with the sprouting system), and had difficulty producing home-grown high-quality forage. SB may have application in small-scale operations, particularly organic farms, with lands where tillable acreage can produce crops of greater value than for livestock feed, or for producers experiencing severe extended drought. Additionally, farms that have an excess of labour may benefit with a sprouted grain system. Each farm must put pencil to paper to determine if implementing sprouted grains in feeding management is a sound management decision, making sure to include all costs in deciding whether money could be better spent growing or purchasing higher-quality forage.

Conclusion Sprouted grain may be a very costly method of producing feed for organic dairy producers in the temperate regions of the USA where high-quality forage production is possible.

Acknowledgements Financial support was provided for this project by The Ceres Trust (Chicago, IL) and by a Northeast Sustainable Agriculture Research and Education (SARE) Partnership grant (#ONE14-224-27806).

References

GriffinT, Conrad Z, Peters C, , Ridberg Rand Tyler EP. 2014. Renewable Agriculture and Food Systems 4,349-363.
Hafla AN, Soder KJ, Brito AF, Rubano MD and Dell CJ 2014. Journal of Dairy Science 97,7856-7869.

Fodder systems and environmental impact of cow milk production in Italy

Giulia Gislon, Luciana Bava, Maddalena Zucali, Alberto Tamburini, Anna Sandrucci

University Of Milan, Milan, Italy

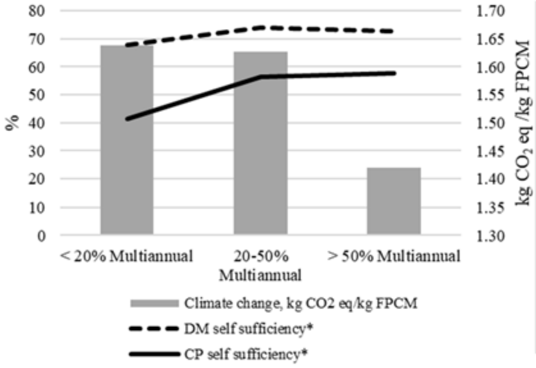
E-mail: luciana.bava@unimi.it

Take home message Fodder systems choices can change the environmental impact of milk production in dairy cow farms. Fodder systems based on permanent and multiannual crops, as grassland and lucerne, can be helpful in mitigating environmental load of milk production compared to systems based on arable crops, as maize or winter cereals.

Introduction Animal feeding, and in particular protein feeding, is a critical point in the livestock sector. The choice of crop system for home-grown fodder production can be crucial in the environmental prospective, it can affect feed self-sufficiency, amount and type of purchased feeds, efficiency of feed conversion, stocking rate but also the use of fertilizers and fuel consumption in field operations. The aim of the study was to evaluate, through a Life Cycle Assessment approach, the environmental impact of milk production in three groups of farms, characterized by different fodder systems.

Material & methods Inventory data about milk production, herd composition, feeding rations, housing system, manure management and crop production from 17 dairy farms of the Po plain were collected. Farms were divided in three groups on the basis of percentage of land with permanent and multiannual fodder crops (grassland or lucerne) on the total fodder agricultural area (Multiannual). Grazing was not adopted by any farm. Feeding ration composition were calculated using CPM-Dairy Ration software. Gas emissions were estimated according to IPCC (2006) and EEA (2009). Environmental impact categories, expressed per kg of Fat and Protein Corrected Milk (FPCM), were estimated with the methods recommended by ILCD Handbook (IES, 2012) and using the SIMAPRO software (Prè Consultants 2014).

Results & discussion The six farms with less than 20% of Multiannual were characterized by the smallest agricultural area, the largest herd size and the lowest dairy efficiency (59 ha, 176 lactating cows and 1.11 kg milk/kg dry matter intake on average, respectively). The most productive herds were in the intermediate group (20-50% of Multiannual; 6 farms) with 27.4 kg FPCM/cow per year, while the last group of farms (5), characterized by >50% of Multiannual, showed the lowest stocking density (3.39 LU/ha) and the lowest use of nitrogen from synthetic fertilizer (22 kg/ha per year).The results of impact categories showed the lowest values in the group of farms with >50% Multiannual. In particular, climate change per kg FPCM decreased as percentage of Multiannual increased (Figure 1); at the same time also feed self-sufficiency, both as dry matter and as crude protein, tended to increase, probably because the farms with >50% Multiannual were able to reduce the quota of high impact purchased feeds (particularly soybean meal, one of the most impactful feed on climate change) without compromising milk production. The same farms used the lowest amount of nitrogen fertilizer (both synthetic and organic), thanks to the low presence of annual crops, with an important restrain of acidification (Figure 2).



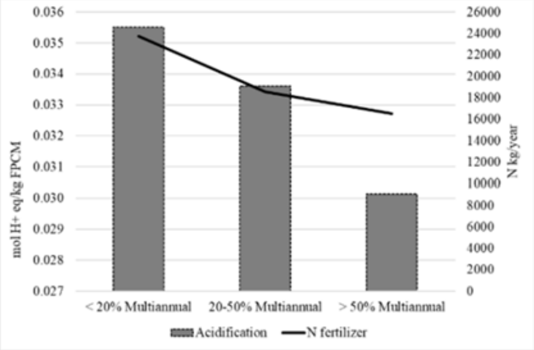


Figure 1 (left) Climate change and self sufficiency, (DM and CP) in the threee groups of farms.

Figure 2 (right) Acidification and N fertilizer load in the three groups of farms.

*DM and CP self sufficiency: percentage of DM or CP produced on farm and the total DM used for animal feeding.

Conclusion Fodder systems characterized by extensive use of permanent and multiannual crops, as grassland and lucerne, can reduce the environmental impact of milk production in comparison to systems based on arable land due to the high amount of feed self-production, both energy and protein feeds, and the reduced use of nitrogen fertilizers.

Acknowledgements The study was part of the project Forage4Climate (LIFE15 CCM/IT/000039) supported by EU commission.

References

EEA, 2009. EMEP/EEA. Air Pollutant Emission Inventory Guidebook 2009. Technical Guidance to Prepare National Emission Inventories, 4.B Animal Husbandry and Manure Management GB2009 update June 2010.
IES. European Commission - Joint Research Centre - Institute for Environment and Sustainability. 2010. International Reference Life Cycle Data System (ILCD) Handbook General guide for Life Cycle Assessment - Detailed guidance. First edition March 2010
IPCC, 2006. IPCC Guidelines for National Greenhouse Gas Inventories. Volume 4: Agriculture, Forestry and Other Land Use. Chapter 10: Emissions from Livestock and Manure Management, pp. 1-87

Simulating the effect of frequency and timing of *Plantago lanceolata* allocation on diurnal urination patterns of grazing dairy cows

Pablo Gregorini, Grant Edwards

Lincoln University, Christchurch, Canterbury, New Zealand

E-mail: pablo.gregorini@lincoln.ac.nz

Take home message Grazing management of plantain swards can help reduce N leaching further.

Introduction The largest contributor to nitrogen (N) leaching from pasture-based dairy farms is the N excreted as urinary N (UN) onto pastures. Mixed swards of ryegrass (*Lolium perenne*) and plantain (*Plantago lanceolata*) reduce UN excretion by dairy cows, decreasing N leaching (Totty *et al.*, 2013). Our objective was to simulate diurnal patterns of N excretion by dairy cows as affected by grazing management, i.e. frequency and timing of allocation of monoculture swards of plantain. We tested the hypothesis that not only the incorporation of plantain to diets reduces UN, but also -and at the same resource allocation- grazing management.

Material & methods We used the MINDY model (Gregorini *et al.*, 2018), which simulates excretion patterns of grazing ruminants. Eight instances of MINDY [initialized as pregnant Friesian dairy cows (500 kg liveweight) in mid-lactation (180 days in milk)] grazed over a term of 20 days in February (summer) under the following treatments: 1) frequency of allocation and 2) timing of allocation. Frequency of allocation was set by strip-grazing (24 hrs. pasture breaks) plantain or ryegrass monocultures for 1, 5 or 10 consecutive days (1D, 5D and 10D), allocated either after the morning (AM) or afternoon (PM) milking, i.e. Timing. In addition, other four MINDY instances grazed either plantain or ryegrass monocultures only (PlO and RgO, respectively) allocated AM or PM. Plantain and ryegrass swards had 30 cm height with an herbage mass of 3,000 kg/ha. Herbage allowance was 30 kg DM/d per cow. Chemical composition (g/kg DM) of plantain and ryegrass herbage was the following: 154, 139 CP; 225, 212 WSC; 370, 455 NDF; 170, 100 Ash, and 120, 198 g DM/ kg fresh herbage. The outputs required from MINDY were: Milk solids production (MS = Fat+CP), N intake (NI), N in milk (NM), UN and faecal N (FN), urinary volume (UV), urinations per day (UD) and N discharged onto pasture (NP) and milking shed (NSh) as proportion of total N excretion.

Results & discussion Table 1 presents model outputs. Compared to RgO, in average, AM and PM PlO reduced UN and urine N concentration by 23 and 15%, and increased Nsh by 10%, while increasing MS by 13%. Further PlO PM, as compared to PlO AM increased MS, Nsh, NM and reduced NI by 3, 10, 15 and 30%, respectively. When swards were allocated PM, 1D, 5D and 10D increased MS, MN, FN and NSh by 3, 9,1, and 60%, while reducing UN by 3.4% compared to 1D, 5D and 10D allocated AM. Coefficient of variation for UN (16, 17%), UN concentration (3, 6%) and NSh (10, 21%) for the mean of AM and PM of 1D, 5D and 10D suggests considerable effects of frequency×timing of Pl allocation.

Table 1 MINDY outputs on the effect of grazing management, *i.e.*, frequency and timing of allocation of monoculture swards of plantain on N excretion and urination patterns by grazing dairy cows.

Variable	PM					AM				
	RgO	PlO	1D	5D	10D	RgO	PlO	1D	5D	10D
MS (g/d)	1,440	1,720	1,640	1,670	1,650	1,380	1,500	1,500	1,480	1,470
NM (g/d)	80	100	90	90	90	70	80	80	80	80
NI (g/d)	440	500	620	510	490	460	560	610	540	520
FN (g/d)	140	200	210	190	180	140	200	200	190	180
UN (g/d)	170	140	200	160	160	180	130	210	160	160
UV (L)	41.3	37.7	56.2	44.6	42.38	42.3	37.3	57.0	52.0	49.4
Ur /d	12	13	21	15	14	13	15	26	23	21
NSh	0.11	0.14	0.11	0.12	0.09	0.11	0.11	0.04	0.05	0.03
NP	0.88	0.86	0.89	0.88	0.91	0.89	0.84	0.96	0.95	0.97

Conclusion Our work supports the premise that including plantain in the diet of grazing dairy cows reduce UN and urine N concentration, and thereby can help reducing N leaching. However, it is not only the dietary plantain content, but also how it is grazed (*i.e.*, grazing management by frequency and timing of pasture allocation), which helps to reduce environmental impact can further while maintaining or increasing MS.

Acknowledgements This work was supported by FRNL programme funded by the New Zealand MBIE.

References

Gregorini P, Provenza F, Villalba J, Beukes P, Forbes M 2018. Journal of Agricultural Science (Cambridge) (In Press)
Totty VK, Greenwood SL, Bryant RH, Edwards GR 2013. Journal of Dairy Science 96,141-149.

The effects of replacing barley grain with *Atriplex halimus L.* or olive cake on growth performance and carcass characteristics of Awassi lambs

Ahmed Aljamal, Belal Obeidat, Mohammed Obeidat, Rami Kridli, Kamel Mahmoud

Jordan University Of Science And Technology, Irbid, Jordan

E-mail: bobeidat@just.edu.jo

Take home message: Testing and using alternative feeds in ruminant feeding is important to reduce dependence on conventional feeds, reduce production costs and, hence, increase profitability. This study showed that using alternative feeds reduced the cost of production without impacting growth and carcass characteristics of lambs.

Introduction: Barley grain is widely used as the major source of energy for ruminants. Although, with the increased global demand, the price of barley grains has increased portentously, exerting pressure on sheep producers especially in arid and semi-arid regions where feed supplementation is essential. Hence, researchers are interested in the possibility of adding alternative feeds in livestock diets. Olive cake (OC) and *Atriplex halimus* L. (ATR) are great feed alternatives that can be fed to livestock to decrease the cost of production, thereby improving profitability. Olive cake, the residue of olive milling following oil extraction, is composed of skin, crushed pulp, stone wall, kernel, and remaining oil. Another possibility is *Atriplex halimus* L. (Mediterranean saltbush), which is a drought and salinity resistant shrub native to Jordan. In previous studies, OC (Awawdeh and Obeidat, 2011; Obeidat, 2017), and ATR (Obeidat *et al*., 2016) were used to replace the wheat straw, the forage source, in sheep diets. In the current study, however, OC and ATR are used to replace part of the barley grain. Therefore, the objectives of the study were to evaluate the effects of dietary inclusion of olive cake and *Atriplex halimus* L. on growth performance, nutrient intake, digestibility, carcass characteristics as well as the cost of production in growing Awassi lambs.

Material & methods: Thirty newly weaned male lambs (initial body weight; BW = 18.0 ± 0.46 kg) were randomly assigned to 1 of 3 treatment diets. Diets were: 1) control (CON), 2) 200 g/kg OC (OC) or 3) 200 g/kg ATR (ATR) of dietary dry matter (DM). The experiment lasted for 87 days (10 days for adaptation and 77 days for data collection). Nutrient intakes were measured daily. Body weight of lambs was measured at the beginning of the study and biweekly thereafter. On day 56, 6 lambs from each group were housed in metabolic cages to evaluate nutrient digestibility and N balance. Total feacal and urine output was collected, weighed, and sampled (10% feces and 5% urine) daily for 5 days. At the end of the study, all lambs were slaughtered to evaluate carcass characteristics and meat quality. Data were analysed using MIXED procedure of SAS (Version 8.1, 2000, SAS Inst. Inc., Cary, NC). For all data, the fixed effects included only treatment. Least square means were separated using appropriate pair-wise t-tests if the fixed effects were significant ($P < 0.05$). Lambs were modelled as a random effect.

Results & discussion: Intakes of DM, crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) were comparable among diets. Ether extract (EE) intake was greater ($P < 0.001$) in OC diet compared with the CON and ATR diets (25, 55 and 24 g/d for CON, OC and ATR, respectively). Average daily gain (ADG) and feed efficiency (DM intake:ADG) did not differ among groups. Cost of gain was reduced ($P < 0.001$) in OC and ATR diets compared with the CON diet (2.45, 1.64 and 1.56 US$/kg for CON, OC and ATR, respectively). Dry matter digestibility was lower ($P < 0.05$) in ATR diet compared with the CON, whereas the OC diet was intermediate (77, 73 and 70% for CON, OC and ATR, respectively). Digestibility of NDF was lower ($P < 0.05$) in the OC diet compared with the CON diet, while the ATR diet was intermediate. Digestibility of CP and ADF and N balance were not different ($P = 0.08$) among diets. Performance and cost of gain results were consistent with results obtained by Obeidat *et al.* (2016) and Obeidat (2017) when ATR and OC were included in the growing lamb's diets. Dressing percentage, carcass composition and dissected leg carcass cut weights were similar among diets. The lack of differences in dressing percentage and carcass cuts can be attributed to the similarity in intake and growth performance among diets.

Conclusion: Results of this study indicate the possibility of olive cake and *Atriplex halimus* L. inclusion at 200 g/kg of dietary dry matter without affecting growth performance and carcass characteristics. Additionally, results showed the economic value of using such products as it reduced the cost of gain compared with the control diet.

Acknowledgements: The authors wish to thank the Deanship of Scientific Research at Jordan University of Science and Technology for the financial support of this project **(**320/2017**)**.

References:

Awawdeh MS, and Obeidat BS 2013. Asian Aust. Journal of Animal Science 26,661-667.
Obeidat, BS 2017. Animal Feed Science and Technology 231,131-137.
Obeidat BS, Mahmoud, KZ Meswadeh JA and Bsoul EY 2016. Small Ruminant Research 137,65-70.

Effects of incorporating opuntia [*Opuntia Ficus-Indica (L.) Mill.*] in conventional green and dry fodders based diets on nutrient intake, utilization and performance of sheep

Asim Kumar Misra[1], Sunil Kumar[1], Kiran Kumar Tirumala[1], Shahid Ahmed[1], Dana Ram Palsaniya[1], Ashutosh Sarker[2], Sawsan Hassan[3], Serkan Ates[3,4], Mounir Louhaichi[3]

[1]ICAR-Indian Grassland and Fodder Research Institute, Jhansi, UP, India, [2]2International Center for Agricultural Research in the Dry Areas (ICARDA), South Asia and China Regional Program, New Delhi, India, [3]3International Center for Agricultural Research in the Dry Areas (ICARDA), Amman, 1195, Jordan, [4]Animal and Rangeland Sciences Department, Oregon State University, Corvallis, Oregon, 97331, USA

E-mail: asimkmisra@gmail.com

Take home message Opuntia is highly palatable and in conjunction with conventional fodder sources can maintain adult sheep in arid and semi arid conditions.

Introduction Semi-arid and arid regions of South-east Asian countries are prone to drought. During summer the harsh agro-climatic condition in these regions, results in progressive denudation of surface vegetation leading to low energy availability and loss of production. However, the opuntia plant remains green even during summer and can serve as a feed resource during scarcity (Sirohi *et al*., 1977; Misra *et al*., 2006). In view of the possible importance of opuntia as scarcity feed in hot semi-arid and arid environments, an experiment was undertaken to assess nutrient intake and utilization in sheep fed opuntia in conjunction with conventional green and dry fodders.

Material & methods Thirty-two local adult female sheep (31.00±0.85 kg body weight (BW) and 2 to 3 years in age) were fed on four experimental diets in equal groups based on comparable age and BW. The diet consisted of chopped (5-8 cm) Opuntia cladodes to provide 20 percent of dietary dry matter (DM) in conjunction with chopped green Napier (*Pennisetum purpureum*) grass (NG), Mediterranean clover (*Trifolium alexandrinum*) hay (MC), lathyrus (*Lathyrus sativus*) straw (LS) and gram (*Cicer arietinum*) straw (GS), *ad libitum*. All experimental animals were supplemented 200 g concentrate supplement (580g/kg groundnut cake, 400g/kg maize grain, 10g/kg mineral mixture and 10g/kg common salt) per head daily. The experimental feeding was continued for 21 days and at the end followed by a 7 days duration metabolic trial. Results obtained for intake, digestibility and plane of nutrition were subjected to analysis of variance procedure of SPSS Base 13.

Results & discussion Opuntia was readily eaten and animals did not allow any sort out of the opuntia quota offered to them. Daily DM intake (kg per 100 kg BW and g per kg $BW^{0.75}$) was higher ($P<0.01$) for Mediterranean clover straw (MC) fed group (4.16kg and 100.12g) and lathyrus straw (LS) fed group (4.35kg and 101.71g) compared to green napier fodder (NG: 3.39kg and 79.31g) and gram straw (GS) fed animals (3.45kg and 82.25g). The total tract apparent digestibility of DM, organic matter (OM) and protein were lower ($P<0.01$) in sheep fed opuntia with gram straw (GS) diet compared to other diets (NG, MC and LS). Similarly, digestibility of fibre fractions (NDF, ADF and Cellulose) also remained lower ($P<0.01$) on GS diet. The total digestible nutrients and digestible protein intake were also lower ($P<0.01$) in GS (48.74 and 3.12 g/ kg $BW^{0.75}$ respectively) compared to other groups, but it was over and above the standard requirements (5.00 and 36.00 g per kg $BW^{0.75}$) in sheep for maintenance (ICAR, 2013). No significant ($P<0.01$) changes in live weights were observed and animals maintained the live weight throughout the experimental period. Problem of laxative effects on opuntia feeding was not observed in the present experiment and the faecal DM content remained between 36.98 to 59.51 percent for the different experimental diets. A laxative effect appears when the volume of opuntia in the diet is high (>50 to 60 % of total dry matter intake). Moreover, opuntia may improve the nutritive value of poor quality roughage due to its high content of soluble carbohydrates, but nutrient intake and utilization on opuntia feeding with fodders having moderate level of protein appears to be more beneficial than the fodders which are either low in protein (<80g per kg DM) or very rich in protein (>120 g per kg DM).

Conclusion It is evident from the results that opuntia is highly palatable and in conjunction with conventional fodder sources can maintain adult sheep during summer in arid and semi arid conditions. Animals maintained live weight without any digestive disturbances such as diarrhoea and bloating.

Acknowledgements Financial assistance provided under project 3- sub project 2, promoting cactus (*Opuntia ficus-indica*) as drought resilient feed resource under different agro-ecological production systems across India within the Indian Council of Agriculture Research (ICAR) – the International Center for Agricultural Research in the Dry Areas (ICARDA) and the CGIAR Research Program (CRP) on Livestock collaboration, is thankfully acknowledged.

References

ICAR 2013. Nutrient Requirement of Animals- Sheep, Goat and Rabbit (ICAR-NIANP), Indian Council of Agricultural Research, New Delhi, India.

Misra A K, Mishra AS, Tripathi M K, Chaturvedi O H, Vaithiyanathan S, Prasad R and Jakhmola R C. 2006. Small Ruminant Research 63, 125-134.

Sirohi SK, Karim SA and Misra AK 1977. Journal of Arid Environments 36, 161-166.

505

The potential of ramie as forage for ruminants: impacts on growth, digestion, ruminal fermentation, carcass characteristics and meat quality of goats

Hongrong Wang[1], Hao Zhang[1], Yao He[1], Shaoxun Tang[2], Lihuai Yu[1]

[1]*Laboratory of Metabolic Manipulation of Herbivorous Animal Nutrition, College of Animal Science and Technology, Yangzhou University, Yangzhou, China,* [2]*Key Laboratory for Agro-Ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Changsha, China*

E-mail: hrwang@yzu.edu.cn

Take home message Ramie by-products are a potential use of ramie as forage for ruminants. The current experiment showed that ramie hay or raw ramie was a better way to use ramie by-products, which benefit to improve growth performance, rumen fermentation, nutrient digestibility and meat quality of goats.

Introduction Ramie has been used as a textile raw material for long time in China, where 200 million tons more ramie by-products are produced annually. Ramie residues become much more interesting, combined with the high yields of ramie in fresh biomass (Kipriotis *et al*., 2015; Dinh *et al*., 2007). However, less information is available documenting the effect of ramie on goat performance and to evaluate it's the potential as ruminant forage. The objective of this study was to investigate the influence of ramie inclusion in feedlot diets provided as hay or silage on growth performance, digestion, ruminal fermentation, and meat quality of goats.

Material & methods The inclusion of ramie forage in diets consisted of 4 treatments: (1) ramie residues silage without bast fiber, (2) raw ramie silage with bast fiber, (3) ramie residues was dried as hay without bast fiber, and (4) whole raw ramie was dried as hay with bast fiber. 48 Liuyang black castrated goats (18±1.0 kg of BW) were divided into four groups with eight replicated goats and offered the experimental diets. Diets were formulated to meet NRC (2007) nutrient requirements of growing castrated goats with 100 g/d ADG and was assigned to 4 treatments: 24% Rice straw + 36% ramie residues silage or 36% ramie hay + 40% concentrate with above 4 treatment ramie forage. The experimental period lasted for 60 days with 14 days adaptation period. The amount of feeds offered and refused was recorded. On d 1 and d 60 of the evaluation of treatments, ADG and DMI were measured. The Goats were housed in individual metabolism cages and assigned to the four previously corresponding to experimental diets. The digestibility trial lasted 7 d after a 5 d adaptation period at d 40 of the experimental period. On the d 60, rumen fluid (20 ml proximately) was collected before morning feeding (0 h) using a flexible stomach tube and a 100 ml syringe for pumping. The pH, NH_3-N, and volatile fatty acid were analysed. At the end of the trial, all animals were slaughtered and carcasses were immediately weighed to obtain the hot carcass weight. The GLM procedure of Statistics (2000) was used. Values were given as means. Differences among means were determined using the Fisher test. *P* values <0.05 were considered statistically significant, and *P* values < 0.10 were considered trends in the data.

Results & discussion There was an increased tendency for ADG in ramie hay treatment than that of ramie silage treatment (*P*=0.067) (Table 1). DMI (P=0.068) and FCR (*P*=0.053) tended to increase in the ramie residues treatment compared to raw ramie treatment. FCR (*P*=0.065) tended to be higher in the ramie silage treatment versus ramie hay treatment.

Table 1 Effects of ramie as hay or silage with or without bast fiber on DMI and growth performance of goats.

Items	Silage		Hay		SEM	P-value		
	SD	SF	HD	HF		T	F	T×F
DMI(g/d)	584.67	551.29	590.79	567.63	11.84	0.870	0.068	1.741
ADG(g/d)	72.50	81.23	73.98	86.74	3.736	0.067	0.042	0.009
FCR	8.06	6.79	7.89	6.54	0.509	0.065	0.053	0.253

The apparent digestibility of DM, CP, NDF, and ADF were significantly increased in the raw ramie treatment compared to ramie residues treatment ($P \leq 0.037$). Although ruminal NH_3-N was similar among treatments, a decrease in ruminal ammonia-N concentration was detected in the raw ramie treatment than that of ramie residues treatment (*P*=0.091). An increased tendency was noted for the molar proportion of butyric acid in the ramie hay treatment compared to ramie silage treatment (*P*=0.062).

Conclusion These results indicate that the ramie hay or raw ramie treatment was better for improving DM intake, the growth performance, rumen fermentation, nutrient digestibility, meat quality of goats than that of ramie silage or ramie residues treatment, respectively. It is suggested that ramie hay or ramie by-products could be used as a source of high quality forage of ruminants.

Acknowledgements The research was supported by the China Special Fund for Agro-scientific Research in the Public Interest (No. 201303144) and the Top Talents Award Plan of Yangzhou University (2016).

References

Dinh VT, Pham BD and Hoang VH 2007. Evaluation of ramie (*Boehmeria nivea*) foliage as a feed for the ruminant. In: Preston R, Ogle B. Proceedings MEKARN Regional Conference.

Kipriotis E, Heping,X, Vafeiadakis T, Kiprioti M and Alexopoulou E 2015. Industrial Crops and Products 68, 126-130.

First and second cut timothy silages in the diets of finishing Hereford bulls

Maiju Pesonen, Arto Huuskonen

Natural Resources Institute Finland (Luke), Ruukki, Siikajoki, Finland

E-mail: maiju.pesonen@luke.fi

Take home message Daily dry matter intake and average daily gain of the finishing Hereford bulls decreased when the second cut timothy silage was used in total mixed ration instead of the first cut silage.

Introduction The importance of grass silage (GS) digestibility for finishing cattle is demonstrated in several studies. In a meta-analysis of Huuskonen *et al.,* (2013) a 1 g/kg dry matter (DM) increase in D-value (digestible organic matter in DM) increased DM intake (DMI) of growing cattle by 7.2 g/d. In dairy cows, Kuoppala *et al.,* (2008) observed a lower DMI of regrowth GS compared to primary growth GS although most of the chemical and digestibility parameters were similar. The aim of the present study was to examine the effects of regrowth GS on performance of finishing bulls.

Material & methods A feeding experiment was comprised of 30 Hereford bulls which were fed a total mixed ration *ad libitum*. At the start the bulls were 304 (±29.5) days old and weighed 369 (±47.5) kg. The bulls were housed in an uninsulated barn in pens, five bulls per pen. A GrowSafe feed intake system was used to record individual feed intakes. The two dietary treatments included either first (GS1) or second (GS2) cut timothy (*Phleum pratense*) silage (550 g/kg DM), rolled barley (435 g/kg DM), and a mineral-vitamin mixture (15 g/kg DM). At the beginning of the experiment the bulls were randomly allotted to pens which were then randomly allotted to two treatments (15 bulls/treatment). Experimental silages were harvested on 25 June and 11 August 2015; GS1 and GS2, respectively. The aim was to have both silages with D-value of 690 g/kg DM. The stands were cut by a mower conditioner and harvested with an integrated round baler wrapper 24 hours after cutting. Both silages were treated with a formic acid-based additive. The relative intake potential of silage DM (SDMI index) was calculated as described by Huhtanen *et al*., (2007). The target for the average carcass weight of the bulls was 350 kg. The bulls were selected for slaughter based on live weight and slaughtered in three batches. The carcasses were classified for conformation and fatness using the EUROP classification. The data were subjected to ANOVA using the SAS GLM procedure. The statistical model included the fixed effect of diet and random effect of slaughtering batch. The effect of pen was used as an error term when differences between treatments were compared because treatments were allocated to animals penned together. Initial live weight was used as a covariate in the model.

Results & discussion Analysed D-values were 697 and 683 g/kg DM for GS1 and GS2, respectively, and close to pre-planned. Due to the rainy weather conditions during the first cut, the DM content of the GS1 was 31% lower compared to the GS2 (229 vs. 331 g/kg). The silages had similar contents of metabolisable energy (ME) (11.1 vs. 10.9 MJ/kg DM) and crude protein (CP) (149 vs. 151 g/kg DM) but the GS2 had 7% higher SDMI index (99 vs. 106) compared to the GS1. The fermentation characteristics of both silages were good. The average duration of the feeding experiment was 167 and 180 days for the GS1 and GS2 bulls, respectively. The use of GS1 increased daily DM (9.93 vs. 9.17 kg DM/d, $P<0.01$), ME (119 vs. 108 MJ/d, $P<0.001$) and CP (1309 vs 1220 g/d, $P<0.01$) intakes compared to the GS2. The average daily live weight gain (LWG) of the GS1 bulls was 11% higher (1717 vs. 1543 g/d, $P<0.01$) compared to the GS2 bulls. Dietary treatments had no effects on feed conversion (5.78 vs. 5.94 kg DM/kg LWG) or carcass characteristics. Based on silage chemical parameters and DMI index, the intake response of the GS2 should have been higher compared to the GS1. Nevertheless, DMI was higher when GS1 ration were used. One possible reason for the decreased DMI in GS2 could be the impaired microbiological quality of herbage and/or the contamination of silage by mycotoxins.

Conclusion Overall, daily DM and energy intakes, as well as growth rates of the finishing bulls, decreased when the second cut timothy silage was used in total mixed ration instead of the first cut silage. Observed difference in ME intake is probably a crucial explanation for the improved live weight gain of the GS1 compared to the GS2 bulls. One possible reason for the decreased DMI could be the impaired microbiological quality of herbage, but definite reasons were difficult to identify.

References

Huhtanen P, Rinne, M and Nousiainen, J 2007. Animal 1, 758-770.
Huuskonen A, Huhtanen P and Joki-Tokola E 2013. Livestock Science 158, 74-83.
Kuoppala K, Rinne, M, Nousiainen J and Huhtanen, P 2008. Livestock Science 116, 171-182.

Effect of heat treatment and lignosulfonate on in situ rumen degradability of canola cake protein

Piotr Micek, Katarzyna Slota, Pawel Gorka

University of Agriculture in Krakow, Department of Animal Nutrition and Dietetics, Krakow, Poland

E-mail: p.gorka@ur.krakow.pl

Take home message Combination of heat treatment and addition of LSO_3 is more effective in protecting canola cake protein from degradation in the rumen than the use of heat treatment only.

Introduction Due to an increase of biodiesel production, canola cake (CC) is a widely available source of protein for livestock. However, a use of CC in diets for high yielding dairy cows may be limited, due to excessive canola protein degradation in the rumen. Due to a lack of heat treatment step when cold press system of fat extraction is used, degradation of CC protein is even greater than the traditionally used canola meal protein in diets for ruminants**.** (McKinnon and Walker, 2009). We hypothesized that a combination of heat treatment and addition of lignosulfonate (by-product of paper industry rich in sugars; LSO_3) to promote the browning reaction will be a more effective method of CC protection from rumen degradation than heat treatment only. Therefore, the aim of this study was to determine the effect of heat treatment and heat treatment in combination with the addition of LSO_3 on CC rumen protein degradation.

Material & methods Cold-pressed CC was left untreated (UT), heated at 90, 110, 130 or 150ºC (HT90, HT110, HT130 or HT150, respectively) or processed with 5% of LSO_3 (on DM basis) and then heated at 90, 110, 130 or 150ºC (LHT90, LHT110, LHT130 or LHT150, respectively). Heat treatment was applied for 60 min and spruce calcium LSO_3 was used. Two non-lactating Holstein cows fitted with rumen cannulas were used to determine the in situ rumen degradation of CP, lysine and methionine. The animals were fed 5.3 kg of DM of grass hay and 2.3 kg of DM of concentrate daily. Rumen degradability was determined using nylon bags (pore size 50 ± 15 µm and internal size 6 × 13 cm; ANKOM, USA) filled with 3 g of fresh sample (ground to pass 1.5 mm sieve). The bags were incubated in the rumen for 0, 2, 4, 8, 16, 24, 48 and 72 h. CP and amino acids were determined using method no. 975.44 (AOAC, 2005) and amino acid analyzer (AAA-400; INGOS, Czech Republic), respectively. Degradability constants (a, b, c) and effective rumen degradability (ERD) of CP, lysine and methionine were calculated according to Ørskov and McDonald (1979) using PROC NLIN of the SAS (version 9.2; SAS Inst. Inc., Cary, NC) with passage rate (k_p) set at 6%/h. Data were analyzed using the MIXED procedure of SAS and two separate models. First model included all treatments and treatment and cow as fixed effects. Second model included effect of temperature of heating, LSO_3 and interaction between these two and effect of cow as fixed effects.

Results Heat treatment and heat treatment in combination with LSO_3 slightly decreased CP content in CC (36.6, 35.6 and 34.0% for untreated, heat treated and heat treated in combination with LSO_3 canola cake, respectively) as well as contents of lysine and methionine (18.1 vs. 15.2 and 5.0 vs. 4.7 g/kg of DM). Soluble fraction (a) of CP was less for treated (heated and heated with LSO_3) than untreated CC (35.3 vs. 55.4% of CP; $P < 0.05$), and decreased with increasing temperature of heating, especially for LHT130 and LHT150 (31.8 and 6.5%, respectively; heating temperature × LSO_3 interaction, $P < 0.01$). The potentially degradable fraction (b) of CP was greater for treated compared to untreated CC (59.9 vs. 38.4%; $P < 0.05$) and was greater for LHT130 compared to HT130 (65.3 vs. 59.5% of CP; heating temperature × LSO_3 interaction, $P < 0.01$). Rate of digestion (c) of CP was less for heat treated in combination with LSO_3 canola cake compared to untreated or only heat treated (5.1, 8.8 and 8.1%/h, respectively; $P < 0.05$). ERD of CP was less for treated than untreated CC (63.0 vs. 78.2%; $P < 0.05$) and was the least for LHT130 and LHT150 (61.0 and 34.9%, respectively; heating temperature × LSO_3 interaction, $P = 0.04$). Soluble fraction of lysine was less for treated than untreated CC (32.3 vs. 51.2% of lysine; $P < 0.05$) and was the least for HT130 and HT150 (32.8 and 8.11%, respectively; heating temperature × LSO_3 interaction, $P < 0.01$). The potentially degradable fraction of lysine was greater for treated compared to untreated CC (50.3 vs. 40.3%; $P < 0.05$). Rate of digestion of lysine was less for treated compared to untreated CC (6.7 vs. 13.6%/h; $P < 0.05$). ERD of lysine was less for LHT130 compared to HT130 (63.9 vs. 70.1%; heating × LSO_3 interaction, $P < 0.01$). Similar results were found for degradation constants of methionine and ERD of methionine was less for LHT110 and LHT130 compared to HT110 and HT130 (69.5 vs. 73.7% and 63.7 vs. 72.4% respectively; heating temperature × LSO_3 interaction, $P < 0.01$).

Conclusion Results of this study indicate that: 1) high temperature of heating (over 130ºC) may be necessary to protect CC protein from degradation in the rumen; 2) combination of heat treatment and LSO_3 may be more effective in protecting CC protein, lysine and methionine from degradation in the rumen than the use of heat treatment only.

Acknowledgements This study was supported by the project No. DS3217/KZiDZ/2017.

References

McKinnon JJ and Walker AM 2009. Canadian Journal of Animal Science 89, 401-408.
Ørskov ER and McDonald I 1979. Journal of Agricultural Science 92, 499-503.

In vitro effect of *Robinia pseudoacacia* leaf extract on gas, methane production and rumen degradability of alfalfa hay

Mustafa Sahin[1], Cagri Ozgur Ozkan[1], Ismail Ulger[2], Emrah Kaya[1], Ali Ihsan Atalay[3], Adem Kamalak[1]

[1]Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Kahramanmaras, Turkey, [2]Erciyes University Seyrani Agriculture Faculty, Kayseri, Turkey, [3]Igdir University, Faculty of Agriculture, Kahramanmaras, Turkey

E-mail: akamalak@ksu.edu.tr

Take home message *Robinia pseudoacacia* leaf extract significantly increased the methane production of alfalfa hay.

Introduction Recently, considerable attention has been diverted into the use of tree leaf extract to improve the efficiency of ruminal fermentation. *Robinia pseudoacacia* is one of the legume plants grown in many parts of world in a wide range of climates, where its leaves have been used as livestock feed and contains substantial amount of tannin. However, there is limited research on the effect of *Robinia pseudoacacia* leaf extract on ruminal fermentation. The aim of the current experiment was to determine the effect of *Robinia pseudoacacia* leaf extract on gas, methane production and degradability of alfalfa hay *in vitro*.

Material & methods Fresh leaves of *Robinia pseudoacacia* were chopped (1-2 cm) and extracted at 10 g leaves / 80 ml of solvent mixture (10 ml methanol, 10 ml ethanol and 80 ml distilled water) for 72 h soaking time. Leaf extract was tested at four doses (0.0, 0.6, 1.2 and 1.8 ml/g DM) of alfalfa hay (500 mg) in five replicates for each treatment using *in vitro* gas production technique for 24 h (Menke *et al.*, 1979). The content of the syringe was completely transferred into a beaker by using 50 ml of NDF solution and refluxed for 1 h and the residue was filtered through a pre-weighed sintered glass crucible.

Methane content of gas produced was determined using the infrared methane analyzer. The truly degraded substrate (TDS), partitioning factor (PF_{24}), microbial protein (MP) and efficiency of microbial protein (EMP) were estimated using the equations suggested by Blümmel and Lebzien (1997) as follows: TDS = Substrate incubated (mg) – the residue (mg) , PF_{24}= TDS / GP_{24}, MP (mg/g DM) = TDS – (GP_{24} X 2.2 mg/ml), EMP = (TDS – (GP_{24} X 2.2 mg/ml))/TDS. Gas production, methane production and true degradability values were determined using a specific blank, which is a syringe without substrate, containing only inoculum, incubation medium and *Robinia pseudoacacia* leaf extract.

Results & discussion As can be seen from Table 1, all doses of *Robinia pseudoacacia* leaf extract has no significant effect on gas production, TDS, PF, MP and EMP whereas *Robinia pseudoacacia* leaf extract significantly increased the methane production of alfalfa hay**.** The increase in gas production is possibly associated with the pythochemicals present in the leaf extract of *Robinia pseudoacacia* which may have a stimulating effect on the methanogenic micro-organism.

Table 1 Effect of *Robinia pseudoacacia* leaf extract on gas, methane production, degradability and microbial yield of alfalfa hay.

Extract Doses	Gas (ml)	Methane (%)	TDS(mg)	PF	MP(mg)	EMP (%)
0.0	92.3	13.0^a	316.3	3.4	113.3	35.8
0.6	94.8	15.0^b	321.7	3.4	113.1	35.1
1.2	95.0	16.1^c	319.4	3.4	110.4	34.5
1.8	95.6	18.3^c	322.3	3.4	112.0	34.7
SEM	1.62	0.65	6.70	0.09	7.52	1.73

^{abc} Column means with common superscripts do not differ (p>0.05), SEM: Standard error of the mean

Conclusion *Robinia pseudoacacia* leaf extract has no significant *(p>0.05)* effect on gas production, TDS, PF, MP and EMP, but significantly increased the methane production of alfalfa hay when incubated with rumen fluid *in vitro*. Therefore *Robinia pseudoacacia* leaf extract was not suggested for use in the mitigation of methane production.

References

Blümmel M and Lebzien P 2001. Livestock Production Science 68, 107-117.
Menke KH, Raab L, Salewski A, Steingass H, Fritz D and Schneider W 1979. Journal of Agricultural Science 92, 217-222.

In vitro effect of *Pyracantha coccinea* seed extract on gas, methane production and degradability of alfalfa hay

Cagri Ozgur Ozkan[1], Mustafa Boga[2], Mustafa Sahin[1], Ali Ihsan Atalay[3], Ozer Kurt[1], Adem Kamalak[1]

[1]*Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Kahramanmaras, Turkey,* [2]*Nigde Omer Halis Demir University, Nigde, Turkey,* [3]*Igdir University, Faculty of Agriculture, Igdir, Turkey*

E-mail: cagri@ksu.edu.tr

Take home message *Pyracantha coccinea* seed extract significantly improved degradability and microbial efficiency of alfalfa hay.

Introduction Recently considerable attention has been given to manipulate the efficiency of ruminal fermentation using the leaf and seed of tree leaves. *Pyracantha coccinea* is one of the shrubs grown in Turkey and produces a considerable amount of seed. However there is no information on the effect of *Pyracantha coccinea* seed extract on ruminal fermentation *in vitro*. The aim of the current experiment was to determine the effect of *Pyracantha coccinea* seed extract on gas, methane production and degradability of alfalfa hay *in vitro*.

Material & methods Fresh, frozen and dried seed of *Pyracantha coccinea* were chopped and extracted at 10 g seed / 80 ml of solvent mixture (10 methanol, 10 ml ethanol and 80 ml distilled water) for 72h soaking time . Seed extract was tested at 1.2 ml/g DM of alfalfa hay (500 mg) in five replicates for each treatment using *in vitro* gas production technique Gas production (GP_{24}) was determined for 24 h after incubation (Menke *et al*., 1979). Methane content of gas produced was determined using the infrared methane analyzer. The content of the syringe was completely transferred into a beaker by using 50 ml of NDF solution and refluxed for 1 h and the residue was filtered through a pre-weighed sintered glass crucible. The Truly degraded substrate (TDS), partitioning factor (PF_{24}), microbial protein (MP) and efficiency of microbial protein (EMP) of alfalfa hay were estimated using the equations suggested by Blümmel *et al.*, (1997) as follows. TDS = Substrate incubated (mg) – the residue (mg), PF_{24}= TDS / GP_{24}, MP (mg/g DM) = TDS – (GP_{24} X 2.2 mg/ml), EMP = (TDS – (GP_{24} X 2.2 mg/ml))/TDS. Gas production, methane production and truly degradability values were determined using specific blank, which is a syringe without substrate, containing only inoculums, incubation medium and *Pyracantha coccinea* seed extract. One-way analysis of variance (ANOVA) was used to determine the effect of *Pyracantha coccinea* seed extract on gas and methane production, and truly degradable substrate of alfalfa hay. Significance between individual means was identified using the Tukey's multiple range tests. Mean differences were considered significant at $p<0.05$.

Results & discussion As can be seen from Table 1 *Pyracantha coccinea* seed extract decreased gas production of alfalfa hay whereas *Pyracantha coccinea* seed extract increased methane production, TDS, PF, MP, EMP of alfalfa hay. The reduction in gas production may be associated with secondary metabolites which has a antimicrobial effect on bacteria, fungi and protozoa. However the shift in PF, MP and EMP would be useful to ruminant animal, even if there is a small increase in energy loss as methane production.

Table 1 Effect of *Pyracantha coccinea* seed extract on gas, methane production, degradability and microbial yield of alfalfa hay.

Extract Doses	Gas(ml)	Methane (%)	TDS(mg)	PF	MP(mg)	EMP (%)
Control	94.4^{a}	18.7^{b}	339.9^{b}	3.6^{b}	132.3^{b}	38.9^{b}
Fresh	91.4^{ab}	23.7^{a}	359.8^{a}	3.9^{a}	158.7^{a}	44.1^{a}
Dried	90.2^{ab}	21.8^{a}	355.7^{a}	3.9^{a}	157.2^{a}	44.2^{a}
Frozen	86.2^{b}	21.9^{a}	356.6^{a}	4.1^{a}	166.0^{a}	46.7^{a}
SEM	1.91	0.91	2.64	0.09	5.62	1.41

^{ab} Column means with common superscripts do not differ ($p>0.05$), SEM: Standard error mean

Conclusion *Pyracantha coccinea* seed extract can be used to improve degradability, PF, MY and EMP of alfalfa hay, even if there are small increase in methane production of alfalfa. However before large implication, further *in vivo* investigations are required to determine the effect of *Pyracantha coccinea* seed extract on the ruminant animal performance.

References

Blümmel M and Lebzien P 2001. Livestock Production Science 68, 107-117.
Menke KH, Raab L, Salewski A, Steingass H, Fritz D and Schneider W 1979. Journal of Agricultural Science 92, 217-222.

Effect of replacing grain concentrate with fibrous by-products on dairy performance of early lactation cows

Abdulai Guinguina[1], Marcia de Oliveira Franco[1,2], Sophie Julie Krizsan[1], Pekka Huhtanen[1]

[1]*Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences (SLU), SE-901 83 Umeå, Sweden,* [2]*Natural Resources Institute Finland (Luke), Green Technology, 31600 Jokioinen, Finland*

E-mail: abdulai.guinguina@slu.se

Take home message Fibrous by-products can replace grain concentrate in early lactation dairy cow feeding.

Introduction The replacement of cereal grains in dairy concentrates with fibrous by-products (BP) has been widely studied and effects on animal performance have been variable (Huhtanen *et al*., 1995; Ertl *et al*., 2015). However, there is lack of evidence on the effect of the lower starch and higher fibre contents of BP on production performance of cows in the early postparturient phase; a critical period characterised by negative energy balance resulting from high demand of energy for lactation but lower dry matter intake (DMI). The aim of this study was to analyse the effect of replacing grain concentrate with fibrous BP on production performance of lactating cows starting from early postpartum**.**

Material & methods The experiment was conducted as a continuous design where daily production and feed intake of cows were measured from calving to 20th week of lactation (WOL). Thirteen multiparous and 9 primiparous Nordic Red cows were randomly assigned to one of two treatments (TRT) of 11 cows each according to parity. Treatments consisted of grass silage supplemented with either a grain mixture (0.33 each of barley, oat and wheat on DM basis) or BP (0.33 beet pulp, 0.30 wheat middlings, 0.02 molasses, 0.25 barley bran and 0.10 oat bran on DM basis). Rapeseed meal and urea were added to make both diets isonitrogenous. The diets were fed *ad libitum* as TMR with forage: concentrate of 60:40. Milk was analysed for composition once a week on WOL 1 to 8 and every other week thereafter using an infrared analyser (MilkoScan FT6000, Foss Electric, Hillerød, Denmark). Daily CH_4 production was recorded with GreenFeed system (C-lock Inc., Rapid City, SD). Data were analysed using PROC MIXED procedure in SAS with fixed effects of TRT, WOL and their interaction (TRT*WOL) and a random cow within TRT effect. Least squares means were reported and significance was declared at $P<0.05$.

Results & discussion There was no TRT effect on DMI, milk or energy corrected milk (ECM) yield and composition, feed efficiency (FE) and CH_4 intensity (CH_4 g/kg ECM, Table1). The substitution of grain concentrate with the BP mixture significantly ($P=0.02$) reduced CH_4 yield (CH_4 g/kg DMI) and tended to reduce total CH_4 production (CH_4 g/d), indicating that high dietary fibre does not always lead to increased CH_4 production. Effect of WOL on all measurements was significant. Both DMI and CH_4 production were lowest at calving and increased progressively until wk 20 (Figure 1). From wk 1 to 2, ECM yield increased sharply, peaked at wk 4, and declined steadily thereafter. The effect of TRT*WOL was not significant.

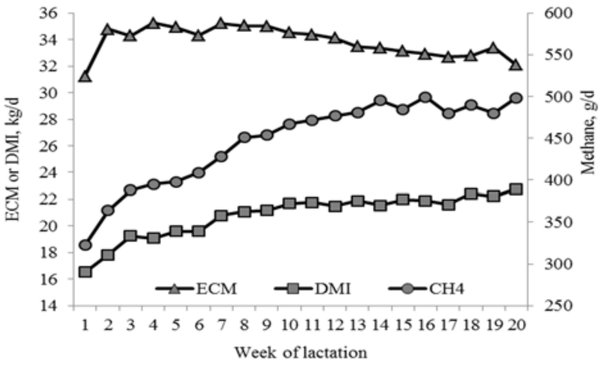


Figure 1 Daily average ECM yield, DMI and CH_4 by week of lactation of Nordic red cows.

Table 1 Effect of concentrate mixture on daily DMI, milk yield and composition, CH_4 emission and FE.

Item	Grain	BP	SEM	*P*
DMI (kg)	20.7	20.9	0.98	0.85
Milk yield (kg)	32.6	31.8	2.68	0.77
ECM (kg)	34.6	33.1	2.83	0.60
Fat (g/kg)	46.1	44.3	1.22	0.15
Protein (g/kg)	33.6	33.2	0.77	0.59
Lactose	45.4	45.4	0.35	0.97
Urea (mg/100mL)	17.4	19.2	1.05	0.10
CH_4 g/d	468	425	20.4	0.05
CH_4 g/kg DMI	22.6	20.4	0.60	0.02
CH_4 g/kg ECM	13.9	13.0	0.89	0.31
FE kg ECM/kg DMI	1.69	1.62	0.10	0.52

Conclusion A complete replacement of grain with by-product as a supplement of a grass silage fed to dairy cows in early lactation reduced CH_4 yield, without impairing production performance.

Acknowledgements This research was supported by grant from FORMAS funding organisation.

References

Ertl P, Zebeli Q, Zollitsch W and Knaus W 2015. Journal of Dairy Science 98. 1225-1233
Huhtanen P, Jaakkola S and Saarisalo E 1995. Animal Science 60, 31-40

The impact of silage additives on undigested NDF and potential digestible NDF of corn, whole barley and alfalfa silages

Mohsen Danesh Mesgaran, Seyyed Mohsen Hosseni, Alireza Vakili, Abbasali Nasserian

Dept. Animal Science, Ferdowsi University of Mashhad, Mashhad, Iran

E-mail: danesh@um.ac.ir

Take home message Inclusion of urea and formic acid to silages decreases undigested NDF (uNDF) and increases potential digestible NDF (pdNDF).

Introduction Silage fermentation is a dynamic process that leads to loss of nutrients; however, it is affected by a variety of biological and technological factors (Kung, 2010). In recent years there has been a focus to enhance silage quality and minimize nutrient losses during ensiling. A critical component for assessing silage quality is fiber digestibility. Increasing fiber digestion results in reduced physical gut fill in the rumen and is related to greater dry matter intake, as well as energy density and milk yield (Oba and Allen, 2000). We hypothesized that Biomin® inoculant, formic acid and urea may alter uNDF and pdNDF. Thus, the effect of silage additives for corn, whole barley and alfalfa on fiber digestion were tested.

Material & methods The whole crop corn (hybrid 700) in d 17 September 2016 at 2/3 milk line of kernel maturity stage, whole barley forage (Hordeum vulgare L.) in d 20 April 2016 at dough stage, and alfalfa forage (Medicago sativa L.) at d 10 May 2016 in second cutting at 40 % flowering, were harvested and chopped. They were then ensiled in laboratory mini-silos, 4-L polyvinyl tubes, and were immediately sealed by polypropylene screw cap on top with a rubber seal. Silage additives were Biomin® inoculant, formic acid and urea. The Biomin® (BioStabil strains) inoculant, was applied in liquid at 800 and 9600 colony forming units per kilogram (cfu/kg) to corn and 800 cfu/kg for whole barley crops. Formic acid was used within the range of four litres per ton in fresh alfalfa. Urea was added to corn and whole barley at 10.8 and 21.6 g/kg based on dry matter. Undigested NDF was determined at intervals of 30 (fast pool), 120 (slow pool), and 240 h. The procedure has been fully described by Goeser and Combs (2009). Undigested NDF was calculated as 100 – IVNDFD (*in vitro* NDF digestibility) where $\text{IVNDFD}_{\%\,\text{NDF}} = [1 - (\text{NDF}_{\text{residual}} - \text{NDF}_{\text{blank}})/\text{NDF}_{\text{sample}}] \times 100$, the amount of pdNDF is calculated from the difference of total NDF and uNDF ($\text{pdNDF}\ \%\ \text{DM} = (100 - \%\ \text{uNDF}_{240\ \text{h}}) \times \%\ \text{NDF}_{\text{sample}}$). Data were analyzed by the generalized linear model (PROC GLM; SAS 9.2). The Dunnett test was used between a control (untreated) and all other means (treated with additives) to compare differences within each crop. The least significant difference in means of uNDF and pdNDF were obtained using the PDIFF option of LSMEANS statement.

Results & discussion Data of undigested NDF and potential digestible NDF of corn, whole barley and alfalfa silages are presented in Table 1. The inclusion of urea to corn and whole barley silages improved fiber digestion by decreasing uNDF concentration at 30 h and increasing potential digestible NDF to corn. Addition of formic acid to the alfalfa silages increased pdNDF at 30 and 240 h. During ensiling, partial hydrolase of fiber with acids may alter ruminal digestibility. So this pre-hydrolase caused NDF to readily be digested in the rumen by increasing surface area available for microbial attack. Weimer (1996) suggested that the buffering effect of silage additive on rumen pH might be a possible explanation for the beneficial effects on fiber digestibility. Inoculation of Biomin did not affect both uNDF and pdNDF at different times of incubation. There were no detectable differences for $\text{uNDF}_{240\text{h}}$ among silages with various additives.

Table 1: The effect of silage additives on undigested NDF and potential digestible NDF of corn, whole barley and alfalfa silages

Items	Corn silage						Alfalfa silage			Whole barley silage				
	$\text{CS}_{0.0}$	$\text{CSU}_{10.8}$	$\text{CSU}_{21.6}$	CSB_{800}	CSB_{9600}	SEM	$\text{AS}_{0.0}$	$\text{AS}_{4.0}$	SEM	$\text{BS}_{0.0}$	$\text{BSU}_{10.8}$	$\text{BSU}_{21.6}$	BSB_{800}	SEM
uNDF_{30}	51.6	48.9*	48.8*	52.4	50.8	1.0	67.7	64.4	1.9	59.2	57.8	55.7*	59.0	1.0
pdNDF_{30}	26.7	29.1*	29.4*	26.7	27.0	0.7	14.1	16.6*	0.2	22.9	23.3	22.2	22.7	0.2
uNDF_{120}	42.6	40.9	41.5	43.8	40.8	1.0	54.7	53.8	0.8	51.0	52.1	48.8*	51.5	0.9
pdNDF_{120}	31.9	33.6*	33.6*	31.6	32.4	0.6	21.8	22.1	0.5	27.5	26.6	28.5	26.9	0.7
uNDF_{240}	31.2	29.9	28.5	31.8	31.2	1.7	46.2	45.7	1.8	39.4	39.2	36.7	39.2	2.2
pdNDF_{240}	38.2	39.9	41.1	38.8	37.9	0.7	24.0	25.0*	0.3	34.1	33.8	35.2*	34.8	0.3

* Within a column, means with an asterisk differ significantly from control ($P< 0.05$). SEM= standard error of means.

Conclusion Inclusion of urea and formic acid as an additive to silages may decrease uNDF and increase pdNDF by providing more energy through a more rapid rate of fermentation. However, it seems that Biomin® inoculant did not influence fiber digestibility.

Acknowledgements Researchers gratefully acknowledge funding from Ferdowsi University of Mashhad, Iran.

References

Goeser JP and Combs DK 2009. Dairy Science 92, 3833-3841.
Kung L 2010. Department of Animal and Food Sciences, University of Delaware Newark, DE, 19717-1303.
Oba M and Allen MS 2000. Dairy Science 83, 1333-1341.
Weimer PJ 1996. Dairy Science 79, 1496-1502.

Sesbania-maize supplementation increased growth of village Bali bull fed on native grasses basal diets in Lombok

Tanda Sahat Panjaitan, Prisdiminggo Prisdiminggo, Luh Gde Sri Astiti

BPTP Balitbangtan NTB, Mataram, NTB, Indonesia

E-mail: tanda_panjaitan@yahoo.com

Take home message Supplementation is essential to stimulate growth of village Bali bull fattened on native grasses in Lombok.

Introduction Bali bulls are traditionally fattened by smallholders with forage collected from rice fields, roadside and vacant land near the village. Growth is low and bulls require a long time to reach slaughter weight (Panjaitan, 2012). A high protein content tree legume, sesbania (*Sesbania grandiflora*), is grown integrated into the paddy field bund while maize is grown as a rotation crop or main crop in wet and rainfed land. A previous study has shown that sesbania supplementation increased the growth of weaned Bali cattle (Dahlanuddin *et al.*, 2013). The objective of this study was to evaluate the response of village Bali bulls fattened with native grasses supplemented with a mix of sesbania and maize.

Material & methods Twenty one growing Bali bulls (14-18 months old and 156 ± 26 kg of initial liveweight (LW)) were randomly assigned to one of 3 treatments diets. The 3 treatments were i) native grasses (NG; CP 135 g/kg DM) *ad libitum*, ii) NG + Sesbania, 5 g dry matter (DM)/kg LW.d (NGS; CP 160 g/kg DM) or iii) NGS + Maize grain, 4 g DM/kg LW.d (NGSM; CP 171 g/kg DM). Native grasses were a mix of grasses, forbs, weed, ferns and others. The study lasted for a total of 120 days, including 30 days for adaptation and 90 days of measurement. The bulls were weighed fortnightly to record LW and to adjust the daily amount of supplements. The digestibility study was conducted for seven days prior to the end of the experiment. Voluntary DM intake was calculated as the difference between feed offered and refusal the following day. Apparent digestibility of DM and organic matter (OM) was measured by total faecal collection. All the data were subjected to analysis of variance according to a completely randomized design.

Results & discussion Supplementation with sesbania or sesbania + maize increased average LWG of young Bali bulls ($P<0.05$), with NGSM further increasing LWG over NGS (Table 1). Supplementations increased total feed intake ($P<0.05$), but had no effect on the intake of the native grass and hence no substitution effect. Supplementation increased DM and OM digestibility of the total diet, with no difference in OM digestibility between the two supplements. LWG was in direct response to the extra energy and CP intake from the supplement. This work has shown a means of increasing LWG in Bali bulls whilst using a mixture of a tree legume and maize supplement.

Table 1 Live weight gain (LWG), DM intake and digestibility of DM and OM of village Bali bulls fed NG, NGS and NGSM. Values are means and s.e. Within rows means with different uppercase letter are significantly different ($P<0.05$)

Parameters	NG	NGS	NGSM
LWG (kg/d)	0.33 ± 0.01^a	0.40 ± 0.02^b	0.52 ± 0.03^c
Intake (g DM/kg LW.d):			
- Basal diet	23.7 ± 0.50	22.8 ± 0.50	22.3 ± 0.56
- Supplements	-	4.84 ± 0.01	8.58 ± 0.13
- Total	23.7 ± 0.50^a	27.64 ± 0.50^b	30.88 ± 0.68^c
DM digestibility (g/Kg)	486 ± 12.9^a	582 ± 7.5^b	631 ± 23.7^c
OM digestibility (g/Kg)	583 ± 9.9^a	661 ± 8.7^b	681 ± 21.1^b

Conclusion Supplementation of sesbania or sesbania + maize increased liveweight gain of young Bali bulls fattened traditionally on native grasses through an increase in total dry matter intake and digestibility.

Acknowledgements We thank the Lekong Siwaq farmer group for implementing the study and financial support from BPTP NTB research collaboration section.

References

Dahlanuddin, Yanuarianto O, Poppi DP, McLennan SR, and Quigley SR 2013. Animal Production Science 54, 915-921.
Panjaitan T. 2012. XVth AAAP Animal Science Congress.

Grazing performance and the use of concentrate in double herds breeding systems with dairy cow and suckling cattle herds in mountain areas: a bioeconomic simulation analysis

Zakary Diakite, Claire Mosnier, René Baumont, Gilles Brunschwig

Université Clermont Auvergne, INRA, VetAgro Sup, UMR 1213 Herbivores, F-63122 Saint-Genès-Champanelle, France

E-mail: zakary.diakite@vetagro-sup.fr

Take home message For plots with geographical constraints, double herds breeding systems with dairy and suckling herds allow a better grazed grass utilization rate, leading to good forage autonomy and good economic performances at farm scale.

Introduction Ruminant production is predominant in mountainous areas. In those areas, some plots of grasslands cannot be mechanized or are difficult to reach. Many mountain breeding systems combine in the same farm, a herd of dairy cows and a suckling cattle herd. We hypothesize that double herds allow a better grazed grass utilization rate than specialized herds for plots with constraints. The aim of this study was to characterise which herd distributions allow the best combination between grazed grass utilization, animal production and economical performances.

Material & methods We used the bio economic optimization model Orfee (Mosnier *et al.*, 2017a) and data from two farm-types with dairy and suckling herds in cruising condition from the INOSYS database (Charroin *et al.*, 2005). Farm_1 is a medium size system with a summer mountain pasture and Farm_2 is a large size system with a fragmented plot and cereal crops. These two farms are representative of the mountain double herd cattle farms. The model was adapted to take into account geographical constraints and the heterogeneity of grassland plots based on the French typology of permanent grasslands (Launay *et al.*, 2011). Simulations are based on the two different farms and five scenarios (described as the percentage of livestock units (LU) in the dairy (D) herd): 100D for a single dairy herd, 75D for a dominant dairy herd, 50D for balanced dairy and suckling herds, 25D for a dominant suckling herd and 0D for a single suckling herd.

Milk yield is fixed, and the use of grassland, facilities and machinery are optimized in the simulations. Biotechnical and economic indicators characterizing the feeding system, the animal and the system performances have been analysed for each scenario. This leads to evaluate simultaneously; operating profit per men work unit (MWU), grazed grass utilization rate (ratio of the amount of grazed grass to the amount of available pasture grass), animal production (milk and meat quantities), forage and concentrate consumption, related with the combination of dairy and suckling herds.

Results & discussion The annual cumulative consumption of forage is around 4 tons of dry matter (tDM) per LU in both farms for all the scenarios. Linked to the decrease of dairy LU in herds (Table 1), we observed a trend corresponding to an increase in the stocking rate and a decrease of concentrates intake per LU. Grazed grass utilization rate and operating profit per MWU reach their maximum for mixed herds systems (between 50D and 25D) as 60% of farms with double herds in the Massif Central (France). These systems that maximize grazed grass utilization also allow good economic performances.

Table 1 Levels of main indicators related to different analysed criteria for scenarios corresponding to the farm-types.

Farm-types	Farm_1					Farm_2				
Scenarios	100D	75D	50D	25D	0D	100D	75D	50D	25D	0D
Total livestock unit (LU)	65	73	80	82	80	113	125	137	132	128
Concentrates (tDM/LU/year)	0.95	0.87	0.76	0.54	0.25	.83	0.85	0.86	0.52	0.33
Grazed grass utilization rate (%)	58	72	85	82	85	72	84	89	100	90
Stocking rate (LU/ha)	0.93	1.04	1.14	1.18	1.15	1.05	1.17	1.28	1.24	1.20
Milk sold per year (1000L/year)	257	215	158	82	0	486	403	302	146	0
Meat sold (1000kg/ year)	9	12	15	17	19	18	26	34	40	45
Operating profit (1000€)/MWU	6.51	7.21	7.64	7.67	5.78	18.82	20.60	21.86	22.34	22.02

Conclusion This study shows that systems with double herds, particularly systems with 25 to 50% dairy cattle LU, maximize the grazed grass utilization rate in a context of mountain grasslands, with constraints of mechanisation and access to dairy cows. These systems that maximize grazed grass utilization also have good animal production and economic performances. However, the structure of the farm plot is essential to specify the optimal herd's distributions, which improve grazing and economic performances.

Acknowledgements This research was supported by grants from Auvergne Region and the French Ministry of Agriculture.

References

Charroin T, Palazon R, Madeline Y, Guillaumin A and Tchakerian E 2005. Rencontres Recherches Ruminants 12, 335-338.
Launay F, Baumont R, Plantureux S, Farrié JP, Michaud A and Pottier E 2011. Éd Paris: Institut de l'Elevage.
Mosnier C, Duclos A, Agabriel J and Gac A. 2017a. Agricultural Systems 157, 202-215.

Characterization of Neutral Detergent Fibre fractions in spring and autumn pasture swards

Michael Dineen[1,2], Brian McCarthy[2], Michael Van Amburgh[1]

[1]*Department of Animal Science, Cornell University, Ithaca, New York, USA,* [2]*Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland*

E-mail: michael.dineen@teagasc.ie

Take home message The nutritive value of Neutral Detergent Fibre differs and this needs to be quantified to understand its potential impact on animal performance

Introduction Numerous animal studies have shown that when forages of different *in vitro* digestibility but similar Neutral Detergent Fibre (NDF) concentration are fed, significant increases in dry matter intake (DMI) and milk production can be achieved (Oba & Allen, 2000). Neutral Detergent Fibre can be fractionated into two pools, one that is unavailable to microbial digestion (uNDF) and a potentially digestible pool (pdNDF) which is NDF minus uNDF. The degradation rate (k_d) of the pdNDF has also been shown to be highly variable between feeds and even within the same plant species (Van Soest, 1994). Therefore, our objective was to characterise the profile of NDF in spring and autumn pasture swards.

Materials & methods The pasture swards assessed were comprised of eight perennial ryegrass only (*Lolium perenne* L.; PRG) and eight PRG/white clover (*Trifolium repens* L.; WC) mixtures. The sixteen samples (n = 8 spring, n = 8 autumn) were analysed for crude protein (CP), aNDFom (NDF analysis conducted with α-amylase and sodium sulfite and ash correction; Mertens, 2002) and 0.5 g dry matter of each sample was fermented with the residues analysed for aNDFom, according to Goering & Van Soest (1970). Residual aNDFom of the fermented samples were obtained at 0, 12, 30, 120, and 240 hours. A glass microfiber filter (934-AH, Whatman) was used in all analyses as suggested by Raffrenato & Van Amburgh (2011), to avoid particle loss and increase recovery. A composite decay model was used to estimate parameters; pdNDF, k_d, and uNDF (Raffrenato & Van Amburgh 2010). The data were analysed using the t-test procedures in SAS 9.4.

Results & discussion Feed chemistry and aNDFom fractionation results are shown in Table 1. Pre-grazing yield and CP were not significantly different across season. However, the aNDFom concentration in autumn swards was elevated. The rate at which the pdNDF pool degraded was faster for Spring compared to Autumn pasture (9.53 versus 7.76 ± 0.6% hour^{-1}, respectively). Furthermore, the extent to which aNDFom was digested was greater for Spring compared to Autumn (9.75 versus 15.50 ± 0.44 % uNDF, respectively). Predictions of the Metabolisable Energy (ME) per kg of dry matter of the swards showed that spring pasture had a higher energy density ($P < 0.01$) and also supplied a higher amount of grams of Metabolisable Protein (MP; $P < 0.01$) to the animal.

Table 1 Effect of season on pre-grazing yield and nutritive value of pasture swards.

Variable[1]	Spring	Autumn	S.E.	*P*-value
Pre-grazing yield (kg DM ha^{-1})	1,691	1,494	137	0.228
Crude Protein (g kg^{-1} DM)	214	190	10	0.106
aNDFom[1] (g kg^{-1} DM)	325	355	8	0.006
Rate of degradation (% hour^{-1})	9.53	7.76	0.60	0.021
uNDF[2] (% aNDFom)	9.75	15.50	0.44	<.001
ME[3] (Mcal kg^{-1} DM)	2.68	2.52	0.04	0.003
MP[4] (g kg^{-1} DM)	121.30	111.90	2.21	0.004

[1]aNDFom = Neutral Detergent Fibre; [2]uNDF = undigested NDF; [3]ME = Metabolisable energy; [4]MP = Metabolisable protein

Conclusion Neutral Detergent Fibre as a fraction can behave differently, even within plant species. The fraction can differ in degradation rates and the extent to which it degrades. Season can affect these characteristics, independent of pre-grazing yield of the plant. Increased degradation of NDF can affect energy supply to the animal through increased volatile fatty acid production and increased flow of microbial protein, while also affecting DMI. Therefore, it is essential that determination of these fractions are included in all basic pasture feed analyses.

References

Goering H K and Van Soest P J 1970. US Agricultural Research Service.
Mertens DR 2002. Journal of the Association of Official Analytical Chemists International 85:1217-1240.
Oba M and Allen MS 2000. Journal of Dairy Science. 83,1333-1341.
Raffrenato E and Van Amburgh M E 2010. Proceedings Cornell Nutrition Conference for Feed Manufacturers 52–65.
Raffrenato E and Van Amburgh ME 2011. Journal of Dairy Science 94, 3613–3617.
Van Soest PJ 1994. Nutritional Ecology of the Ruminant. Cornell Univ Press, Ithaca, NY, USA

Effects of dietary inclusion of selenium yeast on antioxidant indices of Oula sheep

Yuhui Tan, Xiongxiong Cui, Xiang Xiao, Shenghua Chang, Fujiang Hou, Zhaofeng Wang

State Key Laboratory of Grassland Agro-ecosystems, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, China

E-mail: tanyh16@lzu.edu.cn

Take home message Supplementing selenium yeast can improve antioxidant function of grazing Oula sheep.

Introduction Selenium is an essential trace element to maintain metallic functions of animals and feeding animals with low selenium content can cause detrimental effects on growth rate, immune function, fertility and health (Čobanová K *et al*., 2016). Oula sheep is the main livestock that graze on the Qinghai-Tibet Plateau; however, there is little information in the literature on the effects of dietary addition of selenium on the grazing performance of Oula sheep. Therefore, the objective of the present study was to evaluate the effect of supplementing selenium on antioxidant functions of Oula sheep**.**

Materials & methods The study was conducted in Azi Breeding Centre in Maqu of Gansu province in China in August 2017. A total of 24 male Oula sheep (Oula breed) at 1 year age and 31.0±0.64 kg live weight were used in a 4-treatments study, with diet treatments including various selenium levels, achieved by supplementing selenium yeast, at 0, 0.2, 0.4 and 0.8 mg/kg DM. Each treatment had 6 sheep balanced for live weight and age among the treatments. Sheep were housed individually in crates for 34 d with total collection of faeces and urine during the final 6 d. The animals were offered fresh herbages (cut daily at 1500) fed *ad libitum* at 9:00, 11:00, 14:00 and 17:00 h. Water was freely available during the study period. In the final day of the study, a blood sample was taken from the neck vein of each sheep. The prepared serum samples were preserved at -20°C for further analysis of malondialdehyde (MDA) content, activity of superoxide dismutase (SOD), total antioxidant capacity (T-AOC) and activity of glutathione peroxidase (GSH-Px).

Results & discussion The results are presented in Table 1. The activity of GPx in the serum of Oula sheep increased by adding selenium yeast, with the 0.4 ppm group having the highest value, when compared with the control group. Selenium supplementation had no effect on the activity of SOD in 0.2 ppm and 0.8 ppm group when compared with CK, although for 0.4 ppm group the variable was higher and increasing significantly. The addition of selenium yeast significantly increased T-AOC, while decreased MDA content with the decrease being significant for 0.2 ppm and 0.4 ppm group. All data were statistically analyzed by one-way ANOVA.

Table 1 Effects of selenium supplementation on antioxidant variables of Oula sheep.

Treatments	MDA nmol/mL	SOD U/mL	T-AOC mg/mL	GSH-px nmol/min/L
0	0.86a	111bc	3.45b	572b
0.2	0.54b	116b	5.53a	625b
0.4	0.48b	135a	5.49a	1054a
0.8	0.62ab	96c	4.97a	750ab
s.e	0.051	4.23	0.287	62.814
P-value	0.015	0.002	0.02	0.012

Means in the same row with different superscripts differ, $P < 0.05$. P-value is the observed significance level of, the F-test for treatment; SE = standard error of the mean.

Conclusion Dietary addition of selenium can improve antioxidant activity of grazing Oula sheep, with the supplementation of 0.4 ppm in diets being appropriate.

Acknowledgements This research was supported by Program for Changjiang Scholars and Innovative Research Team in University (IRT_17R50), Natural Science Foundation of China (31672472) and the Fundamental Research Funds for the Central Universities (lzujbky-2017-53).

Reference

Čobanová KŠ, Faix I. Plachá K, Mihaliková Z, Váradyová,S, Kišidayová and Ľ Grešáková 2016. Biological Trace Element Research. 175,1-8.

Texas legume shrubs with browse potential

James Muir, Gulten Girgin, Nichole Cherry

Texas A&M AgriLife Research, Stephenville, TX, USA

E-mail: j-muir@tamu.edu

Take home message Texas shrub nutritive values are highly variable among species.

Introduction Shrubs occupy the vegetation layer between the herbaceous and arboreal canopies. They are generally defined as species that predominate from 0.5 to 3 m and have multiple stems at the ground level. Introducing a shrub canopy into rangelands, grasslands, pastures and forests could augment vegetation productivity vis-à-vis ecosystems with a single herbaceous or arboreal canopy (Estell *et al*., 2014). This, in turn, could support greater herbivore mass per area as efficiencies in grazer to browser or bulk to selective ruminant herbivory continuums increase (Muir *et al*., 2015). Leguminous shrubs are of particular interest when looking for species to populate intermediate-height canopies where ruminants browse. For example, there are 26 native genera that fit into the shrub category in Texas USA (Diggs Jr., *et al*., 1999). There are many with potential for cultivated pasture or rangeland restoration potential. Others are less attractive due to low biomass, spines or poisonous seeds or leaves. Our objective was to survey nutritive values and polyphenols of shrub legumes native to north-central Texas, USA to determine if a wider survey of browse potential is merited.

Materials & methods We collected leaf material during July 2017 from two or more distinct populations of *Leucaena retusa Benth., Cercis canadensis L. var. texensis (S. Watson) M. Hopkins, Gleditsia triachanthos L.,* and *Styphnolobium affine (Torr. & A. Gray) Walp.* in northcentral Texas. We assayed these for *in vitro* dry matter digestibility (IVDMD), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL; Van Soest and Robertson, 1980) N, total phenolics (TP) and protein precipitating phenolics (PPP; Naumann *et al*., 2014). We compared these using analysis of variance, where appropriate we used least square means separation, and we considered differences significant at $P \leq 0.05$.

Results & discussion *Leucanea retusa* had 75% greater N content than *S. affine* (Table 1). *Leucaena retusa* also had 28% lower ADF, 53% lower ADL, and 77% lower PPP than *G. triacanthos*. Although it had similar or less total phenolics than *L. retusa, C. Canadensis* had far more biologically active phenolics. For late-season nutritious browse, *L. retusa* shows promise; if biologically active polyphenols are important; for example for internal parasite suppression, *C. canadensis* might be promoted although its low N content may be a limitation.

Table 1 Nitrogen, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), total phenolics (TP), protein precipitable phenolics (PPP) and *in vitro* digestibility (IVDMD) in leaves of four Texas shrubs.

	Leucanea retusa	*Gleditsia triachanthos*	*Styphnolobium affine*	*Cercis canadensis*
		g kg^{-1} dry matter		
N	31.7 A*	26.0 B	22.2 C	11.0 D
NDF	402 A	399 A	451 A	219 B
ADF	258 B	339 A	336 A	287 B
ADL	100 B	211 A	150 B	
PPP	17 D	46 B	25 C	154 A
TP	224 A	117 B	144 B	154 B
IVDMD	286 BC	352 A	318 AB	253 C

*Means in a row with a shared letter do not differ according to the least significant differences ($P \leq 0.05$).

Conclusions Based on the example of four Texas species, there are numerous native leguminous shrubs that land managers can domesticate as shrub browse. Among these, some have challenges such as thorns, spines, poisonous seeds or leaves. Laboratory evaluation may indicate further challenges such as high ADL and phenolic compounds. Of the four shrubs we surveyed in northcentral Texas, *L. retusa* shows the most promise as a ruminant browse.

References

Diggs Jr. GM, Lipscomb BL, and O'Kennon RJ 1999. Botanical Research Institute of Texas, Fort Worth Texas, USA.
Estell RE, Havstad KM, Cibils AF, Anderson DM and Schrader TS 2014 Rangelands 36, 25-31.
Muir JP, Pitman WD, Foster JL, Dubeux Jr. J C 2015 African Journal Range and Forage Science 32, 97-112.
Naumann HD, Hagerman, AE, Lambert BD, Muir JP, Tedeschi, LO and Kothmann MM 2014 Journal of Plant Interactions. 9, 212-219.
Van Soest PJ and Robertson JB 1980 *In* W.J. Pigden et al., (ed.) Proc. Int. Workshop, Ottawa, ON. 12-14 Mar. 1979.

517

Intake and digestibility of capulin leaves (*Prunus salicifolia*) in growing goats

Jose Antonio Ruiz Perez[2], Lizbeth Robles Jimenez[1], Nicolas DiLorenzo[3], Roberto Montes de Oca[1], Jorge Arredondo[1], Manuel Gonzalez Ronquillo[1]

[1]Universidad Autonoma del Estado de Mexico, Toluca, Estado de Mexico, Mexico, [2]Centro de Bachillerato Tecnológico Agropecuario No. 150., Acambay, Estado de Mexico, Mexico, [3]Universidad de Florida, Marianna, Florida, USA

E-mail: mrg@uaemex.mx

Take home message *Prunus salicifolia* can be used as a natural native resource for feeding goat kids.

Introduction Small-scale livestock systems make use of natural forage resources, such as native vegetation, as part of livestock diets. Some of these native forages contain condensed tannins, which reduce methane (CH_4) emissions (Wanapat *et al*., 2015). The objective of this study was to evaluate the effect of the inclusion of *Prunus salicifolia* leaves in diets for growing kids at levels of 0, 15 and 30% of the total diet.

Materials & methods Six Saanen goat kids of three months of age with an average weight of 12.25 ±2.25 kg were used, housed in individual metabolic cages (1.2 x 0.6 m). Animals were fed twice a day at 8:00 and 16:00 h. Three diets were used, which were formulated according to the nutritional requirements of the goats (NRC, 2006). Diets were formulated to contain 14% CP and 2.73 MJ ME/ kg DM using regional foods. A control diet with barley hay (P0) was used, which was replaced by *P. salicifolia* at two levels: 15% (P15) and 30% (P30) of the total diet, respectively. The study was conducted over three periods of 14 days each: 10 days for adaptation to the diet and 4 days later to estimate dry matter intake. Faeces and urine were collected at the end of each experimental period. A repeated 3 x 3 Latin square design was used as the experimental design. A Tukey test was used when significant differences were observed between treatments ($p < 0.05$).

Results & discussion The inclusion of 30% of the leaves of *P. salicifolia* increased DMI, and had the lowest content of NDF and ADF, improving the N utilization of the diet. P30 reduced the N losses compared with P15 and P0. The lower content of NDF and ADF in the diet increase the DM intake, in addition to the tannins present in the *P. salicifolia* plant improving the use of N (Table 1).

Table 1 Chemical composition of the diet (g/kg DM), dry matter intake (DMI, g/LW$^{0.75}$/day), digestibility (g/kg DM) and nitrogen balance (g N/day) of kid goats feed with *Prunus salicifolia* at different levels.

	P0	P15	P30	SEM	P value	Linear	Quadratic
Chemical composition of the diet (g/kg DM)							
CP	140	139	138				
NDF	502	462	421				
ADF	286	260	234				
kg LW$^{0.75}$	6.03	6.74	6.83	0.45	0.1347	0.0714	0.3741
DMI, g/LW$^{0.75}$	76.8^b	79.9^b	94.4^a	9.25	0.0008	0.0004	0.1719
NDF intake	46.9^a	37.1^b	33.4^b	10.12	0.0001	0.0001	0.1842
ADF intake	23.7^a	20.9^b	18.6^b	5.70	0.0001	0.0001	0.1731
Digestibility (g/kg DM)							
DM	511	492	489	122	0.7357	0.4756	0.7439
OM	531	510	508	125	0.7418	0.4812	0.7539
NDF	282^a	249^{ab}	226^b	61	0.0027	0.0006	0.7347
ADF	160^a	140^{ab}	125^b	34	0.0008	0.0002	0.7163
N intake (gN/d)	1.56	1.61	1.91	0.17	0.0911	0.0468	0.3329
N excretion							
Faeces	3.06	2.10	1.56	0.59	0.0550	0.0214	0.6422
Urine	1.14	1.11	0.69	0.36	0.3129	0.1851	0.4688
N balance	-2.64^a	-1.61^b	-0.34^c	0.11	0.0001	0.0001	0.1917

Conclusion The inclusion of *Prunus salicifolia* in kid goats improves DM and N intake without affecting digestibility, improving the N utilization.

Acknowledgements This project was supported by UAEM 4335/2017.

References

NRC (National Research Council).2006. Nutrient Requirement of Goat.
Wanapat M, Cherdthong A, Phesatcha K and Kang S 2015. Animal Nutrition 96-103.

Effect of the essential oil of the leaves of *Callistemon viminalis* on the *in vitro* digestibility of *pennisetum clandestinum* hay in West African Dwarf goat

Hippolyte Mekuiko Watsop, Fernand Tendonkeng, Ferdinand Ngoula, Jules Lemoufouet, Emile Miégoué, Mama Mouchili, Albert Chounna, Pamo Tedonkeng Etienne

University of Dschang, Dschang/ West Cameroon, Cameroon

E-mail: hippolytewatsop87@gmail.com

Take home message The objective of this study is to contribute to the improvement of the digestibility of feed in the ruminants by the use of the vegetable extracts.

Introduction Microbial digestion in the rumen is a major phenomenon whose efficiency can be improved by the use of food additives (Noirot *et al.,* 2007). Many food additives were developed to improve the efficiency of the use of nutrients by decreasing the total quantity of methane or ammonia produced, thus increasing the quantity of energy or nitrogen available for the animal need. However, the risk of the presence of antibiotic residues in milk, the meat and its effects on human health led to its prohibition for a use in animal feeds by the European Union (Aouadi and Ben, 2012). This prohibition caused a renewed interest for the natural substitutes which represent vegetable extracts (tannins, saponins, essential oils).

Materials & methods One adult West African Dwarf goat was used as donor of rumen fluid for the study of the digestibility of *Pennisetum clandestinum* hay associated with different level of *Callistemon viminalis* essential oil. The leaves of *Callistemon viminalis* were collected in the campus of the University of Dschang in February 2016 and were dried with the free air, the shade and the ambient temperature. The samples intended for the extraction of essential oils were crushed. Four rations were used in this study:

- *P. clandestinum* + concentrated + 0mg essential oil (FPc+HECv0)/kg of dry matter: control;
- *P. clandestinum* + concentrated + 100mg of essential oil of *Callistemon viminalis* / kg of dry matter (FPc+HECv100);
- *P. clandestinum* + concentrated + 200mg of essential oil of *Callistemon viminalis* / kg of dry matter (FPc+HECv200);
- *P. clandestinum* + concentrated + 400mg of essential oil of *Callistemon viminalis* / kg of dry matter (FPc+HECv400).

The evaluation of the quantity of gas produced and preparation of reagent were made according to the method and the procedure (*Hohenheim gas method*) described by Menke *et al*., (1979).

Results & discussion The effect of essential oil on the parameters of *in vitro* digestibility is presented in Table 1.

Table 1 Effect of the essential oil of the *Callistemon viminalis* on *the in vitro* digestibility of the *P. clandestinum* hay West African Dwarf goat.

Rations	GP after 24h (ml/500mg)	ME(MJ/k gDM)	MM (mg)	PF (mg/ml)	VFA(mmol /40ml)	IVDMD (%)	IVOMD (%)	NDF-N(g/k gDM)
FPc+HECv0	32.31^b	7.61^b	96.41^b	3.71^a	0.71^b	50.00^b	52.64^b	1.56^a
FPc+HECv100	35.65^a	8.06^a	76.89^d	2.91^b	0.79^a	51.89^a	55.61^a	1.35^a
FPc+HECv200	31.25^b	7.47^b	88.90^c	3.07^b	0.68^b	48.03^c	51.70^b	1.58^a
FPc+HECv400	20.95^c	6.06^c	104.0^a	2.84^b	0.44^c	39.79^d	42.54^c	1.62^a
SEM	0.18	0.02	0.48	0.04	0.00	0.57	0.16	0.05
p	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.32

a,b,c,d: the mean bearing the same letter in the same column are not significantly different (p>0,05). SEM: standard Error of mean; P = Probability; GP = gas production; ME = metabolisable energy; MM = microbial mass; PF = partitioning factor; VFA = volatile fatty acid; IVDMD = *in vitro* dry matter digestibility; IVOMD = *in vitro* organic matter digestibility; NDF-N = residual nitrogen.

This positive effect of the *Callistemon viminalis* essential oil at the dose of 100mg/kg DM is due to their monoterpenes and hydrocarbon compounds which could favorably modulate ruminal fermentations. The negative effect of this essential oil at 400 mg indicates that the intensity of the antimicrobial activity of essential oil is strongly related to the dose used.

Conclusion This study shows that the addition of the essential oil of *Callistemon viminalis* (100 mg/ kg of dry matter) in the rations-based *Pennisetum clandestinum* hay contributes to improve its digestibility in West African Dwarf goat. The addition of 400mg of essential oil in the ration lowered (p<0.05) in a general way the parameters of *in vitro* digestibility.

References

Aouadi D and Ben SH 2012. Rencontre Recherches Ruminants 2012, 19.
Menke KH, Raab L, Salewski A, Steingass H, Fritz D and Schneider W, 1979. Journal of Agricultural Science 93, 217-222.
Noirot V, Moncoulon R, Sauvant D and Bayourthe C 2007. Revue de Médecine Vétérinaire 158, 589-597.

Assessment of quality and rumen degradability of mixed silages of sugarcane tops with whole plants of *Tithonia diversifolia* (Hemsl.) Gray in combination with molasses, fungi and lactobacilli

Raciel Lima Orozco[1,2], Miguel Ángel Arce González[2], Idalmys Bello Morales[3,2], Einar Artiles Ortega[2,4], Veerle Fievez[4]

[1]*Universidad Central Marta Abreu de Las Villas, Centro de Investigaciones Agropecuarias, Santa Clara, Villa Clara, Cuba,* [2]*Universidad Central Marta Abreu de Las Villas, Departamento de Medicina Veterinaria y Zootecnia, Santa Clara, Villa Clara, Cuba,* [3]*MINAG, Instituto de Medicina Veterinaria, Remedios, Villa Clara, Cuba,* [4]*Ghent University, LANUPRO, Ghent, Belgium*

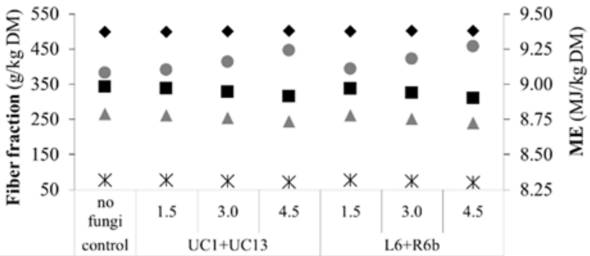
E-mail: raciello@uclv.edu.cu

Take home message Combined, rather than individual, addition of fungi, lactobacillus and molasses enhances quality of mixed silages of sugar cane tops with whole plants of *Tithonia diversifolia*, whereas fungi dose affects rumen degradability.

Introduction Tropical areas show feed deficits, mainly during the dry season (Ojeda, 2000). Hence, the conservation of available feed resources during the end of rainy season plays a key role in the ruminants' feeding systems. Thus, we assessed the quality and rumen degradability of sugarcane (*Sacharum spp.*) tops with whole plants of *Tithonia diversifolia* (Hemsl.) Gray ensiled in combination with either molasses, lactobacillus or fungi as well as their combination as additive.

Materials & methods The labscale silages were made in triplicate (Lima *et al.*, 2014) from sugarcane and *T. diversifolia* crops of two years old harvested at 12 months and 90 d of regrowth, respectively, in Santa Clara, Cuba. Sugarcane tops and *T. diversifolia* forage were mixed in a ratio of 60:40, respectively, and either or not in combination with one of the three following additives or their combination [fungal inoculant [FI; UC1 (*Penicillium sp.*) + UC13 (*Aspergillus sp.*) or *Trichoderma sp.* (L6+R6b)], *Lactobacillus plantarum* (LAB; 3×10^6 colony forming units/g fresh matter (FM)) and molasses (MOL; 39.4 g/kg FM)] or not. Both FI were inoculated at three fungi doses (FD; 1.5, 3.0 and 4.5×10^5 spores/g of FM) in order to prepare 28 treatments [2FI×3FD×2LAB×2MOL+4contol(2LAB×2MOL)]. The chemical proximate content (CPC) prior and after ensiling, as well as the ensilability and the *in vitro* ruminal degradability (IRD) of the silages were determined (Lima *et al.*, 2014). The metabolisable energy (ME) was estimated from CPC (Roche *et al.*, 1999). The ensilability parameters (pH, lactate, acetate, ammonia, ethanol) were used to select the best silages by a full factorial design in the GLM of SPSS with FI, FD, LAB and MOL as factors. The CPC, ME and IRD of the best silages were compared by one-way ANOVA of SPSS and further, a post-hoc Tukey test was performed when $P<0.05$.

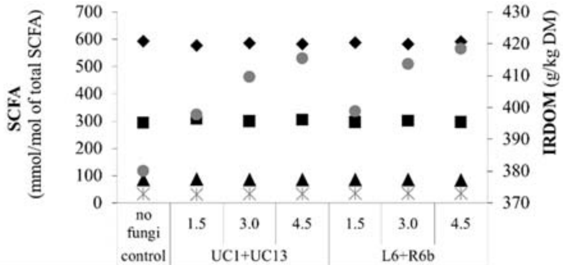
Results & discussion The forages that received a simultaneous addition of all additives showed the best silage quality, independently of FI or FD. However, the FD ($P<0.001$) but not the FI ($P>0.05$) affected the fiber fraction content (Figure 1) and IRD of OM (IRDOM, Figure 2). In the silages with all additives, the contents of DM, CP, EE and Starch were similar ($P>0.05$), but the ADF content was lower ($P<0.001$) in those silages with higher FD by reduction ($P<0.001$) of lignin and cellulose contents. In addition, the silages with higher FD showed higher ($P<0.001$) ME content and better ($P<0.001$) IRDOM, with similar ($P>0.05$) molar proportions of short chain fatty acids produced during a 24 h *in vitro* incubation.



	control	UC1+UC13			L6+R6b		
	no fungi	1.5	3.0	4.5	1.5	3.0	4.5
◆ NDF	498	499	500	501	500	501	502
■ ADF	342^{c}	338^{c}	328^{b}	315^{a}	337^{c}	325^{b}	310^{a}
▲ Cellulose	265^{c}	262^{c}	254^{b}	244^{a}	261^{c}	251^{b}	240^{a}
× Lignin	77.2^{c}	76.4^{c}	74.1^{b}	71.2^{a}	76.1^{c}	73.4^{b}	70.1^{a}
● ME	9.08^{c}	9.10^{c}	9.16^{b}	9.24^{a}	9.11^{c}	9.18^{b}	9.27^{a}

Different superscript letters in the same row show statistical differences (P<0.05; Tukey test)

Figure 1 Content of neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, lignin and metabolisable energy (ME) of mixed silages of sugar cane tops and *T. diversifolia* forage with molasses and *L. plantarum* addition in combination or not with a mixture of two fungi strain (UC1+UC13 or L6+R6b) inoculated at different doses (n=3).



	control	UC1+UC13			L6+R6b		
	no fungi	1.5	3.0	4.5	1.5	3.0	4.5
◆ Acetate	591	576	586	582	587	581	589
■ Propionate	293	308	299	303	294	300	295
▲ Butyrate	84	86	84	84	84	85	83
× Isoacids+Valerate+Caproate	31.7	30.2	31.8	31.3	34.4	33.7	33.1
● IRDOM	380^{d}	398^{c}	409^{bc}	415^{a}	399^{c}	414^{b}	418^{a}

Different superscript letters in the same row show statistical differences (P<0.05; Tukey test)

Figure 2 Short chain fatty acids (SCFA) proportion and *in vitro* rumen degradability of OM (IRDOM) of mixed silages of sugar cane tops and *T. diversifolia* forage with molasses and *L. plantarum* addition in combination or not with a mixture of two fungi strains (UC1+UC13 or L6+R6b) inoculated at different doses (n=3).

Conclusion The silages inoculated simultaneously with molasses, lactobacillus and fungi at doses of 3.0 and 4.5 $\times10^5$ spores/g of FM showed the best silage quality, the lowest ADF content and the highest IRDOM, independently of fungi strain. Thus, in tropical ruminant feeding systems ensiling of mixed forage from rich protein plants and fibrous by-products can be performed when molasses, lactobacillus and adequate fungi strains are simultaneously added prior ensiling.

Acknowledgements This research was supported by grants from VLIR-OUS and UCLV.

References

Lima Orozco R, Van Daele I, Álvarez Hernández U and Fievez V 2014. The Journal of Agricultural Science 152, 967-980.
Ojeda F 2000. In Silage Making in the Tropics with Particular Emphasis on Smallholders, Roma, Italy, p. Paper 8.0.
Roche A, Larduet R, Torres V and Ajete A 1999. Cuban Journal of Agriculture Science 33, 13-19.

Temporal change in rumen parameters of sheep fed either arrowleaf clover, biserrula or French serradella pasture *ad libitum* in late spring-summer

Lucinda Watt[1,2], Gaye Krebs[1,2], John Piltz[2,3], Belinda Hackney[2,4], Michael Friend[2]

[1]*Charles Sturt University, School of Animal and Veterinary Sciences, Wagga Wagga, NSW, Australia,* [2]*Graham Centre for Agricultural Innovation, Wagga Wagga, NSW, Australia,* [3]*NSW Department of Primary Industries, Wagga Wagga, NSW, Australia,* [4]*Local Land Services, Forbes, NSW, Australia*

E-mail: luwatt@csu.edu.au

Take home message Temporal rumen parameters vary when sheep are fed different annual pasture legumes, and data suggests arrowleaf clover may not be a "bloat-free" legume for grazing ruminants.

Introduction No studies have previously investigated temporal rumen parameters of sheep fed second generation annual pasture legumes biserrula (B; *Biserrula pelecinus* L.), French serradella (FS; *Ornithopus sativus* Brot) and new accessions of arrowleaf clover (AC; *Trifolium vesiculosum* Savi) under *ad libitum* feeding conditions. Sheep grazing B pastures are known to develop aversions to the plant (Thomas *et al.*, 2014). Reports have labelled AC as a "bloat-free" legume containing condensed tannins (Thompson, 2005); however, this has not been quantified in new accessions.

Materials & methods Twelve rumen fistulised Merino wethers were fed individually twice daily (08:30 h and 16:30 h) a pre-cut pasture of either AC (cv. Arrotas), B (cv. Casbah) or FS (cv. Margurita) over two periods in a partial cross-over design (B and FS were fed across two periods due to their indeterminate growth pattern, unlike AC; Table 1). The pastures were cut daily. The wethers were arranged in ascending order of LW and then allocated to each pasture on a stratified LW basis in a randomised complete block design. All wethers allocated to AC in Period 1, were randomly assigned (on block) to either B or FS in Period 2. Ruminal fluid collections occurred over a 24 h collection period (day 13) in 3 h intervals, commencing 3 h after the 08:30 feed. Urine and faecal output was collected over a 7 d period to determine the N balance of the wethers fed the varying pastures. A linear mixed model using REML was used to test all response variables. Period was tested in the model for all response variables and included if significant, otherwise it was fitted as a random effect.

Table 1 Experimental design; P= period.

	AC	B	FS
P1 (n = 12 wethers)	6	3	3
P2 (n = 6 wethers)	-	3	3

Results & discussion In Period 1, DMI of wethers fed B (556 ± 87 g/d) was lower ($P < 0.01$) than those fed AC (957 ± 75 g/d) or FS (1039 ± 121 g/d). In Period 2, DMI of wethers fed B (1048 ± 81 g/d) increased ($P < 0.01$) and was similar ($P > 0.05$) to those fed FS (1063 ± 77 g/d), despite the decline ($P < 0.05$) in DOMD of B (0.51 ± 0.03%; plants were senescing) compared to Period 1 (DOMD = 0.67 ± 0.03%; plants were in full-late flower). Ruminal NH_3-N concentrations in wethers fed AC were higher than those fed B or FS (Table 2) with ruminal NH_3-N increasing substantially ($P < 0.001$) in those fed AC shortly after feeding (6-15 h, data not shown). N intake of those fed AC (33 ± 5 g/d) was similar ($P > 0.05$) to those fed FS (24 ± 4 g/d), but was higher ($P < 0.01$) than those fed B (19 ± 4 g/d). Urinary N output of wethers fed AC (6.3 ± 1.2 g/d) was higher ($P < 0.001$) than those fed B (0.9 ± 1.1 g/d) or FS (2.3 ± 1.1 g/d), although N balance did not differ ($P > 0.05$). As shown in Table 2, total VFA, propionic and butyric acid concentrations were higher in those fed AC; whilst those fed B had lower acetic and butyric acid concentrations. The A:P ratio was higher for wethers fed FS. Wethers on the varying pastures differed in rumen pH with AC having the lowest and those fed B the highest. All wethers fed AC showed signs of frothy bloat (excessive foam escaping via the rumen cannula).

Table 2 Rumen parameters over 24 h. Results reported in mmol/L unless specified; [a]Significant differences ($P \leq 0.05$) indicated by differing superscripts.

	AC	B	FS
Total VFA	118.8 ± 12.1^c	73.6 ± 10.5^a	94.2 ± 10.7^b
Acetic	69.1 ± 6.7^b	51.1 ± 6.6^a	66.0 ± 6.8^b
Propionic	25.9 ± 2.5^b	15.8 ± 2.4^a	17.8 ± 2.4^a
Butyric	13.5 ± 1.1^c	4.4 ± 1.0^a	8.2 ± 1.0^b
A:P (ratio)	3.2 ± 0.2^a	3.4 ± 0.2^a	4.2 ± 0.2^b
Ruminal NH_3-N (mg/L)	325 ± 22^c	76 ± 15^a	123 ± 16^b
Rumen pH	6.4 ± 0.1^a	6.9 ± 0.1^c	6.6 ± 0.1^b

Conclusion Low DMI of wethers fed B in Period 1 was likely due to an aversion to the plant during this stage of growth, which was not observed in Period 2 as indicated by the increase in DMI (despite the decline in DOMD). High ruminal NH_3-N of wethers fed AC indicated extensive degradation of soluble proteins in the rumen, which likely contributed to the higher total VFA from deamination of AA and lower rumen pH due to an increase in VFA production (and bloat). Higher propionic acid concentrations are advantageous for liveweight gain; however, high loss of urinary N in wethers fed AC indicated poor N utilisation. Further studies are warranted to measure the incidence of frothy bloat in sheep fed AC pastures.

Acknowledgements This research was supported by funding from MLA, Charles Sturt University and the Graham Centre.

References

Thomas DT, Milton JTB, Revell CK, Ewing MA and Lindsay DR 2014. Grass and Forage Science 70, 374-380.
Thompson RB 2005. NSW Department of Primary Industries, Primefact 102.

Association of molecular structure spectral profiles with chemical and nutrient profiles of feedstock and co-products from bio-oil processing: Comparison crusher plants within Canada and within China as well as between Canada and China

W.M.S. Gomaa[1,2], G.M. Mosaad[2], Peiqiang Yu[1]

[1]Department of Animal and Poultry Science, College of Agricultural and Bioresources, University of Saskatchewan, Saskatoon, Canada, [2]Department of Animal Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt

E-mail: peiqiang.yu@usask.ca

Take home message Comparisons of canola seeds, meal or pellets in Canada, in China, and between Canada and China indicated the presence of significant variations among different crusher plants and processing co-products.

IntroductionAlthough canola has been extensively studied, the relation between its protein molecular structure and the chemical and nutrient profiles remains unclear. In addition, little information is present about canola variations among the different crusher plants, the processing products, and the different producers' countries such as Canada and China. These variations affect the processing-induced molecular structure, chemical composition and, concentration of protein and carbohydrates and availability of nutrients. This study aimed to 1) use molecular spectroscopy as a novel technique to quantify protein molecular structures of canola in relation to its chemical, energy and nutrient profiles, 2) determine and compare (a) protein molecular structure using Fourier transform infrared (FT/IR) molecular spectroscopy technique; (b) chemical composition; (c) energy values; (d) protein and carbohydrate sub-fractions partitioned by the Cornell Net Carbohydrate and Protein System (CNCPS) in canola seeds, meal or pellets from crushing plants in Canada in comparison with China; and 3) find the correlation between protein molecular structural features and nutrient profiles in canola.

Materials & methods Feedstock-Canola seeds and co-products from bio-oil processing (canola meal or meal pellets) were obtained from five different processing plants in Canada as well as from five different processing plants in China (each plant had three different batches of seeds, meal or meal pellet as replicates). Total samples: Canadian seed: 5 (processing plants) × 3 (batches/plant) =15 samples; Canadian meal: 3×2=6 sample; Canadian meal pellet: 3×3=9; Chinese seed: 5×3=15; Chinese meal: 5×3=15. Attenuated Total Reflectance (ATR)-Fourier transform infrared FT/IR molecular spectroscopy was used as a rapid tool to detect protein molecular structures (Yu, 2005; Zhang and Yu, 2014). The data were analysed using Proc Mixed SAS 9.4. Correlation and multiple regression analysis were performed. The model variables selection for regression was carried out using Stepwise Option in SAS.

Results & discussion Our results showed the possibility to characterize protein molecular structure of canola using the molecular spectroscopy. Processing induced changes between seeds and meal were found in the chemical, energy profiles, protein and carbohydrate fractions and protein molecular structure. However, no strong correlation was found between the chemical and nutrient profiles of canola seeds and their protein molecular structure profiles. On the other hand, canola meal had strongly correlated with protein structure in the chemical profile, energy values and, protein and carbohydrate fractions. The TDN, NE for lactation and NE for growth could be predicted with the following equations: TDN1x = 55.82 + 2.83 * Area ratio of amide I and II, NEL p 3x = 1.39 + 0.12 * Height ratios of amide I and II, and NEg = 0.92 + 0.11 * Height ratios of amide I and II, respectively.

Conclusion Generally, comparisons of canola seeds, meal or pellets in Canada, in China, and between Canada and China indicated the presence of some variations among different crusher plants and bio-oil processing products.

Acknowledgements The authors would like to thank Brittany Dyck and Qin Guoqin (Canola Council of Canada) for help sampling canola seed and canola meal in various crushers in Canada and China. The SRP Chair (PY) research programs have been supported by the Ministry of Agriculture Strategic Research Chair (PY) Program, SaskCanola, and Natural Sciences and Engineering Research Council of Canada (NSERC) etc.

References

Yu P 2005. British Journal of Nutrition 94, 655-665.
Zhang X and Yu P 2014. Journal of Agricultural and Food Chemistry 62, 6199-6205

Effect of increasing level of brewers grains in diets of rabbits on carcass quality and economic efficiency

Zakia Harouz-Cherifi[1], Si Ammar Kadi[2], Azedine Mouhous[2], Mokrane Berchiche[2], Thierry Gidenne[3]

[1]Akli Mohand Oulhadj University, Bouira, Algeria, [2]Mouloud Mammeri University, Tizi-Ouzou, Algeria, [3]GenPhySE, Toulouse University, INRA, Toulouse, France

E-mail: cherifiz@yahoo.fr

Take home message Produce acceptable rabbits carcasses with brewers grains, a neglected agro-industrial by-product.

Introduction Brewers grains is an agro-industrial by-product of beer production. According to the Feedipedia database (Heuzé *et al.*, 2017), it can be considered as a good source of protein (24%) and fibre (NDF: 62.4%, ADF: 19.7%). In Algeria this product is inexpensive and locally available (8.37 €/quintal) compared to soybean meal (46 €/quintal). The aim of this trial was to study the possibility to produce acceptable rabbits carcasses with a feed containing high rate of brewers grains and measure its effect on economic efficiency and competition with humain food.

Materials & methods At weaning (35 d. of age), 180 rabbits were divided into three identical groups (body weight = 860 ± 126g) and housed in collectives cages (4 rabbits per cage) until slaughter (77 days). Each of the three groups was fed *ad libitum* either pelleted feed containing 0% (control or B0), 20% (B20) or 30% (B30) of dried brewers grains in partial replacement of soybean meal and to decrease alfalfa and barley rates. The average protein and ADF levels were 14.4 and 15% respectively. After six weeks of fattening, ten rabbits per group were slaughtered (without fasting) in controlled conditions. The weight of full digestive tract, skin and hot carcass was recorded immediately after slaughter. On chilled carcasses, data were recorded: the weight of cold carcass, liver, perirenal fat, inguinal fat and scapular fat. Data were subjected to analysis of variance with R software in order to evaluate the effects of the diet on slaughter parameters.

Results & discussion Growth performance (30g/days) and feed intake (98g/days) were not affected by treatments, but feed conversion was higher in B20 group (3.65 *vs* 3.92) because of its low energy concentration (DE). The incorporation of brewers grains did not impair the slaughter traits (Table 1). Dressing out percentage was the same in all groups (59.4± 0.5% on average for the cold carcass, p>0.05). Economic efficiency (Table 2) was 15% and 41% better in B20 and B30 diets respectively with almost 0,10 and 0,23 Euro (€) more benefit per kg of meat. For the competition of feeds with human food, the values obtained for two indicators SAPAA and PAACoH (Pothin *et al.*, 2016) showed that the diets used in this essay have a low competition on space and resources.

Table 1 Slaughter performance of rabbits in the experimental groups.

	B0	B20	B30	SEM	P
Body weight (BW), g (78days)	2164	2151	2166	38	0.952
Weight of full digestive tract, g	388	428	420	14.48	0.148
Cold carcass weight, g	1294	1265	1289	24	0.665
Liver weight, g	83	77	92	4.44	0.562
Perirenal fat, g	19	20	23	1.65	0.658
Dressing out percentage of cold carcass, %	60.9	59.76	60.1	0.49	0.843

Table 2 Economic performance of rabbit units according to the diet fed to the rabbits.

Parameters	B0	B20	B30
Total weight gain (kg)	1.20	1.16	1.25
Total feed intake/rabbit (kg)	3.52	3.92	3,78
Feed price (€/kg)	0.28	0.23	0.20
Feed cost €/kg	1.01	0.91	0.77
Economic efficiency (%)	283	324	399
Revenue €/kg meat produced	2.84	2.94	3.08

Conclusion These results show that the incorporation of brewers grains at increasing rates does not impair the slaughter performance of rabbits and allows production of carcasses at the same level to those permitted by the control diet, improves the economic efficiency and makes feeds uncompetitive with human food.

References

Heuzé V, Tran G, Sauvant D and Lebas F, 2017. Feedipedia, a programme by INRA, CIRAD, AFZ and FAO.
Pothin A, Méda B and Fortun- Lamonth L 2017. 12ème journées de la recherche Avicole et des Palmipèdes à Foie gras.

Detect molecular spectral features of *vicia faba* varieties in relation to protein metabolic characteristics in ruminant system using advanced synchrotron radiation based infrared microspectroscopy

M.M. Rahman[1], H.H. Zhang[1,2], W.X. Zhang[3], Peiqiang Yu[1]

[1]*Department of Animal and Poultry Science, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, Canada,* [2]*College of Life Science and Engineering, Foshan University, Guangdong, China,* [3]*College of Animal Science and Technology, Henan University of Animal Husbandry and Economy, Zhengzhou, China*

E-mail: peiqiang.yu@usask.ca

Take home message The protein molecular spectral profiles are highly associated with truly digestible nutrients and energy values and can be used as predictors for rapid evaluation of these nutrients in feeds.

Introduction To date there has been very little application of the advanced synchrotron-radiation based molecular spectroscopic technique to study molecular structure make-up in pulse seeds. The objectives of this study were to detect an interactive association between protein molecular structure and nutrition availability in newly developed *vicia faba* varieties. The detailed analysis items included: (a) protein molecular structure profiles, (b) protein chemical profiles, (c) metabolic characteristics of protein, (d) bio-energy values and (e) the relationship between protein molecular structure and protein chemical and metabolic profiles in ruminant system.

Materials & methods Two varieties (cv. Snowdrop and FB9-4) of faba beans differed in tannin content were evaluated. These varieties were developed by CDC (Uni of Sasketchewan, Canada) and grown in Saskatchewan, Canada and provided by Bryan Doig (Ag Specialist, Saskatchewan Canada). Synchrotron radiation-based infrared microspectroscopy (SR-IMS) was applied as a molecular technique (Yu, 2005). Advanced synchrotron radiation based and globar-sourced vibrational infrared microspectroscopy have been developed as a rapid, direct, non-destructive and bioanalytical technique. In contrast to traditional "wet" chemical methods, this technique, taking advantages of synchrotron light brightness (million times brighter than sunlight) and small effective source size, is capable of exploring the molecular chemistry, molecular structure and molecular nutrition at ultra-spatial resolutions (Yu, 2004; 2005). The synchrotron data collection was carried out at Advanced Light Sources (ALS-LBNL, Berkeley). The data were statistically analyzed using the Mixed procedure SAS 9.4. Treatment means were compared using Tukey method. Contrast was carried out between meal and pellets. Correlation Analysis and Multiple regression analysis were performed using PROC CORR and PROC REG procedure of SAS 9.4. A multiple regression with variable selection analysis was carried out using the PROC REG procedure in SAS 9.4 with the following model to determine which protein molecular structure features of faba bean play an important role in determining nutrient supply: $Y = \text{amide I } (A\_I) + \text{amide II } (A\_II) + \text{amide I to amide II ratio } (R\_I\_II) + \alpha\text{-helix } (\alpha\_h) + \beta\text{- sheet } (\beta\_h) + \alpha\text{-helix to } \beta\text{-sheet ratio } (R\_\alpha\_\beta)$. The model used a Stepwise option with variable selection criteria: Slentry = 0·05, Slstay = 0·05. All variables left in the final model are significant at the 0·05 level.

Results & discussion The ratio of both amide I to II area and height were higher ($P < 0.01$), while the ratio of α-helix to β-sheet was lower ($P < 0.05$) in CDC Snowdrop compared to FB9-4. The CP content and the predicted truly digestible nutrients as well as the bioenergy contents did not vary between the two varieties. The CDC Snowdrop had higher ($P < 0.01$) rapidly degradable CP fraction (75.99 vs. 71.45% on CP) and lower ($P < 0.01$) moderately degradable CP fraction (19.43 vs. 22.85% on CP), resulting in higher ($P < 0.01$) rumen degradable protein and lower ($P < 0.01$) rumen undegradable protein content than that of FB9-4 variety. However, the total supply of digestible rumen undegraded feed protein was higher ($P < 0.05$) in FB9-4 than CDC Snowdrop. Strongly positive correlations were found between ratio of α-helix to β-sheet and CP contents ($R = 0.86$, $P < 0.01$) as well as truly digestible CP contents ($R = 0.83$, $P < 0.01$); respectively.

Conclusion The results of this study reveal that the protein are metabolized differently between different type of faba bean varieties and advanced SR-IMS molecular spectroscopy can be used to rapidly delineate protein molecular structures along with their nutritive value in ruminant livestock system.

Acknowledgements The National Synchrotron Light Source (NSLS) and Advanced Light Source (ALS) are supported by the U.S. Dept of Energy. The SRP Chair (PY) Programs fund from the Sask Pulse Growers (SPG), NSERC-Individual Discovery Grant; NSERC-CRD Grant, Saska Ag Strategic Research Chair Program Fund, Ag Development Fund (ADF), SaskMilk, SaskCanola, Sask Forage Network, Western Grain Research Foundation (WGRF), POGA etc are acknowledged.

References

Yu P 2004. British Journal of Nutrition 92, 869.
Yu P 2005. Journal of Agricultural and Food Chemistry 53, 7115–7127.

Effect of the inclusion of field pea on the degradability of the dry matter of the concentrate and the volatile fatty acids in fattening lambs

Mireia Blanco, Pablo José Rufino-Moya, Sandra Lobón, Isabel Casasús, Margalida Joy

Centro de Investigación y Tecnología Agroalimentaria de Aragón, Zaragoza, Spain

E-mail: mblanco@aragon.es

Take home message The effect of the inclusion of field pea in the fattening concentrate of lambs on the degradability of dry matter and proportions of volatile fatty acids is dose-dependent.

Introduction The partial substitution of soya by field pea (*Pisum sativum*), a local source of protein, in the fattening diets of lambs has been encouraged to reduce the dependency of Europe on soya imports. The field pea has a high content of starch, with lower ruminal degradability than barley (Walhain *et al*., 1992). Moreover, the ruminal solubility of the protein of field pea is greater than that of soya in cattle (Vander Pol *et al*., 2009), but there are no studies in lambs. The aim of the study was to evaluate the effect of different proportions of field pea in the concentrate of fattening lambs on *in sacco* digestibility.

Materials & methods Four rumen-fistulated Rasa Aragonesa wethers (55 kg LW) were randomly assigned to four treatments that differed in the inclusion of field pea (0, 10, 20 and 30%) in the fattening concentrate during 4 periods. The concentrates were formulated to be iso-energetic (1.18 MJ/kg FM) and iso-proteic (175 g CP/kg FM). Every 15 days, 2 nylon bags (50 µm) per sample (3 g) were introduced through the fistula and incubated in the rumen for 2, 4, 6, 8, 12 and 24 hours. Ruminal content was extracted to determine pH, ammonia and volatile fatty acids (VFA). The disappearance of dry matter (DM) was studied during incubation and fitted to the equation $y=a+b(1-e^{-ct})$ and N disappearance was studied after 24 h of incubation. Data were analysed using SAS v.9.3. The disappearance of DM was studied with NLIN procedure and the VFA, ammonia and N disappearance with proc GLM with the inclusion of field pea as fixed effect.

Results & discussion Rumen pH was not affected by the inclusion of field pea in the concentrate. The disappearance of DM is reported in Figure 1. The rapidly degradable and soluble fraction (a) was affected by the inclusion of field pea ($P<0.05$) being greater in 30% than in 0% field pea, presenting 10% and 20% intermediate values. The rate of degradation (c) and the slowly degradable fraction (b) were not affected by the inclusion of field pea. The inclusion of field pea did not affect total VFA production but affected the proportions of VFA (Table 1). The percentage of acetic acid was greater in 30% field pea ($P<0.05$). Propionic acid percentage was greatest in 20%, intermediate in 30% and 10% field pea and lowest in 0% field pea ($P<0.001$). Butyric acid percentage was greater in 0 and 10% than in 20 and 30% field pea ($P<0.001$). The inclusion of field pea tended to affect ammonia concentration ($P<0.10$) but did not affect the disappearance of N ($P>0.05$)

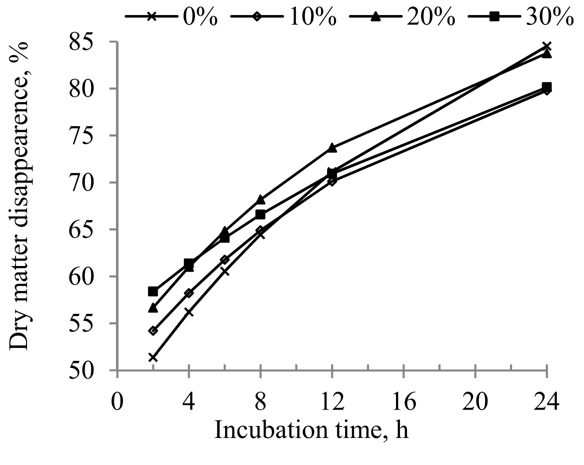


Figure 1 Effect of the inclusion of field pea on dry matter disappearance during incubation.

Table 1 Effect of the inclusion of field pea on total and percentages of volatile fatty acids (VFA), ammonia and nitrogen (N) disappearance.

	0%	10%	20%	30%	P-value
VFA, mmol/l	104	101	103	99	0.36
acetic acid, %	69.9b	69.9b	69.8b	70.6a	0.04
propionic acid, %	13.6d	14.4c	15.6a	14.9b	0.001
butyric acid, %	12.5a	12.2a	11.4b	11.7b	0.001
isobutyric acid, %	1.06a	0.97b	0.91c	0.92c	0.001
valeric acid, %	1.00a	0.92bc	0.93b	0.87c	0.001
isovaleric acid, %	1.96a	1.58b	1.36c	1.11d	0.001
Ammonia, mg/l	105a	83ab	90ab	70b	0.06
N disappearance, %	48.0	29.4	37.3	48.0	0.66

Within a line, means with different letter differ at $P<0.05$

Conclusion The inclusion of field pea affected the dry matter disappearance through the rapidly degradable and soluble fraction (a) and the proportions of VFA.

Acknowledgements Project RTA2014-00032-c02-01 and RZPP2017; INIA and ESF funded M. Blanco contract.

References

Vander Pol M, Hristov AN, Zaman S and Delano N 2008. Journal of Dairy Science 91, 698-703.
Walhain P, Foucart M and Thewis A. 1992. Animal Feed Science and Technology 38, 43-55.

To estimate intestinal truly absorbed protein of alfalfa hay and alfalfa silage using new Dutch System (DVE/OEB)

Parisa Kheyrandish, Mohsen Danesh Mesgaran, Alireza Vakili

Ferdowsi University of Mashhad, Mashhad, Iran

E-mail: p.kheyrandish@gmail.com

Take home message Type of preservation of alfalfa may impact on DVE/OEB content by altering the digestion of protein in small intestine.

Introduction Storage of alfalfa as silage leads to increased dry matter solubility and degradation rate. Storage in form of silage causes anaerobic condition and pH reduction which results in microbiological, biochemical and anaerobic fermentation processes. Alfalfa hay in comparison with alfalfa silage has less amount of crude protein and also has more neutral detergent fiber which is probably due to dissipation of leaf along alfalfa desiccation and hemicellulose hydrolysis during silage fermentation (Nelson and Saiter, 1992). Accordingly, the aim of this study is to assess the effect of alfalfa storage method on chemical composition and intestinal truly absorbed protein of alfalfa hay and alfalfa silage using new Dutch System (DVE/OEB).

Materials & methods Alfalfa was harvested at second cut in half bloom stage, then half of them were dried at 65 ºC for 48 h, using air forced oven, and the rest were ensiled for 28 days. Samples were analyzed for crude protein, neutral detergent fiber (NDF) and acid detergent fiber (ADF) were assayed according to Van Soest *et al.* (1991), Sodium sulfite and alpha amylase were not used in the NDF assay. Ruminal *in situ* incubations were performed according to Ørskov & McDonald (1979). Three lactating Holstein multiparous cows, fitted with a ruminal cannula, were fed a TMR containing 50% Forage and 50% concentrate and passage rate was considered 0.06/h. The DVE value is calculated according to Tamminga *et al.* (1994): DVE = DVBE + DVME - DVMFE, where DVME is the microbial protein synthesized in the rumen and digested in the intestine, DVBE is the feed protein not degraded in the rumen but digested in small intestine, and DVMFE is the endogenous protein losses associated with digestion and OEB value is calculated as follows: OEB = MREN – MREE, where OEB is the Degraded protein balance, MREN is the microbial protein synthesized in the lumen based on available nitrogen and MREE is the microbial protein synthesized in the rumen based on available energy. Data were analyzed as a complete randomized design by GLM procedures of SAS, statical model included effect of treatments (preservation methods) and residual error.

Results & discussion The effects of preservation methods of alfalfa on truly absorbed protein and chemical composition are presented in Table 1. DVE level in alfalfa hay is significantly higher than alfalfa silage. According to the low level of effective protein degradation of alfalfa hay, it can be concluded that a greater part of the protein in alfalfa hay in comparison with alfalfa silage passes the rumen to the small intestine. In alfalfa silage, whereas, a greater part of protein is in the form of non-protein nitrogen and its amount of passage is also lower to the small intestine.

Table 1 Prediction of chemical composition and intestinal truly absorbed protein of alfalfa hay and alfalfa silage using new Dutch System (DVE/OEB).

	Chemical composition				Nutritive value					
Item	CP	NDF	ADF	Ash	DVMFE	DVME	DVBE	DVE	OEB	DVE/CP
Hay	198.00^{a}	421.00	296.00	91.50	2.38	50.03^{b}	88.38^{a}	136.04^{a}	15.77^{b}	0.69^{a}
Silage	192.57^{b}	432.00	312.00	92.83	2.40	59.27^{a}	32.15^{b}	89.07^{b}	69.24^{a}	0.46^{b}
SEM	2.46	7.27	5.23	1.83	0.17	0.41	0.82	1.22	0.92	0.011
P-value	0.0007	0.34	0.09	0.63	0.94	0.0001	0.0001	0.0001	0.0001	0.0001

DVME: Rumen synthesized microbial protein digested in the small intestine, DVMFE: endogenous protein losses in the digestive tract DVBE: Digestion in small intestine of the undergraded feed protein, DVE: truly absorbed protein in the small intestine, OEB: reflects the difference between the potential microbial protein syntheses based on rumen degraded feed CP and that based on energy (rumen fermented OM) available, for microbial fermentation in the rumen.

Conclusion Results indicate that the type of preservation of the forage may impact on DVE/OEB content of alfalfa by altering the digestion of protein in small intestine.

References

Nelson WF and Saiter LD 1992. Journal of Dairy Science 75, 1571-1580.
Tamminga S, Van Straalen WM, Subnel APJ, Meijer RGM, Steg A, Wever CJG and Blok MC 1994. Journal of Livestock Production Science 40, 139–155.
Van Soest PJ, Robertson JB and Lewis BA 1991. Journal of Dairy Science 74, 3583–3597.
Ørskov ER Mcdonald I 1979. Journal of Agricultural Science 92, 499–503.

Validation of prediction equations to estimate rumen-undegradable crude protein in tropical feedstuffs using protein fractionation technique

Khaterine Salazar-Cubillas, Uta Dickhöfer

Universität Hohenheim, Stuttgart, Germany

E-mail: khaterine.salazar-cubillas@uni-hohenheim.de

Take home message Development of specific algorithms is needed for accurate prediction of rumen-undegradable crude protein in tropical ruminant feeds.

Introduction Advanced laboratory techniques exist to estimate feed protein leaving the rumen. However, laboratory techniques and related algorithms have been developed for ruminant feeds commonly used in temperate husbandry systems. Hence, the aim of this study was to validate two previously published undegradable crude protein (UDCP) prediction equations on tropical feedstuffs (concentrates, forage legumes and grasses). In addition, nutritional data was used to generate a best-fit equation for UDCP estimation based on proximate analysis and protein fractionation.

Materials & methods Twenty-six samples of concentrate feeds and forages from six tropical countries were used. *In situ* UDCP was determined using 1.7 g of dry material in a polyester bag with a pore size of 50±15 µm. Samples were incubated in duplicate in the rumen of three cannulated cows following procedures of Shannak *et al*. (2000). Incubation periods were 2, 4, 8, 16, 24, and 48 h. Additionally, non-protein nitrogen (fraction A), soluble protein (fraction B1), insoluble true protein (fraction B2), neutral detergent insoluble nitrogen (fraction B3), and acid detergent insoluble nitrogen (fraction C) were analysed in duplicate (Licitra *et al*., 1996). The UDCP proportions (g/kg crude protein (CP)) were calculated for a rumen passage rate of 5 %/h using equations of Kirchhof (2007) and Shannak *et al.* (2000). Additionally, a linear multiple regression was developed to predict UDCP from concentrations of different CP fractions. Differences in UDCP values between techniques were determined by Tukey tests. Additionally, linear regressions between measured and estimated UDCP values were analysed.

Results & discussion There were no linear regressions between *in situ* UDCP and those estimated by equations of Kirchhof ($r^2 = 0.23$; intercept 161 (standard error (SE) 42.3); slope 0.26 (SE 0.09)) and Shannak ($r^2 = 0.08$; intercept 210 (SE 37.8); slope 0.15 (SE 0.09)). For concentrates, no differences in UDCP estimates were observed between equations; yet, both equations overestimated UDCP proportions in forage legumes (Figure 1). The following model was developed ($r^2 = 0.68$; $p<0.05$, Figure 2):

$$UDCP = 375.8 + 52.1 \times (B3/(B1+B2)) - 3.1418 \times A$$; where UDCP is in g/kg CP and CP fractions are in g/kg DM.

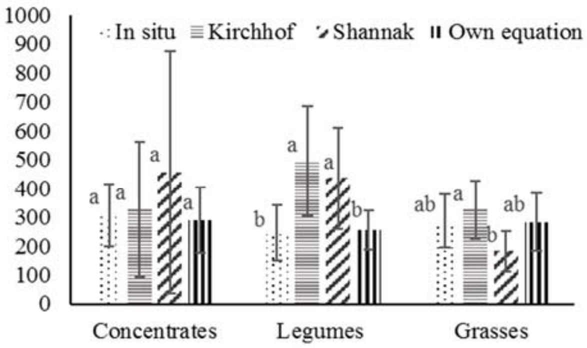


Figure 1 Undegradable crude protein (g/kg crude protein) in concentrates (n = 6), forage legumes (n = 13), and grasses (n = 7) determined by different equations (Arithmetic means; one standard deviation).

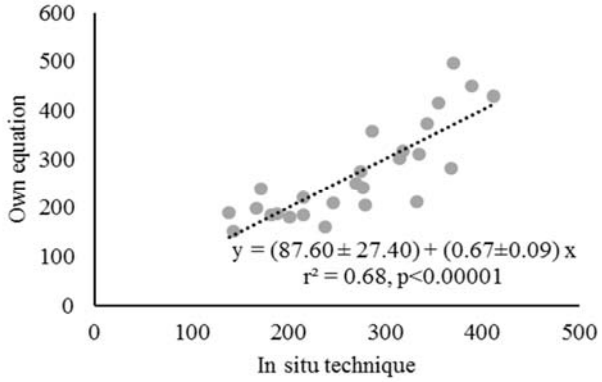


Figure 2 Linear relationship between undegradable crude protein (g/kg crude protein) determined *in situ* or estimated using own prediction equation (mean and SE).

High variations of fractions B2, B3, and C between tropical forage species hampers prediction of UDCP concentration.

Conclusion However, while existing equations developed for temperate forages overestimate UDCP in tropical forage legumes, our specific algorithm allows for accurate prediction of UDCP from chemically determined CP fractions in tropical ruminant feeds.

References

Kirchhof S 2007. Christian-Albrechts-Universität. Kiel, Germany.
Licitra G, Hernandez TM and Van Soest PJ 1996. Animal Feed Science and Technology 57, 347-358.
Shannak S, Südekum, K-H and Susenbeth A 2000. Animal Feed Science and Technology 85, 195-214.

Effect of high-moisture corn or rehydrated corn grain ensiled with or without the use of *L. buchneri* on digestibility and rumen parameters of feedlot Nellore beef cattle

Naiara Caixeta Da Silva[1], Vinicius Morais de Almeida Campos[3], Cleisy Ferreira do Nascimento[1], Michele Aparecida Prado Alves[4], Flávio Dutra de Resende[1,2], Gustavo Rezende Siqueira[1,2]

[1]São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal, São Paulo, Brazil, [2]Agência Paulista de Tecnologia dos Agronegócios, Polo Regional Alta Mogiana, Colina, São Paulo, Brazil, [3]Veterinary, São Paulo, Brazil, [4]Animal scientist, São Paulo, Brazil

E-mail: naiara.caixeta.silva@hotmail.com

Take home message The ensiling of high-moisture corn or rehydrated corn grain improves the efficiency of diet utilization as a result of higher starch digestibility.

Introduction Ensiling ground corn grain after rehydration is a promising technique to improve the nutritional value of corn kernels. This technique is a valuable tool on farms where high-moisture corn cannot be produced. The storage of corn grain increases starch digestibility by promoting proteolysis of the protein matrix; however, corn grain silages have low aerobic stability (Da Silva *et al.*, 2017). Furthermore, little is known about the metabolism of animals fed rehydrated corn grain silage, high-moisture corn, or rehydrated corn grain silage treated with bacterial inoculants. The objective of this study was to evaluate the benefits of ensiling high-moisture corn or rehydrated corn grain silage with or without the use of *L. buchneri* compared with dry ground corn on the digestibility and rumen parameters of feedlot Nellore beef cattle.

Materials & methods The following treatments were tested: 1) dry ground corn (DGC); 2) high-moisture corn (HMC); 3) HMC treated with *L. buchneri* at 1 × 10^5 cfu/g (HMC-LB; strain NCIMB 40788); 4) rehydrated corn grain silage (RCG); and 5) RCG treated with *L. buchneri* at 1 × 10^5 cfu/g (RCS-LB; strain NCIMB 40788). Ten cannulated Nellore bulls (602 ± 94 kg BW) were blocked by initial BW into two Latin squares (5 × 5). The animals were fed an acclimation diet for 15 d and a finishing diet for five 21-d periods. Experimental total mixed ration (TMR) were iso-nitrogenous (crude protein = 14.6% DM of TMR) and contained sugarcane bagasse (12.5% DM of TMR), dried citrus pulp (17.0% DM of TMR), peanut meal (3.99% DM of TMR), a mineral premix (3.99% DM of TMR with 53.4% urea), and DGC, HMC, HMC-LB, RCG, or RCG-LB (62.6% DM of TMR). Starch digestibility was measured for difference between consumed and excreted, divided by the consumed. The data were analysed using the MIXED procedure of SAS. Differences between the means were compared by the *t*-test at 5% probability. Rumen samples were collected on the 21th day of each period at 0, 3, 6, 12 and 18 h after feeding for measured of pH and ammoniacal nitrogen ($N\text{-}NH_3$), data were analysed as repeated measures over time.

Results & discussion Animals fed RCG, RCG-LB, HMC, and HMC-LB had a lower ($p = 0.02$) total dry matter intake (DMI; 6.39, 6.50, 6.41, 6.20 kg/d, respectively) compared with DGC-fed bulls (6.90 kg/d). Silage-fed animals had a higher ($p = 0.01$) DM digestibility (average 808 g/kg for silages) compared with those fed DGC (764 g/kg). The results can be explained by the greater ($p < 0.01$) starch digestibility in RCG, RCG-LB, HMC, and HMC-LB (average 999 g/kg for silages) compared with DGC (961 g/kg). Silage-fed bulls had the lowest rumen pH value at 6 h and did not present $N\text{-}NH_3$ peaks (Figure 1). Bulls fed RCG and RCG-LB had the highest ($p = 0.02$) propionate levels and lowest ($p = 0.02$) acetate/propionate ratios.

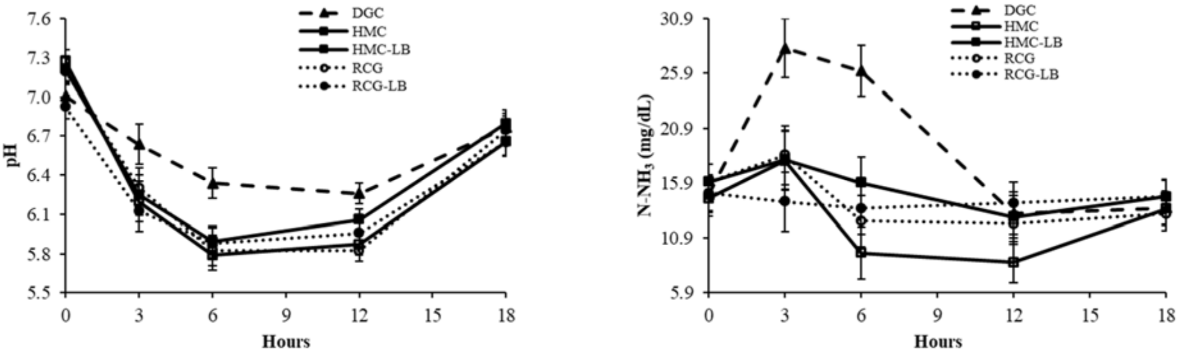


Figure 1 Rumen pH and ammoniacal nitrogen ($N\text{-}NH_3$) in feedlot Nellore beef cattle fed dry ground corn (DGC), high-moisture corn (HMC), HMC treated with *L. buchneri* at 1 × 10^5 cfu/g (HMC-LB; strain NCIMB 40788), rehydrated corn grain silage (RCG), and RCG treated with *L. buchneri* at 1 × 10^5 cfu/g (RCS-LB; strain NCIMB 40788).

Conclusion Feeding grain silages to feedlot beef cattle is recommended because it improves the digestibility of diet. Additionally, rehydrated corn grain silage is an alternative to high-moisture corn, with or without the use of *L. buchneri*, as it has a similar rumen fermentation profile and total starch digestibility.

Acknowledgements The authors thank *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP, grant no. 2013/16720-2) and *Conselho Nacional de Desenvolvimento Científico Tecnológico* (CNPq).

References

Da Silva NC, Nascimento CF, Nascimento FA, de Resende FD, Daniel JLP and Siqueira GR 2017. Journal of Dairy Science 101 4158-4167

Use of *in vitro* gas production technique to evaluate the effects of microwave irradiation on uncrushed whole canola seed nutritive values

Hamid Paya, Akbar Taghizadeh, Ali HosseinKhani, Hamid Mohammadzadeh, Hossein Janmohammadi, GholamAli Moghadam

University of Tabriz, Tabriz, East Azarbayjan, Iran

E-mail: hamid.paya@tabrizu.ac.ir

Take home message Because of rapid heating process, microwave irradiation is being used for feed processing in ruminant nutrition.

Introduction Full-fat oilseeds are often economical sources of protein and energy for lactating dairy cows. However, the protein in raw oilseeds can be highly degradable in the rumen. Soybeans and cottonseed are the most common oilseeds fed to dairy cows although full-fat canola seed is a potential alternative in regions where it is grown (Wang *et al.*, 1997). Heat processing is the most commonly used physical method to protect oilseeds and oilseed meals from ruminal degradation. Recently microwave is being used for feed processing in ruminant nutrition because of higher nutritive quality of final products due to faster heating process along with precise control process (Lashkari *et al.*, 2015).

Materials & methods The canola seed used in the study was a feed-grade product and was provided by a commercial company. Three 500 g samples of whole and uncrushed canola seeds were placed in a Pyrex pan (28×28×6 cm) with 1–2 cm height and were subjected to microwave irradiation (Butane microwave oven BC380W, Iran; emitting a 2450 MHz microwave frequency) at a power of 800 w (1.8 w/g microwave energy) for 4 minutes. Samples (300 mg) were weighed into 100 ml serum vial. Rumen liquor samples were obtained from the two wethers that were fed on a diet comprising (DM basis), 550g/Kg alfalfa hay, 400g/Kg barley grain, 48 g/Kg wheat bran and 2 g/Kg lime stone at maintenance level. Rumen fluid was collected after the morning feeding and filtered through four layers of cheesecloth and flushed with CO_2. Each feed sample was incubated in 5 replicate with 20 ml of rumen liquor and buffer solution (1:2; Fedorak and Hrudey, 1983). Five vials containing only the rumen fluid/buffer solution and no feed sample was included with each test and the mean gas production value of these vials was termed the blank value. Gas production was measured in each vial after 2, 4, 8, 12, 16, 24, 36, 48, 72 and 96 h of incubation using a water displacement apparatus. Rate and extent of gas production was determined for each feed by fitting gas production data to the one component McDonald model: $Y=A\,(1-e^{-ct})$.

Results & discussion The mean values of *in vitro* cumulative gas production "corrected for blank" and estimated kinetic parameters for raw and microwave irradiated uncrushed canola seed are shown in Table 1. There was a difference ($P<0.05$) in gas production among treatment in initial time (until 16 hour of incubation). Microwave irradiated uncrushed canola seed fermented faster than other.

Table 1 *In vitro* gas production characteristics of feed samples incubated in buffered rumen fluid.

	Gas production (ml g^{-1} DM)										Gas production constants	
Feeds[1]	2 h	4 h	6 h	8 h	12 h	16 h	24 h	36 h	48 h	72 h	96 h	*A*	*c*
RCS	20^b	42^b	64^b	83^b	104^b	117^a	137^a	162^a	191^a	212^a	219^a	219^a	0.04^a
MCS	23^a	46^a	69^a	91^a	112^a	121^a	132^a	147^a	164^a	181^a	184^a	176^a	0.08^a
SEM	0.78	1.10	1.47	1.71	1.77	2.50	5.72	9.10	10.5	10.6	11.2	12.7	0.016

$^{a,\,b}$ Means within a column with different subscripts differ ($P<0.05$); *c*: fractional rate of gas production (h^{-1}); *A*: potential gas production (ml/g DM); RCS = Raw Canola Seed; MCS = Microwave irradiated Canola Seed; N=5

Conclusion Heat processing is the most commonly used physical method to protect oilseeds and oilseed meals from ruminal degradation and it can also alter site of nutrient digestion in the gastrointestinal tract of ruminants. It seems that during microwave irradiation, rapid internal heating vaporizes the water inside the seed, increases pressure inside the seed until the seed coat ruptures. Because of seed coat rupturing, the release of nutrient from seeds is increased in initial time of incubation. A critical difference between microwave and traditional dry or wet heat treatments is that the latter use external sources to heat the seed from outside, whereas microwave generates heat from inside the seed. As microwave and micronization are two techniques with similar mechanism of heating process, therefore our results here could be comparable with Wang *et al.* (1997) results which reported that micronization increased canola seed gas production by damaging seed coat and increasing its availability.

Acknowledgements The authors gratefully thank the staff of the Khalat Poshan Research Center of Tabriz University.

References

Fedorak PM and Hrudey SE 1983. Environmental Technology Letters 4, 425-435.

Lashkari S, Azizi O and Jahani-Azizabadi H 2015. Archives of Animal Nutrition 69, 177–186.

Wang Y, McAllister TA, Zobell DR, Pickard MD, Rode LM, Mir Z and Cheng KJ 1997. Canadian Journal of Animal Science 77, 431-440.

Impact of beet pulp form: pellet or chip on the ruminal dry matter degradation and dairy goat performances

Alexis Ferard[1], Didier Coulmier[2]

[1]*Arvalis Institut du végétal, 44370, Loireauxence, France,* [2]*Desialis, 51000, Chalons en Champagne, France*

E-mail: a.ferard@arvalis.fr

Take home message The suppression of the granulation process of beet pulp decreases the short term DM degradability in the rumen which may have positive consequences on the milk fat content of dairy goats.

Introduction Dehydrated sugar beet pulp is common feed for ruminants; essentially used as pellets. The last industrial step in the dehydration process consists in passing the dehydrated overpressed pulp chips into presses to be granulated into 6 or 8 mm pellets. The use of the dehydrated chips directly in a ruminant diet is supposed to preserve the physical fiber quality while having a high digestibility and ingestibility. Two trials were conducted to evaluate the dehydrated beet pulp form: chips or pellets on the *in sacco* ruminal dry matter (DM) degradability and on dairy goat performances.

Materials & methods The *in sacco* studied dehydrated beet samples from 6 French plants. Samples were dried (60°C -72h) and ground through 4 mm screen. DM degradability was measured *in sacco* at La Jaillière experimental farm (FR-44370) for 2 incubation times (IT: 4h and 48h) with 6 replicates (3 cows*2 sets). The effect of the pulp form was analyzed by IT with a model using proc MIXED of SAS 9.3 including fixed effects: pulp form, plant and their interaction, cow and set. The dairy goat trial was lead at the agricultural college of Melle (FR-79500) using 2 groups of 45 goats receiving a high concentrate diet based on *ad libitum* alfalfa hay, complete dairy pellet (1.14 kgDM/d) and maize (0.45 kgDM/d). The two groups received 2 extra meals every day (0.26 kgDM/meal) of dehydrated beet pulp as chips (group 1) or pellet (group 2). Milk production and quality and DM intake were analyzed by ANOVA.

Results & discussion The passage of dehydrated beet pulp chips into the press to be granulated increased the ruminal short term DM degradability for all 6 plants by on average 0.079 ($P<0.0001$) with a standard deviation (SD) of 0.027 to reach 0.340 (SD=0.073). The pulp form hadn't any significant effect on long term DM degradability: 0.949 (SD=0.027) and 0.955 (SD=0.011) respectively for the chips and the pelleted forms. The difference between the two forms was measured very low suggesting that the granulation have a very slight effect on the energetic value of the pulp.

The DM intake of the two groups of goats was not influenced by the form of the beet pulp with an average 2.97 kg DM/goat/day. The hay intake was not affected with on average 0.84 kgDM/d and 0.87 kgDM/d respectively for the chips and pelleted forms. The pulp form didn't have any effect on milk production (on average 3.50 kg/d). The milk fat content increased significantly from 33.1 to 34.5 g/kg ($P<0.05$) with the chips form compared to pelleted form while the milk protein content remained stable at on average 32.1 g/kg.

Conclusion The *in sacco* trial suggested that dehydrated beet pulp form have an impact on the short term ruminal DM degradability by lowering it. The lower DM degradation in the rumen could explain the significant increase in milk fat content observed in the dairy goat where fed with pulp chips in comparison to pellet pulp. The increases in temperature and in pressure at this industrial process stage could partially explain a better nutrient availability in the pellet compared to the not granulated equivalent. These results suggest preferring pulp chips instead of pelleted beet pulp in diets presenting high risks of rumen acidosis.

Acknowledgements The authors would like to thank all the technicians who participated in the experiments at Arvalis La Jaillière and at the Lycée Agricole de Melle.

References

Le Scouarnec J, Coulmier D, Wimmer E and Bouvet D 2016. Rencontres autour des Recherches sur les Ruminants 23, p.58

Effect of sugar cane straw vs wheat straw supply as a source of long fiber on zootechnical performances and ruminal pH of Limousin bulls fed with a concentrate–based diet.

Christine Gerard[1], Erik Sulmont[2], Jean-Marc Francois[3]

[1]*Neovia, Saint Nolff, France,* [2]*Inzo, Argentan, France,* [3]*Urcoopa, Saint Paul, France*

E-mail: cgerard@neovia-group.com

Take home message Sugar cane straw is as efficient as wheat straw as a source of long fiber to achieve high growth performances in concentrate – based fattening diets of Limousin bulls.

Introduction In some tropical countries, the availability of long fiber (straw, hay) to include in ruminant diets is low. Cereal straw, widely used in ruminant diets in Europe or North America, can’t be produced in these countries. Importation of cereal straw is very expensive. Hence, alternative sources of long fiber are sought. In the French department of La Réunion, in the Indian Ocean, many farmers use sugar cane straw (linked to sugar production), especially in concentrate-based fattening diets. However, they suspect that sugar cane straw is not as efficient as wheat straw to ensure proper fiber supply, promote rumination and optimise feed efficiency. Hence a trial was conducted, in a European farm, to compare zootechnical performances and ruminal pH pattern of Limousin bulls fed with the same concentrate-based diet, but receiving wheat straw or sugar cane straw as a source of long fiber.

Materials & methods Fifty-three Limousin bulls (average initial weight = 400 kg), housed in four pens, receiving a fattening diet composed by straw + complete pelleted feed (17% protein, 33% starch, 8% crude fiber) were allocated in two groups, one receiving wheat straw (CTRL, n=22), the other receiving sugar cane straw (CANE, n=21) as long fiber source in the straw rack and for pen bedding. Both straws were of good quality and offered *ad libitum*. Complete feed intake was restricted to 8.8 then 7.9 kg DM/animal/day, to mimic the local feeding program used in La Réunion. Animals were weighed every month until slaughtering. Collective complete feed intake was measured daily and straw intake was estimated by recording the straw bottle weight and the number of days enabling full consumption of the bottle. 9 bulls (4 in the CRTL, 5 in the CANE group) were equipped with ruminal pH probes during 1.5 month during the fattening period. The whole trial lasted 4 months.

Results & discussion Global daily gain measured was high, and not statistically different between both groups: CANE = 1.86 kg/day, vs CTRL = 1.77 kg/day (p-value=0.24).

Average straw intake was respectively 2.4 kg DM/bull/day and 2.2 kg DM/bull/day, for the CANE and the CTRL group, and average complete feed intake during the whole trial period was around 8.50 kg DM/bull/day for both groups.

Feed conversion ratio (complete feed + straw intake / gain) was good for both groups, numerically slightly better for the CANE group (5.89 kg DMI /kg ADG *vs.* 5.98 kg DMI / kg ADG for the CTRL group).

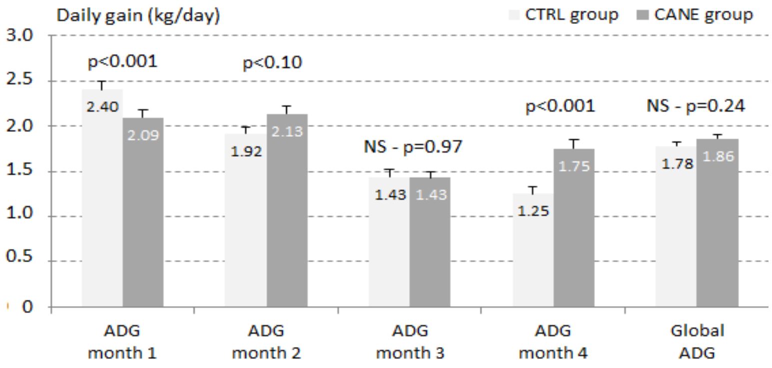


Figure 1 Daily gain (kg/day) per group and period.

Average daily pH was numerically slightly lower (5,90 vs 5,97) and the time for which pH < 6 was slightly higher (866 min/day vs 783 min/day) for the CANE group, compared to the CRTL group.

Conclusion The results showed that high average daily gain and high feed efficiency can be achieved, in European fattening conditions, with sugar cane straw, compared to wheat straw, combined with appropriate complete feed supply. The follow-up of ruminal pH suggests that the supply of sugar cane straw instead of wheat straw doesn’t increase significantly the acidosis risk. The only observed negative point regarding the use of sugar cane straw, compared to wheat straw, was on pen bedding, the cane straw being dustier and less efficient to absorb humidity. It should be interesting to complete these results with the same trial in a tropical environment, to assess the potential impact of the climatic conditions.

Acknowledgements The authors thank the cooperative group URCOOPA (La Réunion) for their participation in this trial and the sugar cane straw supply.

Effect of coconut oil and palm oil on performance and blood lipids of preweaned calves

Fengming Hu, Qiyu Diao, Kai Cui, Hui Tao, Yan Tu

Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China

E-mail: tuyan@caas.cn

Take home message Supplementing coconut oil and palm oil instead of milk fat as the fat sources of MR had no negative effect on performance of calves.

Introduction Fatty acids have functions beyond the role as sources of energy for ruminants. The recent research shows that they can affect performance, immune function and health of calf, especially some short-chain, essential and functional fatty acids. Palm oil as a conventional vegetable oil, is rich in saturated and monounsaturated fatty acids (LCFA), especially palmitic acid and oleic acid. Coconut oil, in contrast with conventional vegetable oil, is a rich source of medium-chain FA (MCFA), especially lauric. The current research of MCFA, LCFA on performance and health of calves is limited. Thus, this trial was aimed to evaluate coconut oil and palm oil on performance and blood lipids of preweaned calves.

Materials & methods Sixty Holstein bull calves (44.6±4.2 kg of BW; 12/treatment) that were 2 to 3 d of age were used to evaluate different sources of fat in five milk replacers (MR) treatments. The MR were A) only whole milk powder (252 g/kg CP, 257 g/kg Fat, of ingredient; DM basis) as fat sources (WM), B) whole milk powder and coconut oil powder (31g/kg CP, 804g/kg Fat, of ingredient; DM basis) provide 1/2 fat respectively (WCM), C) only coconut oil powder as fat sources (CM), D) whole milk powder and palm oil powder (35 g/kg CP,780g/kg Fat, of ingredient; DM basis) provide 1/2 fat respectively (WPM); E) only palm oil powder as fat sources(PM). These five MRs were intended to be isonitrogenous and isocaloric. The liquid diet was fed at 10% live weight and the feeding level was adjusted every two weeks from day 14 to 56 of calves. All calves were fed colostrum and milk during the first week after birth, and then were fed these MRs for a 7-d adaptation period and a 42-d trial period meanwhile with a common calf starter *ad libitum*. Calves were weighed every two weeks until 56 d. The daily intake of the MRs and starter was recorded. Blood samples were taken from the jugular vein from six calves every treatment at 14, 28, 42, 56 d. A 5-point scale was used for daily fecal scoring (Hill *et al*., 2011). The weekly averages of fecal scores were generated per calf for statistical analysis. Data were analysed using Mixed procedure of SAS.

Results & discussion Performance and serum lipids are shown in Table 1. There was a trend for body weight, average daily gain and feed efficiency to decrease as coconut oil or palm oil supplementation increased ($P>0.05$). There was no difference in the MRs intake, starter intake and fecal score ($P>0.05$). The serum TC, HDL-C, and LDL-C concentrations increased ($P<0.05$) as coconut oil increased, whereas there was a decreased trend as palm oil increased ($P>0.05$). There was a same change rule for serum TG and NEFA, but there was no significant difference ($P>0.05$).

Table 1 Performance (n=60) and serum lipids indices (n=30) of calves from 14 to 56 d fed milk replacers with different vegetables oils.

Items	WM	WCM	CM	WPM	PM	SEM	*P*-value		
							MR	Days	MRxDay
Body weight, kg	57.1	56.8	56.0	56.9	54.3	3.92	0.949	<0.01	0.732
Average daily gain, g/d	707.7	684.8	643.5	660.2	553.8	98.81	0.570	<0.01	0.789
MR intake, g/d	598.4	599.1	598.8	608.4	591.5	36.39	0.994	<0.01	0.718
Starter intake, g/d	594.9	646.2	593.0	596.7	582.3	40.51	0.512	<0.01	0.420
Feed efficiency	0.59	0.55	0.54	0.54	0.45	0.071	0.448	0.019	0.687
Fecal score	2.04	1.94	1.93	2.03	1.99	0.094	0.653	<0.01	0.960
TC, mmol/l	1.81^{b}	2.05^{ab}	2.11^{a}	1.81^{b}	1.71^{b}	0.144	0.045	0.435	0.767
TG, mmol/l	0.16	0.17	0.18	0.17	0.14	0.017	0.136	0.063	0.066
HDL-C, mmol/l	0.65^{b}	0.70^{ab}	0.72^{ab}	0.64^{ab}	0.57^{b}	0.047	0.029	0.066	0.919
LDL-C, mmol/l	1.16^{b}	1.29^{ab}	1.43^{a}	1.12^{b}	1.11^{b}	0.087	0.003	0.746	0.476
NEFA, µmol/l	173.4	181.6	183.3	166.5	166.6	8.80	0.171	0.002	0.065

Conclusion Results from this study suggest that supplementing coconut oil and palm oil as the fat sources of MR had no negative effect on performance of calves. Coconut oil powder might increase blood lipids concentrations, and the palm oil powder in milk replacer may affect more the metabolism of preweaned calves.

Acknowledgements This research was supported by Beijing Key Laboratory for Dairy Cow Nutrition.

References

Hill TM, Vandehaar MJ, Sordillo LM Catherman DR, Bateman HG and Schlotterbeck RL 2011. Journal of Dairy Science 94, 3936-48.

Effect of hybrid rye and maize grain processing on *in situ* degradability and intestinal digestibility of starch in ruminants

Patrycja Rajtar, Piotr Micek, Pawel Górka

University of Agriculture in Krakow, Kraków, Poland

E-mail: partycja.wanat12@gmail.com

Take home message Rye starch is more susceptible to fermentation in the rumen than maize starch, but the way of grain processing may significantly alter the kinetics of its digestion.

Introduction Maize silage and maize grain (MG) are currently the most important ingredients of the diets for dairy cows. Unfortunately, due to high prices of MG and some difficulties in maize cultivation, alternative cereal sources are needed. Increasing attention is being given to the hybrid varieties of rye, which are considered to be yield stable and more valuable than traditional ones. However, starch of rye grain (RG) is rapidly degradable in the rumen. This could be reduced by a use of appropriate grain processing (Svihus *et al.* 2005). Therefore, the aim of the study was to determine the effect of processing of RG and MG on effective rumen degradation (ERD) and intestinal digestibility (ID) of starch.

Materials & methods RG of hybrid variety (cultivar Brasetto) and MG (cultivar Podium; KWS LOCHOW Poland) were processed to obtain four degrees of fragmentation: none (whole grain), crushed, and milled to 4 or 1.5 mm. Three Holstein-Friesian cows, fitted with ruminal and duodenal cannulas, were used for the determination of rumen degradation of starch by *in situ* method (2, 4, 8, 16, 24, 48 and 72 h of incubation) using the nylon bag (pore size 50 µm) technique (Ørskov and McDonald, 1979). Intestinal digestibility was carried out according to Peyraud *et al.* (1988). Data were analyzed using the PROC MIXED of the SAS (version 9.2; SAS Inst. Inc., Cary, NC). Statistical model included the effect of cereal type, grain processing and cereal type × grain processing interaction. When significant effect of interaction was showed, means were separated using the PDIFF procedure in SAS with Duncan adjustment.

Results & discussion Processing of grain, regardless of the cereal type, had significant effect ($P<0.01$) on the ERD of starch (rate of outflow from the rumen 0.06 /h, Table 1). The highest ERD of starch were reported for RG and MG milled to 1.5 mm (96.0 and 57.5%, respectively). This effect was primarily due to the largest content of fraction A of starch in the samples, which is a fraction assumed to be immediately degraded in the rumen. In turn, irrespective of grain processing, ERD of starch of RG was higher ($P<0.01$) than that of MG; however, significant interaction ($P<0.01$) was shown.

Table 1 Effect of processing on degradability constants (A, B, C), effective rumen degradability (ERD) and intestinal digestibility (ID) of starch of hybrid rye and maize grain.

Item		Processing	A (%)	B (%)	C (%×h^{-1})	ERD (%)	ID (%)
Cereal	Rye	None (whole grain)	8.8^{ef}	86.9^{ab}	1.6^{d}	18.3^{g}	56.4^{d}
		Crushing	49.3^{b}	49.4^{d}	50.4^{a}	93.5^{b}	73.0^{bc}
		Milling (4 mm)^{1}	37.7^{c}	60.3^{c}	25.4^{bc}	85.5^{c}	87.2^{b}
		Finely milling (1.5 mm)^{1}	71.6^{a}	27.9^{e}	47.7^{ab}	96.0^{a}	100.0^{a}
	Maize	None (whole grain)	1.2^{f}	36.5^{e}	0.5^{d}	4.6^{h}	19.9^{e}
		Crushing	3.5^{f}	96.0^{a}	2.4^{d}	36.9^{f}	58.7^{c}
		Milling (4 mm)^{1}	12.2^{e}	87.3^{ab}	4.3^{cd}	50.5^{e}	56.3^{d}
		Finely milling (1.5 mm)^{1}	21.4^{d}	78.3^{b}	4.2^{cd}	57.5^{d}	98.0^{a}
Effect (*P*-value)		Cereal	<0.0001	<.0001	0.0001	<.0001	<.0001
		Processing	<.0001	<.0001	<.0001	<.0001	<.0001
		Cereal × processing	0.0001	0.0001	0.0003	0.0001	0.0002
SEM			1.66	1.87	0.05	0.21	2.61

$^{a, b, c}$ Means within a column with different superscripts are significantly different ($P \leq 0.01$);
^{1}milled to pass through 4.0 or 1.5 mm sieve size

Conclusion The greater degree of fragmentation, the higher ERD and ID of starch. Rye starch is more susceptible to fermentation in the rumen than maize starch, but the way of grain processing may significantly alter the site and extent of its digestion. Further studies are needed to determine, in detail, the structural factors that affect starch digestibility in processed rye grain and its utilisation by ruminants.

Acknowledgements This study was supported by the project No BIOSTRATEG2/297910/12/NCBR/2016.

References

Ørskov ER and McDonald P 1979. Journal of Agricultural Science 92, 499-503.
Peyraud JL Genest-Rulquin Ch and Vérité R 1988. Reproduction Nutrition Development 28, 129-130.
Svihus B, Uhlen AK and Harstad OM 2005. Animal Feed Science and Technology 122, 303–320.

Horses and cattle grazing a mesophile grassland select vegetation in a complementary way

Géraldine Fleurance[1,2], Laurent Lanore[2], Laurence Wimel[1], Cédric Dubois[1], Bertrand Dumont[2]

[1]IFCE Pôle Développement, Innovation et Recherche, Exmes, France, [2]Université Clermont Auvergne, INRA, VetAgroSup, UMR Herbivores, Saint-Genès-Champanelle, France

E-mail: geraldine.fleurance@inra.fr

Take home message Horses graze shorter patches than cattle do in a mesophile grassland. Mixed-grazing may thus lead to a more homogeneous use of grasslands, with consequences on sward nutritive value and biodiversity.

Introduction Enhancing diversity within animal production systems is a key principle of agroecology to achieve sustainability goals (Dumont *et al*., 2013). In particular, mixing different herbivore species at pasture is assumed to increase vegetation utilisation rate and animal liveweight gain. Few data are available on mixed grazing between horses and cattle in mesophile grasslands; here, we test the hypothesis that the two species grazing together differ in their selectivity.

Materials & methods Study was carried out at the IFCE experimental farm (Chamberet, France, 440m a.s.l) over three grazing seasons (April-October) from 2015 to 2017. Each year, mixed grazing by two 2-yr old saddle horses and three heifers (1.4LU.ha^{-1}) was tested with three replicates. In each plot, animals were grazed together, alternatively in two 1.35-ha subplots. Bites were recorded in May, July and September by scan sampling from dawn to dusk, with one bite observed every 10-min for each individual, and one observation-day per plot. Bites were characterised by sward height (5 classes) and dominant botanical family (4 classes). Diet selection was quantified with Jacobs' indices: $S_i=(c_i-a_i)/(c_i+a_i-2c_ia_i)$ where c_i and a_i are proportions of the bite i in the diet and in the plot, respectively (Fleurance *et al*., 2016). Statistical analyses were carried out with SAS PROC Mixed for repeated measurements using the main effects of species, year, season, their interactions, and plot as random effect. Selection for ($S_i>0$) or against ($S_i<0$) a bite type was assessed with a Student's *t*-test.

Results & discussion Horses with their two sets of incisors selected short vegetative patches (VS) while cattle selected tall vegetative areas (VT). Both species selected areas of intermediate sward height (VI) and rejected low quality areas (Figure 1). These results extend previous observations in less fertile natural grasslands (Menard *et al*., 2002, Cornelissen & Vulink 2015). We confirm that horses select grasses and avoid forbs (Fleurance *et al*. 2016). So did cattle in our experiment. Further analyses of sward composition and of social interaction between cattle and horses will allow explaining this result.

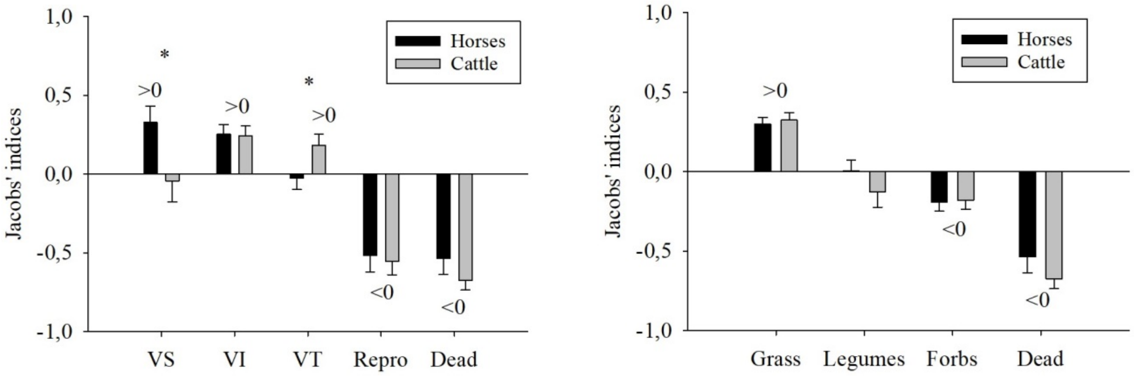


Figure 1 Diet selection by horses and cattle (mean±s.e.) (species×season NS) according to: sward height type (left): vegetative short (VS, ≤4cm), intermediate (VI, 5-8cm), tall (VT, ≥9cm), reproductive (Repro), dead; and dominant botanical family (right). Differences between species: *p<0.05; >0 and <0 is for significant selection for or against this bite type.

Conclusion Our experiment reveals complementary selection by horses and cattle in a mesophile grassland. Using an additional treatment where horses were conducted alone in a rotational system and at the same stocking rate, we will investigate if foraging behaviour of horses was modified by the presence of cattle**.** We will also analyse if mixed grazing reduces sward heterogeneity and species richness, and increases sward feeding value compared to horse grazing alone.

Acknowledgements This research was funded by IFCE, INRA GISA STReP and PSDR4 Auvergne project new-DEAL.

References

Cornelissen P and Vulink JT 2015. Applied Animal Behaviour Science 163, 28-38
Dumont B, Fortun-Lamothe L, Jouven M, Thomas M and Tichit M 2013. Animal 7, 1028-1043.
Fleurance G, Farruggia A, Lanore L, Dumont B 2016. Agriculture, Ecosystems and Environment 231, 255-263.
Ménard C, Duncan P, Fleurance G, Georges J-Y and Lila M 2002. Journal of Applied Ecology 39, 120-133.

A strategic utilization of *Lotus uliginosus* improves sheep superfine wool production of native grasslands based systems

Ignacio De Barbieri, Martín Jaurena, Zully Ramos, Fabio Montossi

Instituto Nacional de Investigación Agropecuaria, Tacuarembó, Uruguay

E-mail: idebarbieri@inia.org.uy

Take home message The productivity and quality of superfine wool production of native grasslands based systems can be increased by the utilization of small areas of oversown legumes.

Introduction Superfine wool production in Uruguay takes place predominantly on Basaltic shallow soils with native grasslands as the main nutritional basis. The possibilities of increasing forage production through oversown pastures are limited to small areas of medium to deep soils. This study sought to evaluate the opportunity of increasing superfine wool production based on native grasslands by the strategic use of oversown *Lotus uliginosus.*

Materials & methods During a two-year period, two feeding strategies were evaluated: i) wethers continuously grazing native grasslands (NG, 4 ha) or ii) wethers continuously grazing native grasslands (3 ha) altering with 50 days of winter access to a native oversown pasture (NOP) with *Lotus uliginosus* (1 ha, 25-50% of legume cover and an average forage on offer of 2840 kg DM/ha). A rotational stocking method (7/14, days stocking period/rest period) was applied when animals grazed the improved pasture. Thirty-two mature Merino wethers were allocated to one of the two replications of either treatment (16 wethers/treatment) based on their live weight (56.7 ±6.2 kg), body condition score (3.2 ±0.4 units), age and breeding values for clean fleece weight and fibre diameter. Stocking rate was adjusted by the put-and-take technique (Mott and Lucas, 1952) at the beginning of each grazing season considering herbage mass, weather forecast and available soil water, with the objective to maintain the herbage height between 5 and 15 cm. Herbage height was assessed fortnightly, while herbage mass was estimated each season (Hayden and Show, 1975). The live weight and condition score of the animals were assessed once a month, while annual fleece weight was recorded at shearing (September) when a mid-side wool sample was taken to evaluate wool quality. Animal and forage data were analysed using a general linear model in a completely randomised experimental design by analysis of variance (SAS, $P<0.05$). The model included year, treatment, and their interaction (the latter was removed when it was not significant).

Results & discussion Native grasslands herbage mass (1410 vs 1553 kgDM/ha for NOP and NG respectively) and height were not affected by treatments, except for average herbage height in winter which was taller for NG than for NOP (Table 1). NG animals were heavier at the beginning of the spring, possibly as the result of a lower grazing pressure in previous seasons. However, that effect was not large enough to alter body condition score, wool production and quality. The inclusion of *Lotus* allowed an annually stocking rate increase of 0.6 wethers/ha (5.9 versus 5.3 wethers/ha for NOP and NG, respectively), explained for the larger carrying capacity of the improved pasture (NOP) during winter than native grasslands (NG), which in turn improved the wool production per hectare by 15%.

Table 1 Effect of two feeding strategies on live weight and body condition score of wethers at the beginning of each season, annual wool production and quality, and season native grasslands sward height (means, sem).

Feeding strategy	NOP	NG	sem
Live weight (kg)			
Summer	54.7	57.4	1.05
Autumn	53.8	55.3	1.21
Winter	54.9	58.1	1.20
Spring	53.9^{b}	57.9^{a}	1.10
Body condition score			
Summer	3.4	3.4	0.07
Autumn	3.1	3.0	0.06
Winter	3.3	3.4	0.05
Spring	3.0	3.0	0.08

Feeding strategy	NOP	NG	sem
Wool traits			
Fleece weight (kg)	4.24	4.14	0.12
Fibre diameter (μ)	17.0	16.7	0.19
Staple length (cm)	8.5	8.3	0.17
Staple strength (N/ktex)	36.2	36.6	0.99
Native grasslands sward height (cm)			
Summer	10.2	10.0	0.63
Autumn	10.7	10.9	0.40
Winter	7.2^{b}	8.6^{a}	0.37
Spring	9.0	8.7	0.48

^{ab}Means within rows and traits with differing letter are significantly different ($P<0.05$)

Conclusion The addition of small areas of oversown pastures to be used strategically during winter allows an increase of wool production per hectare without negative effects on wool quality, in extensive production systems. This intensification implies using slightly larger stocking rates on native grasslands areas of the production system from spring to autumn.

References

Haydock KP and Shaw NH 1975. Australian Journal of Experimental Agricultural 15, 663–670.
MottGO and Lucas HL 1952. VIth International Grassland Congress, 1380–1385.

Effects of dehydrated hazelnut pericarps and pellets of sainfoin as condensed tannins-containing resources on *in vitro* rumen fermentation

Vincent Niderkorn[1], Elodie Gaudin[2], Angélique Quereuil[1], Hervé Hoste[3]

[1]*INRA, Saint Genes Champanelle, France*, [2]*MG2MIX, Châteaubourg, France*, [3]*INRA, Toulouse, France*

E-mail: vincent.niderkorn@inra.fr

Take home message The inclusion of two types of condensed tannins-containing resources in a basal diet decreases DM degradability *in vitro* but potentially reduces pollutant emissions and improves protein use efficiency.

Introduction Some forage legumes and by-products from human food industry with high condensed tannins (CT) contents have been shown promising to control gastrointestinal nematodes and to be part of the solution against multiresistant strains to synthetic anthelmintics in small ruminants (Gaudin *et al*., 2016). The aim of this study was to evaluate the effects of including hazelnut pericarps and pellets of sainfoin in a basal diet on *in vitro* rumen fermentation characteristics.

Materials & methods Four treatments were tested: i) a basal diet consisting in 800 g/kg hay from permanent grassland and 200 g/kg concentrate mix, ii) the basal diet + 48% pellets of sainfoin (PS), iii) the basal diet + 8% freeze-dried hazelnut pericarps (HP), and iv) the basal diet + 24% PS + 4% HP. The diets were prepared to be isotannic (20 g/kg of CT, except for the basal diet) and isoproteic (132 g/kg, adjusted with casein). The substrates were incubated in a batch system containing buffered rumen fluid from sheep at 39 °C (Macheboeuf *et al*., 2014). After 24 h of fermentation, the gas production and composition were recorded, and the fermentation end-product concentrations in the incubation medium and the *in vitro* DM disappearance (IVDMD) were determined. Data were analyzed with the Mixed procedure of SAS using the repetition of fermentation run ($n = 3$) as random factor.

Results & discussion The DM disappearance and the total gas production were negatively affected by the inclusion of PS and HP ($P < 0.001$ and $P = 0.002$, respectively) indicating a loss of fermentability likely due to the composition of PS and HP and/or their CT content (Table 1). On the other hand, both PS and HP had an antimethanogenic effect as shown by the lower methane (CH_4) production and the higher CO_2:CH_4 ratio than for the basal diet ($P < 0.001$). We also observed a reduction in NH_3 concentration ($P < 0.001$) with the three CT-containing resources, suggesting a beneficial reduction of rumen protein degradation, PS being even more efficient than HP. Considering the basal diet + PS + HP, the high concentration of CT in HP compare to PS allowed reducing the incorporation of CT-containing resources for comparable effects on fermentation parameters.

Table 1 *In vitro* rumen fermentation characteristics of a basal diet including pellets of sainfoin (PS) and/or hazelnut pericarps (HP).

Item	Basal diet	Basal diet + PS	Basal diet + HP	Basal diet + PS + HP	s.e.m	*P*-value
DM disappearance (%)	55^a	46^c	49^b	48^b	2.6	< 0.001
Total gas production (mmol/g DM)	8.7^a	8.0^c	8.2^{bc}	8.4^{ab}	0.27	0.002
CH_4 (mmol/g DM)	1.54^a	1.31^c	1.33^c	1.40^b	0.096	< 0.001
CO_2:CH_4 (mol/mol)	4.69^c	5.10^{ab}	5.15^a	5.03^b	0.279	< 0.001
Total VFA (mmol/l)	161	142	164	147	16.9	0.673
NH_3 (mmol/l)	10.0^a	5.5^c	7.3^b	6.6^{bc}	0.54	< 0.001

VFA: volatile fatty acids; s.e.m: standard error of the meansWithin a row, means followed by different letters are significantly different ($P < 0.05$).

Conclusion Under *in vitro* conditions, the inclusion of PS and HP in a basal diet led to lower fermentability and decreased rumen CH_4 production and protein degradability.

Acknowledgements This work was funded by the INRA metaprogram GISA (project STReP). The authors thank the companies MG2MIX (Châteaubourg, France) and Inovfruit (Mussidan, France) which supplied dehydrated pellets of sainfoin and hazelnut pericarps, respectively.

References

Gaudin E, Simon M, Quijada J, Schelcher F, Sutra JF, Lespine A and Hoste H 2016. Veterinary Parasitology 227, 122-129.
Macheboeuf D, Coudert L, Bergeault R, Lalière G and Niderkorn V. 2014. Animal 8, 1797-1806.

Effect of feeding cold-pressed sunflower cake on dairy cows' production performance

Idoia Goiri, Raquel Atxaerandio, Izaro Zubiria, Roberto Ruiz, Aser Garcia-Rodriguez

Neiker, Vitoria-Gasteiz, Spain

E-mail: aserg@neiker.eus

Take home message Cold-pressed sunflower cake is a valuable feedstuff for dairy cows replacing, partially, soybean meal and, totally, palm fat in the concentrate.

Introduction Cold-pressed sunflower cake (SUN) is a cheap by-product of oil-manufacturing which can be obtained on-farm after simple mechanical extraction of the oil. SUN has been shown to have higher crude fat content than those of conventional solvent and expeller meals and a relative high content of protein. In addition, the fatty acid profile of SUN makes it a promising feedstuff to obtain healthier milk (Amores *et al*., 2014). Therefore, the objective of the present study was to assess the effect of feeding SUN on dairy cows' performance.

Materials & methods Ten dairy cows were used in a crossover design with two treatments (CTR, SUN) and two 63-day experimental periods. Concentrates were CTR and SUN (230 g kg^{-1} sunflower cold pressed cake, in place of portions of the soybean meal and total palm fat) and were individually fed. Concentrates were formulated to provide similar amounts of energy (1 UFL), protein (190 g kg^{-1}) and fat (60 g kg^{-1}). Forage was group fed *ad libitum*. Cows were milked twice daily at an automatic milking system, on four different days/period individual samples of milk were taken for fat, protein and lactose analysis. Quantities of concentrate offered and refused were recorded on an individual basis. The dry matter intake and apparent organic matter digestibility were estimated using chromium oxide as external marker and acid insoluble ash as internal marker. Cows received ten grams of Cr_2O_3, stored in gelatin capsules at 0730 h and 1800 h during 10 days, collecting feces on the last three. Individual blood samples were collected for non-esterified fatty acids, blood urea nitrogen and beta hydroxybutyrate determinations. Cows were weighted at the beginning and the end of each experimental period. Data were analysed using the MIXED procedure of SAS, with fixed effects of concentrate, sequence of treatments, period, breed and the initial record measured at week 0 as covariate, and the random effect of pair (cow). For milk production data analysis, the same model but for repeated measures was used.

Results & discussion SUN did affect neither milk yield, nor milk composition (Table 1). Although some studies related feeding sunflower oil with milk fat depression (Zened *et al*., 2012) we did not see this effect. SUN also did not affect intake, digestibility or daily weight gain. Plasma metabolites were also not affected by the concentrate used.

Table 1 Milk productive performance, intake, apparent digestibility and plasma metabolites of lactating cows fed SUN.

Item	CTR	SUN	SEM	P-value
Milk yield (kg day^{-1}*)*	21.1	20.7	0.33	0.233
Fat (g kg^{-1})	40.1	37.1	0.18	0.101
Protein (g kg^{-1})	30.5	31.8	0.11	0.266
Lactose g kg^{-1})	48.5	49.5	0.05	0.056
Intake (kg day^{-1})				
Dry matter intake	20.1	19.9	0.78	0.813
Forage dry matter intake	15.4	15.2	0.76	0.867
Apparent organic matter digestibility (g kg^{-1})	763.3	744.7	20.02	0.381
Daily weight gain (g day^{-1})	-30.1	-93.6	114.4	0.594
Plasma metabolites				
Beta hydroxybutyrate (mmol L^{-1})	0.742	0.680	0.0476	0.235
Non-esterified fatty acids (mmol L^{-1})	0.188	0.176	0.0264	0.670
Blood urea nitrogen (mg dL^{-1})	24.7	23.8	3.75	0.816

CTR: control, SUN: cold pressed sunflower cake, SEM: standard error of the mean.

Conclusion Cold pressed sunflower cake can be used to formulate a concentrate for dairy cows, replacing totally saturated palm fat and partially soybean meal, without affecting productive and physiological traits.

Acknowledgements This research was supported by RTA2015-00022-C03-02.

References

Amores G, Virto M, Nájera AI, Mandaluniz N, Arranz J, Bustamante MA, Valdivielso I, Ruiz de Gordoa JC, García-Rodríguez A, Barron LJ and de Renobales M 2014. Journal of Dairy Research 81, 410-416

Zened A, Troegeler-Meynadier A, Najar, T andEnjalbert F 2012. Journal of Dairy Science 95, 5916-5926.

Effects of replacing maize by citrus pulp in a sheep diet in Rusitec fermenters

María José Ranilla[1,2], Jairo García-Rodríguez[1], Sonia Andrés[2], Francisco Javier Giráldez[2], María Dolores Carro[3]

[1]*Universidad de León, León, Spain,* [2]*Instituto de Ganadería de Montaña ULE-CSIC, Grullero, León, Spain,* [3]*Universidad Politécnica de Madrid, Madrid, Spain*

E-mail: mjrang@unileon.es

Take home message Replacing maize by citrus pulp in the diet of sheep increased digestibility and propionate production when tested in the Rusitec system.

Introduction Citrus (*Citrus* spp.) is one of the most important fruits crop worldwide, and a large part of its production is processed, resulting in large quantities of by-products (USDA-FAS, 2010). Citrus pulp is the solid residue that remains after fresh fruits are squeezed for their juice, and it could be used as a cereal substitute in ruminant feeding due to its high energy content and digestibility (Heuzé *et al.*, 2017). The objective of this study was to assess the effects of replacing maize by citrus pulp in a dairy sheep diet on ruminal fermentation in Rusitec fermenters.

Materials & methods Four Rusitec fermenters were used in a cross-over design in two 14-day incubation periods. Two 50:50 forage:concentrate diets were used, with the concentrate containing either maize (M; 20% fresh matter basis) or being totally replaced by citrus pulp (C). On days 9, 10, 11 and 12 of incubation, the pH was measured and samples of solid and liquid contents were taken to determine apparent digestibility of diet and daily production of volatile fatty acids (VFA). Data were analysed by analysis of variance with repeated measures using the procedure MIXED of SAS. The effects of diet, incubation period, sampling day and their interactions were considered fixed, whereas fermenter was considered a random effect.

Results & discussion As shown in Table 1, total VFA production was unaffected ($P>0.05$) by diet, although propionate production was higher ($P=0.003$) in fermenters receiving the C diet. Accordingly, acetate/propionate ratio was lower ($P<0.001$) when fermenters were fed the diet containing citrus pulp. Dry matter apparent digestibility was higher ($P<0.01$) for C than for M diet (0.740 vs 0.711 g/g), but neutral detergent fibre digestibility was lower for C diet (0.332 vs. 0.392 g/g; $P<0.001$). In addition, pH values in C fermenters were higher ($P<0.001$) than in M ones.

Table 1 Effects of replacing maize (M) by citrus pulp (C) in a dairy sheep diet on ruminal fermentation in Rusitec fermenters

	M	C	SEM	P
Total volatile fatty acids, mmol/d	91	88	3.7	0.708
Acetate, mmol/d	43	41	1.7	0.158
Propionate, mmol/d	15	17	0.8	0.003
Butyrate, mmol/d	18	19	0.9	0.273
Other VFA[1], mmol/d	15	11	0.7	<0.001
Acetate/Propionate, mol/mol	2.83	2.40	0.096	<0.001
Digestibility, g/g				
Dry matter	0.711	0.740	1.3624	0.006
Neutral detergent fibre	0.392	0.332	1.4522	<0.001
pH	6.12	6.40	0.059	<0.001

[1] Calculated as the sum of isobutyrate, isovalerate, valerate and caproate.

Conclusion Replacing maize by citrus pulp in the diet did not negatively affect ruminal fermentation in Rusitec fermenters,and improved dry matter apparent digestibility and VFA profile. The results of this study indicate that by-products from juice industry could be included in ruminant's diets, promoting better ruminal fermentation.

Acknowledgements This work was supported by the Spanish Ministry of Economy and Competitiveness (Project AGL2016-75322-C2-2-R).

References

USDA-FAS, 2010. Citrus: World Markets and Trade. July 2010 Citrus Update. Foreign Agriculture Service – USDA
Heuzé V, Tran G, Hassoun P and Lebas F., 2017. Citrus pulp, dried. Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. https://www.feedipedia.org/node/680 Last updated on January 1, 2017, 17:52

Effects of replacing barley straw and maize silage by olive cake in a dairy sheep diet in Rusitec fermenters

Jairo García-Rodríguez[1], María Dolores Carro[2], Francisco Javier Giráldez[3], Sonia Andrés[3], María José Ranilla[1,3]

[1]*Universidad de León, León, Spain,* [2]*Universidad Politécnica de Madrid, Madrid, Spain,* [3]*Instituto de Ganadería de Montaña ULE-CSIC, Grulleros, León, Spain*

E-mail: mjrang@unileon.es

Take home message Replacing part of the forage by olive cake in the diet of dairy sheep had no negative effects on *in vitro* ruminal fermentation.

Introduction Spain is the world's largest producer of olive oil. Olive oil extraction generates several by-products that can be used to feed animals, particularly cakes and pomaces. Olive cake (OC) could be an ingredient for ruminant feeding due to its high fibre and medium protein contents (FEDNA, 2010). Olive cake also contains bioactive compounds with antimicrobial and antioxidant activities (Uribe *et al.*, 2014) that could be beneficial for the animals. The objective of this study was to assess the effects of replacing part of the forage by olive cake in a dairy sheep diet on ruminal parameters in Rusitec fermenters.

Materials & methods Four Rusitec fermenters were used in a cross-over design in two 14-day incubation periods. A 50:50 forage:concentrate diet (CON) was formulated, with forage containing alfalfa hay, maize silage and barley straw in proportions of 40, 40 and 20%, respectively. The forage in the experimental diet (OC) was composed by alfalfa hay, maize silage, barley straw and OC in proportions of 40, 13.3, 13.3 and 33.3%, respectively. After an 8-d adaptation period, on days 9, 10, 11 and 12 samples of solid and liquid contents were taken to determine apparent digestibility of diet and daily production of volatile fatty acids (VFA), and to measure pH. Statistical analysis was done by an analysis of variance with repeated measures using the procedure MIXED of SAS. Fermenter was considered a random effect, and diet, incubation period, sampling day and their interactions were considered fixed effects.

Results & discussion As shown in Table 1, total VFA was lower (P=0.003) in fermenters receiving the OC diet (88 vs 94 mmol/d for OC and CON diets respectively), mainly due to a reduction in acetate and other VFA (calculated as the sum of isobutyrate, isovalerate, valerate and caproate) production. However, both dry matter and acid detergent fibre apparent digestibilities were higher (P<0.001) for OC than for CON diet, which could be partly related to the greater (P=0.016) pH values and the higher microbial protein synthesis (data not shown) observed for OC diet.

Table 1 Effects of replacing part of barley straw and maize silage in the control diet (CON) by olive cake (OC) in a dairy sheep diet on ruminal fermentation in Rusitec fermenters.

	CON	OC	SEM	P
Total VFA, mmol/d	94	88	1.2	0.003
Acetate, mmol/d	45	41	0.6	0.005
Propionate, mmol/d	20	19	0.4	0.070
Butyrate, mmol/d	17	17	0.4	0.710
Other VFA, mmol/d	12	11	0.2	<0.001
Acetate:Propionate, mol/mol	2.25	2.16	0.047	0.460
Digestibility, g/g dry matter				
Dry matter	0.709	0.745	0.3803	<0.001
Neutral detergent fibre	0.377	0.337	0.8881	0.401
Acid detergent fibre	0.182	0.282	0.6721	<0.001
pH	6.06	6.22	0.115	0.016

Conclusion In Rusitec fermenters, replacing part of the barley straw and maize silage of the forage in the diet by olive cake slightly decreased daily total VFA production, but improved diet apparent digestibility. These results suggest that olive cake has potential to be included as a forage in diets for ruminants.

Acknowledgements Funding from the Spanish Ministry of Economy and Competitiveness is gratefully acknowledged (Projects AGL2016-75322-C2-1-R and AGL2016-75322-C2-2-R).

References

FEDNA. http://www.fundacionfedna.org/tablas-fedna-composicion-alimentos-valor-nutritivo Last update November 2016.

Uribe E, Lemus-Mondaca R, Vega-Galvez A, Zamorano M, Quispe-Fuentes I, Pasten A and Di Scala K 2014. Food Chemistry. 147, 170-176.

Chemical composition and *in vitro* ruminal fermentation of *Brassica* vegetables

Trinidad de Eván[1], María José Ranilla[2,3], Andrea Vintimilla[1], Carlos N Marcos[1], Javier González[1], Jairo García-Rodríguez[2], María Dolores Carro[1]

[1]*Universidad Politécnica de Madrid, Madrid, Spain,* [2]*Universidad de León, León, Spain,* [3]*Instituto de Ganadería de Montaña ULE-CSIC, Grulleros, León, Spain*

E-mail: mjrang@unileon.es

Take home message Cabbages and Brussels sprouts are good sources of protein and energy for ruminants.

Introduction The production of fresh vegetables is consistently growing, making available increasing amounts of by-products and wastes. These by-products could be used for animal feeding, thus generating economic benefits for the farmers and contributing to alleviate the environmental problems associated with their elimination, but information on their nutritive value is limited. Therefore, the objective of this study was to investigate the potential of using market wastes from four *Brassica* species as feed for ruminants.

Materials & methods Three samples of Brussels sprouts (*Brassica oleracea* var. *gemmifera),* white cabbage (*Brassica oleracea* var. *capitate* f. *alba)*, savoy cabbage (*Brassica oleracea* var. *capitata* f. *sabauda)* and red cabbage (*Brassica oleracea* var. *capitata* f. *rubra)* were obtained in different weeks at local markets in Spain. Samples were dried at 45ºC and ground to 1 mm before analyses of chemical composition. Samples (200 mg) were incubated *in vitro* with buffered rumen fluid (20 ml) from four rumen-fistulated sheep and total volatile fatty acid (VFA) and NH_3-N concentrations were determined after 24 h of incubation. Samples of sugar beet pulp and wheat DDGS were also fermented for comparative purposes. Data were analyzed as a mixed model with vegetable as fixed effect and inoculum as a random effect.

Table 1 Chemical composition (g/100g of dry matter) and *in vitro* ruminal fermentation of *Brassica* vegetables.

Item	Crude protein	Ether extract	Sugars	Neutral detergent fiber	Acid detergent fiber	Total VFA (µmol)	Acetate/ Propionate (mol/mol)	NH_3-N (mg/l)
Brussels sprouts	15.1^{a}	2.90^{a}	41.4	17.5^{a}	10.3^{a}	1632	2.04^{b}	284^{a}
White cabbage	19.5^{b}	3.57^{ab}	35.3	23.5^{ab}	15.4^{b}	1636	2.55^{a}	356^{b}
Savoy cabbage	20.7^{b}	4.36^{b}	34.2	28.0^{b}	17.3^{b}	1612	2.57^{a}	412^{c}
Red cabbage	19.8^{b}	4.54^{b}	27.2	25.4^{b}	17.6^{b}	1673	2.60^{a}	349^{b}
SEM[1]	0.59	0.220	3.35	1.54	0.75	19.6	0.031	8.6
p value	<0.001	0.002	0.095	0.007	<0.001	0.186	<0.001	<0.001
Sugar beet pulp	9.44	0.80	13.5	48.0	24.2	1700	2.60	139
Wheat DDGs	32.9	4.61	6.97	29.5	11.2	1311	1.61	357

[a,b,c] within each column, means in the same row with different superscript differ (P<0.05); [1] standard error of the mean

Results & discussion Dry matter content ranged from 6.60 in red cabbage to 16.3 g/100 g in Brussels sprouts. Chemical composition (Table 1) was in the range of that reported previously (Ngu and Ledin, 2005; Wadhwa and Bakshi, 2013). Savoy and red cabbage had greater ($p < 0.05$) crude protein (CP), ether extract (EE), neutral detergent fiber (NDF) and acid detergent fibre content than Brussels sprouts. However, there were no differences between white cabbage and Brussels sprouts in EE and NDF content. No differences ($p = 0.186$) were detected among vegetables in total VFA production, but acetate/propionate ratio was lower ($p < 0.05$) for Brussels sprouts compared with cabbage samples, which was related to the high sugar content of Brussels sprouts. All cabbage samples had greater ($p < 0.05$) NH_3-N concentrations than Brussels sprouts, which is consistent with the greater CP content of cabbages. Total VFA production of all vegetables was similar to that of sugar beet pulp, but greater than the values measured for wheat DDGS. Acetate/propionate ratio for cabbages (2.55 – 2.60) was similar to that of sugar beet pulp (2.60), but Brussels sprouts had lower values (2.04).

Conclusion The different types of cabbage and Brussels sprouts are good sources of crude protein and sugars for ruminants, and were extensively fermented *in vitro*. Further research is required to improve the knowledge on the nutritive value of these by-products and to discard potential negative effects of secondary compounds from *Brassica* vegetables on animal health and production.

Acknowledgements Funding from the Spanish Ministry of Economy and Competitiveness is gratefully acknowledged (Projects AGL2016-75322-C2-1-R and AGL2016-75322-C2-2-R).

References

Wadhwa M and Bakshi MPS 2013. RAP Publication 2013/04.
Ngu NT and Ledin I 2005. Asian-Australasian Journal of Animal Science 18, 197-202.

Methane production *in vitro* as influenced by tropical plants containing hydrolysable tannins

Moufida Rira[1,2], Diego P Morgavi[1], Lucette Genestoux[1], Inès Sakhri[2], Sihem Djibiri[2], Philippe Lecomte[3,4], Michel Doreau[1]

[1]INRA UMRH, Saint-Genès Champanelle, France, [2]Ecole Nationale Supérieure de Biotechnologie, Nouvelle ville Ali Mendjeli, Algeria, [3]SELMET, Univ Montpellier, CIRAD, INRA, Montpellier SupAgro, Montpellier, France, [4]CIRAD, UMR SELMET, PPZS, Dakar, Senegal

E-mail: moufida_r@yahoo.fr

Take home message Hydrolysable tannins can be a promising way to reduce enteric methane production.

Introduction Methane (CH_4) is the second largest contributor of greenhouse gases after dioxide carbone in the atmosphere. Enteric CH_4 from ruminants accounts for 39% of greenhouse gas emissions by livestock (Gerber *et al.*, 2013). A meta-analysis by Jayanegara *et al.* (2012) showed that condensed (CT) and hydrolysable tannins (HT) contained in tropical plants may reduce CH_4 production in the rumen. However, information on a possible anti-methanogenic effect of HT is scarce. The aim of the present study was to evaluate the effectiveness of *Acacia nilotica*, a tropical plant rich in HT, to reduce CH_4 production *in vitro*.

Materials & methods Leaves (AL) and pods (AP) of *Acacia nilotica* harvested in Senegal were tested. Hydrolysable tannins content was determined by the rhodanine method for gallotannins and by the potassium iodate method for ellagitannins. Two experiments were carried out to evaluate the effects of HT on rumen fermentation using an *in vitro* batch technique (Rira et al., 2015). In each experiment, four series of 24-h incubations were performed using rumen contents of 4 sheep fed a tropical grass. Total gas and CH_4 production were measured. A first experiment tested different levels of replacement a tropical forage (Ctl, natural grassland based on *Dichanthium spp)* by AL on one hand, AP on another hand: 0:100, 25:75, 50:50, 75:25 and 100:0. The second experiment tested the combination of AL and AP without Ctl, in different proportions: 100:0, 75:25, 50:50, 25:75, and 0:100. Total gas and methane production data were analysed using mixed models where source and replacement rate of HT (experiment 1) and AL:AP ratio (experiment 2) were the fixed effects; sheep was the random effect in both experiments.

Results & discussion The two parts of *Acacia nilotica*, especially pods, have a high content of HT but contain low concentration of fiber (Table 1). The inclusion of HT from AL and AP inhibited fermentation in a dose-dependent way, as observed by the lower gas production ($P < 0.01$, Figure 1A). Likewise, CH_4 production decreased by the addition of AL and AP ($P < 0.01$, Figure 1B). The inhibitory effect of AL and AP on fermentation and CH_4 production was significant from a 50% inclusion level. Gas production was slightly but significantly lower with AP than with AL inclusion ($P = 0.026$). Although AP was richer in HT than AL, their anti-methanogen effect did not differ ($P > 0.05$). No interaction between level of inclusion and source of HT was observed. Compared to Ctl, a 100% level of HT (average of AP and AL) decreased gas and CH_4 production by 29 and 59%, respectively. Combination of two sources of HT had no significant effect on fermentation and on CH_4 production ($P>0.05$, Table 2).

Table 1 Crude protein, NDF and hydrolysable tannins content (g/kg DM) of *Acacia nilotica* leaves (AL) and pods (AP).

	AL	AP
Crude protein	115	140
NDF	159	227
Gallotannins	31	84
Ellagitannins	147	266

Table 2 Effect of the leaves:pods ratio of *Acacia nilotica* on gas and methane (mL/24 h) production *in vitro.*

	100:0	75:25	50:50	25:75	0:100
Gas	25.60	27.08	26.88	26.55	23.26
CH_4	1.41	1.59	1.29	1.86	1.75

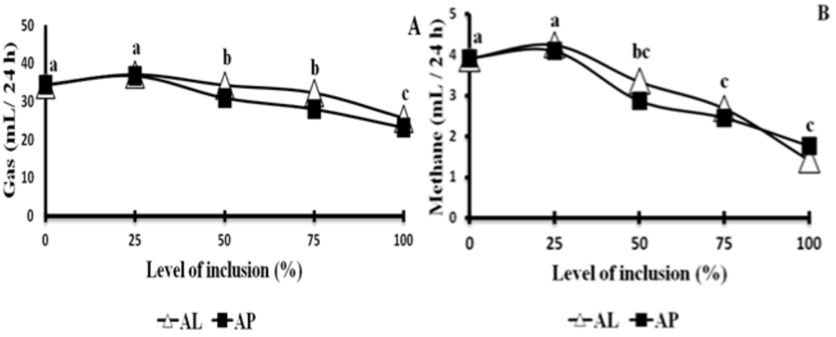


Figure 1 Effect of *Acacia nilotica* leaves (AL) and pods (AP) at different levels of inclusion, on 24-h *in vitro* total gas (A) and methane (B) production. Means with different superscripts significantly differ ($P<0.05$).

Conclusion *Acacia nilotica*, a HT-rich plant, can be used for reducing enteric CH_4 emission. Further work should evaluate the effect of *A. nilotica* inclusion in diets on *in vivo* digestibility before recommending plants containing HT in sustainable CH_4 mitigation strategies.

References

Gerber PJ, Steinfeld H, Henderson B, Mottet A, Opio C, Dijkman J, Falcucci A and Tempio G 2013. FAO, Rome, Italy.
Jayanegara A, Leiber F, Kreuzer M 2012. Journal of Animal Physiology and Animal Nutrition. 96, 365–375.
Rira M, Morgavi DP, Archimède H, Marie-Magdeleine C, Popova M, Bousseboua H and Doreau M 2015. Journal of Animal Science, 93, 334-347.

Effect of fungal treatment on the chemical composition and *in vitro* gas production of saffron residues

Vahid Kardan Moghadam[1], Mohammad Hassan Fathi[2], Mostafa Yousef Elahi[1]

[1]*University of Zabol, Zabol, Iran,* [2]*University of Birjand, Birjand, Iran*

E-mail: iranmanesh1824@yahoo.com

Take home message The objectives of this study were to determine the chemical composition and estimating nutritive value saffron residues using *in situ* and *in vitro* gas production technique.

Introduction Saffron residues which are consisted of stamen, leaves, sepal and petal are produced in large-scale in Iran, the largest saffron producer in the world, as the total production of them is about 87000 tons annually. In most of the studies, it was determined that digestibility's of the lignocellulosic residues can be increased by the enzymes produced by fungi and become suitable to use in ruminant nutrition. Fungal treatment as a biological method has been recently considered as a promising method for improving the nutritive value of straw (Levin and Forchiassin, 2003).

Materials & methods Saffron residues were collected (approximately 6 months after saffron flowering) from a field in Birjand, Iran. They were chopped to 3-4 cm long pieces and soaked for 24h in pasteurized cold water and inoculated with *P. florida* mycelium. The inoculated saffron was packed into polyethylene bags of 5kg (three replications for each treatment). Mycelium incubation was done in open and aerobic condition at room temperature of 22±5 and relative air humidity of 65±5. After 64 days, the residues were removed from the fermentation chamber, air dried and used for chemical analysis (AOAC, 1990) and *in vitro* gas production (Menke and Steingass, 1988). Dry samples from each plot were milled in a hammer mill through a 2mm sieve for chemical analysis and 1mm sieve for gas production. Three replications for each experiment). Experimental data were analyzed based on one-way design.

Results & discussion Fungal treatment also affected chemical composition of the substrate (Table 1). There was an increase in CP content after treatment, while NDF and ADF contents decreased. In this study the results of fungal treatment effects on the chemical composition of saffron residues is in agreement with other studies (Jalk *et al.*, 1998; Magnigo *et al.*, 2004). These studies have reported that fungus decrease cellulose, hemicellulose and lignin content. Gas production volume of saffron residues in all of the incubation times were affected by fungus culture and differences between them were significant (Table 2).

Table 1 Chemical composition (% DM) of saffron residues treated with *P. florida* (mean± SE).

	DM	CP	ASH	NDF	ADF
Control	93.97±1.10	6.63±0.07	5.23±0.62	45.9±0.62	38.01±1.81
Treated	67.69±1.68	14.85±0.59	6.71±0.18	28.23±1.05	26.67±0.27
Sig	***	***	NS	***	**

Table 2 *In vitro* gas production (ml/200mg DM) and estimated parameters of saffron residues incubated with *P. florida* (mean± SE).

Treatment	Incubation times (h)								Estimated parameters			
	2	4	8	16	24	48	72	96	b (%)	c (%/h)	OMD* (%)	ME** (MJ/Kg DM)
Control	8.9±1.37	17.3±1.4	26.4±2.11	38.3±2.9	40.2±3.27	48.3±2.89	50.6±3.0	51.6±2.09	49.8±2.5	0.091±0.009	53.9±2.95	8.0±0.45
Treated	25.2±0.2	35.1±0.2	45.5±0.46	61.2±0.2	67.2±0.45	71.0±0.47	77.9±0.1	78.8±0.26	73.9±0.26	0.138±0.001	81.7±0.61	12.2±0.09
Sig	***	***	***	***	***	***	***	***	***	***	***	***

* $OMD=14.88 + 0.889 \times GP + 0.45 \times CP + 0.0651 \times XA$ ** $ME= 2.20 + 0.136 \times GP + 0.057 \times CP + 0.0029 \times CP^2$

Conclusion It was concluded the saffron residues as suitable mushroom compost can improve nutritive value of saffron resides in comparison with the untreated saffron residues. However, more research is needed to provide information on the best methods for utilization of fungi treated residues by ruminants and also more work may be required before application to *in vivo* studies.

References

AOAC. 1999. In: Cunnif P (Ed.), Official methods of analysis of the association of official analytical chemists, 16th ed. AOAC International, Gaithersburg, MD, USA.
Jalk D, Nerud F and Siroka P 1998. Folia Microbiologica 43, 687-689.
Levin LA and Forchiassin VA 2003. International Biodeterioration & Biodegradation, 52, 1-5.
Magingo FS, Oriyo NM, Kivaisi AR and Danell E 2004. Mycologia, 91, 197-204.
Menke KH and Steingass, H 1988. Animal Research and Development 28, 7-55.

Measuring and predicting the voluntary intake of mountain permanent grassland hays in sheep and heifers

Bertrand Deroche[1,2], Yves Arrigo[3], Lorène Salis[2], Mickaël Bernard[4], Marc Barbet[4], Massoud Aoun[1], René Baumont[2]

[1]Idnea, Sautron, France, [2]INRA, UMR Herbivores, Saint-Genes-Champanelle, France, [3]Agroscope, Posieux, Switzerland, [4]INRA, Herbipôle, Saint-Genes-Champanelle, France

E-mail: bertrand.deroche@inra.fr

Take home message NDF and crude protein content are promising predictors of voluntary intake of mountain permanent grassland hays.

Introduction The estimation of the voluntary dry matter intake (VDMI) of forage is essential to feed ruminants. In France, VDMI of grass and legume forages is measured in sheep, which is the standard animal, and used to predict VDMI of cattle. Moreover, VDMI is related to digestibility measured in sheep as well as to chemical components of forages (Baumont *et al*, 2018). These relationships were established mainly for single species forages. In a rich species grassland, differences between species can affect the feed value of forage (Bruinenberg *et al,* 2002). Thus, these links need to be investigated on multispecies forages, such as mountain permanent grassland hays.

Materials & methods Twelve hays were prepared for this study: in Switzerland (Agroscope Posieux), 2 plots of contrasting botanical diversity (high and low) were harvested at 2 vegetative cycles (1 and 3) and barn-dried in 2015; in France (INRA Laqueuille), 2 plots differing in fertilization level (high and low) were harvested at 2 maturity stages (early and late) and field-dried in 2015; the same plots were harvested at early maturity stage and field-dried or barn-dried in 2016. VDMI was measured using Latin square designs (4 hays per Latin square) with 8 Prim'Holstein heifers (15 months, 368 kg in average) and 8 male castrated Texel sheep (between 1 and 2 years old, 55 kg in average). *In vivo* organic matter digestibility (OMd) was measured in sheep experiments. The links between the chemical composition (crude protein: 107 ± 26 g/kg DM, NDF: 584 ± 77 g/kg DM, ADF: 314 ± 41 g/kg DM), the OMd measured in sheep and the VDMI measured in sheep and heifers were analysed through regressions (PROC REG) using SAS 5.1.

Results & discussion VDMI measured in heifers (100 ± 11 g/kg body weight$^{0.75}$) was closely linked to VDMI measured in sheep (66 ± 8 g/kg body weight$^{0.75}$) (Figure 1). OMd measured in sheep (62 ± 7 %) was also highly linked to sheep VDMI, but to a lesser extent to heifer VDMI (Table 1). Higher links were found between single chemical composition criteria (crude protein, NDF and ADF) and VDMI measured in sheep and heifers than between OMd measured in sheep and VDMI (Table 1). This could be due to the two Swiss hays that were rich in dandelion (90%) or in legumes (50%) and had the highest VDMI despite an OMd comparable or lower to those of the other two Swiss hays rich in grasses.

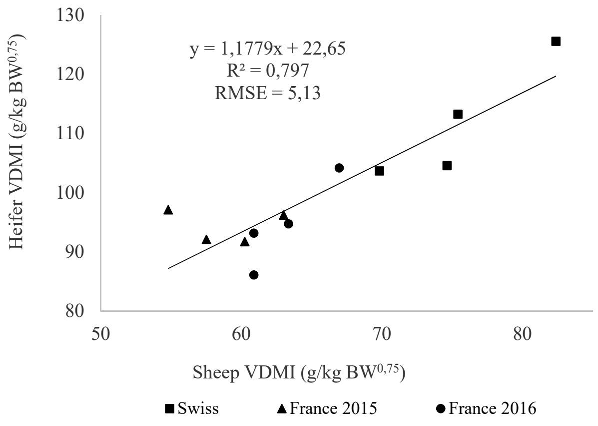


Figure 1 Relationship between heifer and sheep VDMI.

Table 1 Links between heifer or sheep VDMI and chemical components or OMd (n=12).

	Heifer VDMI (g/kg BW $^{0.75}$)	Sheep VDMI (g/kg BW $^{0.75}$)
Sheep OMd (%)	R² = 0.464 RMSE = 8.34	R² = 0.732 RMSE = 4.47
Crude protein (g/kg DM)	R² = 0.731 RMSE = 5.90	R² = 0.897 RMSE = 2.77
NDF(g/kg DM)	R² = 0.837 RMSE = 4.60	R² = 0.899 RMSE = 2.75
ADF(g/kg DM)	R² = 0.801 RMSE = 5.08	R² = 0.924 RMSE = 2.38

Conclusion Despite a small number of samples, extrapolation of the VDMI from sheep to heifers is possible for hays from rich species grasslands. For prediction purpose, chemical composition criteria seem more promising than OMd. A higher number of hays is needed to confirm these conclusions.

Acknowledgements This research was supported by grants from IDENA and the “Association Nationale de la Recherche et de la Technologie”. The authors are grateful to the staff of INRA UE 1414 Herbipôle and Agroscope Posieux.

References

Baumont R, Sauvant D, Maxin G, Chapoutot P, Tran G, Boudon A, Lemosquet S and Nozière P 2018. In: INRA feeding system for ruminants, pp. 411-440. Wageningen Academic Publishers, Wageningen, the Netherlands.
Bruinenberg MH, Valk H, Korevaar H and Struik PC 2002. Grass and Forage Science. 57, 292-301.

From feedlot to grazing: residual effect of feeding high grain diets on further grazing performance of beef calves

Alvaro Simeone, Virginia Beretta, Agustín Carrocio, Sergio López, Matías Orcasberro, Juan Vilaró

University of the Republic, Paysandú, Uruguay

E-mail: asimeone@adinet.com.uy

Take home message When deciding about lot-feeding early-weaned beef calves it is relevant to consider the carryover effect of this management on the subsequent grazing performance.

Introduction In pasture-based beef cattle systems, lot-feeding spring-born early-weaned beef calves with a high concentrate diet during summer is a strategy used to improve 180-days-old liveweight (LW) compared to traditional grazing management (Beretta *et al*., 2015). However, this raises the question as to the possible negative residual effect of feedlot feeding on further grazing performance, and on whether it could be overcome by the way the transition between diets is made. This experiment evaluated the effect of the feeding system during summer (FS) and the transition strategy for removing concentrate from the diet (T), on autumn grazing performance and pasture utilization by early-weaned calves.

Materials & methods Forty-eight Hereford calves (67.0±15.0 days old, 80.3 kg±15.1 kg) were randomly allotted to one of four treatments in a factorial arrangement 2×2. During summer (98 days) calves were handled in one of two FS: *ad libitum* supply of an all-concentrate feedlot diet (FL), or grazing a mixed grass-legume pasture supplemented with a commercial energy-protein concentrate (19% CP, 1 kg DM/100 kg LW) (PS). For transition to grazing autumn pastures, half the animals in each FS had concentrate gradually removed during the last 14 days (GT), while the other half, had concentrate abruptly removed on the last day (AT). During autumn (10 weeks, wk) all treatments strip-grazed the mixed grass-legume pasture (forage allowance 8 kg/100 kg LW), each replication in an independent paddock (n= 3/ treatment, 4 calves/ replication). Calves were weighed weekly and pasture utilization determined. During wk-2 and wk-8 apparent OM digestibility was estimated *in vivo* (using acid insoluble ash as internal marker), and on wk-1, 2, 4 and 8, calves were observed for day grazing activity (GA). Data was analysed according to the following general model: $Y_{ijk}=\mu+FS_i+T_j+(FS\times T)_{ij}+e_{ijk..}$ Week effect and its interactions were included for GA analysis.

Results & discussion Table 1 summarizes main results. Calves from FL showed lower mean LW gain compared to PS but they were still heavier by the end of autumn. The difference in LW gain between feeding systems was maximum during de first 14-d of autumn grazing ($P<0.01$) and became smaller as time progressed, disappearing after 42 days ($P>0.05$), (Figure 1). Transition only affected GA, with a significant interaction FS×T×wk ($P<0.01$): on wk-1, calves from FL with AT grazed less compared to those with GT (0.45 vs. 0.63 probability of occurrence, $P<0.05$), while no effect was observed for PS calves (AT: 0.57 vs. GT: 0.55, $P>0.05$). Differences in GA were not registered after wk-2.

Table 1 Effect of summer feeding system (FS)[1] and transition strategy[2] on autumn grazing performance of Hereford calves (days 1-70): liveweight (LW), pasture utilization (PU), OM digestibility (OMD) and grazing activity (GA) probability.

	FS		P-value[3]		
	Feelot	PS	FS	T	FS×T
LW, kg (day 1)	211.0	147.0	**	ns	ns
LW gain, kg/d)	0.097	0.335	**	ns	ns
LW, kg (day 70)	217.8	170.5	**	ns	ns
PU, %	51.6	55.3	ns	ns	ns
OMD %	69.4	75.0	*	ns	ns
GA (probability)	0.57	0.54	ns	**	ns

[1]Feedlot *vs.* supplementation on pasture (PS). [2]Gradual vs. abrupt removal of concentrate. [3] ns: $P>0.05$, * $P<0.05$, **$P<0.01$

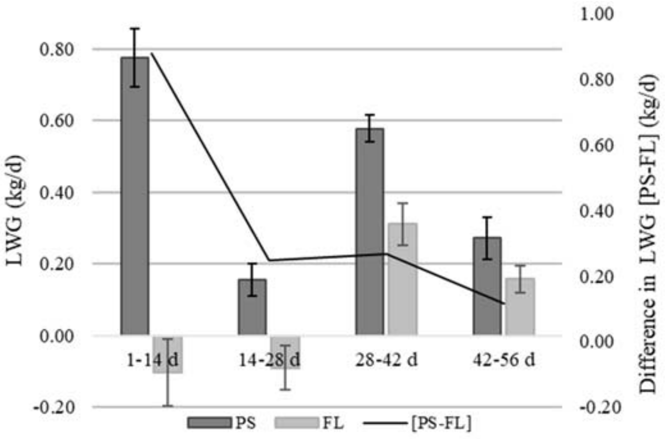


Figure 1 Effect of summer feeding system (feedlot, FL or pasture supplementation, PS) on liveweight gain (LWG) during autumn grazing period (1 to 70 days).

Conclusion Lot-feeding early-weaned calves during summer reduces calf growth rate when going out to pasture in autumn compared to calves coming from pasture grazing, and this effect is not overcome by removing gradually the concentrate from the diet. A combination of behaviour and digestion factors appear to be involved in this response.

References

Beretta V, Simeone A, Henderson A, Iribarne R, Silveira B 2015. 66th Meeting of the European Federation of Animal Science, Warsaw, Poland

Using self-feeders for winter supplementation of beef calves grazing oats pastures

Alvaro Simeone, Virginia Beretta, Maria Belen Algorta, Gonzalo Iruleguy, Inés López

University of the Republic, Paysandú, Uruguay

E-mail: asimeone@adinet.com.uy

Take home message Using self-feeders for grain supplementation of beef weaned calves grazing oats is a viable alternative

Introduction Grain supplementation of cattle grazing winter pastures has shown to enhance animal growth rates (Simeone *et al*, 2003). Using self-feeders could contribute to reduce labour cost associated to supplement distribution, however, animal performance and pasture utilisation could be impaired compared to daily grain supply (where offered quantity may be easily controlled), and this effect could be influenced by grazing management, as well. The present experiment evaluated the effect of forage allowance (FA), supplementation and supplement delivery system on winter grazing performance and pasture utilization by beef calves grazing oats.

Materials & methods Forty-eight Hereford calves (192.3±6.9 kg) gazing an oat pasture (*Avena byzantina* cv. RLE 115, 32 ha) were randomly distributed in 12 groups and 6 treatments (n=2/treatment, 4 calves/replication). Treatments had a factorial arrangement resulting from the combination of 2 FA (2.5 or 5.0 kg DM/100 kg liveweight, LW) and 3 supplementation strategies (S): supplementation with ground sorghum grain (1.0 kg DM/100 kg LW) offered daily in conventional troughs (DS) or using self-feeders (SF), and a control without supplementation (CON). In SF, supplement was always available but sorghum grain was mixed with NaCl (111 g/kg mixture) for grain intake regulation at 1.0 kg DM/100 kg LW (Rich *et al*. 1976). Calves in SF and DS were gradually introduced to grain intake during 7 days. Each replication grazed an independent paddock, in 7-days strips for 70 days. Calves were weighed every 14 days after 12 h fasting. Weekly, supplement (SDMI) and forage (FDMI) dry matter intake were estimated as the difference between offered DM and refusals. Supplement conversion ratio (SCR) was calculated as the quotient between SDMI and the difference in weight gain between the supplemented treatment and CON. Data was analysed according to a randomized plot design, following the general model: $Y_{ijk} = \mu + FA_i + S_j + (FA \times S)_{ij} + b_1 LWI + e_{ijk.,}$ with initial LW as covariate.

Results & discussion Average pre-grazing DM biomass and sward height were 2336 ± 713.7 kg/ha and 26.9 ± 3,6 cm, respectively. Pasture crude protein, NDF and ADF contents were 147, 524 and 236 g/kg DM, respectively. Table 1 summarizes the effect of FA and S on mean winter calf performance. A higher FA increased LW gain and FDMI. Supplementation increased LW gain while no differences were observed due to S, being this response independent of FA. A significant interaction FA×S was only observed for SDMI: calves form SF showed lower SDMI compared to DF, but this difference was larger for calves grazing with higher FA (FA=5.0: 1.0 *vs*. 0.50 kg/100 kg LW; FA=2.5: 1.0 *vs*. 0.58 kg/100 kg LW; P<0.01). SCR was not statistically affected by FA or S. However, observed lower mean values for calves grazing with high FA or supplemented in self-feeders reflect higher FDMI and lower SDMI, respectively.

Table 1 Effect of forage allowance (FA) and supplementation strategy with sorghum grain to Hereford calves grazing oats during winter (70 days), on liveweight gain, dry matter intake, pasture utilisation and supplement conversion ratio.

	FA (kg/100 kg LW)		Supplementation (S)[1]			P-value[3]		
	2.5	5.0	CON	DS	SF	FA	S	FA×S
Liveweight (LW) gain (kg/d)	0.37	0.65	0.37^b	0.60^a	0.56^a	**	**	ns
Residual sward height (cm)	6.31	11.4	7.15^b	9.10^{ab}	10.3^a	**	*	ns
Pasture utilisation (%)	70.8	54.8	68.6^a	59.7^b	60.2^b	**	**	ns
Pasture intake (kg/ 100 kg LW)	2.18	3.12	3.16^a	2.35^b	2.44^b	**	**	ns
Supplement intake (kg/ 100 kg LW)	0.79	0.75	--	1.00	0.54	**	**	**
Supplement conversion ratio[2]	6.89	9.31	--	8.96	7.24	ns	ns	ns

[1]CON: no supplementation. DS: daily supply 1 kg/100 kg LW, SF: supplementation in self-feeders with NaCl for intake regulation. [2] SCR= Supplement DM intake/response in weight gain due to supplement intake. [3] **P<0.01. ns: P>0.05

Conclusion Results suggest that using self-feeders for grain supplementation of Hereford calves grazing oat pastures may replace daily hand feeding without affecting animal performance or pasture utilisation. Variations in supplement intake may occur associated to FA and quantity of salt mixed with the grain for intake control.

References

Rich TD, Armbruster S, Gill DR 1976. Historical Materials from University of Nebraska-Lincoln Extension. Paper 274.
Simeone A, Beretta V, Rowe J 2002. Animal Production in Australia. 24, 213 – 216.

Impact of perennial ryegrass ploidy and white clover inclusion on herbage nutritive value

Clare Guy[1,2], Deirdre Hennessy[1], Trevor J. Gilliland[2,3], Fergal Coughlan[1], Bríd McClearn[1,2], Michael Dineen[1], Brian McCarthy[1]

[1]*Teagasc Moorepark, Fermoy, Ireland,* [2]*The Institute for Global Food Security, Belfast, UK,* [3]*Agri-food and Biosciences Institute, Hillsborough, UK*

E-mail: clare.guy@teagasc.ie

Take home message Tetraploid swards had a superior nutritive value compared with diploid swards, and including white clover in grazing swards improved herbage nutritive value.

Introduction Herbage nutritive value can differ between perennial ryegrass (*Lolium perenne* L.: PRG) ploidies as tetraploid cultivars can have a superior herbage nutritive value compared with diploid cultivars (Gilliland *et al*., 2005). Including white clover (*Trifolium repens* L.: WC) can further improve herbage nutritive value compared with grass-only swards (Dewhurst *et al*. 2009). The nutritive value of grass-only swards tends to decline toward the latter end of the grazing season due to advanced maturity, whereas grass-WC swards maintain sward nutritive value throughout the grazing season (Dewhurst *et al*. 2009). Improving herbage nutritive value through the use of grass-WC swards and tetraploid swards could lead to a higher dry matter (DM) intake rate (Dewhurst *et al*. 2009). The objective of this study was to examine the effect of PRG ploidy and WC inclusion on herbage nutritive value.

Materials & methods A 3-year farm-systems grazing study (43.6 ha) commenced in 2014 on 1 and 2-year old swards. The study was a 2 × 2 factorial design, consisting of two PRG ploidies (diploid, tetraploid) and two WC treatments (with WC, without WC), resulting in four sward types: diploid-only; tetraploid-only; diploid-WC; tetraploid-WC. Four diploid (sowing rate: 30 kg/ha) and four tetraploid (sowing rate: 37 kg/ha) cultivars were sown with and without WC (50% Chieftain; 50% Crusader; sowing rate: 5 kg/ha) in five blocks. Nitrogen fertiliser application was 250 kg N/ha/year and each sward type was rotationally grazed at a stocking rate of 2.75 cows/ha. Herbage sub-samples, collected when measuring pre-grazing herbage mass, were freeze dried and milled through a 1 mm sieve. Sub-samples were selected from treatments at four time points (February-March, mid-May to mid-June, mid–June to mid-July, September) across the year. Samples were analysed for DM, acid detergent fibre (ADF), neutral detergent fibre (NDF), crude protein (CP) and in-vivo organic matter digestibility (OMD). Analyses were undertaken using general linear models (PROC GLM) in SAS 9.4, with year, PRG ploidy, WC inclusion, time point and PRG ploidy × WC inclusion interaction as fixed effects and block as a random effect.

Results Herbage nutritive value is presented in Table 1. Tetraploid swards had consistently greater OMD ($P < 0.01$; + 10 g/kg DM) and lower structural fibre ($P < 0.001$; NDF: - 18 g/kg DM; ADF: - 16 g/kg DM) contents than diploid swards. White clover inclusion led to higher CP ($P < 0.001$; + 40 g/kg DM) and greater OMD ($P < 0.001$; + 22 g/kg DM) contents than grass-only swards. Grass-WC swards (diploid-WC, tetraploid-WC) had lower NDF and ADF contents ($P < 0.001$; NDF: - 47 g/kg DM; ADF: - 17 g/kg DM) than grass-only swards (diploid-only, tetraploid-only) due to lower WC stem proportions (Buxton, 1996). This can lead to increased DM intake and utilisation (Ayres *et al*., 1998). Time point had an effect on herbage nutritive value ($P < 0.001$); OMD and CP declined between spring and autumn, from 786 to 738, and 236 to 215 g/kg DM, respectively. Structural fibres increased from spring to autumn; NDF and ADF values increased from 427 to 442, and 256 to 261 g/kg DM, respectively. Grass-WC and tetraploid swards had a lower DM content than grass-only and diploid swards, respectively ($P < 0.001$; grass-WC: 17.2%; grass-only: 19.3%; diploid: 18.7%; tetraploid: 17.8%). Year had an effect ($P < 0.05$) on all herbage nutritive value characteristics except CP content.

Table 1 Comparison of herbage nutritive values between the four sward types; diploid-only (DO), tetraploid-only (TO), diploid-WC (DC) and tetraploid-WC (TC) over three years (standard error in parentheses).

	DO	TO	DC	TC	P[1]	C[2]	Yr3	Time point	P × C
CP (g/kg DM)	194.4 (3.27)	197.6 (3.30)	233.8 (3.29)	240.0 (3.33)	NS	***	NS	***	NS
OMD (g/kg DM)	755.0 (3.84)	760.6 (3.87)	772.0 (3.85)	786.6 (3.91)	**	***	***	***	NS
NDF (g/kg DM)	463.9 (4.26)	445.3 (4.29)	416.8 (4.29)	398.5 (4.34)	***	***	***	***	NS
ADF (g/kg DM)	270.6 (3.16)	256.8 (3.18)	255.8 (3.18)	238.2 (3.21)	***	***	***	***	NS

[1]P = Ploidy; [2]C = White clover; [3]Yr = Year; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and NS = non-significant

Conclusion White clover inclusion resulted in improved nutritive value, and varying sward composition with perennial ryegrass ploidy led to improved nutritive value in tetraploid swards.

Acknowledgements The authors gratefully acknowledge the Teagasc Walsh Fellowship for financial support.

References

Ayres J, Nandra K and Turner A 1998. Grass and Forage Science 53, 250-259.
Buxton DR 1996. Animal Feed Science and Technology 59, 37-49.
Dewhurst RJ, Delaby L, Moloney A, Boland T and Lewis E 2009. Irish Journal of Agricultural and Food Research 167-187.
Gilliland T, Agnew R, Fearon A and Wilson F 2005. XX International Grassland Congress: Offered Papers. Wageningen Academic Publishers, 227.
Statistical Institute of Analysis (SAS) 2013. Users guide: statistics. Version 9.4. SAS Institute, Cary, NC, USA.

The effect of partial substitution of rapeseed meal and faba beans by *Spirulina platensis* microalga on phosphorus use efficiency in dairy cattle milk production

Marjukka Lamminen[1,2], Anni Halmemies-Beauchet-Filleau[1], Tuomo Kokkonen[1], Seija Jaakkola[1], Aila Vanhatalo[1]

[1]*University of Helsinki, Department of Agricultural Sciences, Helsinki, Finland,* [2]*Helsinki Institute of Sustainability Science, Helsinki, Finland*

E-mail: marjukka.lamminen@helsinki.fi

Take home message Faba beans and microalgae have potential to increase phosphorus use efficiency if milk production response could be boosted e.g. by more balanced amino acid supply.

Introduction Phosphorus (P) is necessary nutrient for both plants and animals, but also the major cause of eutrophication when present in environment at excessive amounts. The P load of animal production can be strongly influenced by dietary P concentration, i.e. selecting feed resources to optimise P supply. The aim of this study was to investigate the effects of rapeseed meal or faba beans (*Vicia faba*) as sole protein feed or partially substituted by *Spirulina platensis* microalga on animal performance and the efficiency of P utilisation (PUE) in milk production (P in milk:P intake). It was hypothesised that faba beans decrease P intake and result in lower P secretion in environment due to lower P content of faba beans than rapeseed meal. Further, inclusion of spirulina in the diet was hypothesised to decrease P intake and increase PUE because of lower P to protein ratio in spirulina than in rapeseed meal and faba beans and isonitrogenous protein supplementation of diets.

Materials & methods Eight multiparous, mid-lactating Finnish Ayrshire cows, four of which were rumen cannulated, were used in a replicated, balanced 4×4 Latin square study with 2×2 factorial arrangement of treatments. Treatments consisted of two basal protein feeds (rapeseed meal or faba bean; RSM and FB, respectively) as sole protein feed or 50% their crude protein substituted by spirulina (RSM-SPI and FB-SPI, respectively). Cows were offered ad libitum total mixed ration consisting of second cut grass silage, barley, experimental protein feed, sugar beet pulp, minerals and vitamins. The concentrate:forage ratio was 45:55 on all treatments and N supply was identical from protein feeds. The P content of feeds, milk, faeces and urine were analysed with ICP-OES and data was subjected to analysis of variance using the MIXED procedure of SAS (version 9.4).

Results & discussion The P concentration of rapeseed meal, faba beans and spirulina were 8.56, 6.10 and 9.34 g/kg DM, respectively. Partial substitution of basal protein feed by spirulina decreased DM intake. Faba beans induced lower milk production than rapeseed meal. Milk yield was decreased on RSM diets by spirulina inclusion but increased on FB diets probably due to improved AA supply, especially increased methionine intake (+4.9 g/d) by spirulina inclusion on FB diets. The use of faba beans resulted in lower dietary P concentration (3.76 vs. 3.90 g/kg DM, $P<0.001$, respectively), P intake and higher P digestibility (325 vs. 271 g/kg, $P<0.05$, respectively) than rapeseed meal. Spirulina inclusion in the diet decreased P intake on both basal diets due to decreased DM intake on spirulina diets and lower dosage of protein-rich spirulina. The excretion of P in milk was lower and the concentration of P in faeces tended to be lower on faba beans than rapeseed meal diets. There were no differences in PUE between faba beans and rapeseed meal due to lower milk yields on faba bean diets but spirulina tended to increase PUE on both basal diets (on average +7.2%).

Table 1 Effect of substitution of rapeseed meal (RSM) and faba beans (FB) by *Spirulina platensis* (SPI) on P utilisation.

	Treatments				P-value[1]		
	RSM	RSM-SPI	FB	FB-SPI	BP	SPI	BP × SPI
Dry matter intake, kg/d	23.3	22.8	23.1	22.3	0.198	0.037	0.500
Milk yield, kg/d	31.0	30.0	28.5	29.7	<0.001	0.857	0.002
P intake, g/d	91.7	88.1	86.6	83.8	0.003	0.022	0.757
P in milk, g/d	27.6	28.7	25.2	25.9	0.016	0.346	0.821
P in urine, mg/l	9.64	9.55	9.48	8.89	0.324	0.413	0.550
P in faeces, g/kg DM	10.6	10.3	9.62	9.82	0.066	0.882	0.508
P in milk:P intake (PUE)	0.303	0.327	0.291	0.310	0.230	0.073	0.831

[1] BP=RSM vs. FB as basal protein feed; SPI=RSM and FB vs. RSM-SPI and FB-SPI; BP × SPI=interaction of BP and SPI.

Conclusion The environmental P load of dairy cattle production can be decreased by dietary P optimisation. PUE is highly dependent on milk yield, and both faba beans and microalgae have potential to increase PUE especially if milk production response could be boosted e.g. by improving balance of amino acid supply.

Acknowledgements This research was funded by Ministry of Agriculture and Forestry in Finland and Raisioagro Ltd., Finland.

Effects of a low concentrate diet on production and metabolism in early lactation Holstein and Swedish Red dairy cows

Johanna Karlsson, Rolf Spörndly, Mikaela Lindberg, Kjell Holtenius

Swedish University of Agricultural Sciences, Uppsala, Sweden

E-mail: johanna.karlsson@slu.se

Take home message Early lactating dairy cows fed a low concentrate ration, < 4 kg dry matter (DM)/day, based on by-products and high-quality forage *ad libitum* maintained an almost as high milk production as cows fed up to 13 kg DM/day of the same concentrate mixture.

Introduction Ruminants have a unique ability to produce milk and meat from fibrous feed and by-products not suitable for human consumption. However, high-yielding cows are generally fed a high proportion of human edible concentrates in their rations, especially in early lactation (Swensson *et al*., 2017). Most production related disturbances in early lactation occur due to energy deficiency and metabolic imbalance (Esposito *et al*., 2014; Drackley & Cardoso, 2014). The aim of the present study was to investigate the performance of early lactation cows fed forage *ad libitum* combined with a limited amount of concentrate based on by-products compared with cows fed a conventional ration of the same concentrate. It was hypothesized that cows offered a low concentrate ration in early lactation would produce less milk and the metabolic status would be compromised. It was also hypothesized that Holstein cows would produce more milk but be in a less favourable metabolic status when fed low concentrate rations.

Materials & methods Out of totally 100 dairy cows, 85 cows were randomly allocated to a low concentrate (LC) ration while the remaining 15 cows were subjected to a higher concentrate (HC) ration as a control. The groups were unbalanced due to a parallel genetic study on the LC cows. Breeds were Holstein (n = 44, LC = 36, HC = 8) and Swedish Red (n = 56, LC = 49, HC = 7). Concentrate was gradually increased with 0.4 kg DM per day in both groups, from 2.6 kg DM at calving to 4.4 and 13.0 kg DM/day, respectively. The concentrate was by-product based (NDF = 349, CP = 180, starch = 68 g/kg DM, and 13.2 ME MJ/kg DM). The forage was a grass/clover silage (NDF = 450, CP = 136 g/kg DM, and 11.6 ME MJ/kg DM) fed *ad libitum*. Feed intake was individually recorded and the cows were milked twice a day. Milk samples and blood plasma were collected in lactation week 2, 4 and 6. Plasma was analysed for glucose, insulin, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB) and insulin-like growth factor 1 (IGF-1). Body condition score (BCS) was estimated after each milking using a 3D camera. The data was analysed with PROC MIXED in SAS 9.4.

Results & discussion During the first 6 weeks of lactation the cows fed LC tended (P = 0.06) to have a lower energy corrected milk (ECM) production compared with the HC (32.8 kg ECM and 34.8 kg ECM, in LC and HC, respectively). There was no overall difference in DM intake (DMI) or in plasma concentrations of glucose, insulin, NEFA or IGF-1 but the LC group had higher plasma concentration of BHB (P < 0.001). There was a diet * week effect for ECM (P < 0.001), DMI (P < 0.001) and BCS (P = 0.04). There was a tendency (P = 0.06) for a diet * week effect for insulin concentration in blood plasma (Figure 1). There were no significant breed * diet interactions (P > 0.10) for milk yield, measured metabolites or hormones.

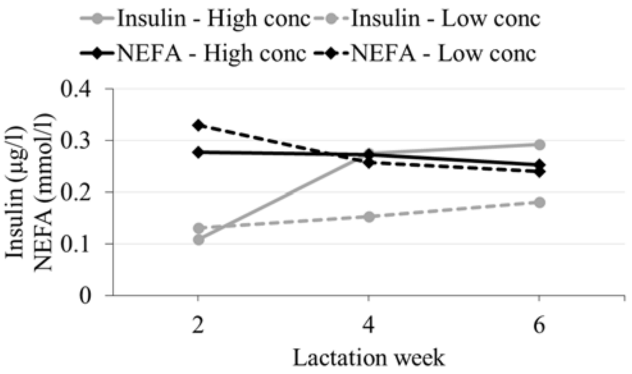


Figure 1 Least square means for insulin and non-esterified fatty acids (NEFA) for high respectively low concentrate ration in dairy cows lactation week 2, 4 and 6.

Conclusion The LC cows had less rapid increase in ECM yield and DMI compared to the HC cows. The cows fed LC compensated the low concentrate allowance by a markedly higher forage intake and thus the total DMI did not differ between treatments. The LC cows lost more in body condition, however the loss was not reflected by higher plasma NEFA concentrations. Both breeds apparently responded equally to the LC diet.

Acknowledgements This research was financed by Formas (The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning) and Swedish Farmers Association for Agricultural Research.

References

Drackley JK and Cardoso FC 2014. Animal 8, s1, 5-14.
Esposito G, Irons PC, Webb EC and Chapwanya A 2014. Animal Reproduction Science 144, 60-71.
Swensso C, Lindmark-Månsson H, Smedman A, Henriksson M and Modin Edman AK 2017. Journal of the Science of Food and Agriculture 97, 4890-4897

Nutritive value of fresh common ash (*Fraxinus excelsior)* leaves for growing rabbits

Farid Djellal[1], Si Ammar Kadi[2], Azeddine Mouhous[2], Hocine Guermah[3], Thierry Gidenne[4]

[1]Département d'Agronomie, FSNV, Université Ferhat Abbas, Sétif, Algeria, [2]Département des sciences agronompiques, Université M. Mammeri, UN1501, Tizi-Ouzou, Algeria, [3]Département d'Agronomie, M'sila, Algeria, [4]GenPhySE, Université de Toulouse, INRA, INPT, INPT, ENVT, Castanet Tolosan, Touilouse, France

E-mail: fariddjellal@yahoo.fr

Take home message Identification and assessment of local alternative sources for rabbit feeding.

Introduction Feed supply is the main constraint to livestock production development in Algeria, while appears a renewal interest for former resources of diets. Thus, alternative feeding systems are aiming to mobilize neglected natural resources. The genus Fraxinus (Oleaceae) is distributed mostly in the temperate regions and the subtropics of the Northern Hemisphere. The main problem to use the ash leaves in rabbit nutrition is the ignorance of its nutritive value. Therefore, the aim of the present study is to evaluate the nutritive value of fresh ash leaves (*Fraxinus excelsior*), harvested in autumn, for growing rabbits.

Materials & methods Ten local Algerian white rabbits, weaned at 35d old (BW: 881± 54g) individually caged, were used to assess to determine the nutritive value, by direct method, of fresh Ash leaves (*Fraxinus excelsior*) harvested in autumn season. Samples of Ash tree leaves were collected throughout the digestibility trial period mixed and stored in polyethylene bag at –20 ºC until the chemical analysis. After 12 days adaptation period, rabbits were used for the digestibility trial following the European reference method described by Perez *et al.* (1995). The chemical analyses were performed at INRA of Toulouse (UMR 1388 GenPhySe, France). Because only one diet was used in this study, it was not allowed to use mean comparison. Thus, the results are presented with mean and standard error.

Results The crude protein (CP) content was moderate, 147 g/kg DM for a fresh forage. The daily average intake of DM was 97 g, corresponding to 106 g/kg LW$^{0.75}$ (Table 1). With this daily consumption, rabbit did not show symptoms of digestive troubles like diarrhea or other during the entire test and achieve a moderate growth. The apparent digestibility coefficient of Ash leaves energy was 70 %. The DE corresponds to 11.86MJ/kg DM with a standard error of 0.36 calculated by the equation proposed by Villamide (1996) for estimation the energy values of feed ingredients by direct method and the DP was 79.72 with a standard error of 03.96 (Table 2).

Digestible energy of Ash leaves was higher than of olive leaves and alfalfa 15 (respectively 5.94 and 8.22 Mj/kg DM; Maertens *et al*., 2002). Digestible protein of those leaves (79.72 g/kg DM) was clearly higher than that of mulberry leaves (16.38 g/kg DM; Deshmukh *et al*., 1993) but also than alfalfa 15 (10.02 g/kg DM; Maertens *et al*., 2002).

Table 1 Body weight, feed intake and digestibility coefficient of the fresh ash leaves (*Fraxinus excelsior*) given as a sole ration for growing rabbits.

	Weight and feed intake		Digestibility (%)	
	Mean	SE	Mean (%)	SE
Initial body weight (g)	816	21		
Final body weight (g)	881	24		
Feed intake (g DM/day)	118.5	06		
Feed intake (g as fed/day)	232.3	13		
Digestibility coefficient				
Dry Matter (DM)			67.1	2.01
Organic matter (OM)			65.2	2.10
Crude protein (CP)			60.9	3.04
Neutral detergent fibre (NDF)			50.1	3.40
Acid detergent fibre (ADF)			43.9	4.02
Gross Energy			61.9	2.62

SE: standard error

Table 2 Nutritive value of fresh Ash leaves (*Fraxinus excelsior*).

	Dietary nutritive value	
	Mean	SE
DE (MJ/Kg DM)[1]	11.86	0.50
DP (g/Kg DM)[2]	79.72	3.96

DE : digestible energy. [2] DP : digestible crude protein

Conclusion The rabbits consume comfortably the Ash leaves (*Fraxinus excelsior*) harvested at autumn season. Nutritive value of those leaves was 11.86±0.50 MJ DE/kg DM and 79.72±03.96g DP/kg DM. Thus, Ash leaves can be useful ingredients in rabbit feeding.

References

Deshmukh SV, Pathak NN, Takalikar DA and Digraskar SU 1993. World Rabbit Science 1, 67-69

Maertens L, Perez JM, Villamide M, Cervera C, Gidenne T and Xiccato G 2002. World Rabbit Science 10, 157-166

Perez JM, Lebas F, Gidenne T, Maertens L, Xiccato G, Parigi-Bini R, Dalle Zotte A, Cossu ME, Carazzolo A, Villamide MJ, Carabaño R, Fraga MJ, Ramos MA, Cervera C, Blas E, Fernàndez-Carmona J, Falcao ECL, and Bengala FJ 1995. World Rabbit Science 3, 41-43.

Villamide MJ 1996. Animal Feed Science and Technology 57, 211-223.

Levels of wet distillers grains for F1 Angus-Nellore bulls finished in feedlot: dry matter intake and performance

Mateus S. Ferreira[1], Maria Betânia Niehues[2], Laís A. Tomaz[2], Luiz Antonio Fogaça[2], Pedro V. R. Paulino[3], Cyntia L. Martins[2], Mário Arrigoni[2], Otávio R. Machado Neto[1,2]

[1]São Paulo State University, School of Agricultural and Veterinarian Sciences, Jaboticabal, São Paulo, Brazil, [2]São Paulo State University, School of Veterinary Medicine and Animal Science, Botucatu, São Paulo, Brazil, [3]Cargill Animal Nutrition, Campinas, São Paulo, Brazil

E-mail: otaviomachado@fmvz.unesp.br

Take home message Wet distillers grains may partially replace corn in beef cattle diets.

Introduction The use of corn for ethanol production has increased in Brazil, enabling the use of wet distillers grains (WDG) in animal nutrition. There is no research on the use of WDG in diets for F1 Angus-Nellore bulls. In Brazil, this is a growing genetic group which often receives diets with high starch content and it may cause ruminal acidosis. Research with Angus-Nellore cattle is still necessary once this group is more susceptible to ruminal acidosis than purebred Angus animals. Furthermore, there is little information about de-oiled WDG, which was used in the present study. The use of WDG may be advantageous since they present high digestibility fibre. Therefore, the aim was to evaluate the effect of different levels of WDG on the dry matter intake (DMI) and performance of feedlot cattle.

Materials & methods A hundred F1 Angus-Nellore bulls with initial average body weight of 369 ± 48 kg and average age of 24 months were allocated in a completely randomized blocks design in group pens with 5 animals in each, with five pens per treatment where they were fed for 129 days. The WDG used in this study contained 320 g of CP/kg DM and 43 g of ether extract/kg DM. The diets presented: 148.4, 161.7, 191.0 and 220.0 g of CP/kg DM; 627.6, 540.4, 434.3 and 328.3 of non-fibre carbohydrates (g/kg DM); and 162.3, 234.6, 308.3 and 382.1 of NDF g/kg DM, according to the increasing levels of WDG (0, 150, 300 and 450 g/kg DM, Table 1). Statistical analyses were performed through SAS statistical software, using the Mixed procedure with linear, quadratic and 0 vs. WDG contrasts.

Table 1 Composition of experimental diets with different levels of distillers grains.

Ingredients (g/kg of DM)	0	150	300	450
Sugarcane bagasse	71.6	71.1	71.1	71.1
Tifton-85 hay	42.0	42.0	42.0	42.0
Ground corn	748.9	652.7	520.0	387.3
Soybean meal	103.3	47.7	29.3	10.9
Wet distillers grains (WDG)	-	150	300	450
Potassium chloride	-	2.30	3.40	4.50
Mineral-vitaminic supplement	34.2	34.2	34.2	34.2

Results DMI increased with the inclusion of WDG when compared to the control treatment, and it can be due a lower subacute acidosis probability. The replacement of corn by WDG tended to increase ADG (P = 0.06). When converted to ketone bodies, the excess protein in WDG diets is used as energy source which may have contributed to the ADG in these diets (Klopfenstein *et al.*, 2008). Animals fed WDG had higher slaughter weight (Table 2). The cost of 129 days on feed was US$ 278.8; 273.8; 257.6 and 241.4 per head according to the increasing levels of WDG. Despite its low fat content, de-oiled WDG can increase the profitability of feedlot operations when used to replace corn in high energy diets.

Table 2 Performance of F1 Angus-Nellore bulls fed different WDG levels.

	WDG levels (g/kg of DM)					Effects		
Item	0	150	300	450	EPM	Linear	Quadratic	0 vs WDG
Initial body weight (kg)	369	370	370	369	22.9	0.94	0.65	0.82
Dry matter intake (kg/d)	10.74	11.53	11.44	11.35	0.332	0.13	0.09	*
Average daily gain (kg/d)	1.80	1.91	2.01	1.90	0.090	0.11	0.10	0.06
F. Efficiency (kg ADG/kg DMI)	0.169	0.165	0.176	0.170	0.010	0.56	0.86	0.85
Slaughter weight (kg)	601	616	630	615	18.8	0.11	0.06	*

Conclusion High inclusion of WDG does not impair the performance and increases dry matter intake.

Acknowledgements This research was supported by grants from FAPESP, São Paulo, Brazil.

References

Klopfenstein TJ, Erickson GE and Bremer VR 2008. Journal of Animal Science 86, 1223-1231.

Nutritional evaluation of *Calotropis* for ruminants

Dongmei Xu[1,2], Lu Ma[1,4], Liansheng Zhao[1], Ying Ma[1], Jianchu Xu[5,1], Bill Weiss[3], Dengpan Bu[1,4]

[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science,Chinese Academy of Agricultural Sciences, Beijing, China, [2]Lanzhou University, Lanzhou, China, [3]The Ohio State University, Wooster, USA, [4]CAAS-ICRAF Joint Laboratory on Agroforestry and Sustainable Animal Husbandry, World Agroforestry Centre, East and Central Asia, Beijing, China, [5]Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China

E-mail: budengpan@caas.cn

Take home message The *Calotropis* can be a candidate feedstuff for ruminates.

Introduction The *Calotropis* as an evergreen shrub with high biomass production and grows well in a variety of soils and different environments. It has been used for medicinal purposes and as substitute for paper, strings and cloth. However, there is little information about the nutritional value of *Calotropis* when fed to ruminants. The purpose of the study was to evaluate the nutrient value of the *Calotropis* and explore its potential as a new feed resource for ruminants.

Materials & methods Samples of the *Calotropis* (included both leaves and stems) were collected and oven-dried at 65 °C for 72 h and ground through 1.0 mm Wiley mill for chemical, energy and digestibility analysis. We measured the chemical compositions of the *Calotropis* and computed the protein and carbohydrate fractions by using the Cornell Net Carbohydrate and Protein System (CNCPS) model. *In vitro* gas production with an automated trace gas recording system was measured to estimate the metabolic energy concentration of *Calotropis*. In situ ruminal degradation was determined by incubating bags (3 g of sample) in the rumen for 0, 2, 4, 8, 12, 18, 24, 30, 48, 72 h. After incubation bags were taken from the rumen and washed with cold tap water and oven-dried, and then the residue was assayed for DM, NDF, ADF and CP. The effective degradability of DM, NDF, ADF and CP were calculated by the equation: ED = a+b[c/(c+k)], here c= 0.05 h^{-1}.

Results The nutrient components of the *Calotropis* are shown in Table 1. Metabolic energy was estimated using gas production data and ME ranged from 1.83 to 2.20 Mcal/kg. The degradability of DM, CP, NDF and ADF is exhibited in Figure 1. The DM and CP degraded faster than fiber, and the degradability of DM and CP reached 90 % by about 18 h and the fiber reached about 70 % by 24 h. When calculated by exponential equation, the effective degradability (ED) of DM was 73.04 %, CPD was 82.46 % DM and the NDFD and ADFD was 56.7 and 53. 5 % DM respectively.

Table 1 Chemical composition of stem and leaves together sample of the *Calotropis*.

Item	g/100g, % DM
DM	95.16±0.083
NDF	28.19±0.463
ADF	17.80±0.284
CP	25.95±0.412
EE	3.86±0.087
Ash	17.14±0.069
Starch	2.72±0.138
NDFIP	4.17±0.013
ADFIP	1.16±0.357
SCP	13.60±0.412
NPN	10.38±1.416
ADL	0.64±0.223

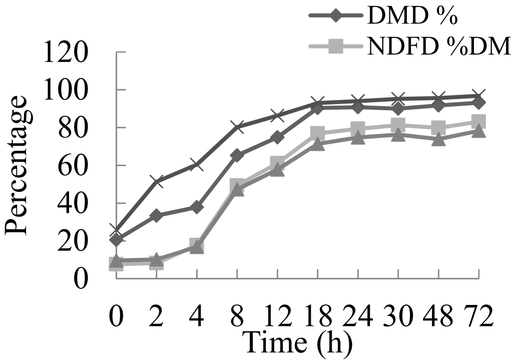


Figure 1 Dynamic ruminal degradability for DM, NDF, ADF and CP of the *Calotropis.*

Conclusion The *Calotropis* contained much moderate concentrations of crude protein and fiber, and the fiber had high in situ digestibility. Estimated ME concentrations were less than values for average corn silage. These data indicate *Colotropis* has potential as a feedstuff but *in vivo* studies are needed.

Acknowledgements This research was supported by the Agriculture Science and Technology Innovation Program (ASTIP-IAS07), Chinese Academy of Agricultural Science and Technology Innovation Project（CAAS-XTCX2016011-01) and Beijing Dairy Industry Innovation Team (BJDIIT) .

551

Relationship between propanol content and fermentation parameters in silage

Maksim Runin, Marko Kass, Andres Olt

Estonian University of Life Sciences, Tartu, Estonia

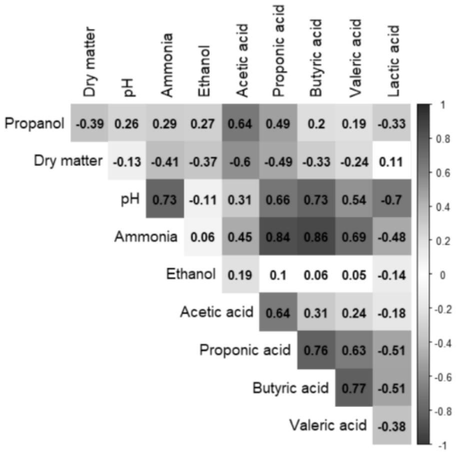
E-mail: maksim.runin@gmail.com

Take home message High propanol concentration is more likely to appear in silages with low dry matter, and it is related with propionic and acetic acid concentrations.

Introduction High quality forage and balanced feeding of herbivores are essential for animal health and production. Silage is the main feed component for dairy cattle and its quality is very important. Previous study showed that high concentrations of propanol in ration can negatively influence milk fat percentage (Raun, Kristensen 2012). As propanol content in silage is not routine analysed, there is limited data about propanol concentration in silage. The objective of study was to evaluate propanol contents in silages, and to examine relationships between propanol and other silage fermentation parameters.

Materials & methods 1,678 grass and maize silage samples were collected over a one-year period from dairy farms in Estonia. Chemical analysis of samples was determined by routine analytical procedures. Fermentation parameters (acids, alcohols) were measured using gas chromatography. For statistical analysis only grass silage samples (n=540) with propanol concentration more than 0.5 g/kg of dry matter (DM) were taken, confirming fermentation with propanol as an end product. Correlation analysis was performed (R statistics) to find correlations between propanol and other fermentation parameters. In addition, samples were grouped based on criteria (DM, cuts, additives or ensiling technology) and samples with reported criteria were used in group analysis. Mean propanol concentrations were found within the groups to determine the effect of criteria on propanol concentration in the silage.

Results & discussion Mean propanol concentrations in all samples was 1.3 (SD=3.0) g/kg DM and in grass silage samples chosen for statistical analysis 3.65 (SD=4.2) g/kg DM. Correlation analysis (Figure 1) showed correlation between propanol and acetic acid (r=0.64, p<0.01) and propionic acid (r=0.49, p<0.01). Statistical analysis within the groups (Figure 2) showed significant difference in mean propanol concentration within DM group (p<0.01 between all three pairwise). Mean propanol content was lower in big bales silages (n=24) compared to other (clamp and pile) ensiling methods (p<0.01). No effect of cut, DM or additives was found for big bales due to small number of samples.



	Dry matter	pH	Ammonia	Ethanol	Acetic acid	Proponic acid	Butyric acid	Valeric acid	Lactic acid
Propanol	-0.39	0.26	0.29	0.27	0.64	0.49	0.2	0.19	-0.33
Dry matter		-0.13	-0.41	-0.37	-0.6	-0.49	-0.33	-0.24	0.11
pH			0.73	-0.11	0.31	0.66	0.73	0.54	-0.7
Ammonia				0.06	0.45	0.84	0.86	0.69	-0.48
Ethanol					0.19	0.1	0.06	0.05	-0.14
Acetic acid						0.64	0.31	0.24	-0.18
Proponic acid							0.76	0.63	-0.51
Butyric acid								0.77	-0.51
Valeric acid									-0.38

Figure 1 Correlation matrix of fermentation parameters. Intensity of grey shows the strength of correlation. Correlation coefficients brought inside the boxes. White background refers to p-value >0.05.

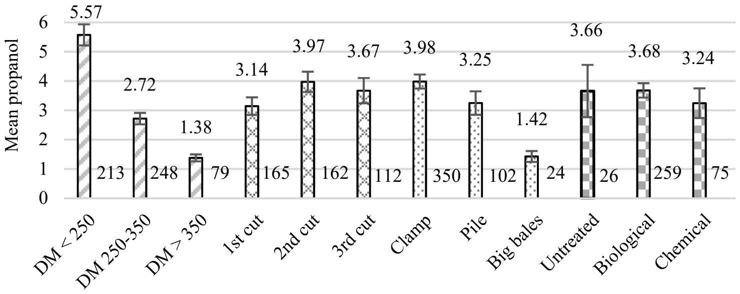


Figure 2 Mean propanol concentration in groups. Top numbers show mean propanol concentration of group, bottom number – number of samples in group.

Conclusion A correlations between propanol, acetic and propionic acid concentrations was found. Higher propanol content is more likely to be produced in silages with low amount of DM. Average propanol concentration of silages ensiled in big bales is found to be less than in other types of ensiling technologies. Differences in usage of additive in ensiling process have not been found.

Acknowledgements The study was funded by the Estonian Rural Development Plan measure 16.2 of the Estonian Ministry of Rural Affairs. We are extremely grateful for for the cooperation of the staff of Torma POÜ.

References

Raun BML and Kristensen NB 2012. Livestock Science. 144, 37–47.

Identification of a first set of criteria for a multicriteria evaluation of diets for ruminants

Gaëlle Maxin, Pierre Nozière, René Baumont

Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, Saint-Genès-Champanelle, France

E-mail: gaelle.maxin@inra.fr

Take home message Twenty-three criteria are proposed to assess the impacts of diets for dairy ruminants on animal production, animal health, environment and milk quality.

Introduction To meet the sustainability challenge, ruminant diet evaluation concept has to cover new objectives other than feed value. Some feed evaluation systems have already begun to evolve by proposing multiple animal responses regarding wastes and animal health (INRA, 2018), but none of these systems propose an exhaustive set of criteria for assessing all impacts on animal health, environment or product quality. The objective of this work was to define criteria to assess the diets for ruminant on the 4 categories of impacts (animal production and health, environment, and quality of the animal products) using collaborative construction.

Materials & methods Literature search and expert consultations were performed to define and organize the set of criteria. Different experts were consulted: scientists specialized in the different aspects of ruminant production and farm advisors. A first set of criteria was obtained; this set was then underwent a second round of consultation with 9 experts from very different spheres (research, vet, technical institute and animal production company) in order to correct/complete the criteria. We chose to perform the evaluation at the animal scale to fit better with the control level of farmers. Thus, the set of criteria had to consider the effects of feeding on the four impacts (production, health, environment and product quality) considered at animal scale. We only detailed here the set of criteria adapted to dairy ruminants.

Results & discussion Twenty-three criteria ranked in the four categories of impacts and 11 dimensions are proposed to assess the different impacts of diets for dairy ruminants. For animal production, 3 criteria were identified (Table 1). For animal health, only health problems related to animal feeding were considered, excluding very specific health problems and those in which nutrition plays only a minor part compared with farm conditions or genetics. Defining criteria of product quality was more complex, as they depend on animal and the purpose of the product. Also, origin of quality is complex and multifactorial. Thus, in this first study we focused on milk quality excluding derived products and on criteria on which feeding has a marked and proven effect. At animal scale, environmental impacts imply essentially wastes. Nutrients losses that cause environmental concerns are phosphorus, nitrate, methane and ammonia. Thus, criteria retained were methane, nitrogen and minerals emissions.

Table 1 Dimensions and criteria proposed to assess the impacts of diets on dairy ruminant production and health, wastes and milk quality.

Impacts	Dimensions and criteria identified
Animal production	Milk production: insure intake to cover energy requirements (C1); cover protein (C2), minerals and micronutriment (C3) requirements
Animal health	Toxicity: avoid antinutritional compounds (C4); avoid risks linked to feed conservation (C5); avoid minerals excess (C6)
	Digestive diseases: limit risk of acidosis (C7); bloat (C8); alkalosis (C9); ketosis (C10)
	Parasitism: supply optimal quantity of tannins (C11)
	Oxidative stress: supply optimal quantity of antioxidants (C12)
	Reproduction: boost the reproductive performances (C13)
Milk quality	Nutritional quality: improve the protein (C14) and the fat contents (C15); the fatty acid profile (C16) and the micronutrients content (C17)
	Sensorial quality: avoid feedstuffs causing unpleasant flavour (C18); obtain a desirable color (C19)
	Milk stability: boost the antioxidant quantity (C20)
Environment	Wastes: limit methane emitted (C21); reduce nitrogen (C22) and minerals (C23) released

Conclusion We proposed a first set of criteria to assess the impacts of diets on animal production and health, environment and milk quality. The next steps will be to identify the potential indicators that need to be collected at feedstuff scale to ensure the conformity of diets with each criterion. This work constitutes the first step required to develop a multiobjective formulation tool of diets.

Acknowledgements The authors thank all experts they consulted for help in building this first set of criteria.

References

INRA, 2018. Wageningen Academic Publishers, Wageningen, The Netherlands

In vitro degradability and nutritional variability of tropical forage according to grazing systems and maturity levels

Adibe Luiz Abdalla[1], Gabriel Zanuto Sakita[1], Wilian dos Santos da Costa[1], Vagner Ovani da Silva[1], Beatriz Elisa Bizzuti[1], Alessandra Aparecida Giacomini[2]

[1]*University of São Paulo, Centre for Nuclear Energy in Agriculture, Piracicaba, São Paulo, Brazil,* [2]*Animal Science Institute, Nova Odessa, São Paulo, Brazil*

E-mail: wiliancosta@usp.br

Take home message Grazing strategies and forage management are potential tools to achieve livestock sustainability.

Introduction Tropical forages are the most abundant ruminant feed for grazing worldwide, and different pasture management have been proposed to enhance livestock production. The nutritional value of forages change during their growth stages, influencing nutrients availability according to the stage of maturity. Our aim was to evaluate the degradability and nutritional values of *Brachiaria brizantha* cv. Marandu in different grazing systems and two distinct maturity levels.

Materials & methods Samples from randomly selected sites were harvested at Instituto de Zootecnia (IZ, Nova Odessa, Brazil) in four different grazing systems: Continuous grazing (CG), Crop-livestock integration (CL), Forest-pasture integration (FP) and Consortium of *B. brizantha* with *Macrotilloma axillare* (CS). The samples were dried (72 h at 60°C); stems, leaves and senescence materials were separated, weighted (to determine their proportions), gathered and grinded (1 mm). Regarding senescence stage (% of senescence material), each sample was classified into Before Senescence (BS; ≤8 %) and After Senescence (AS; >8%). Chemical composition parameters were determined according to AOAC (2011) and Van Soest *et al.* (1991). For the *in vitro* gas production (IVGP), forage samples and ruminal content were incubated for 24 h (Bueno *et al*., 2005). The fermentation gas pressure (psi) was measured at 4, 8, 12 and 24 hours then converted to mL of gas (GP). Accumulated gas (mL/g dry matter (DM)) values were submitted to the France model (France *et al*., 1993) in order to estimate colonization time (L, h), potential gas production (A, mL), gas production rate (µ, mL/gDM/h) and time to achieve half of the potential GP (T ½, h). After 24 h the IVGP was stopped and solid residue was washed with neutral solution for determining *in vitro* truly degraded organic matter (TDOM) (Goering and Van Soest, 1970). Statistical analysis of chemical composition and IVGP data were performed using Sisvar ®. France model parameters were estimated using the nonlinear procedure (PROC NLIN) of SAS® v9.3. The values obtained were submitted to analysis of variance. Fixed effects of grazing systems, maturity levels and their interaction were tested and for all procedures P<0.05 level of significance was used.

Results & discussion Chemical composition and IVGP parameters in the different grazing systems and maturity levels are present in Table 1. Lower values of neutral detergent fibre (NDF), acid detergent fibre (ADF), TDOM, net gas (NetGP) and net methane ($NetCH_4$) productions were found for CG treatment when compared to the others evaluated grazing systems (P<0.01). Higher values of soluble carbohydrates explain higher NetGP found for CS and CL treatments. Also, among grazing systems, differences in L and T ½ values were found (P<0.05; data not shown). Regarding maturity levels, higher values of NDF, ADF, crude protein (CP), TDOM, NetGP and $NetCH_4$ were found in the BS treatment (P<0.01). In addition, better ruminal environment for microbial colonization was found in BS treatment, fact that is reflected in lower (P<0.05) L value when compared to AS (0.47 and 0.59 ± 0.038, respectively). There was no significant (P>0.05) interaction between grazing systems and maturity levels.

Table 1 Chemical composition and IVGP parameters in the different production systems and maturity levels evaluated.

Parameters	Grazing systems				S.E.	P value	Maturity levels		S.E.	P
	CS	FP	CG	CL			BS	AS		value
NDF (g/kg DM)	632.4a	685.8a	764.3b	656.5a	17.4	.0003	639.1a	707.0b	12.6	.0013
ADF (g/kg DM)	350.8a	371.8a	439.6b	375.0a	14.1	.0028	355.1a	400.1b	10.2	.0063
CP (g/kg DM)	118.6a	137.6a	66.1b	107.1ab	12.0	.0050	125.8a	96.8b	8.7	.0309
TDOM (g/kg)	540.9a	493.0a	399.9b	510.3a	18.4	.0004	526.1a	465.8b	13.4	.0051
NetGP (ml/g DM)	108.1a	91.1bc	82.3c	100.0ab	2.82	.0000	102.9a	91.5b	2.0	.0010
$NetCH_4$(ml/g TDOM)	4.8a	3.8a	2.9b	4.21a	0.2	.0004	4.43a	3.7b	0.1	.0090

a,b,c - Means followed by same letters in the same row do not differ according to Tukey test (P>0.05); S.E. - Standard Error.

Conclusion Both grazing systems and maturity levels affected the degradability and nutritional value of *B. brizantha*.

Acknowledgements Animal Nutrition Laboratory (CENA/USP) for their assistance and IZ for providing the forage samples.

References

AOAC. 2011. Official Methods of Analysis of AOAC International.
Bueno ICS, Cabral Filho SLS, Gobbo SP, Louvandini H, Vitti DMSS and Abdalla AL 2005. Animal Feed Science and Technology 123, 95-105.
France J, Dhanoa MS, Theodorou MK, Lister SJ, Davies DR and Isac D. 1993. Journal of Theoretical Biology 163, 99-111
Goering, HK and Van Soest PJ 1970. Forage fiber analysis. Agricultural Handbook. .
Van Soest PJ, Robertson JB and Lewis BA 1991. Journal of Dairy Science 74, 3583-3597.

Inclusion of insect meals *Hermetia illucens* and *Tenebrio molitor* in a diet for dairy cows: effect on *in vitro* ruminal linoleic and linolenic acid biohydrogenation

Andrea Cabiddu[1], Olga Gachiuta[2], Giovanni Molle[1], Paolo Bani[2]

[1]*Agris, Animal Production Science Department, Bonassai, Olmedo, Italy,* [2]*DIANA - Dipartimento di Scienze Animali, della Nutrizione e degli Alimenti (Università Cattolica Sacro Cuore), Piacenza, Italy*

E-mail: acabiddu@agrisricerca.it

Take home message Addition of insect meal in a conventional dairy cow diet did not affect ($P > 0.05$) ruminal linoleic and linolenic acid biohydrogenation.

Introduction The use of insects in animal feed could improve the sustainability of animal diets and contribute to meet the growing global demand for livestock products. The costs of conventional feed resources such as soymeal and fishmeal are very high and moreover their availability in the future will be limited. Insect rearing could be a part of the solution. In a scenario of a continuous increasing demand by consumers of animal products with high standard nutritional value the inclusion of insect meal could be of interest due to their high level of unsaturated fatty acid. The aim of this research was to evaluate by *in vitro* test the influence of HI and TM meal inclusion in diet for dairy cow on ruminal biohydrogenation of linoleic (BC182) and linolenic (BC183) acids.

Materials & methods Two full fat (FF) or defatted (DEF) insect larval meals (*Hermetia illucens* (HI) and *Tenebrio molitor* (TM)) were studied. Five treatments were adopted: in a control diet (CTR, typical dairy cow diet based on 44% concentrates and 56% roughage (corn silage+hay), calcium salts of palm fatty acids and soybean protein concentrates were substituted for HI-FF, HI-DEF, TM-FF or TM-DEF to keep the diet isolipidic (5.9% CF, DM basis) and isonitrogenous (16.8% CP, DM basis). Percentage of inclusion of insect meals ranged from 3.31 to 4.84, on DM basis. Each substrate was incubated over two time points (0 and 6 h) with buffered rumen inoculum from 3 rumen cannulated heifers (2 replicates/heifer) according to Ahmed *et al.* (2013). Substrate (2 g DM) were used and 11 ml of medium plus 9 mL of rumen inoculum prepared according to Cabiddu *et al.*, (2010) was added. Incubation was carried out into 60 mL tubes for 0 and 6 h. Data (6 replicates /treatment) was analysed to test the effect of dietary treatments on BC182 and BC183 by two models GLM, 1. to compare CTR diet to meal including insect meals; and 2. to assess the fixed effects of insect species, fat level and their interaction.

Results & discussion Content of ether extract (EE) in HI-FF, HI-DEF TM-FF and TM-DEF meals were 36.26, 3.58, 31.55 and 3.58% DM, respectively. Higher levels of unsaturated fatty acid were found in TM than in HI meal (75% vs 45% FAME). Level of PUFA in HI-FF diet was increased by 30% and in TM-FF diet by 45% compared to CTR one. Compared to CTR diet, linolenic acid was 28 and 51% higher in HIFF and TMFF, respectively. Linolenic acid content increased by 34% in HIFF without any major change in TMFF. Overall, the inclusion of insect meal (either FF or DEF) did not affect BHC182 and BHC183 ($P > 0.50$, data not shown). The interaction between insect and fat level was significant in treated samples (Table 1). Using TM-DEF increased significantly BC182 and BC183. The bigger increase of biohydrogenation in TM is probably due to the higher level of PUFA in TM compared to HI.

Table 1 Effect of inclusion of *Hermetia illucens* (HI) and *Tenebrio molitor* (TM) either as full fat (FF) or defatted (DEF) meal in the diet of a dairy cows on *in vitro* ruminal biohydrogenation (BH) of C18:2 and C18:3 (values expressed as percent of initial fatty acids).

	Treatments				Sem	Effects		
	HI-FF	HI-DEF	TM-FF	TM-DEF		I	F	I*F
BC182	25.01 ^{a}	20.40 ^{a}	15.08 ^{a}	46.39 ^{b}	3.30	ns	*	**
BC183	28.56 ^{a}	31.37 ^{a}	22.18 ^{a}	52.64 ^{b}	3.16	ns	**	**

Sem = standard error of mean; I = insect species; F= fat; a, b, c = superscript letters within the same row represent significant differences between treatments; * $P < 0.05$; ** $P < 0.01$

Conclusion Overall, the inclusion of insect meal in a convention dairy cow diet did not influence the ruminal BC182 and BC183. Different process of insect larval meals (FF or DEF) associated to the different PUFA profile found in HI and TM could strongly modify the ruminal BH pathway with different magnitude.

References

Ahmed S, Minuti A and Bani P 2013.Italian Jouranl of Animal Sciences 12, 359-365

Cabiddu A, Salis L, Tweed JKS, Molle G, Decandia M and Lee MRF 2010. Journal of the Science of Food and Agriculture 90, 829–835

Ammoniated alkaline barley as a raw material in concentrates fed rumen cannulated Norwegian Red dairy cows: a pilot study evaluating effects on rumen pH and digestibility

Stine Gregersen Vhile[1], Egil Prestløkken[1], Dag-Kristoffer Forberg[1], Inger Johanne Karlengen[2]

[1]*Norwegian University of Life Sciences, Ås, Norway*, [2]*Norgesfôr AS, Oslo, Norway*

E-mail: stine.vhile@nmbu.no

Take home message Ammoniated alkaline barley is a suitable raw material and can be used in concentrates for dairy cows with no negative effect on rumen pH or total tract digestibility.

Introduction Sustainable milk- and meat production based on a high degree of self-sufficiency and local feed resources is a prioritized political goal in Norway. However, in 2015, 95% of the protein- and 27% of the carbohydrate raw materials used in concentrates to land based production animals were imported. The objective of the present study was to evaluate the effect of a novel raw material, based on locally produced ammoniated alkaline barley, in concentrates to dairy cows on rumen pH and total tract digestibility. Ammoniated alkaline barley is produced by mixing mature, whole grain and Home n´Dry® pellets. The mix is stored under plastic for three weeks while a chemical process takes place and ammonia is released. The treatment results in an alkaline, nitrogen-enriched product that can be used as a raw material in concentrates for ruminants.

Materials & methods One feeding experiment with six rumen cannulated dairy cows of the Norwegian Red breed was conducted at the Experimental Farm of the Norwegian University of Life Sciences, Ås, Norway. The experimental animals were blocked by days in milk (DIM) and by yield and divided into three dietary treatments in a 3 x 3 Latin Square design with two animals per treatment. The dietary treatments were grass silage (*ad libitum*) and one of the concentrates (i) Con A added ammoniated alkaline barley (ii) Con B added maize, soybean meal and beet pulp (positive control) and (iii) Con C added urea and barley (negative control). The concentrates were formulated to be isocaloric (NEl/kg DM) and isonitrogenous (CP/kg DM). Con A and Con C were similar in starch and sugar content. The experimental period was 63 days, divided into three periods consisting of a 14-day basal period where the animals were fed 11 kgs of concentrate, followed by a seven-day-period with increasing levels of concentrates. On day 12 in each period pH was measured continuously for 24 hours. A 72-hour-period of total collection of faeces and urine was performed from day 12 to 14. Differences among treatments were analysed using analysis of variance (ANOVA) by the GLM procedure SAS (2000) using cow, period and treatment as fixed effects. The feeding experiment was in accordance with Norwegian legislation controlling experiments with animals. All results presented in this summary are from the basal period.

Results & discussion There were no significant differences in total tract digestibility or rumen pH between the dietary treatments ($P \geq 0.05$). However, interesting numerical differences in total tract digestibility and rumen pH were observed. This could indicate either an alkaline effect in the rumen making the rumen pH more favourable for fermentation or a direct effect of the alkaline treatment on digestibility of the nutrients in the alkaline barley product. Con B was expected to be the higher in rumen pH than Con A and Con C due to the composition and the chemical characteristics of the concentrates. Con B contained starch from maize with a slower degradation rate in the rumen compared to starch from barley. In addition, Con A and Con C were higher in total starch and sugar content compared to Con B because the latter contained beet pulp. Con C had numerically longer periods with rumen pH below 5.8 compared to Con A and Con B measured over a 24-hour-period.

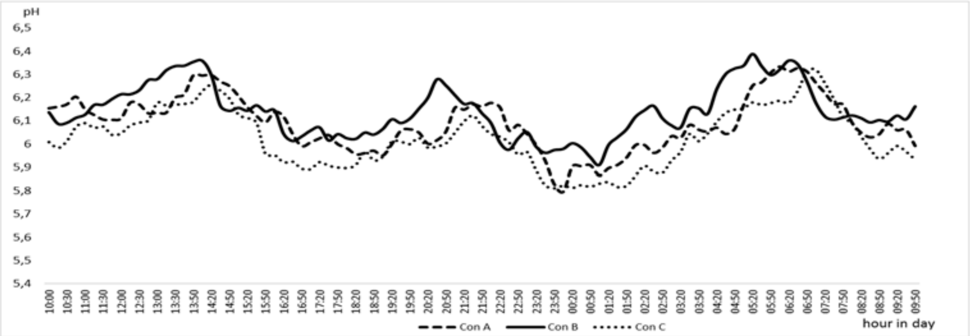


Figure 1 Effect of different different isocaloric and isonitrogenous concentrates fed rumen cannulated dairy cows (n=6) on 24-h rumen pH. Con A: Added ammoniated alkaline barley, Con B: Added maize, soybean meal and beet pulp, Con C: Added barley and urea.

Conclusion Ammoniated alkaline barley is a suitable raw material that can be used in concentrates for dairy cows with no negative effect on rumen pH or total tract digestibility. In addition, interesting indications on increased digestibility and rumen pH from this pilot study should be investigated further in a larger and longer lasting production trial with dairy cows.

Acknowledgements This research was supported by the Norwegian Agricultural Agency, Norgesfôr AS and Strand Unikorn.

In situ rumen degradability and *in vitro* gas and methane production of tannin-rich plants from tropical origin

Moufida Rira[1,2], Diego P Morgavi[1], Lucette Genestoux[1], Angélique Quereuil[1], Harry Archimède[3], Emmanuel Tillard[4], Michel Doreau[1]

[1]INRA UMRH, Saint Genès Champanelle, France, [2]Ecole Nationale Supérieure de Biotechnologie, Nouvelle ville Ali Mendjeli, Algeria, [3]INRA URZ, Petit Bourg, Guadeloupe, [4]CIRAD, Saint Pierre, Reunion

E-mail: moufida_r@yahoo.fr

Take home message Some tropical plants containing condensed and hydrolysable tannins may reduce enteric methane production while maintaining a high rumen dry matter degradability.

Introduction Trees and shrubs play an important role in ruminant nutrition in tropical areas, but their intake and nutritive value is often limited by anti-nutritional factors such as tannins (Waghorn, 2008). However, a moderate content of condensed tannins (CT) can reduce methane production without adverse effect on rumen fermentation. In opposition, the effect of hydrolysable tannins (HT) is scarcely known. This study investigated the in situ ruminal degradation and *in vitro* fermentation and methane production of 6 tropical tannin-rich plants containing different proportions of CT and HT.

Materials & methods Tropical tannin-rich plants consisted of leaves of *Calliandra calothyrsus* (CC) from Réunion Island, of *Leucaena leucocephala* (LL). *Gliricidia sepium* (GS), *Manihot esculenta* (ME), *Musa sp* (MS), both from Guadeloupe Island, and of *Acacia nilotica* pods (AN) from Senegal. Condensed tannins were determined by the butanol-HCl method with 3 fractions: free, bound to proteins and bound to fibre. Hydrolysable tannins content was determined by the rhodanine method for gallotannins and by the potassium iodate method for ellagitannins. *In situ* rumen degradability was measured by the nylon bag technique using three rumen cannulated Holstein cows. Bags containing plants were incubated in the rumen for 0, 3, 6, 12, 24, 48 and 96 h. Kinetics of degradation was fitted to an exponential model with lag time (McDonald, 1981) and dry matter degradability was calculated using a passage rate of 0.04 h^{-1}. *In vitro* gas and methane production were measured in a batch system for 24 h of incubation with rumen fluid from 4 sheep in 4 successive runs. Data were submitted to an ANOVA with a mixed model; means were compared using a Tukey test.

Results & discussion All plants had a high CT level (Table 1), which limits the level of inclusion in diets for preventing adverse effects on ruminant nutrition. A large fraction of CT was bound to proteins, ranging between 32 and 61% for AN et GS, respectively. Hydrolysable tannins concentration was high for AN, moderate for CC and negligible for other plants.

Table 1 Tannin content and composition of tropical tannin-rich plants.

	AN	CC	MS	GS	LL	ME
Total condensed tannins, g/kg DM	160	360	130	110	180	170
Condensed tannins bound to proteins, % of total	32	35	45	61	46	57
Condensed tannins, bound to fibre, % of total	10	12	26	34	24	7
Gallotannins, g/kg DM	84	6	traces	traces	traces	traces
Ellagitannins, g/kg DM	266	27	4	9	13	12

Dry matter degradability and gas production were higher for GS, ME, LL and AN than for CC and MS. These latter had a low degradation rate in the rumen (Table 2). The low degradability of CC might be explained by the high CT content. In contrast, low degradability of MS seems to be due to other factors. Lowest methane production was recorded for AN, CC and MS; AN was the only plant that reduced methane production without affecting gas production.

Table 2 Methane, *in vitro* fermentation and *in situ* dry matter degradability of tropical tannins rich plants.

	AN	CC	MS	GS	LL	ME	SEM	*P* value
In vitro total gas production, mL / 24h	23.26^{b}	16.25^{c}	11.90^{c}	28.01^{a}	24.28^{ab}	27.95^{a}	1.438	< 0.001
In vitro methane production, mL / 24h	1.75^{c}	1.66^{c}	1.41^{c}	3.54^{a}	2.59^{b}	4.00^{a}	0.165	< 0.001
In situ degradation rate (c), h^{-1}	0.046^{b}	0.025^{b}	0.032^{b}	0.078^{ab}	0.090^{ab}	0.203^{a}	0.0145	< 0.001
In situ dry matter degradability, %	65.42^{a}	30.84^{c}	32.24^{c}	64.92^{ab}	63.20^{b}	65.46^{a}	0.484	< 0.001

Conclusion AN, GS, LL, ME can serve as potential source of feed in spite of their CT. *Acacia nilotica* pods can be used as anti-methanogen plant included in diets owing to its high *in vitro* fermentation and *in sacco* degradability. Additional studies are necessary using various vegetative stages and varieties before drawing definitive conclusions about these plants.

References

McDonald IM 1981. Journal of Agricultural Science 96, 251-252.
Waghorn G 2008. Animal Feed Science and Technology 147, 16-139.

Supplementing with grass silage or restricting pasture: strategies to overcoming short term feed deficits in the diet of early lactation dairy cows in early spring

Aisling Claffey[1,2], Tommy Boland[2], Luc Delaby[3], Emer Kennedy[1]

[1]*Teagasc Moorepark AGRIC, Fermoy, Ireland,* [2]*UCD School of Agriculture and Food Science, Dublin, Ireland,* [3]*INRA, Saint-Gilles, France*

E-mail: aisling.m.claffey@teagasc.ie

Take home message Offering early lactation cows 60% of their required pasture allowance for up to two weeks, during periods of grass deficit, can result in the production of similar quantities of milk compared to cows offered grazed grass and grass silage.

Introduction Supplementation with grass silage, has traditionally been used to manage early spring pasture deficits; however, it is more costly and of lower nutritive value than grass (Dillon *et al.*, 1995). The purpose of this study was to determine the effects of restricting pasture allowance (PA) or supplementing with grass silage over a six week period in early lactation in comparison to unrestricted PA.

Materials & methods From March 14, 2016, 105 cows were assigned to one of six treatments for a period of six weeks in early lactation. Cows were randomised based on breed, parity, calving date and pre-experimental production. The control (C) treatment was allocated a 100% PA to maintain a residual above 3.5 cm. For week 1-2, two treatments were replicated and offered 60% PA (E60; Figure 1). From week 3 to 6, one E60 treatment returned to 100% PA (E60x2) while the other treatment remained at 60% PA for 6-weeks (E60x6). During week 1-2, the remaining replicated treatments were allocated 50% PA by day and *ad libitum* grass silage by night (M60 and L60). From week 3-6, M60 cows were offered a 60% PA for two (M60x2) or four weeks (M60x4). The replicated L60 treatment remained on *ad libitum* grass silage by night during weeks 3-4 and from week 5 was offered a 60% PA for two weeks. Fresh PA was allocated after each milking; above 3.5 cm. Fresh silage was offered each night. The silage fed was of good quality; 75 % dry matter digestibility and 12 % crude protein. Milk yield was recorded daily while composition was measured on a weekly basis. Milk yields were summed after two, four and six weeks for cumulative analysis. Variables associated with production were analysed using PROC MIXED in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The model contained terms for treatment, days in milk and pre-experimental production which was centred within breed and parity.

Results & discussion Milk production (MP) was higher for the C cows throughout the experiment; after 6 weeks they had produced 114 kg milk cow^{-1} more than all other treatments demonstrating that an all pasture diet is more beneficial than supplementing with grass silage or restricting PA. After two weeks MP was greater for cows offered pasture by day and grass silage by night than those offered a restricted PA (Figure 2a), however, during weeks 2-4 an increased PA allowed the E60x2 treatments to increase their MP and produce a similar yield to M60 and L60 (587 kg milk cow^{-1}) after four weeks which indicates that imposing a two week restriction on cows does not impair MP when compared to cows offered grass by day and silage by night. The MP of E60x6 was lower than all other treatments after four weeks (551 kg milk cow^{-1}). Cumulative MP at six weeks was similar for the E60x2, M60x2, M60x4 and L60 treatments (875 kg milk cow^{-1}, Figure 2b). The M60x2 and M60x4 did not differ to the E60x6 (853 kg milk cow^{-1}) after six weeks. Cows offered silage, however, produced milk at a higher cost than those offered grass only.

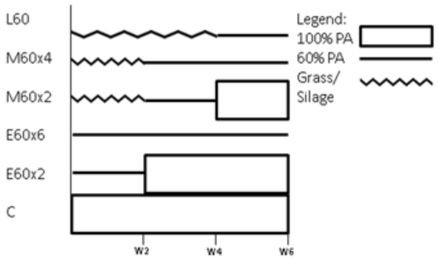


Figure 1 Treatment description.

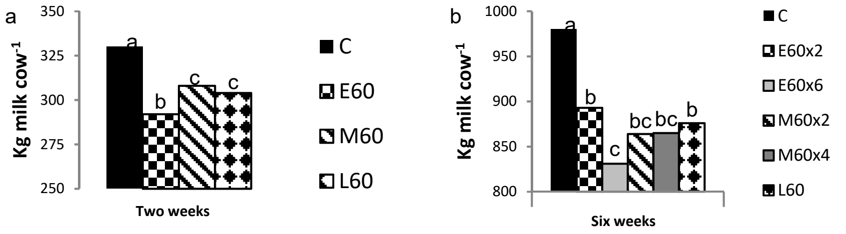


Figure 2 Cumulative milk production (kg milk cow^{-1}) at week 2 (a) and 6 (b).

Conclusion An all pasture diet is more beneficial than supplementing with grass silage or restricting PA. There were no MP differences between cows offered grass silage or those offered reduced PA for up to two weeks. Greater MP losses occur with extended restrictions. This demonstrates that if a short-term feed deficit were to arise in spring, cows could be restricted at pasture for up to two weeks and produce milk at a lower cost than cows fed a proportion of silage.

References Dillon P, Crosse S, Stakelum G and Flynn F 1995. Grass and Forage Science 50, 286-299.

Effects of low-moisture sugarcane molasses-based block supplementation on rumen fermentation parameters in Nellore steers fed low quality forage

Rayanne Costa[1], Jane Ezequiel[1], Yury Granja-Salcedo[1], Edivilson Castro Filho[1], Robson Barducci[1], Sérgio Pereira Junior[1], Andresa Feliciano[1], João Pedro Bertoco[1], Júlia Rodrigues[1], Luis Felipe Artioli[2]

[1]FCAV/UNESP, Jaboticabal - SP, Brazil, [2]MUB Animal Nutrition, Guararapes - SP, Brazil

E-mail: rayannevianacosta@gmail.com

Take home message Low-moisture sugarcane molasses-based block decreases pH variations, making it suitable for fiber degradation.

Introduction Sugarcane molasses has been used in the supplementation of low quality pasture ruminants in tropical countries as an energy source, since they are readily fermentable carbohydrates, a carrier for non-protein nitrogen and feed additives, in addition to large amounts of sugarcane molasses are available as a cheap source of energy supplement (Kunkle *et al.,* 2000). The optimization of the ruminal environment due to the supplementation with multinutritional blocks based on sugarcane molasses has shown to increase the consumption of low quality roughage (Freitas *et al.*, 2003), however, little is known about its impact on the fermentation profile. The objective of this study was to evaluate the effects of low-moisture, sugarcane molasses-based block (LMB) supplementation on ruminal metabolism of beef cattle fed low quality forage.

Material & methods The experiment was conducted using six cannulated bovines in the rumen, distributed in double Latin square 3 × 3. The treatments were constituted in the hay supply of *Brachiaria brizantha cv*. Marandu as exclusive bulk (CP = 35g e NDF = 729g/kg DM) with three supplements: protein mineral salt (SMP, CP = 460 g/DM, US$ 0.58/Kg), protein energy supplement (SPE, CP = 290 g/kg DM, US$ 0.40/Kg) and MUB (MUB® Nutrição Animal; Guararapes, Brazil; CP = 460 g/kg DM, US$ 1.46/Kg). The treatments SMP and SPE were arranged in a slush form, while MUB was arranged in solid block form. The SMP and MUB were offered *ad libitum*, while the SPE was given according to the body weight of the animals (0.2% BW), in order to simulate the Brazilian reality of each type of supplement used. After 14 days of adaptation, the ruminal content collections were fasted (time 0), 2, 4, 6, 8, 10, 12 and 14 hours after feeding. The pH was determined immediately with a digital potentiometer. The NH_3-N was determined according to the methodology described by AOAC (1990). The data were analyzed in software R® as a double Latin square 3 × 3 with time-repeated measurements using the Tukey test, with a significance of 5%.

Results & discussion Total DM intake was higher in animals supplemented with SPE (3.91 kg DM/d) when compared to MUB (3.17 kg DM/d) and SMP (3.06 kg DM/d) (P <0.001). Supplement intake of SPE (0.545 kg DM/d) was higher than SMP (0.147 kg DM/d) and MUB (0.175 kg DM/d) (P=0.0013). Animals that were fed with MUB and SMP had a higher variation of N-NH_3 concentrations throughout the day (Figure 1). There was supplement × time interaction in the ruminal concentrations of NH_3-N (P<0.001). Thus, SPE presented lower concentrations 6 h after feeding when compared to MUB and SMP. At 8h after feeding SMP presented higher than SPE (P = 0.005) and at 12h MUB presented lower concentration than SMP and SPE (P = 0.006). The mean pH of MUB (7.18) was higher when compared to SPE (7.01) and SMP (7.07) (P <0.0001). The MUB and SMP had little variation over time (Figure 2). Values above pH 6.2 of ruminal liquid are considered as the minimum limit for adequate fermentation of the fiber (Orskov, 1988).

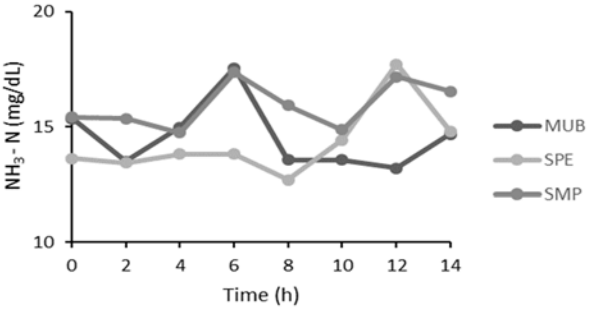


Figure 1. Concentration of amnonium nitrogen depending on the treatments and collection time

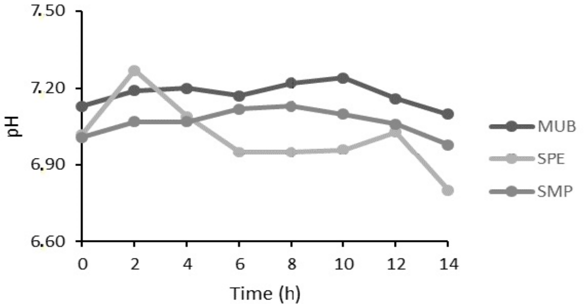


Figure 2. Ruminal pH depending on the treatments and collection time

Conclusion The variations in NH_3-N concentrations, throughout the day, remained above the minimum limit. The MUB decreases pH variations, making it suitable for fiber degradation. Diets with high fiber content with adequate granulometry stimulate a higher rumination rate and higher saliva production, which leads to increased dilution of ruminal contents and buffering, maintaining adequate conditions for the development of cellulolytic bacteria and protozoal.

Acknowledgements This research was supported by a scholarship from the National Council for Scientific and Technological Development (CNPq).

References

Association of Official Analytical Chemists - AOAC. 1990. 16.ed
Orskov ER 1988. Zaragoza: Acribia,
Kunkle W E, Johns JT, Poore MH, and Herd DB, 2000. Journal of Animal Science, 77, E-Suppl, 1-11.
Freitas S, Patiño, HO, and Mühlbach PRF, 2003. Brazilian Journal of Animal Science, 32, 1508-1515.

Holstein genetic strain and feeding system affects feeding behaviour strategy of dairy cows during late lactation

Alejandro Mendoza[1,2], Gonzalo Fernández[2], Santiago Fariña[1], Alsiane Capelesso[3], Cecilia Cajarville[3], Rodolfo Ungerfeld[4]

[1]*Programa de Producción de Leche, Instituto Nacional de Investigación Agropecuaria, Semillero, Colonia, Uruguay,* [2]*Departamento de Bovinos, Facultad de Veterinaria, Universidad de la República, Libertad, Uruguay,* [3]*Departamento de Nutrición, Facultad de Veterinaria, Universidad de la República, Libertad, Uruguay,* [4]*Departamento de Fisiología, Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay*

E-mail: amendoza@inia.org.uy

Take home message Feeding system has a more marked effect on cow behaviour than cow genetic strain.

Introduction There is an opportunity to improve the income over feed cost per cow through a higher pasture intake, but it is not clear the suitability of dairy cows that have been genetically selected with different objectives (*i.e.*, different levels of milk solids) for such a feeding system. The objective of this experiment was to evaluate the effects of feeding systems with different levels of grazed pasture on the cow´s behavior as well as how they interact with the genetic strain of the cow.

Material & methods The experiment was carried out at the Estación Experimental of INIA "La Estanzuela" (Colonia, Uruguay, 34º33' S, 57º69' W), comparing 2 Holstein genetic strains: North-American (NA, n=60) or New Zealand (NZ, n=60). Within each strain, cows were assigned to 2 feeding systems after calving: Maximum Pasture (MP) or Fixed Pasture (FP). In MP cows were offered the maximum amount of pasture allowed by the weekly pasture growth rate and stocking rate (12.6 and 15.6 kg dry matter [DM]/d above 5 cm for NZ and NA, respectively, offered in 2 equal strips per day), and were supplemented with concentrate at the milking parlor (5.9 and 6.4 kg DM/d for NZ and NA, respectively). In FP, grazed pasture was restricted to ≈33% of the diet (5.8 and 7.3 kg DM/d above 5 cm for NZ and NA, respectively) and was offered between afternoon (post-meridiem; p.m.) and morning (ante-meridiem; a.m.) milking, and cows were supplemented with a TMR (12.7 and 14.7 kg DM/d for NZ and NA, respectively) in a feed pad between a.m. and p.m. milking. Cows in all treatments were allocated according to calving date and parity, and within strain, for previous lactation yield, BW and estimated breeding values for milk solids. During spring (average days in milk = 208±20), time spent eating (pasture or TMR) or ruminating was estimated by recording behaviors in 15 cows/treatment (including 5 primiparous) during 3 consecutive days using instantaneous sampling (Martin and Bateson, 1993) with 5-min scan sampling in two periods: between a.m. (05:30) and p.m. (15:30) milking (period I), and for 2 hours after p.m. milking (period II). At the beginning of the recording period, sunrise and sunset occurred at 05:33 and 19:44, respectively). Bite rate was visually recorded in each cow during 1 min every 30 min for 2 hours during period II, during 2 consecutive days. Data from period I and II were analyzed separately using the MIXED procedure of SAS (version 9.4; SAS Institute Inc. Cary, NC, USA) with a model of analysis of variance that included the fixed effect of genotype, feeding system, and their interaction.

Results No feed refusals were observed in the feed pads where FP cows were managed during period I. Although not measured, it is clear from the amounts of feed offered during this period that FP ate more DM than MP. However, during period I, FP cows spent less time eating TMR than MP cows grazing pasture, particularly NA cows, but there were no differences in time spent ruminating between feeding strategies, although NZ cows ruminated 10 min less than NA cows. During period II, FP spent 18 min less time grazing and 19 min more ruminating than MP cows (Table 1). Bite rate during period II was not affected by the feeding strategy, but was greater in NZ compared with NA (27.0 vs 22.3 bites/min; SEM = 2.0).

Table 1 Effect of cow genotype (G) and feeding system (FS) on eating and ruminating behaviour.

	NZ		NA		SEM	P > F		
	FP	MP	FP	MP		G	FS	G x FS
Period I								
Eating, min	160.1b	233.6a	120.4c	234.4a	4.3	<0.01	<0.01	<0.01
Ruminating, min	147.2	140.8	156.3	151.9	3.0	0.03	0.25	0.82
Period II								
Eating, min	85.0	98.6	83.5	104.9	4.7	0.44	<0.01	0.20
Ruminating, min	29.0	8.6	26.8	9.4	2.3	0.76	<0.01	0.52

Conclusion Offering a TMR between a.m. and p.m. milking allowed FP cows to reduce the time spent feeding compared with cows that grazed in the same period. Irrespective of cow genetic strain, during the 2-h period after p.m. milking when all cows grazed the same pasture, FP cows still spent less time grazing and more time ruminating than MP cows.

References

Martin and Bateson P 1993. Measuring behavior: an introductory guide. 2nd ed. pp. 222. Cambridge University Press, Cambridge, UK.

Can digital camera images provide useful information for pasture management?

Elvira Sales-Baptista[1,2], Isabel Ferraz-de-Oliveira[1], Sónia Carrilho[4], José Lopes de Castro[1,2], João Serrano[1,3], Manuel Cancela d'Abreu[1,2]

[1]Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Évora, Portugal, [2]Universidade de Évora, Departamento de Zootecnia, Évora, Portugal, [3]Universidade de Évora, Departamento de Engenharia Rural, Évora, Portugal, [4]Universidade de Évora, Mestrado em Zootecnia, Évora, Portugal

E-mail: elsaba@uevora.pt

Take home message Indices from proximity imagery were best correlated with pasture quality than with biomass.

Introduction The assessment of availability and nutritional quality of Mediterranean native pastures is a major challenge. Being multi-species they also present plant communities of high heterogeneity both in the vertical and horizontal planes. Pasture structure also changes along season as different plant species with different phenology mature. Therefore, frequent data collection with non-destructive methods, such as ground-based images (Inoue *et al*, 2014) should facilitate repeated measures across time on the same spot enabling the evaluation of spatial distribution of biomass along with insights on the evolution of pastures. The aim of this study was to determine the potential of the visible spectrum from digital images as a surrogate for biomass availability and quality of native pastures, compared with traditional clipping methods and other reflectance methods (NDVI).

Material & methods Sampling was conducted on a native pasture (2.3 ha), located at University of Évora, Portugal (38° 32.2′ N; 8° 01.1′ W). The site was grazed by 15 adult, non-lactating Black Merino ewes, equipped with Global Navigation Satellite System (GNSS) receivers. From April to mid-June pasture samples were collected on a weekly basis on 3 patches (400 m^2 each) identified as the preferential grazing sites on the previous 24 hours. Percentage time spent grazing per hour was the criteria used to select preferential grazing sites. Inside each patch, 3 sampling points were randomly assigned using a 0.25 m^2 frame. Before pasture sample clipping at each sampling point, multispectral bands of the area surrounding the frame were acquired (proximity sensor OptRx® AOS, Ag Leader, Iowa, USA) and a set of two nadir images captured at 0.8 m above the ground and centred with the frame (commercially available "action" camera mounted on a pole, GoPro, Inc., San Mateo, CA, USA). Vegetation within the frame were then clipped, stored in plastic bags for dry matter, crude protein and NDF determination. Image analysis was performed with spatial analysis tools from the software ADI (version 1.3.7) (www.dew.globalsystemsscience.org) and red, green and blue profile extracted and used for calculating several visible spectrum indices (Greenness Index (GI), Green Leaf Algorithm(GLA), RGB Greenness (RGBG) and Green-Red Vegetation Index(GRVI).

Results & discussion The indices used to determine plant greenness (Fig.1) obtained both from the proximal sensor and from digital imagery, have shown similar temporal trends. For instance RGBG obtained from digital images is highly correlated with NDVI from the proximal sensor ($r^2 = 0.94$). All the imagery indices provided poor estimations of green biomass (*e.g.,* for GRVI r^2=0.07; P=0.005). However RGBG index relates significantly with NDF content (r^2=0.57; P< 0.001; Fig 2), and with Crude Protein (r^2=0.46; P<0.001) evidencing the effect of phenological state of the pasture.

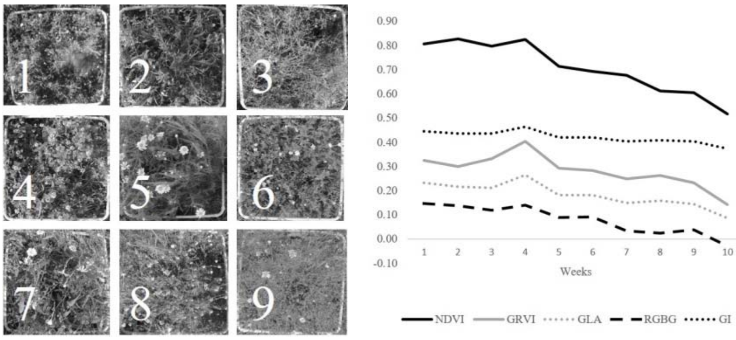


Figure 1 Temporal changes in pasture and in vegetation indices.

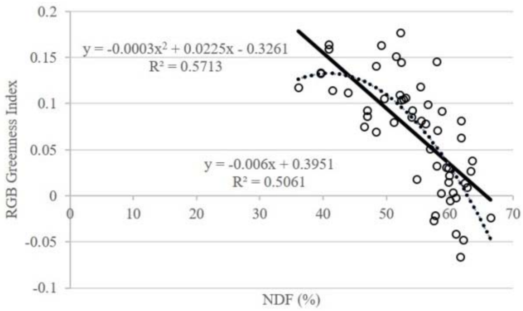


Figure 2 NDF estimates from images.

Conclusion Pasture ground-based imagery is an easy-to-perform and useful methodology for long-term *in situ* observations, and is a promising tool to estimate pasture quality parameters. Further developments include coupling with other sensors so that it may also be useful for estimation of biomass availability.

Acknowledgements This work was funded by National Funds through the FCT under the Project UID/AGR/00115/2013.

References

Inoue T, Nagai S, Kobayashi H and Koizumi H 2015. Ecological informatics, 25, 1-9.

561

Enhancement of ruminal acetate production by supplementing cellulose acetate, a new feed additive candidate

Yuto Watabe[1], Satoshi Koike[1], Yutaka Suzuki[1], Shu Shimamoto[2], Yasuo Kobayashi[1]

[1]*Graduate School of Agriculture, Hokkaido University, Sapporo, Japan,* [2]*DAICEL Corporation, Tokyo, Japan*

E-mail: watawata@anim.agr.hokudai.ac.jp

Take home message Cellulose acetate has potential as a feed additive candidate for ruminant animals to enhance ruminal acetate production through alteration of rumen microbiota.

Introduction Cellulose acetate (CA) is a semi-synthetic polymer obtained through the esterification of acetic acid with cellulose, *i.e.*, part of the hydroxyl group of cellulose is substituted with an acetyl group, changing physical properties such as solubility and viscosity. CA is mainly used as an industrial material to produce textiles, cigarette filters, and others. However, its use as an animal feed ingredient or additive has not been evaluated. We focused on CA as a new cellulosic feed source for ruminants. In the present study, a series of *in vitro* studies were carried out to determine how CA supplementation affects rumen fermentation and microbiota.

Material & methods Batch culture studies were carried out to select CA suitable for feed use and to define its optimal supplementation level. Strained rumen fluid from 4 sheep was mixed with equal amounts of McDougal's buffer in screw capped test tubes with a butyl rubber stopper into which grass hay and concentrate (2:1) with fibre source such as cellulose (control), water-soluble CA (WSCA) or insoluble CA were placed. Each fibre source was supplemented at 10% of total substrate. Tubes (n=4 per treatment) were incubated at 39℃ for 24 h to determine fermentation and microbial parameters. Then, the dose response of these parameters to different supplementation levels of WSCA (0, 7.5, 15, 22.5 and 30% in total feed substrate) were tested in the same manner. Continuous culture system (Rusitec) having 8 fermenters was operated and effect of WSCA supplementation was evaluated on the above rumen parameters. Mixture of strained rumen fluid from two Holstein cows was mixed with the above buffer in 5:8 (650 ml /fermenter) to which a daily feed (9 g grass hay and 3 g concentrate) was given by a nylon bag. At the same feeding time, cellulose (5.1 g) was supplemented to 4 fermenters (control), while a half of the cellulose was replaced by WSCA for the supplementation to another 4 fermenters (treatment). The supplementation level of WSCA was set at 15% of total feed (>15% showed increase of acetate production; see results). Incubation lasted for 7 days and the culture samples weres taken during the last two days at 3 h intervals. Digestibility, fermentation and microbial parameters were determined.

Results & discussion In batch culture studies, WSCA was shown to increase ruminal acetate level. This increase was observed in a dose-dependent manner, indicating that WSCA supplementation at >15% of total feed has a notable impact on acetate production (Figure 1). In Rusitec study, WSCA increased acetate level, together with higher abundance of genus *Prevotella* and *Prevotella ruminicola* (Figure 2). Dry matter digestibility and total gas production were not affected. Therefore, the results suggest that WSCA supplementation at 15% of total feed (minimum level to alter fermentation pattern) can increase acetate level with no negative effect on feed digestion, and this could be related to activation of *Prevotella* species. As ruminal acetate is involved in milk fat synthesis, WSCA can be considered as a feed additive candidate suitable for dairy cattle.

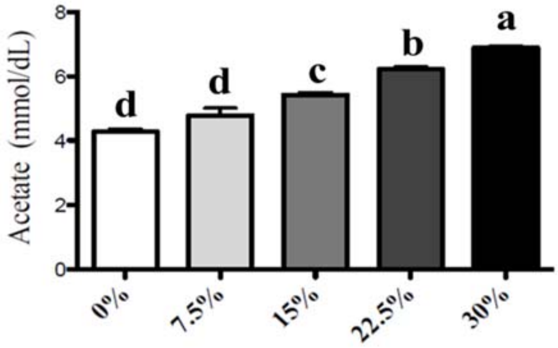


Figure 1 Response of acetate production to different levels of WSCA supplementation in batch culture$^{a, b, c, d}$ Means with different superscripts differ.

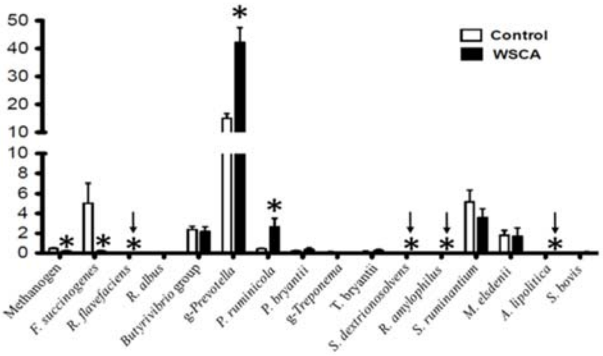


Figure 2 Relative abundance (%) of rumen representative bacterial species and groups in Rusitec*. Differ between treatments ($P < 0.05$).

Conclusion WSCA supplementation at 15% of total feed can increase acetate production without adverse effect on feed digestion and fermentation, based on the results from Rusitec study. This favourable change could be related to activation of *Prevotella* species *in vitro* system.

Acknowledgements Financial support from JSPS (16K15020) is highly appreciated.

The effect of peanut vine partially replacing corn silage in a total mixed ration on growth performance and carcass characteristic of small-tailed Han sheep

Xue-Meng Si, Zhen-Wei Zhang, Yan-Lu Wang, Wei-Kang Wang, Wu-Song Chou, Hong-Jian Yang

State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China

E-mail: yang_hongjian@sina.com

Take home message Peanut vine as a high-quality roughage has high utilization value in sheep feeding practice.

Introduction The peanut planting area in eastern China is around 466.3 ha as reported in 2016, and its harvest often results in large yields of peanut vines. Peanut vine hay on a dry matter basis contains 7.5-8.8% crude protein, 1.18-1.75% crude fat, 39-62 % neutral detergent fibre, 33-39% acid detergent fibre and 12-16% ash content depending on varieties present. Its use is increasing because it is recognized as a high-quality, low-price roughage (Zhang *et al.,* 2010). Corn silage with good palatability, high starch and digestible fibre has been well accepted in ruminant diets (Khan *et al.,* 2015), but the use of peanut vine hay in sheep diets needs more attention. Therefore, this study attempted to determine the effects of peanut vine partially replacing corn silage in total mixed rations on growth performance and carcass characteristic of small-tailed Han sheep.

Material & methods Forty small-tailed Han sheep with similar initial live weights were randomly assigned into two treatments. The control group was fed *ad libitum* a corn silage based ration (CS), and the another group was fed *ad libitum* a forage combination of 75% corn silage and 25% peanut vine hay (PV). Each treatment had five pens and four sheep in each pen. The forage:concentrate ratio in both total mixed rations (TMRs) was adjusted to 70:30 in 1-30 days, 60:40 in 31-60 days and 50:50 in 60-90 days. Live weight was recorded every 15 days to calculate average daily gain (ADG), and the amount of TMR offered and orts was recorded daily to calculate dry matter intake (DMI) per pen. Feed conversion ratio was calculated as DMI divided by ADG. After 90 days of feeding, sheep were slaughtered to determine dressing rate and *longissimus dorsi* muscle composition. Net revenue was calculated by the difference between live body weight gain income and feed cost for each treatment group. Economic feed efficiency was calculated by the net revenue in PV divided by the net revenue in CS. Data were subjected to analysis of variance with the GLM procedure of SAS (1999). Least square means were compared with a multiple comparison test (Tukey/Kramer), and significance was declared at $P<0.05$.

Results & discussion Peanut vine replacing 25% corn silage in TMR of PV sheep did not alter ADG, but increased DMI by 9.1%. Consequently, feed conversion ratio increased to 8.8%. PV sheep had a decreased carcass mass, dressing rate and meat:bone ratio by 4.6%, 8.6%, and 3.2%, respectively. Crude protein content of *longissimus dorsi* muscle increased by 0.9%, but fat content significantly decreased by 16.7%. When total feed cost and live weight gain income were used to make economic evaluations of sheep fattening, the PV ration increased net revenue by more than 77 Chinese Yuan (approximately 9.9€), and economic feed efficiency was increased by 41% compared with the CS diet. These results suggest that the inclusion of peanut vine hay in rations can promote feed intake without negative effects on the growth performance of sheep. Although there is negative effect of the inclusion of peanut vine on carcass characteristics, fattening sheep with this feed was economically beneficial.

Table 1 Effect of peanut vine (PV) replacing 25% of corn silage (CS) on growth performance, carcass characteristic economic feed efficiency of small-tailed Han Sheep.

Items	CS	PV	S.E.M	*P* value
Initial weight (kg/head)	21.4	21.3	0.28	0.99
Final weight (kg/head)	43.1	43.7	0.79	0.83
ADG (g/d)	258	266	9.6	0.52
DMI (kg/d)	1.20	1.31	0.05	0.09
Feed : Gain ratio	4.64	5.05	0.22	0.07
Carcass mass (kg/head)	23.8	22.7	0.72	0.52
Dressing rate (%)	42.1^a	38.5^b	0.02	0.04
Meat : bone ratio	3.20	3.08	0.18	0.32
Longissimus dorsi composition				
Dry matter (%)	25.7^b	26.0^a	0.53	0.02
Crude protein (%)	17.4^b	18.3^a	1.02	0.01
Crude Fat (%)	4.2^a	3.5^b	0.65	0.04
Ash (%)	1.25	1.28	0.13	0.37
Evaluation of economic feed efficiency				
Feed cost (CNY/head)	247	184		
Gain income (CNY/head)	434	448		
Net revenue (CNY/head)	187	264		
Feed efficiency (%)	100	141		

Means in a row with different superscript letter differ ($P<0.05$)

Conclusion Using peanut vine to replace 25% corn silage in TMRs of sheep showed a beneficial effect on feed intake though no significant improvement occurred for body weight gain. The dietary inclusion of peanut vine seems to decrease dressing rate of sheep and significantly decreases fat content of *longissimus dorsi* muscle. However, sheep fattening with dietary inclusion of peanut vine was favourable in terms of economic feed efficiency.

Acknowledgements This study was supported by whole corn silage popularization and application project (2015-2017) of Ministry of Agriculture of China.

References

Khan NA, Yu P, Ali M, Cone JW and Hendriks WH 2015. Journal of the Science of Food & Agriculture 95, 238-252.

Zhang, F, LiKY, Wang XQ, Wang K, Ma SL, Zhang XT, Wu ZJ and Liu XH 2010. Journal of Hebei Agricultural Sciences (*In Chinese*) 17, 72-73.

Feeding behaviour of F1 Angus-Nellore cattle fed different levels of wet distillers grains

Maria Betânia Niehues[1], Mateus S. Ferreira[2], Laís A. Tomaz[1], Luiz Antônio Fogaça[1], Pedro V.R. Paulino[3], Cyntia L. Martins[1], Mário Arrigoni[1], Otávio R. Machado Neto[1,2]

[1]*São Paulo State University, School of Veterinary Medicine and Animal Science, Botucatu, São Paulo, Brazil,* [2]*São Paulo State University, School of Agricultural and Veterinarian Sciences, Jaboticabal, São Paulo, Brazil,* [3]*Cargill Animal Nutrition, Campinas, São Paulo, Brazil*

E-mail: otaviomachado@fmvz.unesp.br

Take home message High levels of wet distillers grains raise daily time spent eating, number and size of meals.

Introduction Several factors are related to the regulation of dry matter intake in ruminants. High concentrations of starch and energy in the diet can generate differences in dry matter intake. A higher proportion of rumen degradable starch is associated with an increase in organic acids production and considerable reduction in acetate/propionate ratio. These factors may affect the feeding behaviour of cattle in feedlots. Little is known about how the inclusion of wet distillers grains, a high fibre and protein by-product, influences feeding behaviour (*Swanson et al.*, 2014). Therefore, the aim was to evaluate the replacement of corn and soybean meal by increasing levels of WDG on the feed behaviour of Angus-Nellore cattle.

Material & methods One hundred F1 Angus-Nellore bulls with initial average body weight of 369 ± 48 kg and average age of 24 months were allocated in a completely randomized block design in group pens with 5 animals in each, with five pens per treatment where they were fed for 129 days. The WDG contained 320 g of CP/kg DM, 578 g NDF/kg DM and 43 g of ether extract/kg DM. The diets presented: 148, 161, 191 and 220 g of CP/kg DM; 627, 540, 434 and 328 of non-fibre carbohydrates (g/kg DM); and 162, 234, 308 and 382 of NDF g/kg DM, according to the level of WDG (0, 150, 300 and 450 g/kg DM). The content of NDF-roughage was 90 g/kg DM in all treatments. Diets were provided twice a day and daily intake was obtained by weighing the offered diet and leftovers. Every five minutes, during three periods of 24 hours, animals were assessed for feeding behaviour. The observations were performed at the 7th (D-7), 52th (D-52) and 93rd (D-93) days of the experiment, according to Robles *et al.* (2007). Time spent eating (EAT), ruminating (RUM), resting (minutes) and number of meals per day were assessed. DMI for each pen was measured at the collection day. Meal length (minutes) was calculated by dividing EAT by the number of meals/day. Dry matter intake per meal (kg) was calculated by dividing DMI by the average number of meals per pen in each day. EAT and RUM data were used to calculate the time needed to eat one kg DM (MINDM; EAT/DMI) and rumination rate (DMRR; RUM/DMI) expressed in minutes per kg of DM. Data were analysed as repeated measures using the MIXED procedure of SAS with linear, quadratic and 0 *vs.* WDG contrasts and are presented in Table 1

Results & discussion There was a linear effect on EAT with the inclusion of WDG, which can be an effect of changes in dietary carbohydrate source and, consequently, in the ruminal environment. Fermented corn starch in the rumen generates propionate and WDG fibre generates acetate. The lower DMI in the diet without WDG is probably due to a higher propionate production in the rumen, since propionate has greater hypophagic effect than acetate. After a meal, the increased portal flux of propionate and uptake by liver increase hepatic oxidative reactions which induce satiety resulting in smaller meals. RUM and DMRR were not affected by the treatments, probably because the diets presented the same content of roughage NDF. The effect of WDG on MINDM is a consequence of changes in the numbers of meals and dry matter intake per meal.

Table 1 Feeding behaviour of F1 Angus-Nellore bulls fed different levels of wet distillers grains.

	WDG levels (g/kg DM)					Effects		
	0	150	300	450	EPM	Linear	Quadratic	0 *vs.* WDG
EAT (min/d)	158	159	175	194	7.18	***	0.22	0.03
RUM (min/d)	258	261	259	256	11.98	0.97	0.95	0.89
Resting (min/d)	1019	1014	1001	984	14.46	0.07	0.69	0.25
Meals (day)	13.8	12.7	14.2	16.1	0.88	*	0.10	0.58
DMI (kg/day)	11.02	11.83	11.21	11.74	0.32	0.15	0.55	*
Meal length (min)	12.2	13.7	13.0	12.2	0.89	0.85	0.18	0.43
DMI per meal (kg)	0.782	0.955	0.845	0.769	0.06	0.59	0.05	0.31
MINDM (min/kg DM)	14.7	13.9	16.2	17.1	0.74	***	0.23	0.23
DMRR (min/kg DM)	23.7	22.8	23.6	22.5	1.26	0.53	0.91	0.53

Conclusion Different levels of WDG grains change the eating behaviour of F1 Angus-Nellore bulls.

Acknowledgements This research was supported by grants from FAPESP, São Paulo, Brazil.

References

Robles V, González LA, Ferret A, Manteca X and Calsamiglia S. 2007. Journal of Animal Science 85, 2538-2547
Swanson KC, Islas A, Carlson ZE, Goulart RS, Gilbery TC and Bauer ML. 2014. Journal of Animal Science 92, 2531-2537

Simulating the effects of harvesting strategies and lucerne-grass binary mixtures on dairy farm profitability and agro-environmental performance in Canada

Veronique Ouellet[1], Jean-Philippe Laroche[1], Gaetan Tremblay[2], Gilles Belanger[2], Doris Pellerin[1], Martin Chantigny[2], Philippe Seguin[3], Edith Charbonneau[1]

[1]Departement des sciences animales, Universite Laval, Quebec, Quebec, Canada, [2]Agriculture and Agri-Food Canada, Soils and Crops Quebec Research and Development Centre, Quebec, Quebec, Canada, [3]Department of plant science, Mcgill University, macdonald campus, Saint-Anne-de-Bellevue, Quebec, Canada

E-mail: veronique.ouellet.6@ulaval.ca

Take home message Harvesting lucerne-timothy binary mixture at the recommended lucerne stage of early bloom is still the most profitable option for Canadian dairy farms. Tall fescue is a potential alternative to timothy in lucerne-grass mixture on Canadian dairy farms.

Introduction To optimize dairy farm profitability, producers should focus on maintaining cow productivity while decreasing the proportion of concentrates in the diet. When feeding forages of high digestibility, less concentrates are required to produce a given output of milk. However, producing forages of higher digestibility entails cutting forages at an earlier stage of development than that usually recommended; this leads to lower dry matter (DM) yield and stand life (Bélanger *et al.*, 2001), and higher operation costs. Our first objective was to evaluate how lucerne-timothy harvesting strategies (HS) influence dairy farm profits, N and P balance, and greenhouse gas emissions. Our second objective was to determine which lucerne-grass binary mixture is the most profitable on Canadian dairy farms under the best HS determined in objective 1.

Material & methods The linear programming model N-CyCLES was adapted to compare two different HS: frequent cuts at an early lucerne developmental stage (early bud) producing highly digestible forages along with an autumn cut (HS1); and less frequent cuts at the recommended lucerne developmental stage (early bloom) producing higher forage DM yields of slightly lower digestibility along with no autumn cut (HS2). The model was then adapted to compare 4 lucerne-grass (timothy, tall fescue, meadow fescue, brome) binary mixtures under the most profitable HS. All simulations were conducted on two typical Canadian dairy farms located in contrasting climate areas using averaged data (2010-2014): Central Canada farm of 71 cows and 100 ha of cropland and Maritimes farm of 63 cows and 135 ha of cropland. Forage DM yield and nutritive value were derived from field trials conducted at representative climate locations. The model optimized feeding, cropping area, and manure management as a single unit of management.

Results & discussion Harvesting strategies (HS1, HS2) influence DM yields and nutritive value of lucerne-timothy forages with lower yields and higher nutritive values achieved under HS1 (Table 1). Results of the simulations showed highest dairy farm profits with HS2 for the farms of the two areas (Table 1). This is explained by the greater income arising from higher forage yields produced under HS2 than HS1. Moreover, P balance, and total greenhouse gas emissions were comparable between the two HS for both farms (Table 1). Nitrogen balance was lower under HS2 for both farm when compared to results obtained under HS1 (Table 1). Under HS2, for the Maritimes farm, lucerne-tall fescue obtained the highest net income. Under HS2, for the Central Canada farm, only slight net income variations were observed between the tested lucerne-grass binary mixtures.

Table 1 Effects of two harvesting strategies (HS1, HS2) on forage DM yield and nutritive value of lucerne-timothy binary mixtures, and on dairy farm net income, greenhouse gas emission, and P and N balance at two locations in Canada.

	Maritimes		Central Canada	
	HS1	HS2	HS1	HS2
Forage dry matter yield (Mg/ha)	4.8	6.3	4.6	7.3
Forage crude protein (g/kg DM)	163	159	178	170
Forage neutral detergent fibre (g/kg DM)	491	527	485	522
Forage neutral detergent fibre digestibility (g/kg DM)	669	605	732	641
Net income ($/100 kg FPCM[1])	12.1	15.8	22.4	24.8
Crop income ($/100 kg FPCM)	3.0	5.0	10.2	12.1
CO_2 eq. (kg/FPCM)	1.4	1.4	1.3	1.3
N balance (g/kg FPCM)	11.4	8.9	12.7	11.5
P balance (g/kg FPCM)	1.6	1.4	0.4	0.4

[1]FPCM: Fat and protein corrected milk

Conclusion These results suggest that harvesting lucerne-timothy binary mixtures at the recommended lucerne stage of early bloom is more profitable than harvesting more frequently at an earlier developmental stage aimed at higher nutritive value. Agro-environmental performances were comparable under the two tested HS. The lucerne-tall fescue mixture was the most profitable for the Maritimes farm whereas the 4 tested mixtures had a similar effect on the Central Canada farm.

Acknowledgements This research was funded by Agriculture and Agri-Food Canada and Dairy Farmers of Canada as part of the Dairy Science Cluster initiative, and by FRQNT-Novalait-MAPAQ for the portion on grass species. The first author received a scholarship from the Natural Sciences and Engineering Research Council of Canada.

References

Bélanger G, Michaud R, Jefferson PG, Tremblay GF and Brégard A 2001. Canadian Journal of Plant Sciences. 81, 577-585.

Rich protein feeds in Mixed Crop-Livestock Systems in tropical areas: A literature review of available resources and their use by livestock.

Harry Archimede[1], Denis Bastianelli[2], Audrey Fanchone[1], Jean-Luc Gourdine[1], Louis Fahrasmane[1]

[1]*INRA, Petit bourg, Guadeloupe,* [2]*CIRAD, Montpellier, France*

E-mail: harry.archimede@inra.fr

Take home message The choice of protein resources consumed for livestock in Mixed Crop-Livestock Systems (MFCS) is not made solely on the basis of their feed value and productivity. Decisions are taken on the basis of a multi-criteria assessment responding to different ecosystem services provided by these resources.

Introduction MFCS are diverse and include some agroforestry systems. The resilience of tropical agro systems to climate change and their potential to contribute to food security depends partly on the development of agro-ecological practices. The characteristics of MFCS potentially allow the development of agro-ecological practices. More than 60% of the world population live in MFCS that produce at least 40% of the food (Herero *et al.,* 2010). Improving the performance of these systems is therefore an important target for increasing the global supply of food, including animal proteins. In MFCS, plants have several functions other than feed: nutraceutical, service plant (soil cover, green fertilizer, repulsion of pathogens...). Among these resources, legumes also have the characteristic of being able to fix atmospheric nitrogen and soluble phosphorus in the soil, making them available for other plants. The aim of this paper is to present the main protein-rich resources available in MFCS.

Material & methods The primary goal of MCLS is to produce food for humans. In MCLS, livestock are mainly reared on non-food biomass. Feed strictly dedicated to livestock is an adjustment component of these MCLS. These systems are more or less complex and rich in plant species depending on pedo-climatic, historical, economic and socio-cultural criteria. The most complex MCLS consist of several vegetation strata (tree, shrub, herbaceous) with sub-annual to multiannual operating cycles. The literature review has been performed on potential rich protein feeds coming from all the plant strata using Web of Science data base. It is not an exhaustive study but a typology. Some representative resources coming from the different plant strata have been selected on the basis of the importance of their use and the availability of published data on their feed value. The availability of the most abundant feed biomass, which can be used by livestock, is often discontinuous because they are based on the production cycles of food crops. The optimal use of animal "feed" requires the implementation of preservation strategies. Some of these are also presented.

Results & discussion In MCLS, the dual crops, which overall valorisation satisfies the needs of man and livestock, are strategic: seeds and vines of legumes, leaves and tubers of energetic crops (*e.g., Ipomeas batatas, Manihot esculenta Xanthosoma sagittifolium*). Wines and leaves, widely used in tropical regions, have crude protein (CP) values above 14% Dry Matter (DM) but high variabilities are registered depending on harvest and storage conditions. Peas with a CP content above 25% DM are generally not intended for animals but humans. However some species like *Vigna unguiculata*, *Cajanus cajan*, *Lablab purpureus* are used as livestock feed in some areas. There is a great diversity of forage trees, *e.g., Erythryina variegala, Gliricidia sp Leucaena spla, Morus alba.* It is a heterogeneous resource because only leaves and young stems are consumed by animals while their production is accompanied by a variable fraction of indigestible stem. Moreover, some secondary metabolites can limit their use. The protein contents of these resources are generally high: 15 to 30 CP% DM. However, a smaller number of forage trees, often food trees, are present in MCLS although they have important agronomic and environmental benefits in integrated systems by their ability to fix nitrogen. Herbaceous legumes are also poorly represented in these systems as they develop in specialized livestock systems.
The development of appropriate technologies mainly based on the use of heat to outweigh some antinutritional factors, will fully value the potential of many protein resources. Moreover, certain technologies, such as complete food blocks which is expanding in India, have been developed to take into account of the seasonal diversity of resources and to formulate rations that are more balanced in nutritional terms.

Conclusion Livestock may have diversified protein sources linked to agro-ecological practices, without competition with human food. These resources can be diverse from one region to another if they are derived from local biodiversity.

References

Herero M, Thornton PK, Notenbaert AM, Wood S, Msangi S, Freeman HA, Bossio D, Dixon J, Peters M, van de Steeg J, Lynam J, Parthasarathy Rao P, Macmillan S, Gerard B, McDermott J, Seré C and Rosegrant M 2010. Science. 327, 822-5.

Nitrogen balance of Flemish giant rabbit fed *Azolla pinnata* as a substitution for copra meal

Ristianto Utomo, Nafiatul Umami, Cuk Tri Noviandi, Aditya Permadi

Universitas Gadjah Mada, Yogyakarta, Indonesia

E-mail: ristiantoutomo@ugm.ac.id

Take home message *Azolla pinnata* has potency as an alternative protein source for rabbits.

Introduction The availability of feedstuffs for rabbits in Indonesia is still limited. Copra meal, as one of the main source of protein for rabbits, is also easily spoiled during storage due to its high fat content. Therefore, alternative feedstuffs with a similar nutritive value, more available with a less or similar price, and easy to get is needed as a substitution for copra meal. Copra meal contains about 200 g/kg crude protein, meanwhile *Azolla pinnata* contains 200 – 300 g/kg crude protein (DM) (Cohen *et al*., 2002; Katayama *et al*., 2008). Thus, a study that determines the effects of substituting copra meal with *Azolla pinata* on nitrogen balance in the diets of Flemish giant rabbits was performed.

Material & methods By following a completely randomized design, 20 male Flemish Giant rabbits at 3 months old and, 1,534±277 g body weight were divided into 4 treatment groups (Az0: 150 g/kg copra meal, without *Azolla*, Az50: 100 g/kg copra meal and 50 g/kg *Azolla*, Az100: 50 g/kg copra meal and 100 g/kg *Azolla*, and Az150: 150 g/kg *Azolla,* without copra meal). All dietary treatments were pelleted and offered *ad libitum* for 50 days. Nitrogen balance was determined based on total collection of faeces and urine. All data were analysed for variance and continued with Duncan's new multiple range test for any significant differences.

Results & discussion Although the feed consumption and dry matter digestibility data showed increasing trends numerically, no significant differences were detected by any statistical means (Table 1). These results are due to the crude fibre content of copra meal and *Azolla pinnata* being similar (144.0 *vs.* 178.6 g/kg; Hartadi *et al*., 2005). The similar crude protein content between copra meal and *Azolla pinnata* (210.0 *vs.* 215.8 g/kg; Hartadi *et al*., 2005) also lead to similar results of N consumption among all treatments (Table 1). Thus, the N retention also showed relatively similar results.

Table 1 Feed consumption, dry matter digestibility, N consumption, N faeces, N urine, and N retention of Flemish giant rabbit fed *Azolla pinnata* as substitution for copra meal

Variables	Az0	Az50	Az100	Az150
Feed consumption (g DM/d)	79.6 ± 15.54	78.6 ± 9.77	83.1 ± 16.96	84.3 ± 9.95
Dry matter digestibility (g/kg)	678.1 ± 60.50	681.0 ± 91.00	684.1 ± 56.50	686.0 ± 67.40
N consumption (g DM/d)	2.14 ± 0.43	2.11 ± 0.29	2.25 ± 0.47	2.28 ± 0.27
N faeces (g DM/d)	0.44 ± 0.11	0.47 ± 0.08	0.52 ± 0.15	0.49 ± 0.14
N urine (g DM/d)	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
N retention (g DM/d)	1.65 ± 0.25	1.58 ± 0.26	1.66 ± 0.34	1.73 ± 0.21

Az0: 150 g/kg copra meal, without *Azolla*, Az50: 100 g/kg copra meal and 50 g/kg *Azolla*, Az100: 50 g/kg copra meal and 100 g/kg *Azolla*, and Az150: 150 g/kg *Azolla,* without copra meal.

Conclusion It can be concluded that *Azolla pinnata* can be used as substitute for copra meal up to 150 g/kg without negatively affecting the performance of the Flemish Giant rabbit.

Acknowledgements This research was supported by grants from Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia.

References

Cohen MF, Meziane T, Tsuchiya M and Yamasaki H 2002. Aquatic Botany 74, 181-187.
Katayama N, Yamashita M, Kishida Y, Liu CC, Watanabe I and Wada H. 2008. Acta Astronautica 63, 7-10.
Hartadi H, Reksohadiprodjo S and Tillman AD 2005. Tabel Komposisi Pakan Untuk Indonesia. Gadjah Mada University Press, Yogyakarta.

Fodder trees as an alternative resource to feed ruminants: voluntary intake and *in vivo* digestibility of white mulberry (*Morus alba*) and common ash (*Fraxinus excelsior*) leaves in sheep

Cécile Ginane[1], Mickaël Bernard[2], Véronique Deiss[1], Donato Andueza[1], Jean-Claude Emile[3], Sandra Nowak[3], Camille Béral[4]

[1]INRA UMR Herbivores, Saint-Genès-Champanelle, France, [2]INRA Herbipôle, Saint-Genès-Champanelle, France, [3]INRA Ferlus, Lusignan, France, [4]Agroof, Anduze, France

E-mail: cecile.ginane@inra.fr

Take home message Leaves of common ash and white mulberry represent a feed alternative to grass, of great value, for ruminants.

Introduction In breeding systems of domestic herbivores that aim to maximize grazing (*e.g.,* to reduce inputs), the challenge is to feed the herd all year round with local forage resources. In temperate regions, the increasingly frequent summer droughts often force breeders to cope with scarcity and/or a sharp decrease in quality of pasture resources while animals are producing. In this context, fodder trees may represent a highly available resource, but many questions remain about the range of feed value (Hejcmanová *et al.,* 2014; Emile *et al.*, 2017), as well as about their acceptability by animals, which are essential conditions for their use on the field.

Material & methods In late summer 2017, we used 18 castrated Texel wethers (18 months, 61.6 ± 6.9 kg), housed in individual pens and allocated to three treatments (n=6) based on diets offered: a 1st cycle permanent grassland hay, or fresh leaves of common ash (*Fraxinus excelsior*) or mulberry (*Morus alba*) trees (Table 1). Tree leaves were harvested twice a week and stored at +4°C for up to 4 days. All sheep received hay for 11 days (period 1). Then, after 4 days of transition, two-thirds of them received one of the two fodder trees for 15 days while the others still received the grassland hay (period 2). *In vivo* digestibility was measured over the last 6 days. Animals were fed *ad libitum* (10 and 15% of refusals for hay and fodder trees, respectively) with a daily calculation of offered forages based on the amounts individually ingested on the previous day. During the digestibility period, samples of offered forages, refusals and faeces (total collection) were individually collected, weighed and dried. We analysed samples for contents in crude protein (CP, Kjeldhal method), neutral detergent fibre (NDF) and acid detergent fibre (ADF). We used mixed models (SAS EG 5.1) to assess the effect of forages (and period for dry matter intake (DMI)) on digestibility and intake data.

Results Daily dry matter intake of hay were initially similar between groups but increased between periods 1 and 2 in groups that changed their diet towards tree leaves ($p = 0.006$). Dry matter intake of leaves was about 1.5 times greater than of hay with no difference between tree species (Table 1). Organic matter digestibility (OMD) was lowest for hay and highest for mulberry ($p = 0.0001$, Table 1). Similarly to DMI, digestible organic matter intake (DOMI) was far lower with hay than with fodder trees, with no difference between common ash and mulberry ($p = 0.003$, Table 1).

Table 1 Chemical composition, intake level and digestibility of experimental forages (n=6).

Experimental forages	DM (g/g)	OM (g/g DM)	CP (g/kg DM)	NDF (g/kg DM)	ADF (g/kg DM)	DMI (kg/d)	OMD	DOMI (g/kg $BW^{0.75}$)
Pasture hay	0.90	0.90	91	588	314	1.54^a	0.54^a	34.0^a
Common ash leaves	0.34	0.91	147	326	193	2.13^b	0.63^b	56.8^b
Mulberry leaves	0.34	0.84	108	202	117	2.16^b	0.68^c	59.7^b

DM: dry matter; OM: organic matter; DMI: dry matter intake; OMD: organic matter digestibility; DOMI: digestible organic matter intake; BW: body weight. Means within a column with different superscripts differ at $p = 0.05$.

Conclusion In late summer, when the need for alternatives to low available standing grass can be great, leaves from common ash or mulberry appear to be good options compared to pasture hay or to ageing swards. Their chemical characteristics are comparable to those of early summer permanent grassland (INRA, 2018) and their palatability is high, as reflected by DMI, even when they are offered as the sole feed for several days. These intakes made the digestible organic matter intake expressed by sheep fed with common ash and mulberry leaves, more than twice as high as the 23 g/kg $BW^{0.75}$ considered to cover the maintenance requirements of adult sheep (INRA, 2018). Thus, despite CP and organic matter digestibility values, such fodder trees are suitable alternative forages, including for high producing animals.

Acknowledgements The authors thank the PARASOL project funded by Ademe, and the staff of UE Herbipôle and UMR Herbivores

References

Emile JC, Delagarde R, Barre P, Niderkorn V and Novak S 2017. Grassland Science in Europe. 22, 548-550.
Hejcmanová P, Stejskalová M and Hejcman M. 2014. Vegetation History and Archaebotany. 23, 607-613.
INRA, 2018. INRA feedings system for ruminants. pp 640.Wageningen Academic Publishers, The Netherlands.

Relations between *in vitro* organic matter digestibility and lignin in grassland forb species

Anjo Elgersma

Independent scientist, Wageningen, The Netherlands

E-mail: anjo.elgersma@hotmail.com

Take home message Differences in *in vitro* organic matter digestibility among forb species could not be fully explained by lignin content or degree of cell wall lignification.

Introduction In dicotyledonous species the relationship between chemical composition and digestibility differs from that of grass, which is often used as a research standard. Therefore, predictions of the digestibility of species-rich forage can be inaccurate. Søegaard *et al.* (2011) and Elgersma *et al.* (2014) showed that in spring, *in vitro* organic matter digestibility (IVOMD) for chicory (*Cichorium intybus*) was comparable with that of grass, but showed a relatively large decrease towards summer; an even greater decline was found in ribwort plantain (*Plantago lanceolata*). Plantain had a lower IVOMD than chicory, while that of salad burnet (*Sanguisorba minor*) was lowest. In contrast, caraway (*Carum carvi*) maintained its high IVOMD from spring to summer. Relationships between IVOMD and neutral detergent fiber (NDF) were shown (Elgersma *et al.,* 2014), but not with NDF compounds such as acid detergent lignin (ADL). This paper aims to show such relationsips as they might increase insight into IVOMD differences among forb species.

Material & methods Pure stands were established with salad burnet, caraway, chicory, and ribwort plantain in 2008. Swards were cut on 29 May, 9 July, 21 August and 23 October 2009 and 31 May, 13 July, 19 August and 21 October 2010. Data on contents of lignin, cellulose and hemicellulose were published earlier (Elgersma *et al.*, 2014). This paper further analyses the IVOMD data in relation to lignin content of DM and to the lignin proportion of cell walls.

Results & discussion The IVOMD and ADL content were negatively related (Fig. 1A); slopes of regression lines were similar among forbs, while the intercept was lower for salad burnet than for caraway and ribwort plantain ($P < 0.05$). The ADL content in ribwort plantain was higher ($P < 0.05$) than in other forbs, as was the degree of cell wall lignification ($P < 0.05$; Fig. 1B). Ribwort plantain had on average a lower IVOMD than caraway and chicory ($P < 0.05$), but a similar level as salad burnet (636, 743, 715 and 641 g/kg OM, respectively) (Elgersma *et al.,* 2014). Thus in salad burnet other factors than lignin or lignification must be associated with IVOMD. The higher IVOMD of caraway ($P < 0.05$) than of ribwort plantain and salad burnet could not be explained by the degree of cell wall lignification as there were no relations, either among or within species (Fig. 1B).

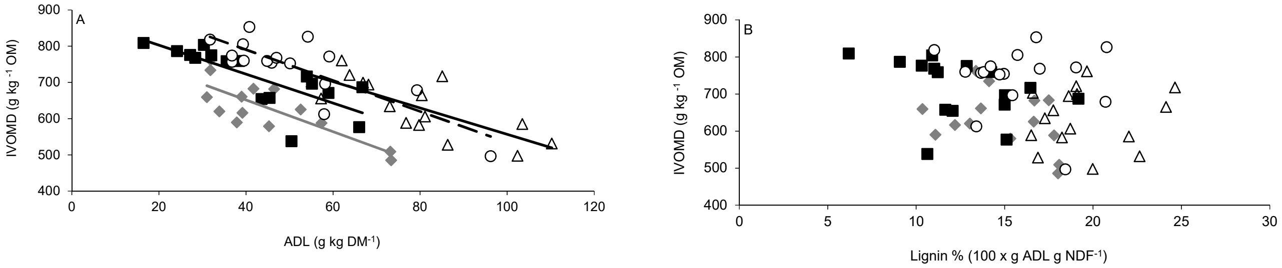


Figure 1 Relationships between IVOMD and lignin content (A) and lignin proportion in cell walls (B) of four species (♦ salad burnet, ■ chicory, O caraway, Δ ribwort plantain); R^2 of regression lines: 0.64, 0.59, 0.67 and 0.58, respectively ($P < 0.05$).

At comparable levels of IVOMD, caraway had more cell wall lignification than chicory, and ribwort plantain more than salad burnet. Assuming that lignin is indigestible, this implies that in caraway and ribwort plantain the lignin present in the cell walls is less an obstruction to fermentation than in chicory and salad burnet. This could be due to physical/spatial reasons, *e.g.,* a different anatomical distribution of lignin in cell wall tissues, and/or to chemical factors, *e.g.,* a different bonding of lignin to other compounds.

Conclusion More research is needed to understand the lignification and composition of cell walls in grassland species.

References

Elgersma A., Søegaard K and Jensen SK. 2014. Grass and Forage Science 69, 705-716.

Søegaard K, Eriksen J and Askegaard M. 2011. Proceedings of the Third Scientific Conference of ISOFAR, Vol. 1 Organic Crop Production, pp. 190–193. Gyeonggi Paldang.

Bali x Hissar cattle fed *Leucaena leucocephala* supplemented with maize grain grew faster than Bali cattle

Dahlanuddin Dahlanuddin[1], Tanda Sahat Panjaitan[2], Sofyan Sofyan[1], Dennis P Poppi[3], Simon P Quigley[3]

[1]*Faculty of Animal Science University of Mataram, Mataram, NTB, Indonesia,* [2]*Assessment Institute for Agricultural Technology, Narmada, NTB, Indonesia,* [3]*School of Agriculture and Food Sciences, The University of Queensland, Gatton, Qld, Australia*

E-mail: dahlan.unram@gmail.com

Take home message Bali x Hissar cross cattle grew faster than Bali cattle, but only by 0.1 kg/day, when fed leucaena supplemented with maize grain.

Introduction The growth rate of Bali cattle (*Bos javanicus*) within traditional management systems is very low (0.2 kg/day; Dahlanuddin *et al.*, 2012). A fattening system based on leucaena (*Leucaena leucocephala*) significantly increased liveweight (LW) gain (LWG) to 0.4 kg/day (Panjaitan *et al*., 2013). As a result, a cattle fattening system based on leucaena being fed as the main or sole diet component has recently become common practice for smallholder farmers in dry areas of eastern Indonesia. Leucaena is high in crude protein [CP; 240 g CP/kg dry matter (DM)] and in a combination with a highly fermentable energy source would further utilise the CP and also extend the use of a limited supply of leucaena in the system. The current system has traditionally used Bali cattle which have a small mature size (220 kg, non-pregnant females under traditional management) and low LWG potential (0.7 kg/day). As more leucaena becomes available there is a need to evaluate whether it is more efficient to feed leucaena to Bali cattle or to their cross with Hissar (*Bos indicus*) which have a higher mature LW (400 kg, non-pregnant females) and potentially have a higher LWG potential.

Material & methods Ten Bali bulls (204.7 ± 3.0 kg) and 10 Bali x Hissar bulls (151.0 ± 5.5 kg) were fed leucaena hay (240 g CP and, 96 g crude fibre/kg DM) *ad libitum* supplemented with maize grain (96 g CP and 74 g crude fibre/kg DM) at 10 g DM/kg LW.day. Mineral mix (Ultra Mineral) was included at 10 g/kg of maize grain offered. The experiment consisted of a 15 day adaptation period followed by a 120 day measurement period. Variables measured were intake and digestibility of DM, the concentration of ammonia nitrogen (NH_3-N) in the rumen and LWG. All data were analysed using t-test (SAS, 1999).

Results & discussion Bali x Hissar bulls grew at a higher rate than the Bali bulls (Table 1). This was most likely due to the higher intake and digestibility of DM by Bali x Hissar bulls resulting in a higher metabolisable energy intake. Supplementation with maize grain resulted in a lower concentration of NH_3-N in the rumen of Bali x Hissar bulls suggesting better microbial capture of degraded protein.

Table 1 Live weight gain (LWG), intake and digestibility of dry matter (DM) and rumen ammonia concentration of Bali and Bali x Hissar bulls fed leucaena hay *ad libitum* supplemented with maize grain at 10 g DM/kg LW.day.

Variables	Bali	Balix Hissar	P value
LWG (kg/day)	0.66 ± 0.02	0.78 ± 0.03	0.01
Feed intake (g DM/kg LW.day):			
- Leucaena	11.0 ± 0.6	13.2 ± 0.4	0.01
- Maize grain	10.4 ± 0.1	11.5 ± 0.2	0.01
- Total	21.4 ± 0.6	24.7 ± 0.5	<0.01
Rumen ammonia-N (mg/L)	318 ± 17.6	205 ± 22.5	<0.01
Digestibility of DM (%)	59.8 ± 2.2	66.5 ± 1.5	0.02

Conclusion Bali x Hissar bulls grow faster than pure Bali bulls when fed a high quality diet based on *Leucaena leucocephala* supplemented with maize grain. However, feed intake of Bali x Hissar bulls was higher than that of the pure Bali bulls. The nutritive requirements of Hissar cattle and their crosses are unknown and require further research to optimise production.

Acknowledgements This experiment was part of a research project funded by the Australian Centre for International Agricultural Research).

References

Dahlanuddin D, Yulianto TB, Priyanti A, Poppi DP and Quigley SP 2012. Animal Production 14, 173-179.

Panjaitan T, Fauzan M, Dahlanuddin D, Halliday MJ and Shelton HM 2013. Proceedings of the 22nd International Grassland Congress. Sydney, Australia.

SAS (1999) 'SAS/STAT user's guide. Version 8. SAS Institute: Cary, NC.

Comparative study of lamb response to vetch summer grazing, cereal residue and commercial diet

Sourour Abidi, Salah Benyoussef, Omar Maamouri

INRA Tunisia, EL Menzah, Tunis, Tunisia

E-mail: sourour.abidi@yahoo.fr

Take home message Dried vetch biomass provided a valuable alternative to cereal stubble and complementation under Conservative Agriculture (CA).

Introduction Nowadays, there is an urgent need to reverse soil degradation and to improve crop and livestock productivity at the farm level, as well as to maintain food security in Tunisia. Conservation Agriculture (CA) can be helpful in enhancing crop productivity, resource use efficiency, and soil health. However, the demand for crop residues as animal feed are among the major constraints for the adoption of CA technologies. Thus, the objectives of this trial are to test the value of summer grazed vetch as an alternative to wheat residues and to compare lamb response to vetch, cereal residues and a commercial diet.

Material & methods The common vetch (cultivar Mghila) has been used in this experiment. Thirty Barbarine lambs with a mean age of seven months, an average weight of 22.5 kg were divided into three groups; each group received one of the following treatments: T1: Vetch grazing during the morning (3 h) and evening (2 h), T2: Wheat stubble grazing during the morning (3 h) and evening (2h) and 400 g barely, T3: Oaten hay *ad libitum* and 600 g commercial concentrate. Vetch and residue biomass, nutritive value and animal growth was assessed every two weeks. Animal behavior were studied once during the grazing period.

Results & discussion Animals grazing vetch spent more time feeding than those on cereal residue (75 % vs 47 %) and less time walking (22 % vs 47 %). However, lambs on stubble spent practically the same time feeding and walking throughout the morning grazing period. This behavior proves the preference of lambs for the vetch diet and therefore its better palatability. This behavior is similar to that described by Dumont *et al.* (2001).

Initial vetch biomass was about 4.5 T DM ha^{-1} and then dropped to 2.5 T DM ha^{-1} in an asymptotic way as grazing goes on through the grazing period. The high CP content of the vetch confirms the interest of this forage alternative. This content dropped in a remarkable way throughout the grazing period. This decrease is explained by the gradual disappearance of seeds. This finding corroborates that of Rebolé *et al.* (2004). DG average was about 168 g day^{-1} for lambs in sheepfold, 68 and 57.1 g day^{-}for lambs on vetch and stubble, respectively.

Focus on DG cost of the three groups; we noticed that the cost of one kg of meat is the cheapest with vetch lambs grazing. On stubble, the cost of one kg produced meat is similar to meat produced with commercial diet

Conclusion It can be concluded that during summer period, dried vetch biomass provided a valuable alternative to cereal stubble and complementation which is rich in energy and protein and should sustain moderate growth performances of growing lambs.

Acknowledgements This trial was conducted under the framework of the CLCA project.

References

Dumont B, Meuret M, Boissy A and Petit M 2001. Fourrages. 166, 213-238.
Rebole A, Alzueta C, Ortiz LT, Barro C, Rodriguez ML and Caballero R 2004. Spanish Journal of Agricultural Research 2, 550-557.

Effect of selective herbicides on the effective degradability of nitrogen of forages issued from two permanent grasslands across the first growth cycle

Donato Andueza[1], Silvia Baizan[2], Fabienne Picard[1], Julianne Pourrat[1], Gaëlle Maxin[1]

[1]*INRA-UMRH, 63122 Saint-Genès Champanelle, France,* [2]*SERIDA, Apdo 13 33300 Villaviciosa (Asturias), Spain*

E-mail: donato.andueza@inra.fr

Take home message Botanical composition of permanent grassland influences the effective nitrogen degradability (END) of forages obtained from these grasslands.

Introduction Herbicide application on permanent grasslands is a common agricultural management practice in Europe. In general, these products are applied against forbs, which are considered as weeds. The use of these products influences the yield and biodiversity of grasslands but also the forages' nutritive value, issued from these plots. In general, END of grasses differs from that of dicotyledonous plants (Balde *et al.,* 1993). It was hypothesized, that changes in the botanical composition of permanent grasslands influences the END of forages issued from these surfaces. The aim of this work was to study the effect of herbicide application against forbs on the END of forage issued from two permanent grasslands across the first growth cycle.

Material & methods The study was carried out on two adjacent permanent grasslands (2 ha each) located at Saint-Genes Champanelle, Puy-de-Dôme, France (45°43'N, 3°1'E; 870 m asl). The first one composed of 86% of grasses (GRG; mainly *Holcus lanatus* and *Lolium perenne*) and 13% of forbs (mainly *Taraxacum officinale*, *Heracleum sphondylium* and *Daucus carotta*) and the other one composed of 78% of grasses (GRF; mainly *Dactylis glomerata* and *Holcus lanatus*) and 21% of forbs (mainly *Knautia arvensis* and *Heracleum sphondylium*). Half of each grassland was treated with herbicide (2-4 D and MCPA) the precedent autumn in order to eliminate the forbs. The swards, treated with herbicide (GRG- and GRF-) and untreated (GRG+ and GRF+), were sampled four times during the primary cut in spring: at 400, 800, 1200 and 1600 degree-days (°D). Forage samples were oven-dried, and ground at 1-mm screen. Then, N rumen degradation was measured with the *in situ* technique (Ørskov and McDonald, 1979). Finally, END in each animal was calculated for a k of 6 % h^{-1}. END values were submitted to ANOVA according to a repeated measurements model including the effects of grassland, herbicide treatment, sampling period and animal. Period was considered as repeated measures and cow as random effect.

Results & discussion The evolution of END of both grasslands, treated and untreated with herbicides was significantly different ($P<0.05$). For the GRG, the END of forage issued from the sward treated with herbicide was higher than that of forage issued from the untreated sward, particularly at 800 and 1200°D, which decreases the protein value (INRA, 2007) and increases N environmental losses. However, for the GRF, the END of forage issued from the untreated sward was higher than that of treated sward at 1200°D. No significant differences were found for the other dates. For both, the grasslands and the herbicide treatments, END were higher ($P<0.001$) at 400 and 800°D than at 1200 and 1600°D, confirming the influence of maturity stage on the END of forages (INRA, 2007). When the END predictions obtained by INRA (2007) and the values found in this experiment, were compared, the obtained bias (0.23) was non-significant.

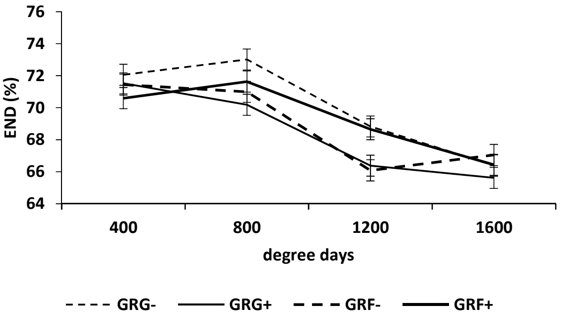


Figure 1 Evolution of effective nitrogen degradability (END) of two permanent grasslands with or without application of herbicide across the first cycle of growth (mean ±sem).

Conclusion The effect of herbicide application on END of permanent grasslands is variable according to their botanical composition. Generally, forages' END was increased (which is not recommended) or did not change in the periods when grasslands were used by livestock (400-800°D). The application of herbicides would not be systematic in permanent grasslands. More research is required in order to determine if differences of END between forages of permanent grasslands, without or with the application of herbicides, are due to the species of grasses or forbs, or their proportions in the grasslands.

Acknowledgements This research was supported by the New-DEAL project funded by PSDR4-Auvergne

References

Balde AT, Vandersall JH, Erdman RQ, Reeves III JB and Glenn BP 1993. Animal Feed Science and Technology. 44, 29-43.
INRA, 2007. Alimentation des bovins, ovins et caprins Besoins des animaux – Valeurs des alimentes, France, 307pp.
Ørskov E R and McDonald I 1979. The Journal of Agricultural Science. 92, 499-503.

Effect of tree density on the evolution of phenological stage of agroforestry permanent grasslands

Donato Andueza[1], André Guittard[2], Julianne Pourrat[1], Mickael Bernard[2], Fabienne Picard[1]

[1]*INRA-UMRH, 63122 Saint-Genès Champanelle, France,* [2]*INRA-Herbipôle, 63122 Saint-Genès Champanelle, France*

E-mail: donato.andueza@inra.fr

Take home message The level of shadow of silvopastoral grasslands affects the phenological stage of forage.

Introduction Silvopasture is an agroforestry practice of planned interactions among trees, forages and ruminants. This association can be an agroecological alternative. It could be designed for creating synergies between vegetal species. However, based on the nutritive value of grass, the effect of shadow trees on the chemical composition of temperate forages is unclear (Buergler *et al.*, 2006). In addition, the effect of the forestry density on the nutritive value is poorly known. The shadow of silvopastoral could influence both the morphogenesis and the chemical composition of forages. In this study the aim is to investigate the evolution of the phenological stage of permanent grasslands across the first growth cycle as an indicator of plant morphogenesis in response to tree density.

Material & methods Three permanent grasslands located at Saint Genès Champanelle Puy-de-Dôme, France (45°43'N, 3°1'E; 870 m asl) were used; One plot did not contain trees (T0) and the other plots contain cherrywood (*Prunus avium*) and maple (*Acer campestre*) at two densities 60 (T+) and 150 (T++) trees/ha, respectively. Trees were planted in 1989. The level of shadow for T0, T+ and T++ were 1 %, 40% and 84% respectively. Grasslands were sampled (n=3) every 2 weeks between 5th May and 3rd August in 2016 across the first growth cycle. Botanical composition of grasslands was determined on 6 samples per grassland (Figure 1). T0 was characterized by high proportions of *Agrostis capillaries*, *Festuca rubra* and *Lolium perenne* whereas the main species of T+ grasslands were *Agrostis capillaries* and *Trisetum flavescens*. T++ was characterized by high proportions of *Agrostis capillaries*, *Festuca rubra*, *Holcus lanatus Arrhenaterum elatius* and *Lolium perenne*. Phenological stage was determined according to the method proposed by Moore *et al.,* (1991) on 75 random grass tillers. The mean stage of plant development by weight (MSW), for each sample, was calculated by: MSW = Σ (C_i D_i)/D, where C_i, is the code of stage i, (1.5= Vegetative leaf development; 2.5 = Elongation; 3.5=Inflorescence emerged; 4= Caryopsis visible) (where, D_i the total dry weight for tillers in stage i, and D the total dry weight for all tillers. Data for MSW estimates underwent repeated measures ANOVA including the effects of grassland, sampling date and replicate.

Results & discussion The evolution of phenological stage of T0 and T+ across the first growth cycle was similar, whereas forage belonging to T++ grassland was less developed compared to those on the other grasslands. The proportion of tillers in the vegetative stage was higher in T++, which explains the different evolution pattern. These phenological stage differences between grasslands could influence the chemical composition and nutritive value of forages issued from these grasslands but they are not only related to the botanical composition.

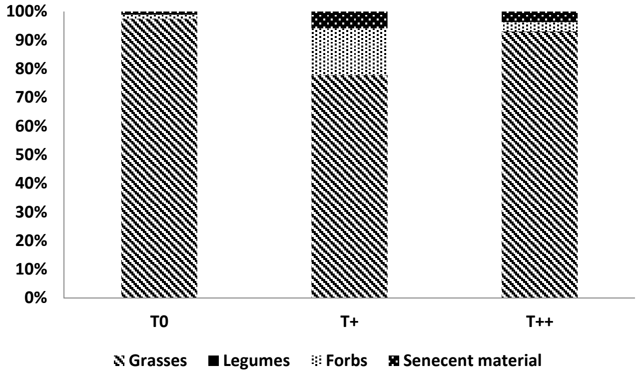


Figure 1 Botanical composition of grasslands.

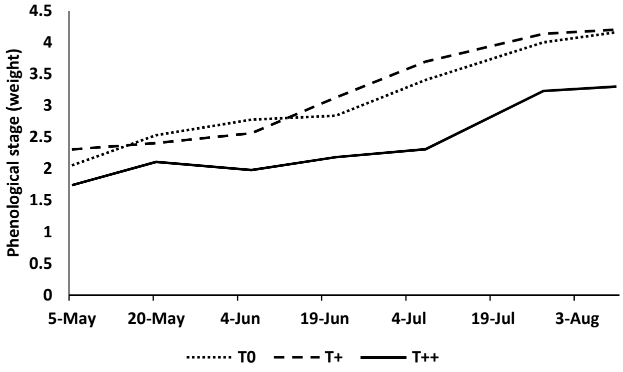


Figure 2 Evolution of phenological stage of grasslands across the first growth cycle.

Conclusion Shadow level of forages in agroforestry influences their growth and thus their phenological stage.

Acknowledgements This research was supported by the PARASOL project (15-60-C0025) funded by Ademe, France. Authors want also acknowledge the staff of UE Herbipôle for their technical collaboration.

References

Buergler A, Fike JH, Burger JA, Feldhake CM, McKena JR and Teutsch CD 2006. Agronomy Journal. 98, 1265-1283.
Moore KJ, Moser LE, Vogel KP, Waller SS, Johnson BE and Pedersen JF 1991. Agronomy Journal. 83, 1073-1077.

Effects of microwave irradiation on *in vitro* ruminal and post-ruminal disappearance of canola seed

Hamid Paya, Akbar Taghizadeh, Ali Hosseinkhani, Hamid Mohammadzadeh

Univversity of Tabriz, Tabriz, Iran

E-mail: hamid.paya@tabrizu.ac.ir

Take home message Microwave irradiation as heating treatment can be used in ruminant nutrition for reducing crude protein degradation in rumen

Introduction A number of different oilseed crops such as oil seed soybean, sunflower, and canola are grown around the world. Oil seeds are a major source of raw materials such as fat, protein, carbohydrate with potential applications as nutritional and functional foods. Many studies have been performed to decrease the ruminal degradability of protein in oilseed using heat treatment such as roasting, micronization, because suppression of protein degradation in the rumen improves protein utilization (Fathi Nasry *et al.,* 2008). Recently microwaves are being used for feed processing in ruminant nutrition because of the higher nutritive quality of the final products due to the faster heating process along with the precise control process.

Material & methods Canola seeds were supplied from a local commercial supplier at Eastern Azerbaijan in northwest Iran. Triplicate 500 g samples of canola seeds were placed in a Pyrex pan (28×28×6 cm) with 1 to 2 cm height and were subjected to microwave irradiation at a power of 800 W (1.8 w/g microwave energy) for 4 min. Evaluation of ruminal and post-ruminal disappearance was carried out according to the procedure of Gargallo *et al.* (2006). Approximately, 5 g of each sample (raw and microwave treated canola seeds) was weighed into a 5×10 cm Dacron polyester nylon bag with 50 µm pore size (four bags per each sample) and suspended in the rumen of two cannulated wethers for 12 h. Bags were then removed and washed with a washing machine, dried in a force air oven at 55 ºC for 48 h and weighed. Samples from each bag were taken for N analysis using the Kjeldahl method. After weighing, the residue (0.5 g) was placed in an *in situ* bag (5×5 cm with pore size 50 µm) and placed in an ANKOM daisy incubator for the determination of post-ruminal digestibility. Briefly, samples were incubated in pepsin/HCl solution for 1 h in a Daisy incubator, followed by incubation in a pancreatin/HK2PO4 solution for 24 h. After 24 h, bags were washed with washing machine. Bags were then dried in a forced air oven at 55 ºC for 48 h. The dry weights of the samples and bags were recorded and bags were opened and contents were pooled by sample for crude protein analysis. Data on ruminal and post-ruminal dry matter and crude protein disappearance were subjected to one-way analysis of variance using the analysis of variation model (ANOVA) of SAS.

Results & discussion The disappearance of post-ruminal and total tract DM of microwave irradiated canola seeds was significantly ($p<0.05$) increased compared to the raw seeds. The disappearance of ruminal CP of microwave irradiated canola seeds was significantly ($p<0.05$) reduced compared to the raw seeds but post-ruminal disappearance of CP was increased ($p<0.05$). No significant difference between the two raw and microwave irradiated canola seeds in total tract CP disappearance.

Table 1 Ruminal, post-ruminal and total tract DM and CP disappearance (%) modified three step procedure.

	Ruminal	Post-ruminal	Total tract
Dry Matter			
Raw canola seed	20.44^a	39.36^b	59.81^b
Microwave irradiated canola seed	20.98^a	42.83^a	63.82^a
SEM	0.787	0.523	0.638
Crude Protein			
Raw canola seed	26.46^a	36.30^b	62.77^a
Microwave irradiated canola seed	22.68^b	42.59^a	65.27^a
SEM	0.637	0.627	0.679

Conclusion The significant reduction in the amount of disappearance of CP by microwave irradiation showed it appeared to increase by-pass protein from canola seed and thereby increase the amount of available CP in the small intestine, provided that the digestibility of by-pass protein does not decrease with microwave irradiation. Consequently, these processing methods appear to shift the site of CP digestion from the rumen to the small intestine and increase the amount of undegraded CP digested in the small intestine.

References

Fathi Nasri MH, France J, Danesh Mesgaran M and Kebreab E. 2008. Livestock Science 113, 43–51.

Gargallo S, Calsamiglia, S and Ferret A. 2006. Journal of Animal Science 84, 2163–2167.

Effect of two forage allowances offered to beef heifers grazing native pastures during mid and late gestation on placental efficiency and calf performance at birth

M. Soledad Orcasberro[2], Laura Astigarraga[1], Pablo Soca[3], Andrea Alvarez-Oxiley[1]

[1]*Facultad de Agronomia-UDELAR, Montevideo, Uruguay,* [2]*Facultad de Agronomia-UDELAR, Cerro Largo, Uruguay,* [3]*Facultad de Agronomia-UDELAR, Paysandú, Uruguay*

E-mail: sorcasberro@fagro.edu.uy

Take home message Beef cows under a nutritional restriction during mid and last gestation might present a placenta efficiency compensation allowing no differences in phenotypic measurements in the calf at birth.

Introduction Beef production systems in Uruguay are based on natural pastures without supplementation, so they are influenced by the high climate variability within and between years, which determines important variations in the energy supply, the main nutrient limiting the breeding process (Berretta, 1994). Consequently, during late gestation (winter), cows may be exposed to a nutritional restriction that determines a decrease in energy supply to the calf. This effect is even more pronounced in primiparous cows because of the requirement for their own growth. As a result, the placental and fetal development can be compromised, affecting the birth of the calf as well as its development trajectory and subsequent productive and reproductive performance in adult life (Funston *et al.,* 2010). The aim of the study was to evaluate the effect of two native forage allowances (Allen *et al.,* 2011; Sollenberger *et al.,* 2015) on primiparous cows in continuous grazing, during mid (autumn) and late gestation (winter), on placental efficiency and calf performance at birth.

Material & methods Two treatments of forage allowance (FA) were evaluated, FA8: 8 (autumn) and 4 (winter) kg DM/kg LW *vs* FA4: 4 (autumn) and 4 (winter) kg DM/kg LW, with 20 primiparous cows (10 per treatment, Hereford and Aberdeen Angus), in second third of gestation at the beginning of the trial (May). FA was adjusted monthly, through the appraisal of available forage and the “put and take” method to adjust animal live weight (LW) to the intended FA. Every month, experimental cows were weighed and body condition score (BCS, 1-8 scale) determined. At calving (September), placenta and calves were weighed. Cotyledons were individually separated, counted, weighed and measured to determine its area per placenta. Within 24 hours postpartum, body measurements were made: length of head (H); distance between shoulders (SH); rump width (RW); body length (BL); thorax circumference (TC); front leg length (FLL); front leg circumference (FLC); hind leg length (HLL); hind leg circumference (HLC). Placenta data and calves body measurements were analysed using ANOVA. Cows LW and BCS were analysed with the Mixed procedure of SAS (2001), as repeated measures.

Results Cows LW and BCS averages did not differ between FA treatments (Table 1), but they decreased during winter (LW: 370±9.1 kg vs 290±8.6 kg and BCS: 4.5±0.09 vs 3.7±0.08, in autumn and winter respectively, $P<0.0001$). Neither placental weight, cotyledon number per placenta nor cotyledon weight per placenta varies between treatments. However, cotyledon area was higher in FA4 than FA8 as well as placenta efficiency that tended to be higher (Table 1). Calf birth weight (Table 1), and body measurements H (22 cm), SH (9.5 cm), RW (12.5 cm), BL (54.5 cm), TC (73.5 cm), FLL (68.0 cm), FLC (19.5 cm), HLL (73 cm), HLC (22.8 cm), were similar between treatments ($P>0.05$).

Table 1 Adjusted differences per treatment.

	FA8 (n=10)	FA4 (n=10)	*P-value*
Cow LW (kg)[1]	328 ± 11.5	332 ± 11.5	0.8095
Cow BCS	4.06 ± 0.11	4.06 ± 0.11	0.969
Calf Birth LW (kg)	31 ± 1.2	32 ± 1.3	0.7032
Placental weight (kg)	4.3 ± 0.11	3.6 ± 0.13	0.404
Cotyledon number per placenta	113.6 ± 13.26	94.6 ± 12.84	0.068
Cotyledon weight per placenta (kg)	1.63 ± 0.257	1.42 ± 0.461	0.413
Cotyledon area (m^2)	0. 38 ± 0.05	0.42 ± 0.09	0.047
Placenta efficiency [2]	7.2 ± 1.45	8.7 ± 1.52	0.056

[1]Adjusted for uterine weight [2](calf weight/placenta weight)

Conclusion While the different FA during mid-gestation had no effect on dam body weight or BCS, the results suggest that there was compensation on cotyledon area and placenta efficiency as expected on a nutritional restriction during mid and late gestation, which allowed that no differences in phenotypic fetal measurements were observed (birth weight and body measurements).

Acknowledgements This research was supported by grants from the National Agency for Research and Innovation (ANII) and the Faculty of Agronomy-UDELAR.

References

Allen VG., Batello C, Berretta J, Hodgson J, Kothmann M, Li X, McIvor J, Milne J, Morris C, Peeters A and Sanderson M 2011. Grass Forage Science. 66, 2–28.
Berretta E. 1994. Utilización y manejo de pastizales. Montevideo, Diálogo 40/ IICA-PROCISUR
Funston RN, Larson DM and Vonnahme KA 2010. Journal of Animal Science. 88 (13 Suppl)
Sollenberger L, Moore J, Allen V and Pedreira C 2005. Crop Science. 45, 896–900.

575

Using the GreenFeed system to measure enteric methane emission from the Belgian Blue and Limousine young bulls and steers in pasture

Alexandre Mertens, Virginie Decruyenaere, Didier Stilmant, Michaël Mathot

CRAW, Libramont, Belgium

E-mail: a.mertens@cra.wallonie.be

Take home message CH_4 emission by grazing steers and bulls are positively correlated to the available grass digestibility.

Introduction While ruminant production systems are criticized for being large contributors to global warming, the benefits of grazing in terms of environmental pressure and feed-food competition are still under investigation. In this context, identifying the levers to improve greenhouse gas (GHG) balance in grazing systems is essential. In this preliminary approach, we explore the link between cattle enteric methane emission and the quality of available grass for two breeds (Limousine (LIM) and double purpose Belgian Blue (BB)) and two animal types (bull and steer) in organic farming.

Material & methods The experiment took place in the experimental farm of CRAW (Mathot *et al*, 2016), in Libramont (Belgium), with a herd consisitng of eight BB and eight LIM (four steers and four bulls for each breed). A rotational grazing system (five paddocks with instantaneous stocking rate of 24.6 heads per ha) was applied between June and September 2017. During this period, cattle ate only grazed grass. The sward surface height (30 cm x 30 cm aluminium plate, 2.122 kg/m²) and available grass quality (organic matter digestibility measured by SPIR) was measured twice a week. In addition, the cattle had access to a GreenFeed (GF) unit (Zimmerman *et al*, 2011) that measures enteric methane emissions (Bird-Gardiner *et al*, 2017). The GF was parametrized to provide up to five pellet drops with 40 s intervals. A visit is recorded when the head of the animal is close to the GF for more than two minutes. A regression is then performed using all GF visits and the estimated digestibility.

Results & discussion The initial weight and average daily weight gain (ADG) are presented in Table 1 All animals but two LIM (one bull and one steer) visited the GF with a good frequency. Few animals, in particular in the BB steers group had a very low ADG. Explanation for these low performances are still under investigation. The mean and standard deviation of the number of visits and the daily CH_4 emission are shown in Table 1. The groups differed but the BB steers showed comparable ADG and daily enteric methane emissions, with larger dispersion for the BB steers. To reduce the impact of the initial weight on the result, we compared the quality of available grass with the daily methane emission per kilogram of metabolic weight. A significant correlation ($p<0.001$, $n=659$) is observed with an R^2 of only about 0.05. This is due to the high variability between each individual measurement including high daily and inter animal variability. The data, aggregated by values of digestibility are visible on the Fig. 1. This correlation could be explained by the increase in digestibility that induce higher feed intake and consequently methane emission. This fact can not be confirmed because ingestion was not recorded. However, digested organic matter was approached through C ($C\text{-}CO_2$+$C\text{-}CH_4$) emissions by cattle, which increase with digestibility. Contrastingly, grass with high digestibility could potentially decrease the CH_4 emission per unit of digested organic matter. Now, we did not observe any relationship between digestibility and CH_4/total C emitted by cattle as measure with GF. This is probably due to the limited number of data.

Table 1 Mean value and standard deviation for the initial weight, average daily gain, number of visits to the GF unit and measured enteric methane emission.

	Initial weight (kg)	ADG (kg/day)	Visits per head	CH4 (g/day)
BB steers	397±71	0.36±0.16	69.5±33.3	168±16
BB bulls	398±71	0.62±0.19	27.5±15.2	170±17
LIM steers	388±40	0.78±0.08	42.3±23.5	182±22
LIM bulls	384±30	0.70±0.06	48±9.8	188±19

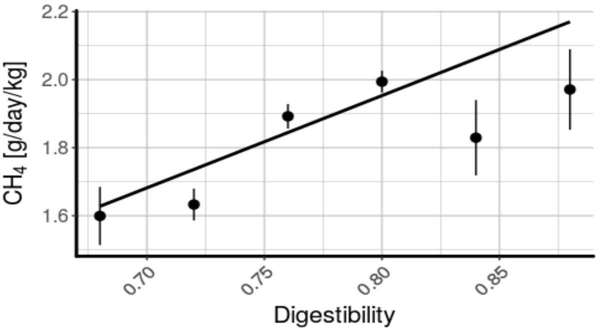


Figure 1 Aggregated (steps of 0.4) data of daily enteric methane emission per metabolic weight measured as a function of the organic matter digestibility of the available forage.

Conclusion The GreenFeed system allows the measuring of enteric methane emissions during the pasture period. We observe a correlation between these emissions and the quality of the available forage. These results are preliminary and could be improved by adding regular weighing and faeces analysis in order to characterise grass digestibility and intake.

Acknowledgements This study is a part of the AUTEFEL project, funded by the Moerman fund of the CRA-W.

References

Bird-Gardiner T, Arthur PF, Barchia IM, Donoghue KA and Herd RM 2017 Journal of Animal Science 95, 4391-4398
Mathot M, Decruyenaere V, Lambert R and Stilmant D 2016, Agriculture, Ecosystem and Environment 233, 94-105
Zimmerman. P, Zimmerman S, Utsumi S and Beede D 2011. Journal of Dairy Science. 94 (E-Suppl. 1), 856

Palm kernel expeller and soy bean hulls have a delayed *in vitro* dry matter degradation compared to other ruminant feeds

Lasse Sembach[1], Rajan Dhakal[1], Hanne Hansen[1], Nicolaj Nielsen[2], Mette Nielsen[1]

[1]*University of Copenhagen, Copenhagen, Denmark,* [2]*NorFor, Aarhus, Denmark*

E-mail: lasse.emil.sembach@sund.ku.dk

Take home message Results from *in vitro* fermentation trials suggest that ranking of feeds according to their degradation kinetics depend on the retention time in the rumen, which may need to be accounted for in modern feed evaluation systems.

Introduction The Nordic feed evaluation system (NorFor) includes the fractional degradation rates of starch, NDF, crude protein and a rest fraction as factors when calculating feed degradability in the rumen. These fractions are all assumed to be degraded by a constant percentage per hour (Volden and Larsen, 2011). It is thereby assumed that all concentrate feedstuffs follow the same type of degradation curve, with the largest degradation rate occurring at the start of the incubation period in the rumen. To evaluate the accuracy of this assumption, gas production over a 48-hour period and dry matter degradation after 9 and 48 hours of fermentation were examined in 12 concentrate feedstuffs in two *in vitro* fermentation trials.

Material & methods The feedstuffs were ground to pass a 2 mm sieve using a hammer mill. 500 mg samples were weighed into 100 ml glass jars. 10 feedstuffs were tested in quadruples in each trial, while 2 feedstuffs (sugar beet pulp) were run in triplicates. Rumen fluid with particulate matter was collected in the morning from two overnight fasted rumen cannulated Jersey heifers, transported in 4 pre-heated thermo bottles and filtered through 2 layers of cheesecloth after arrival at the lab within 45 min after collection. The rumen fluid was then added to a pre-heated (39°C) buffered mineral solution (Mencke and Steingass, 1988), that was continually kept anaerobic by flushing with CO_2. 90 ml of the buffered rumen fluid was dispensed into each 100 ml glass jar, which was placed in a preheated (39°C) thermo-shaker with 40 rotations per minute. The ANKOM^{RF} Gas Production System was used to register the gas production every 10 minutes for each sample during a 48-hour fermentation period, with the gas set to be released at a pressure of 0.0517 bars. Half of the samples were removed and the medium filtered through pre-weighed F57 filter bags (ANKOM Technology) after 9 hours of incubation, and the remaining samples after 48 hours of fermentation. After drying, the weight of undegraded residue withheld in bags was determined, corrected for microbial weight gain in bags and used to calculate dry matter degradation.

Results & discussion The results confirmed our suspicion that degradation curves vary considerably between concentrate feeds normally used in dairy cattle rations, and the maximum rate (ml minute^{-1}) of gas production peaked at different time points. Palm kernel expeller and soybean hulls (NDF > 63 % of dry matter) had a delayed gas production and dry matter degradation response, compared to wheat bran (NDF > 47 % of dry matter), where more than half of the dry matter degradation occurred during the first 9 hours of fermentation. Therefore, as shown in Table 1, the ranking of feeds with respect to dry matter degradability differed, when they were evaluated after 9 hours of fermentation as compared to after 48 hours. In high-yielding dairy cows ingesting over 25 kg DM, rumen retention times may be as low as 6-9 hours for water soluble nutrients and concentrate feeds in general.

Table 1 Percentage of dry matter degraded at 9 and 48 hours in an IVGP trial.

		9 h			48 h	
	n	lsmean	S.E.	n	lsmean	S.E.
Palm kernel expeller	2	15.5 A	1.5	1	50.1 A	2.2
Soybean hulls	2	25.3 B	1.5	2	87.1 EF	1.5
Rapeseed meal 2	2	31.5 BC	1.5	2	73.1 BC	1.5
Sugar beet pulp[a]	2	35.3 CD	1.5	1	91.5 EF	2.2
Rapeseed meal 1	2	41.8 DE	1.5	2	78.0 CD	1.5
Wheat bran	2	44.7 EF	1.5	2	67.9 B	1.5
Sunflower meal 1	2	45.5 EF	1.5	2	73.0 BC	1.5
Sugar beet pulp[b]	2	49.9 EFG	1.5	1	86.9 DEF	2.2
Sunflower meal 2	2	51.6 FG	1.5	2	77.7 CD	1.5
Triticale	2	57.1 GH	1.5	2	92.3 F	1.5
Rye kernels	2	61.9 HI	1.5	2	93.1 F	1.5
Wheat meal	2	63.3 HI	1.5	2	82.1 DE	1.5

[a] Drum dried sugar beet pulp. [b] Steam dried sugar beet pulp. Least square means within a column with different letters are significantly different (P<0.05).

Conclusion Concentrate feedstuffs follow very different degradation curves during rumen fermentation, which must have implications for their energetic value in diets for high-yielding dairy cows, where rumen retention times are low. It should be validated in feeding trials, whether this needs to be accounted for in future feed evaluation systems.

Acknowledgements This research was supported by Norfor – The Nordic feed evaluation system.

References

Mencke KH and Steingass H 1988 Animal Research and Development 28, 7 – 55.

Volden H and Larsen M 2011 Digestion and metabolism in the gastrointestinal tract. In NorFor - The Nordic feed evaluation system (ed. H Volden), pp.59–80. EAAP –European Federation of Animal Science, vol 30. Wageningen Academic Publishers, Wageningen

Fattening rabbits with simplified feed made from *Sulla flexuosa* hay, fig-tree leaves and wheat bran

Si Ammar Kadi[1], Azeddine Mouhous[1], Farid Djellal[2], Thierry Gidenne[3]

[1]Département des sciences agronomiques, faculté des sciences biologiques et sciences agronomiques, Université M. MAMMERI UN1501, Tizi-Ouzou, Algeria, [2]Département d'Agronomie, FSNV, université Ferhat ABBAS,, Sétif, Algeria, [3]GenPhySE, Université de Toulouse, INRA, INPT, ENVT, Castanet Tolosan, Toulouse, France

E-mail: kadisiammar@yahoo.fr

Take home message Use locally available raw material for feeding rabbits.

Introduction The high price of the feed is one of the factors that slow down the development of rabbit breeding in Algeria. This feed is made from imported raw materials, mainly dehydrated alfalfa and soybean meal. The use of alternative feed sources available locally is then strongly desired. This study aimed to test the possibility of fattening rabbits with a simplified feed composed of only raw materials available locally: wheat bran, sulla hay (*Hedysarum flexuosum*) and fig-tree leaves (*Ficus carica*).

Material & methods Two pelleted diets were formulated to meet requirements of growing rabbits; the first is the commercial diet used as control [Soybean meal (13%), Dehydrated alfalfa (35%), Barley (20%), Wheat bran (30.5%), Sodium chloride (0.5%) and Premix (%)] while the second is the experimental diet which was formulated with the aim to contain minimal and only locally available raw materials especially without Soybean meal and dehydrated alfalfa [Sulla hay (25%), Fig-tree leaves (15%), Wheat bran (58,5%), Sodium chloride (0.5%) and Premix (%)]. Fifty-six Algerian white population rabbits were used. At weaning (35 d, 674±390 g), rabbits were divided in two groups of 28 (individually caged) and received *ad libitum* one of the two diets during six weeks. Control of body weight and feed consumption was weekly while control of mortality was daily. The chemical analyses were performed for experimental diets, fig-tree leaves and sulla hay. Data were subjected to analysis of variance with SAS software in order to evaluate the effect of the diet on rabbits fattening performances.

Results & discussion Ash content in simplified diet was higher due to the appreciable contributions of fig tree leaves and sulla hay (Table 1). In addition, crude protein was the inferior limit of the recommendations for this type of feed.

Table 1. Chemical composition of experimental diets

	Control diet	Simplified diet	Fig-tree leaves	Sulla hay
Dry matter (g/kg brut)	913	882	879	885
Crude ash	73.7	127.3	165.5	142
Crude protein (N×6.25)	169.8	148.7	128.1	165.7
NDF	357.6	421.3	306.0	486.4
ADF	193.5	188.6	172.1	345.3
ADL	43.2	46.6	150.1	90.3
Gross energy, MJ/kg	18.37	18.46	16.40	17.02
Digestible energy, MJ/kg	11.43[µ]	12.63[µ]	-	8.9[*]
Digestible protein, g/kg	148[#]	149[#]	-	71.1[*]

[µ] According to Maertens *et al.* (1988); [*]According to Kadi *et al.* (2011) [#] According to Villamide and Fraga (1998);

Table 2. Feed intake, growth and feed conversion

	Control diet	Simplified diet	*SEM*	*P*
Period 35-56 d				
Body weight at 35 d, g	601	702	75	0.55
Body weight at 56 d, g	1411	1537	85	0.504
Daily weight gain, g/d	39	39.9	0.98	<0.001
Daily feed intake, g/d	102.8	100.3	5.60	0.78
Feed conversion rate, g/g	2.63	2.66	0.12	0.055
Period 56-77 d				
Body weight at 77 d, g	2058	2267	73	0.091
Daily weight gain, g/d	31.4	35.3	1.15	0.034
Daily feed intake, g/d	126.2	133.8	4.15	0.047
Feed conversion rate, g/g	3.92	3.82	0.16	0.35
Period 35-77 d				
Daily weight gain, g/d	35.3	37.67	0.77	<0.001
Daily feed intake, g/d	113.9	123.8	4.98	0.20
Feed conversion rate, g/g	3.23	3.27	0.12	0.067

The daily weight gain obtained in this trial (Table 2) are within the range of what has been recorded in this population of rabbits (Kadi, 2012). Simplified diet permitted performances at the same level that of control one. Moreover, for whole period (35-77d), growth rate permitted by simplified was significantly higher (37.6 *vs* 35.3 g/d). Feed conversion ratio was in the standards and at the same level in the two groups.

Conclusion The combination of fig-tree leaves with Sulla hay, in replacement of dehydrated alfalfa, soybean meal and barley, permit appreciable performances at the same level of those of the control diet. These results have to be confirmed with a larger number of rabbits under real breeding conditions.

References

Kadi S, A. 2012. Doctoral thesis, Mouloud MAMMERI university, 143p.
Kadi SA, Guermah H, Bannelier C, Berchiche M and Gidenne T 2011. World Rabbit Science 19,151-159.
Maertens L, Moermans R and De Groote G. 1988 Journal of Applied Rabbit Research 11, 60-67.
Villamide M J and Fraga MJ 1998. Animal Feed Science and Technology 70, 211–224

Meat production in grass based system with bulls and steers of double purpose Belgian Blue and Limousine breeds: ammonia emissions from their manure at barn and store

Michaël Mathot[1], Alexandre Mertens[1], Richard Lambert[2], Didier Stilmant[1], Virginie Decruyenaere[1]

[1]*Centre wallon de Recherches agronomiques, Libramont, Belgium,* [2]*Université catholique de Louvain, Louvain-la-Neuve, Belgium*

E-mail: m.mathot@cra.wallonie.be

Take home message Ammonia emissions from barn and manure stores fluctuate strongly with feeding strategies but when related to cattle production, variation in live weight gain compensate the differences observed per ton of manure produced.

Introduction Ruminant production systems and more particularly specialized meat production systems are strongly criticized for their environmental impacts. In this context, grass based feeding management, potentially inducing low food feed competition or higher carbon sequestration in soils, should be investigated notably regarding systems efficiency (nutrient outputs in products/nutrients in feed) and gaseous emissions. Therefore, we are evaluating four contrasting organic farming systems based on grassland leading to meat production from two animal types (steers or bulls) from two breeds (Limousine (L) and double purpose Belgian Blue (BB)). This evaluation is performed on animal performances and gaseous emissions at barn and during manure storage. In this contribution preliminary results about ammonia emissions are presented.

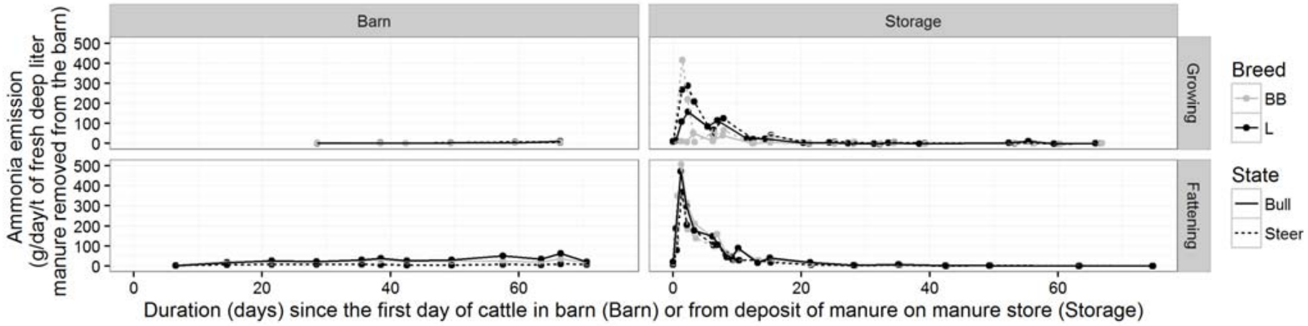


Figure 1 Daily emissions of ammonia at barn and during manure storage related to amount of manure removed from the barn and then stored.

Material & methods From November 2016 to April 2017, four groups (BB_Steer, BB_Bull, L_Steer and L_Bull, n=2 per group) were raised in separated fully strawed flow chambers with accumulation of the manure. They were fed according to their requirement considering two consecutives feeding phases (growing and fattening, duration *ca*. 66 days each). For a given type the two breeds received the same diet. The two phases differ in the amount of concentrate in the diet. The diets (75% forages on dry matter [DM] basis) were relatively similar for both types, as illustrated by target crude protein (CP) concentration of the diet, at growing stage, with 107 for steers and 120 g CP/kg DM for bulls. The difference was higher during the fattening phase with 119 for steers and 163 g CP/kg DM for bulls. For the bulls, during the fattening phase, the forage proportion was decreased to 50% on DM basis. After each phase, the manure was removed from the barn to be stored, per animal type and breed, for *ca* 70 days on concrete storage infrastructures allowing individual covering of the manure with dynamic chambers. Both in barn and in manure stores, NH_3 emissions were measured using wet acid trap technique. This experiment is currently repeated but only preliminary results are presented (mean±standard deviation).

Results & discussion On average 1.0±0.6 (4.7±0.9) and 1.3±1.0 (4.9±0.5) t DM (t fresh matter [FM]) of manure were produced and stored for growing and fattening phases respectively. However, the last day at barn, BB_Bull manure at growing phase was accidentally wet leading to lower dry matter concentration (0.17 *vs* 0.24 DM/FM). As a preliminary approach, this treatment was kept in the data presented. Ammonia emissions varied strongly (Figure 1) in time and with phases in barn and manure stores. They amounted to 0.2±0.1 kg NH_3/t fresh manure from the barn during the growing phase while 1.1±0.6 kg NH_3/t fresh manure was produced during finishing phase. Similarly, emissions from manure stores were of 1.1± 0.8 and 2.0±0.4 kg NH_3/t fresh manure, respectively. During the finishing phase, both emissions at barn and store were higher for bull manure compared to steer manure leading globally to 1.7 times more emissions of NH_3 per ton of fresh manure produced. These results are in accordance with the total ammonium and total nitrogen concentrations of fresh manure that are both significantly ($p<0.01$) and positively correlated to the ammonia emissions per tons of fresh manure stored. However when considering the cattle performances, no major trends are observed (BB_Steer: 67 , BB_Bull: 49 , L_Steer: 60, L_Bull: 68 g NH_3/kg live weight gain) due to compensation of differences in emissions per amount of manure by variation in cattle average daily live weight gains ranging from 0.9 to 1.5 kg over the whole trial.

Conclusion Observation of higher ammonia emissions per ton of fresh manure produced in the bull systems did not induce higher emissions by unit of live weight produced.

Acknowledgments This study is part of the AUTEFEL project, funded by the Moerman fund of the CRA-W

Incorporation of multispecies swards into an intensive sheep grazing system

MB Lynch[1], C Grace[1], H Sheridan[1], S Lott[2], E Brennan[2], R Fritch[1], TM Boland[1]

[1]*School of Agriculture and Food Science, University College Dublin,, Belfield, Dublin 4, Ireland,* [2]*University College Dublin Lyons Farm, Lyons Estate,, Celbridge, Naas, Co. Kildare,, Ireland*

E-mail: bridget.lynch@ucd.ie

Take home message Multispecies swards improve ewe and lamb performance, produce similar herbage at lower nitrogen input compared to grass only. However maintaining the proportions of herbs is a challenge under intensive sheep grazing.

Introduction Grazing multispecies swards (containing herbs and legumes; MSS) has been shown to improve lamb performance (Kemp *et al.,* 2010) and MSS have the potential for increased biomass production at lower inputs of nitrogen (Jing *et al.,* 2017), thus increasing system profitability. In addition, herb containing mixtures have shown potential to reduce intestinal parasites (Marley *et al.,* 2003). However, grazing animals make complex foraging decisions, grazing selectively from a diverse sward, potentially altering the botanical composition of the sward, through selection of the most nutritious species, leading to poor persistency of these species (Jing *et al*., 2017). The objective of this study was to examine the impact of incorporating MSS into an intensive sheep grazing system.

Material & methods Four farmlets were established at UCD Lyons Farm (53˚17´N, 6 ˚31´W); perennial ryegrass receiving 163 kg N per hectare per year (N/ha/y) (PRG); PRG and white clover sward at 90 kg N/ha/y (PRGWC); a six species sward (two grasses, two legumes, two herbs) at 90 kg N/ha/y (6S) and a nine species sward (three grasses, three legumes, three herbs) at 90 kg N/ha/y (9S). An area of 9.6 ha was divided into five blocks and each block divided into four paddocks, with one paddock per block randomly assigned to each sward type resulting in four farmlets. Each farmlet was managed in a 5-paddock rotational grazing system, with 30 twin-rearing ewes at a stocking rate of 12.5 ewes/ha from turnout after lambing until lambs were slaughtered. Lambs were weighed and faecal egg count was measured fortnightly. Target pre-grazing herbage mass was 1200 kg dry-matter (DM)/ha and target post grazing sward height was 4 cm for all rotations. Herbage samples were collected for DM yield, botanical and chemical composition determination at each grazing. Data were analysed using the GLM procedure of SAS 9.4. The model used included the effect of sward type.

Results Annual DM production of the 6S and 9S swards did not differ from the PRG sward despite the lower N application. Herb proportions decreased in the 6S (0.20 vs 0.07; $P < 0.05$) and 9S swards (0.17 vs 0.07; $P < 0.05$) from 2015 to 2016 resulting in increased proportions of grass species in these swards ($P < 0.05$), in addition sward chemical composition was largely similar between sward types. The 6S sward was denser than all other swards and herbage utilisation was highest in the 6S and 9S swards ($P < 0.05$) (Table 1). Lambs grazing the PRG sward required more days to reach slaughter weight than lambs grazing all other sward types ($P < 0.001$). Lambs grazing the 6S and 9S swards required fewer anthelmintic treatments than lambs grazing the PRG or PRGWC swards ($P < 0.0001$).

Table 1 The effect of sward treatment on herbage production (kg DM/ha), density (kg DM/cm/ha) and utilisation, weaning weight (kg), the average daily gain (ADG; g/day), days to slaughter, and number of anthelmintic treatments administered.

Parameter	PRG	PRGWC	6S	9S	SEM	P-value
Herbage production	9891^{ab}	9120^{a}	10329^{b}	9898^{ab}	514.2	<0.05
Herbage density	245^{a}	237^{a}	260^{b}	243^{a}	6.43	<0.05
Herbage utilisation	0.84^{a}	0.88^{b}	0.93^{c}	0.91^{c}	0.012	<0.05
Weaning weight	30.9^{a}	32.5^{b}	33.3^{c}	32.1^{b}	0.42	<0.05
ADG from birth to slaughter	222^{a}	239^{b}	242^{b}	244^{b}	6.6	<0.05
Days to slaughter	181^{a}	167^{b}	168^{b}	168^{b}	3.7	<0.001
No. of anthelmintic treatments	2.7^{a}	2.1^{b}	1.4^{c}	1.5^{bc}	0.06	<0.0001

Conclusion The PRGWC, 6S and 9S swards produced similar annual herbage production from lower N input compared to PRG swards, however maintaining the proportions of herbs is a challenge under intensive sheep grazing. Grazing multispecies swards improved ewe and lamb performance and reduced the requirement for chemical anthelmintics.

Acknowledgements This project was funded by the Irish Department of Agriculture, Food and the Marine: RSF: 11/S/147.

References

Jing J, Soegaard K, Cong WF and Eriksen J 2017. Plos one, 12**,** 169-208.
Kemp PD, Kenyon PR and Morris ST 2010. Revista Brasileira de Zootecnia, 39**,** 169-174.
Marley C, Cook R, Keatinge R, Barrett J and Lampkin N 2003. Veterinary parasitology, 112**,** 147-155.

Faba beans can replace soybean meal and rapeseed meal in diets for dairy cows

Nicolaj P. Hansen, Marianne Johansen, Martin R. Weisbjerg

Aarhus university, AU Foulum, Dep. of Animal Science, Tjele, Denmark

E-mail: martin.weisbjerg@anis.au.dk

Take home message Faba beans are equivalent to soybean and rapeseed meal with respect to feed intake and milk yield.

Introduction The yield of faba beans (*Vicia faba* L.) under Danish conditions has increased considerably in recent years. So has the demand of locally produced feeds and especially protein supplements for dairy cows. This experiment tested the effect of replacing soybean meal and rapeseed meal with either untreated or toasted faba beans as a protein supplement on dry matter (DM) intake (DMI), milk yield (MY) and energy corrected milk yield (ECM).

Material & methods Two repeated 4x4 Latin square design trials were carried out simultaneously – each using 16 primiparous and 24 multiparous Danish Holstein cows. Cows within the trial were blocked according to parity and days in milk (99 ± 63, mean ± SD). Each experimental period lasted for 21 days. Cows were housed in a free cow traffic system with access to an automatic milking system (AMS) capable of feeding concentrates and weighing refusals after each visit. Intake of partial mixed ration (PMR) was recorded for each meal using the Insentec RIC system. The PMR was fed *ad libitum* and consisted of 23.4, 30.5, 30.1, 9.5, 1.3 and 0.9 % on DM basis of treatment concentrate, grass silage, maize silage, sugar beet pellets, rolled barley and palm fatty acids, respectively. In treatment concentrates, faba beans were replaced by an isonitrogeneous mix of rolled wheat (W) and soybean meal (S) or rapeseed meal (R). Treatment concentrates in trial 1 were untreated faba beans (UF), toasted faba beans (TF), 42:58 mix of S and W (SW), and 50:50 mix of TF and SW (TFSW). Treatment concentrates in trial 2 were UF, TF, 61:36 mix of R and W (RW), and 50:50 mix of TF and RW (TFRW). The total diets were planned to have a crude protein content of 160 g/kg DM. Data for DMI, MY and ECM (3.14 MJ/kg, fat and protein energy corrected) for the last 7 days in each experimental period was averaged per cow. Means of response variables were analysed using the Proc Mixed procedure in SAS using treatment, parity (primi- and multiparous), period and all 2-way interactions as fixed effects and cow as random effect. Across trials, effect of toasting faba beans and comparison of soybean meal with rapeseed meal were evaluated in an overall model, including trial as random effect.

Results & discussion ECM of SW and RW did not differ from that of UF or TF within trial. In trial 1 however, total DMI was higher in UF compared to SW. In trial 2, milking frequency was lower for RW compared to UF and TF, whereas total DMI did not differ between treatments. Milk fat yield was lower in SW compared to UF. Milk protein yield was lower in TF in both trials compared to all other treatments. In the overall model for both trials (results not shown), milk protein concentration and yield in TF were lower than all other treatments although not altering ECM. The expected effects of increasing dietary metabolisable protein content by toasting faba beans (Lund *et al.*, 2004) turned out to be negative on milk protein concentration (3.53 % for UF and 3.45 % for TF) and were in accordance to Mogensen *et al.* (2010). In addition, lower milk protein yields were found in TF compared to UF (1173 *vs.* 1215 g/day, respectively). In the overall model, no differences in DMI, MY or ECM were found between SW and RW.

Table 1 Effect of protein supplementation on feed intake and milk yield.

Variable	Trial 1						Trial 2					
	UF	TF	SW	TFSW	SEM	P-value	UF	TF	RW	TFRW	SEM	P-value
n	40	40	40	40			38	39	36	38		
Milkings/day	2.37	2.39	2.39	2.36	0.10	0.94	2.49^a	2.41^a	2.24^b	2.39^{ab}	0.08	<0.01
DMI, kg/day	22.6^a	22.2^{ab}	21.9^b	22.5^{ab}	0.47	0.04	23.1	22.8	22.7	23.2	0.40	0.12
ECM, kg/day	35.0^a	33.9^b	34.3^{ab}	34.6^{ab}	1.15	0.05	36.4	36.0	35.7	36.4	0.90	0.19
Fat, g/day	1422^a	1372^{ab}	1357^b	1394^{ab}	48	0.03	1465	1466	1418	1454	40	0.05
Protein, g/day	1190^a	1149^b	1225^a	1196^a	38	<0.01	1240^a	1196^b	1255^a	1253^a	30	<0.01

Conclusion Faba beans could replace soybean meal and rapeseed meal without affecting ECM, whereas total DMI increased when replacing soybean meal. Toasting of faba beans decreased milk protein concentration and yield.

Acknowledgements The study was funded by the Danish Milk Levy Fund (Mælkeafgiftsfonden).

References

Lund P, Weisbjerg MR and Kristensen T 2004. Journal of Animal and Feed Sciences 13, 143-146.

Mogensen L, Vestergaard JS, Fretté X, Lund P, Weisbjerg MR and Kristensen T. 2010. Livestock Science 128, 123-132.

Performance and nutrient utilization of calves fed diets containing agro-industrial by-products and *Enterolobium cyclocarpum* leaves

Christopher Onwuka, Bukola Ayilara, Adebayo Oni, Moses Arigbede

Federal University of Agriculture, Abeokuta, Abeokuta, Ogun State, Nigeria

E-mail: chysonka@yahoo.com

Take home *Enterolobium cyclocarpum* is a browse plant that can be obtained all year round even in dry season and which could be fed to calves to improve nutrient utilization and livestock performance.

Introduction Alternative feed resources and crop residues are locally available for use to increase livestock production in Nigeria. This study was conducted to evaluate the performance and nutrient utilization of yearling calves fed diets containing agro - industrial by-products and a browse plant (*Enterolobium cyclocarpum*).

Material & methods Leaves of *Enterolobium cyclocarpum* harvested were sun-dried and milled to 2mm sieve size. The ground leaves were included in concentrate rations at different levels. Agro-industrial by-products (wheat offal, palm kernel meal and cassava peels) were used to formulate the concentrate diets. Sixteen male cross-bred, White Fulani X Muturu yearling calves, 10-11 months of age, weighing 75-80kg were used for the 112 day study. The animals were randomly assigned to four treatment groups of *Enterolobium cyclocarpum* leaf meal (ECLM)-based concentrate diets in a completely randomised design arrangement. The treatment diets 1, 2, 3 and 4 contained 0, 5, 10 and 15g/100g ECLM, respectively (Table 1). The animals were each served 3kg concentrate at 09.00hours daily with fresh *Panicum maximum* as basal diet and water available *ad-libitum*. Each calf was weighed at the commencement of the experiment and thereafter fortnightly. Faecal samples voided were collected during the last seven days of the digestibility trial, weighed, dried at 60°C for 48 hours and were used in calculating the digestibility of each nutrient. The data were subjected to one-way analysis of variance (ANOVA).

Results & discussion Highest weight gain ($P<0.05$) and the least feed intake occurred at 10g/100g ECLM inclusion level (Table 2). Digestibility coefficients increased with increasing dietary levels of Enterolobium. The feed conversion ratio decreased ($p<0.05$) with increasing ECLM levels up to 10g/100g.

Table 1 Chemical composition of the experimental diets (g/100g).

Parameters	1 0 ECLM	2 5	3 10	4 15
Dry matter	65.00	90.31	90.71	90.85
Crude protein	16.20	17.78	17.59	17.44
Crude fibre	10.52	15.06	15.48	16.23
Cellulose	27.60	30.59	31.22	36.24
Hemicellulose	18.00	23.09	22.79	18.63

Table 2 Performance characteristics of calves fed ECLM-based concentrate diet.

Parameters	1 0 ECLM	2 5	3 10	4 15	SEM
Initial Weight (kg)	75.00	78.33	76.00	76.67	1.16
Final Weight (kg)	90.13	96.90	95.43	92.23	1.54
Weight gain (kg/day)	0.18^{c}	0.22^{ab}	0.23^{a}	0.19^{bc}	0.01
Concentrate intake(kg)	1.78	1.75	1.62	1.63	2.56
Grass intake (kg)	2.46	2.58	2.56	2.60	1.65
Crude Protein dig. (%)	77.00^{b}	78.81^{ab}	81.59^{a}	78.34^{ab}	0.67
Feed Intake (kg/day)	4.24	4.34	4.18	4.23	0.03
FCR	23.67^{a}	19.73^{bc}	18.01^{c}	22.84^{ab}	0.82

^{abc} means in the same row with different superscripts are significantly different $P<0.05$). Treatment 1= 0, 2= 5, 3=10 and 4= 15 g/100g ECLM inclusion levels respectively.

Conclusion ECLM inclusion up to 10 g/100g in concentrate diets had a positive effect on performance of yearling calves. In conclusion, *Enterolobium cyclocarpum* leaves could be used up to 10 g/100g level of inclusion as supplement with other cheap agro-industrial by-products in concentrate diets.

Acknowledgements The authors acknowledge the support of FUNAAB farm staff.

References

AOAC 2000. 20th ed. AOAC International, Arlington, VA, USA.
Adams MC, Luo J, Rayward D, King S, Gibson R and Moghaddam G H 2008. Animal Feed Science and Technology 145, 41-52.

Understanding factors associated with grazing efficiency of perennial ryegrass

Tomas Tubritt[1,2], Nicky Byrne[1,2], Trevor Gilliland[2,3], Luc Delaby[4], David Cummins[5], Michael O'Donovan[1]

[1]Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland, [2]Institute of Global Food Security, Queen's University Belfast, Belfast, UK, [3]Agri-food and Biosciences Institute, Large Park, Hillborough, UK, [4]INRA, UMR production du lait,, 35590 St. Gilles, France, [5]Department of Agriculture, Food and the Marine, Crop Evaluation and Certification Division, Ballyderown Farm, Kilworth, Co. Cork, Ireland

E-mail: ttubritt@hotmail.com

Take home message This study showed that differences in grazing efficiency exist between perennial ryegrass varieties and identified traits that could be used by breeders as selection criteria for superior grazing varieties.

Introduction Grazing efficiency refers to the ease at which grass swards are grazed to a desired post-grazing height typically <4 cm. Increased grazing efficiency of grass varieties is desirable as it affects the ease of management of grass swards and influences the level of grass utilisation on-farm. Current mechanical simulated grazing variety evaluation protocols do not take grazing efficiency into account. The objective of this study was to identify traits within perennial ryegrass (PRG) (*Lolium perenne*) that influence grazing efficiency.

Material & methods Fifty-five perennial ryegrass varieties with various heading dates were sown in August 2014 in a randomised complete block design in 3 replicates (165 plots). Each variety was sown in a 3m x 7m plot. Plots were rotationally grazed by lactating dairy cows from February to October in both 2015 and 2016. On average sixty-five cows grazed the 0.95 ha paddock by block when herbage mass was estimated to be 1300 kg DM ha^{-1}. Dung pats were removed from the plots after each grazing and a total of 252 kg N ha^{-1}, 47 kg N ha^{-1} and 197 kg N ha^{-1}was applied to plots over both years. Prior to grazing pre-height measurements were taken in each plot using a Jenquip rising plate meter. Post-grazing residual heights (PGRH) were taken as a measure of grazing efficiency of each variety. Three selected herbage samples were taken from each plot cut at 4 cm with a garden hand shears (ACCU 60, Husqvarna AB, Sweden). A sub-sample (0.1 kg) was oven dried at 60˚C to obtain a dry matter value for each plot. Near infrared spectroscopy (NIRS) was used to obtain dry matter digestibility (DMD), water soluble carbohydrates (WSC) and crude protein (CP). Sward morphological characteristics were also quantified by collecting tillers from each plot. Fifty tillers were used to measure tiller mass and the pre-grazing extended tiller height (ETH) and extended sheath height (ESH). Free leaf lamina (FLL) was calculated by subtracting ESH from ETH. Data was analysed using the PROC MIXED and PROC GLM procedure (SAS) with sward height, sward quality and sward morphological characteristics used as variables in the model.

Results Tetraploids achieved lower PGRH than diploids, 3.8cm *vs* 4.1cm (Table 1). This is likely due to the contributory effects of the traits examined. Pre-grazing height was shown to have a significant negative effect (p<0.001) on PGRH and while tetraploids achieved lower PGRH, more herbage (0.3cm) was also removed from this group. FLL (+1.5), tiller mass (+0.02), DMD (13.4 g kg DM^{-1}) and CP (+2.4 g kg DM^{-1}) was greater for tetraploids than diploids and these characteristics were shown to have a significant positive effect (p<0.001) on grazing efficiency. FLL is a trait of interest as it is a reliable trait that is easily measured. Therefore, it is a trait that could be selected for improved grazing efficiency. Increased tiller mass is indicative of reduced tiller density due to less competition between plants for resources. It is easier for cows to graze such swards as reduced bulk density reduces the shear strength required to graze the sward. Swards with increased levels of green leaf, DMD and CP are shown to have greater grazing efficiency.

Table 1 Sward characteristic differences between ploidy and heading date groups under animal grazing.

	Ploidy			Heading date			
Trait	Diploid	Tetraploid	Significance	Intermediate	Late	Significance	s.e
Herbage removed	5.1	5.4	***	5.3	5.2	**	0.03
Post-grazing characters							
Post-Grazing Residual (cm$^+$)	4.1	3.8	***	3.9	4.0	**	0.03
Pre-grazing Characters							
Pre-Grazing height (cm$^+$)	9.4	9.0	***	9.3	9.2	**	0.14
Free leaf lamina (cm)	19.0	20.5	***	20.0	19.5	NS	0.16
Tiller mass (g DM^{-1})	0.06	0.08	***	0.07	0.07	NS	0.001
DMD (g kg^{-1} DM)	819.8	833.2	***	824.0	829.0	***	1.75
CP (g kg^{-1} DM)	176.4	178.8	**	178.3	176.8	NS	1.82

$^+$ Compressed height $P > 0.05$ ^{NS} $P < 0.05$ * $P < 0.01$ ** $P < 0.001$ ***

Conclusion Differences between variety ploidy and heading dates for sward canopy and quality traits influenced the grazing efficiency of these varieties with ploidy having the greatest effect. Some of these characteristics like sward quality are already tested for in routine variety evaluations. Others like FLL and tiller mass are shown in this study to be easily selected for. It is therefore possible in the future for plant breeders to develop varieties with higher grazing efficiency by selecting varieties with improved performance in the traits identified in this study.

Effect of dairy cow diet on milk and milk solids production in a spring calving dairy system

Deirdre Hennessy, MaryAnne Hurley, Stephen McAuliffe

Teagasc AGRIC, Moorepark, Fermoy, Co Cork, Ireland

E-mail: deirdre.hennessy@teagasc.ie

Take home message Milk production is greater from total mixed ration (TMR) compared to pasture based diets. Grass-clover based diets had higher milk production than grass-only based diets.

Introduction Dairy cow diet is one of the main factors influencing milk production. In grass-based systems the quality of the diet is variable and milk production per cow is often lower than when feeding total mixed ration (TMR) diets (Kolver and Muller, 1998; Bargo and Muller, 2002). There is renewed interest in incorporating white clover (*Trifolium repens* L.; clover) into grass-based milk production systems in Ireland due to its ability to fix nitrogen (N), improve pasture quality and utilisation, and increase milk production (Egan *et al*., 2017). The objective of this experiment was to examine the effect of feeding system (TMR, grazed grass-only (GO) and grazed grass-clover (GC)) on dairy cow milk and milk solids yield.

Material & methods A full lactation farm systems experiment (February to November) was undertaken at Teagasc, Moorepark, Fermoy, Co. Cork, Ireland in 2015, 2016 and 2017. The experiment had three treatments: TMR (grass silage, maize silage, concentrate), GO receiving 250 kg N/ha and GC receiving 250 kg N/ha. In February of each year, Holstein Friesian spring calving dairy cows from the Moorepark herd were blocked on calving date, breed, parity, pre-experimental milk yield (MY) and pre-experimental milk solids (MS) yield and randomly allocated to one of the three treatments (n = 18, 18 and 17 in 2015, 2016 and 2017, respectively). Cows remained in their treatment groups for the entire lactation. The GO and GC treatments were stocked at 2.74 cows/ha in a closed farm system and rotationally grazed their respective farm-lets. Cows on the TMR treatment were housed in cubicles with rubber mats and fed at 08:30 h each day using a Keenan diet feeder (Keenan, Borris, Carlow, Ireland). Cows were fed *ad-libitum* to approximately 10% refusal levels via electronically controlled individual feed bins (Griffith Elder and Company Ltd, Suffolk, England in 2015 and 2016, and Hokofarm Group B.V., Voorsterweg 28, 8316PT Marknesse, Netherlands in 2017). Milking took place at 07:30 and 15:30 h daily. Milk yield was measured daily and MS (fat, protein) weekly. Data were analysed using PROC MIXED in SAS.

Results & discussion The TMR treatment had significantly greater ($P < 0.001$) daily MY and MS yield compared to the GC and GO treatments (Table 1), most likely due to the higher and more consistent quality of the TMR diet compared to the pasture diets (Kolver and Muller, 1998; Bargo *et al.,* 2002). Daily MY and MS yield was greater ($P = 0.063$ and $P=0.073$, respectively) on GC compared to GO. Cumulative milk yield was significantly less on the GO and GC treatments compared to TMR. The average annual sward clover content of the GC treatment was 22%.

Table 1 Daily and cumulative milk production from cows grazing grass-only receiving 250 kg N/ha (GO), cows grazing grass-clover receiving 250 kg N/ha (GC), and cows fed on a total mixed ration diet indoors (TMR) over three full lactations (2015, 2016 and 2017).

	GO	GC	TMR	SEM[1]	TRT[2]
Milk yield (kg/cow/day)	21.3^{a}	23.4^{a}	27.3^{b}	0.83	<0.001
Milk solids yield (kg/cow/day)	1.76^{a}	1.90^{a}	2.15^{b}	0.060	<0.001
Milk fat content (g/kg)	47.6^{a}	47.4^{a}	45.1^{b}	1.73	<0.001
Milk protein (g/kg)	36.4^{a}	36.7^{a}	33.8^{b}	0.61	<0.001
Cumulative milk yield (kg/cow)	6099^{a}	6570^{a}	7800^{b}	253.7	<0.001
Cumulative milk solids yield (kg/cow)	492^{a}	519^{a}	607^{b}	18.02	<0.001

[1]SEM=Standard Error of the Mean, [2]TRT=Treatment. Treatments with the same letter in a row are not significantly different to each other.

Conclusion Feed system had a significant effect on milk and MS yield; the TMR treatment had greater milk production than both of the grazing groups.

Acknowledgements This research was funded by the Irish Dairy Levy administered by Dairy Research Ireland. S. McAuliffe and M. A. Hurley are in receipt of a Teagasc Walsh Fellowship.

References

Bargo F. and Muller LD 2002. Journal of Dairy Science 85, 2948–2963.
Egan M, Lynch MB, and Hennessy D. 2017. Journal of Agricultural Science 155, 657–668.
Kolver ES and Muller LD 1998. Journal of Dairy Science 81, 1403–1411.

The wonder of willow tannin-rich tree (*Salix* spp. *Salicaceae*): a potentially valuable tree fodder for ruminants

Mairead Campbell[1], Andreas Foskolos[3], Sokratis Stergiadis[2], Eve Richardson[2], Chris Humphrey[2], Chris Drake[2], Irene Mueller-Harvey[2], Katerina Theodoridou[1]

[1]Queen's University, Institute for Global Food Security, Belfast, UK, [2]University of Reading, School of Agriculture, Policy and Development, Animal, Dairy and Food Chain Sciences, Reading, UK, [3]Aberystwyth University, Wales, UK

E-mail: k.theodoridou@qub.ac.uk

Take home message Willow is a promising alternative animal feed to improve protein utilization and reduce methane emissions.

Introduction Nowadays, it is important to adopt more efficient and environmentally sustainable ruminant feeding systems. One approach to these issues, is the use of sources that contain condensed tannins (CT), able to bind to proteins and protect them from ruminal degradation and reduce methane emissions. Willow is a tannin-rich tree fodder (Falchero *et al.* 2011) and shows a great potential in animal nutrition. However, data on the nutritive value of willow as animal feed are scarce. This study investigated, for the first time, the effect of willow varieties and plant organ on CT quantity and the effect of their CT on *in vitro* methane production.

Material & methods Representative samples (n=15) from five willow varieties were collected from three replicate blocks in the cultivation area of AFBI at Loughgall, Co. Armagh, N. Ireland. Samples were freeze-dried and ground at 1-mm screen. Nitrogen (N) in willow was analysed by Dumas method. *In*-Situ Thiolysis, using chromatography/mass spectrometry, used to assess mean degree of polymerisation (mDP), and ratio of procyanidins to prodelphinidins (PC/PD), and *cis/trans*-flavan-3-ols. HCl-butanol-acetone free flavanols analysis, using UV-Vis spectrophotometer used for CT content. The *in vitro* batch fermentation system (Theodorou *et al.* 1994) was used to evaluate the effect of willow on gas and methane production compared to lucerne, a non-CT legume. ANOVA residual maximum likelihood analysis was performed using variety (Tables 1) and organ (Table 2) as fixed factors and tree as random factor using SAS (version 9.4) (SAS Institute, 2016).

Results & discussion N content among the varieties was similar with average value 32.41 (g/kg DM). Terranova had the lower CT content among willow varieties while leaves had the higher CT content among the plant organs. The methane production was lower ($p<0.001$) for willow compared to lucerne a non-CT legume (Figure 1).

Table 1 Qualitative analysis and CT (g/100 gr) content of five willow varieties.

Willow variety							
	Beagle	Endeavour	Olof	Resolution	Terranova	SEM	P-value
mDP	10.90^a	12.0^a	11.5^a	11.3^a	8.20^b	0.78	<0.001
PC/PD	0.30^b	0.36^b	0.33^b	0.37^b	0.75^a	0.08	<0.001
cis/trans	1.24^a	1.45^a	1.29^b	1.36^b	1.76^a	0.14	0.041
CT content	0.08^a	0.08^a	0.09^a	0.09^a	0.06^b	0.01	0.003

Table 2 Qualitative analysis and CT (g/100 gr) content of plant organs in all willow varieties.

Plant Organ					
	Whole Plant	Leaves	Stems	SEM	P-value
mDP	10.3^b	9.1^b	13.0^a	0.64	<0.001
PC/PD	0.41	0.39	0.47	0.07^b	0.462
cis/trans	1.27^b	0.93^c	2.05^a	0.11	<0.001
CT content	0.09^a	0.11^b	0.05^c	0.01	<0.001

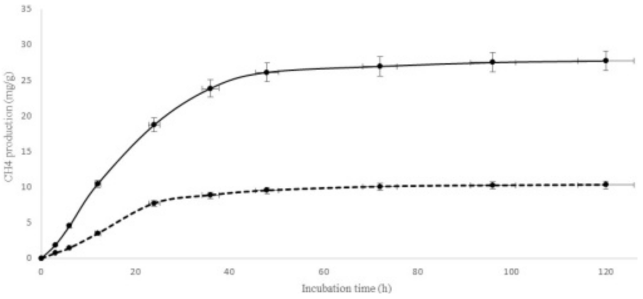


Figure 1. CH_4 (ml/g) produced off willow compared to lucerne.

Conclusion Results demonstrate that willow influence rumen fermentability and as a result reduce methane production. It needs to select the most suitable variety and harvest strategy to optimise the beneficial effect of introducing willow in ruminant nutrition.

Acknowledgements This project was supported by the Department for the Economy (DFE), Northern Ireland, UK.

References

Falchero L, Brown RH, Karp A, Hanley S, Shield I and Mueller-Harvey I 2011. Advances in Animal Biosciences. 2, 410.
Theodorou MK, Williams BA, Dhanoa MS, McAllan AB and France J 1994. Animal Feed Science and Technology 48, 185-197.

Rumen parameter of West African dwarf goats offered concentrate supplement containing varying levels of *Leucaena leucocephala* leaves

Oludotun Adelusi, Adebayo Oni, Victoria Ojo, Yemisi Aderinboye, Christopher Onwuka

Federal University of Agriculture, Abeokuta, Nigeria

E-mail: adelusioludotun@gmail.com

Take home message Utilization of *Leucaena* leaves influenced rumen fermentation for better nutrient absorption.

Introduction The genetic potential of goats for growth, reproduction and lactation is dependent on adequate nutrition especially during the dry season when there is little or no quality forage for consumption. Browse species like *Leucaena leucocephala* and agro-industrial by-products like cassava peels, can serve as a rich protein, energy and fibre source for goat sustenance during this period (Odeyinka, 2001; Fayemi *et al.*, 2011). Thus, this study tested the ruminal response of goats fed concentrate supplement with cassava and *Leucaena* as the major ingredients.

Material & methods Twenty-four West African dwarf goats weighing 8.5±0.24 kg housed in individual pens were used for this study that lasted for 70 days. Concentrate supplements were formulated to contain four levels of *Leucaena* leaves (0, 5%, 10% and 15% on dry matter basis) as dietary treatments and set out in a completely randomised design. Cassava peel make up 40% of each diet, while the other ingredients included dried brewers grain, palm kernel cake, oyster shell and salt. The four treatments contained six goats each that were fed a basal diet of *Panicum maximum* and the concentrate supplement, while water was provided *ad libitum*. Rumen fluid was collected prior to treatment application for baseline measurements of rumen parameters. On the final day of the experiment rumen fluid was collected (0 and 6 hours post-feeding) from the goats using oesophageal tubes and used for the analysis of rumen ammonia nitrogen, volatile fatty acids (Siedlecka *et al.*, 2008) and microbial count (Galyean, 1989). Data obtained were subjected to one-way ANOVA.

Results & discussion The values obtained for volatile fatty acids decreased ($p < 0.05$) as the level of *Leucaena* increased across the diets (Table 1), with the exception of propionic acid ($p = 0.57$) and butyric acid ($p = 0.85$) which were not affected. Rumen NH_3-N values reduced ($p = 0.05$) from 2.16 mg/dL to 1.05 mg/dL at 0 and 10 % levels of *Leucaena* respectively. Microbial count of the rumen was not affected ($p > 0.05$) by the various inclusion levels of *Leucaena* (Table 2).

Table 1 Rumen fermentation parameters of WAD goats fed levels of *Leucaena leucocephala*

Leucaena levels	0 %	5 %	10 %	15 %	SEM
Lactic acid (g/dL)	1.15^a	0.92^b	0.95^b	0.86^b	0.039
Acetic acid (g/dL)	0.65^a	0.30^b	0.30^b	0.27^b	0.061
Propionic acid (g/dL)	0.85	0.70	0.71	0.66	0.048
Butyric acid (g/dL)	0.06	0.04	0.05	0.04	0.007
NH_3-N (mg/dL)	2.16^a	1.64^a	1.05^b	1.20^{ab}	0.275

Table 2 Rumen microbial count of WAD goats levels of *Leucaena leucocephala*

Leucaena levels	0 %	5 %	10 %	15 %	SEM
Bacteria (× 10^6 cfu/ml)	0.90	0.93	0.73	0.69	0.128
Protozoa (×10^3 cells/ml)	0.27	0.33	0.40	0.47	0.040
Fungi (× 10^6 cfu/ml)	0.00	0.03	0.03	0.07	0.019

Conclusion Results showed that inclusion of *Leucaena* leaves in the concentrate diets of goats as additional protein source facilitated adequate utilization of dietary energy denoted by better absorption of volatile fatty acids which is the major energy source to the ruminant animal. The microbial count of the rumen remained unchanged and unaffected by the inclusion of *Leucaena,* thus maintaining the microbial balance of the rumen, an important factor for proper degradation and utilization of feed ingredients.

Acknowledgements This research was carried out at the Directorate of University Farms, FUNAAB, Nigeria.

References

Fayemi PO, Onwuka CF, Isah OA, Jegede AV, Arigbede OM and Muchenje V 2011. African Journal of Agricultural Research 6, 4081-4085

Galyean M. 1989. Laboratory Procedure in Animal Nutrition Research. Department of Animal and Life Science, New Mexico State University, Las Cruces. 107–122pp

Odeyinka, S.M. 2001. Nigerian Journal of Animal Production, 28, 61-65

Siedlecka EM, Kumirska J, Ossowski T, Glamowski P, Gołębiowski M, Gajdus J, Kaczyński Z and Stepnowski P 2008. Polish Journal of Environmental Studies 17, 351-356

Grass-only and grass and clover seasonal sward structure effects on *in vivo* dry matter digestibility and intake on individually housed sheep

Maryanne Hurley[1,2], Tommy Boland[2], Deirdre Hennessy[1]

[1]*Teagasc, Moorepark, Fermoy, Ireland,* [2]*University College Dublin, Dublin, Ireland*

E-mail: mary-anne.hurley@ucdconnect.ie

Take home message Dry matter digestibility is subject to change throughout the grazing season in both grass-only and grass and clover swards.

Introduction The nutritive value of grass-only (GO) swards tends to decline towards the latter end of the grazing season due to advanced maturity (Enriquez-Hidalgo *et al*., 2016), while grass-white clover (*Trifolium repens* L.) (GC) swards maintain sward nutritive value longer into the grazing season (Frame and Newbould, 1986). Including clover in the sward can affect voluntary dry matter intake (VDMI) and DM digestibility (DMD). The objective of this experiment was to determine the effect of grass-only (GO) and grass-clover (GC) on DMD and VDMI, when offered fresh to individually housed sheep in spring, summer and autumn.

Material & methods An *in-vivo* 2×2 Latin square design experiment with two treatments, GO and GC, and two periods (P1 and P2) was undertaken using 12 Texel sheep in Spring (April), Summer (June/July) and Autumn (September). Each period had a 6-day acclimatisation stage and a 6-day measurement stage. Sheep were individually housed and offered fresh herbage daily fed *ad libitum*. The quantity of herbage offered to and refused by each sheep was weighed daily to calculate VDMI. The DMD was calculated once faeces were collected and dried. Pre-cutting herbage mass was measured twice weekly. A 200 g sample of fresh herbage was dried to estimate daily herbage DM content. Leaf, stem and dead proportions of GO and GC, and sward clover content of the GC sward, were measured daily during the measurement period. Data were analysed using Proc Mixed in SAS with terms for treatment, season and the treatment×season interaction. Season and treatment were the fixed effects and the individual sheep were the random effect.

Results & discussion Sward clover content, on a DM basis, was 19.6% in Spring, 17.92% in Summer and 51.54% in Autumn. There was no significant effect of treatment on DMI or DMD (Table 1). The DMD was significantly ($P < 0.001$) greater in Spring than in Summer and Autumn. There was a significant season × treatment effect on leaf proportion; which was greater on GO compared to GC in Spring. Sward leaf content was greatest in Spring and dead content was least in Spring. There was a significant ($P < 0.05$) treatment × season effect on grass stem content which was greater on GO compared to GC in Autumn. Stem content was greater in Spring and Summer than in Autumn. These differences in leaf, stem and dead contents contributed to the season effects on DMD. Herbage quality analyses will provide further information.

Table 1 Herbage DMI, DMD, sward leaf, stem, dead and clover content in Spring, Summer and Autumn on GO and GC.

	Treatment	DMI (kg day^{-1})	DMD (g kg^{-1})	Leaf (%)*	Stem (%)	Dead (%)	Clover (%)
Spring	Grass	1.99	814^{a}	77.5^{a}	17.3^{a}	6.2^{a}	-
	Grass-clover	2.0	817^{a}	71.3^{abc}	18.4^{a}	9.0^{a}	19.66^{a}
Summer	Grass	1.95	749^{b}	62.1^{bc}	25.9^{b}	11.6^{b}	-
	Grass-clover	1.90	769^{b}	64.4^{bc}	24.5^{b}	11.3^{b}	17.92^{a}
Autumn	Grass	1.86	740^{b}	72.0^{ab}	16.0^{b}	12.5^{b}	-
	Grass-clover	2.00	750^{b}	76.5^{ab}	10.7^{c}	12.9^{b}	51.54^{b}
	s.e.m.	0.077	9.1	2.84	1.08	1.71	4.141
	Treatment	N.S.	N.S.	N.S.	<0.01	N.S.	n/a
	Season	N.S.	<0.001	< 0.001	<0.001	< 0.01	<0.001
	Season × Treatment	N.S.	N.S.	< 0.05	<0.05	N.S.	n/a

*the sum of leaf, stem and dead components does not always equal 100% due to rounding; n/a = not applicable; Letters within columns which are the same are not significantly different to each other

Conclusion Clover inclusion did not have a significant effect on DMI or DMD in this experiment, likely due to the high quality of the GO swards maintained through frequent cutting to maintain low herbage masses (<1600 kg DM ha^{-1}). Season had a significant effect on DMD. Leaf percentage was greater in Spring compared to the other two seasons.

Acknowledgements This research was funded by the Irish Dairy Levy. M. A. Hurley is in receipt of a Teagasc Walsh Fellowship.

References

Enriquez-Hidalgo D, Gilliland TG and Hennessy D. 2016 Grass and Forage Science 71, 559-574.
Frame J and Newbould P 1986 Advances in Agronomy 40, 1-88.

Residual feed intake is positively related to beta hydroxybutyrate measured in blood plasma for adult Merino ewes

Sarah Blumer[1], Graham Gardner[1], Mark Ferguson[2], Andrew Thompson[1]

[1]*Murdoch University, Perth, Western Australia, Australia,* [2]*neXtgen Agri Ltd, Christchurch, New Zealand*

E-mail: s.blumer@murdoch.edu.au

Take home message Residual feed intake (RFI) is an expensive and difficult trait to measure so related indicator traits would be useful for the selection of more efficient animals. Beta hydroxybutyrate is positively related to RFI in adult ewes.

Introduction Mediterranean farming regions in Australia typically experience long dry spells during summer and early autumn, and the decline in the quantity and quality of paddock feed can lead to significant liveweight (LW) losses in the adult ewe flock. Stocking rate is the main driver of farm profitability, and selecting ewes to be more feed efficient could potentially enable farmers to maximise stocking rates without compromising reproductive performance. Beta hydroxybutyrate (BHB) has previously been suggested as a useful indicator trait for RFI and efficiency given that it is independent of growth traits and has been demonstrated as repeatable across a number of stages of maturity (Kelly *et al,* 2010). We measured RFI in adult Merino ewes fed a poor quality diet and investigated a range of metabolites that might be useful indicator traits, including BHB.

Material & methods Details of this experiment have been previously reported (Blumer *et al*, 2016). Briefly, 64 Merino ewes were held in single pens for 71 days and were fed a low quality barley chaff treated with urea to increase protein and molasses to increase palatability (5.1 MJ of ME per kg dry matter). After an introductory period the ewes were split into 2 groups (balanced for LW and condition score) and then fed either *ad libitum,* or restricted to about half of their maintenance requirements with the aim of LW loss. LW data was collected twice weekly and feed refusals were measured daily. On day 54, a 6-hour serial bleed was carried out (following a 12-hour fast). Samples for BHB were collected on the hour into heparinised tubes (x 6 samples), the plasma harvested and stored at -20 degrees for later analysis.

Using starting LW and LW change, ME intake was modelled as the dependent variable using general linear models in SAS (for ewes consuming the *ad libitum* diet; n=30). The residuals (RFI) from this model were used to describe efficiency of the individual in relation to the cohort. RFI was then analysed in relation to the mean concentrations of BHB.

Results BHB in plasma was positively associated with RFI ($P<0.05$, Fig. 1). Ewes with lower concentrations of BHB in plasma were more efficient than the cohort. An increase in BHB concentration of 0.02 mmol/L was associated with increased feed requirements of 0.15 MJ of ME per day for ewes to maintain LW. BHB explained 13% of the variation in RFI.

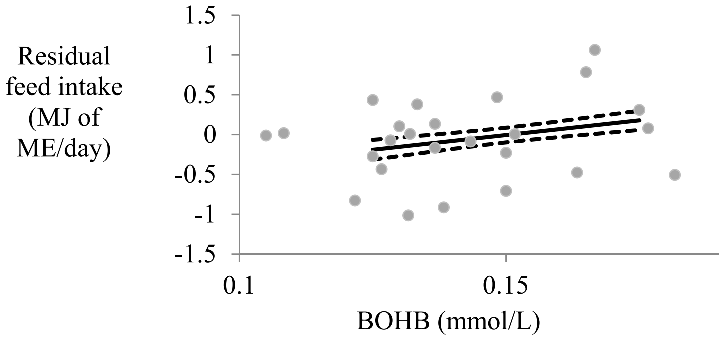


Figure 1 Modelled relationship between BHB in plasma and residual feed intake for adult Merino ewes fed a poor quality diet (±SE; grey dots represent raw BHB data).

Conclusion BHB is a product of fatty acid metabolism via the breakdown of acetoacetate in the liver (ketogenesis), and by the oxidation of butyrate in the rumen wall (the main site of BHB production in a normally fed animal). During undernutrition ketogenesis becomes the main determinant of BHB levels and whole system ketogenesis increases (Chilliard *et al*, 2000). This might suggest that the less efficient animals consuming a poor quality diet in our study were in a permanent state of undernutrition. However, BHB has a number of roles and associations, which could be implicated in feed efficiency including: regulation of intake in ruminants (Laeger *et al*, 2010); increasing in response to fat mobilisation (Kelly *et al*, 2010); and, inhibiting lipolysis (Laeger *et al*, 2010). BHB could be a useful indicator of efficiency in sheep, however the mechanism is unclear and requires further understanding.

Acknowledgements This research was supported by the Australian Government's Cooperative Research Centre Program via the CRC for Sheep Industry Innovation.

References

Blumer S, Gardner G, Ferguson M, and Thompson A 2016. Animal Production Science 56, 789-796.
Chilliard Y, Ferlay A, Faulconnier Y, Bonnet M, Rouel J, and Bocquier F 2000. Proceedings of the Nutrition Society 59, 127-134.
Kelly AK, McGee M, Crews D H, Sweeney T, Boland TM, and Kenny DA 2010. Journal of Animal Science 88, 3214-3225.
Laeger T, Metges CC, and Kuhla B 2010. Appetite. 54, 450-455.

Comparing and combining proxies for methane emissions of lactating Holstein Friesian cows

Sanne van Gastelen[1], Kasper A. Hettinga[2], Jan Dijkstra[1]

[1]*Wageningen University & Research, Animal Nutrition Group, Wageningen, The Netherlands,* [2]*Wageningen University & Research, Product Quality and Design Group, Wageningen, The Netherlands*

E-mail: sanne.vangastelen@wur.nl

Take home message Milk fatty acids alone can predict enteric methane emissions of dairy cows better than lactation stage characteristics alone and dietary composition alone, while combining proxies has an advantage over a single proxy.

Introduction There are several techniques to estimate or measure enteric methane (CH_4) production of dairy cows, but none of these techniques are suitable for large scale precise and accurate measurements. Therefore, the potential of proxies (*i.e.,* indicators or animal traits that are correlated with enteric CH_4 production) for CH_4 emission of dairy cows gained interest. The objectives of the present study were (i) to compare the CH_4 prediction potential of characteristics generally available on farm (*i.e.,* lactation characteristics and dietary composition) with that of milk fatty acids (MFA) which require complex and expensive analysis, and (ii) to determine if a combination of proxies results in a higher CH_4 prediction potential than that of single proxies.

Material & methods Data from 9 experiments (all from Wageningen University & Research, The Netherlands) with individual observations of 218 lactating cows and 30 dietary treatments were used. Milk was sampled and CH_4 emissions were measured for 3 consecutive days in climate respiration chambers. Lactation characteristics of the cows [*i.e.,* parity, days in milk, milk yield (kg/d), milk protein, fat and lactose content (all g/100 g milk), milk urea content (mg/dL), somatic cell count (natural logarithm of cells/ml)], and the milk fatty acid (MFA) profile (elucidated with gas chromatography) were recorded, and the experimental diets were analysed to determine the chemical composition [DM (g/kg), ash, NDF, ADL, ADF, fat, starch (all in g/kg DM), gross energy (MJ/kg DM), and the NDF-to-starch ratio (dimensionless)]. Methane emissions were expressed as production (g/d), yield (g/kg dry matter intake; DMI), and intensity (g/kg fat- and protein-corrected milk; FPCM). Parity was 2.7 ± 1.38, days in milk was 179 ± 85.2, dietary CP was 176 ± 40.1 g/kg DM, dietary NDF was 380 ± 49.9 g/kg DM, DMI was 16.3 ± 2.18 kg/d, FPCM yield was 25.9 ± 5.06 kg/d, CH_4 production was 366 ± 53.9 g/d, CH_4 yield was 22.5 ± 2.10 g/kg DMI, and CH_4 intensity was 14.4 ± 2.58 g/kg FPCM (average ± SD). Multivariate models were developed using a stepwise procedure (PROC GLMSELECT) with CH_4 emissions (*i.e.,* production, yield, and intensity) as the independent variable and stepwise selection of only MFA, only lactation characteristics, only dietary composition, or a combination of these. The significance level for a variable to enter or stay in the model was 0.01 and 0.05, respectively, and was based on the relation between the variable and CH_4 emission. All models were evaluated with the PROC REG procedure in terms of multicollinearity (variation inflation factor >10), but no multicollinearity was observed for any of the CH_4 prediction models. The CH_4 prediction models were evaluated using the coefficient of determination (R^2) analysis and the concordance correlation coefficient (CCC) analysis (Lin, 1989).

Results & discussion Milk fatty acids alone had a greater prediction potential for CH_4 emissions than lactation characteristics alone and dietary composition alone (Table 1). The combination of MFA with lactation characteristics resulted in the highest prediction potential for both CH_4 production and CH_4 intensity. The combination of MFA with both lactation characteristics and dietary composition resulted in the highest prediction potential for CH_4 yield.

Table 1 The coefficient of determination (R^2) and concordance correlation coefficient (CCC) analysis of the prediction equations for methane emission

	CH_4 production (g/d)		CH_4 yield (g/kg DMI)		CH_4 intensity (g/kg FPCM)	
Item	R^2	CCC	R^2	CCC	R^2	CCC
Milk fatty acids (MFA)	0.54	0.72	0.40	0.59	0.62	0.77
Lactation characteristics (LC)	0.43	0.61	0.09	0.18	0.33	0.50
Dietary composition (DC)	0.18	0.30	0.24	0.39	0.29	0.46
LC + DC	0.52	0.70	0.26	0.42	0.53	0.70
MFA + LC	0.72	0.84	0.44	0.62	0.71	0.84
MFA + DC	0.54	0.72	0.40	0.59	0.66	0.81
MFA + LC + DC	0.72	0.84	0.48	0.66	0.71	0.84

Conclusion The MFA profile as a single proxy has a moderate to good prediction potential for CH_4 emission of dairy cows, whereas the potential of lactation characteristics alone and dietary composition alone is much lower. Combining proxies, in particular MFA and lactation characteristics, enhances the CH_4 prediction potential, illustrating that combining proxies has an advantage over a single proxy for CH_4 emissions of dairy cows.

References

Lin LIK. 1989. Biometrics 45,255-268.

Quebracho (*Schinopsis balansae*) extract in beef cattle fed high-roughage total mixed ration affects manure gas emissions

Aaron Norris[1], Luis Tedeschi[1], Kenneth Casey[2], Jose Dubeux[3], Jamie Foster[4], James Muir[5], William Pinchak[6]

[1]Texas A&M University, College Station, TX, USA, [2]Texas A&M AgriLife Research, Amarillo, TX, USA, [3]North Florida Research and Education Center, Marianna, FL, USA, [4]Texas A&M AgriLife Research, Beeville, TX, USA, [5]Texas A&M AgriLife Research, Stephenville, TX, USA, [6]Texas A&M AgriLife Research, Vernon, TX, USA

E-mail: a_norris@tamu.edu

Take home message The addition of quebracho condensed tannins to high-roughage diets mitigates manure greenhouse gas emissions.

Introduction Animal agriculture faces challenges meeting the demands of an increasingly stringent consumer populous, thus, necessitating means of minimizing non-natural feed additives and mitigating emissions of greenhouse gases (GHG). This has generated a global interest in the use of natural compounds as rumen modulators. Condensed tannins (CT), one such class of compounds, have shown promise for specific classes of livestock due to the potential for improving N efficiency and decreasing enteric CH_4 production. However, manure on pasture contributes approximately 15% of agriculture's global GHG inventory (Smith *et al.,* 2014), thus reducing GHG from manure is essential. Therefore, we investigated the impact of feeding differing rates of quebracho CT (QT; *Schinopsis balansae*) upon beef cattle manure emissions of CO_2. CH_4, and N_2O.

Material & methods English crossbred steers (n=8; 443 ± 20 kg) were provided a high-roughage total mixed ration (CP=105 and aNDF=462 g kg^{-1} DM) once daily at 17 g kg^{-1} body weight. Treatments were the addition of QT to base diets at rates of 0, 15, 30, or 45 g kg^{-1} DM (QT_0, QT_{15}, QT_{30}, QT_{45}) with two animals (replications) per treatment. Manure (900 g) from each animal x treatment combination was placed in chamber collars in duplicate. Vented static chamber technique was utilized. Sampling occurred between 0900 and 1100 h on days 0, 1, 2, 4, 6, 8, 10, 12, 14, 17, 21, 24, 28, 31, & 35 following manure application. Gas samples were collected at 0, 12, 24, and 36 min following chamber closure. Gas samples were analyzed using a gas chromatograph equipped with an electron capture, flame ionization, and thermal conductivity detectors. Daily CO_2. CH_4, and N_2O fluxes were calculated from slopes of gas concentrations *versus* time at time zero. Individual cumulative gas production and CO_2 equivalents (CO_2e = N_2O+CH_4) were calculated using summation of measured fluxes and adjusted for baseline soil flux. Cumulative fluxes per unit of manure DM (g kg^{-1}) were calculated for individual gases and CO2e, in addition N_2O emission factors were determined. Analyses of variance were performed using a random coefficients model in which animal within treatment was considered a random effect and statistical significance was assumed at $P \leq 0.10$.

Results & discussion CO_2 and N_2O cumulative emissions differed among treatments (P=0.09 and 0.0004), but there were no differences for CH_4. For both CO_2 and N_2O, QT_0 demonstrated the highest flux and QT_{45} had the lowest (Figure 1A). The same trend occurred for CO_2e as QT_{45} emitted 48% less CO_2e than QT_0 (P=0.08). Emission factors for N_2O demonstrated a separation of treatments in which QT_0 and QT_{15} had greater N emitted in the form of N_2O in comparison to QT_{30} and QT_{45} (P=0.0001; Figure 1B).

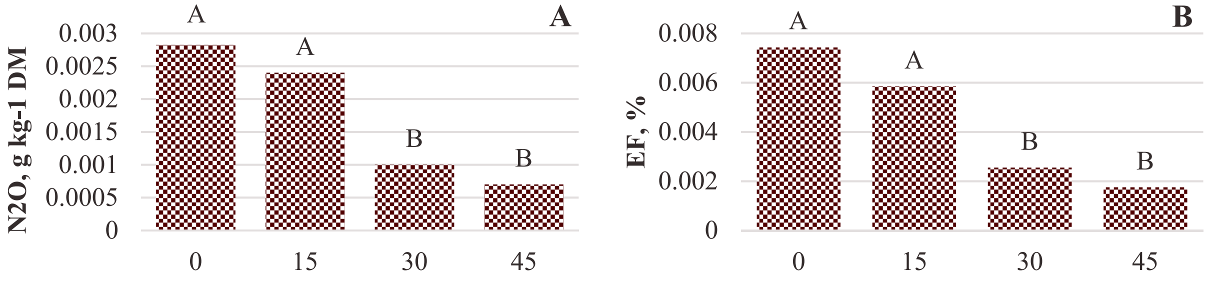


Figure 1: N_2O flux (**A**; g kg^{-1} DM) and Emission Factor (**B**; %) for different levels of dietary QT (g kg^{-1} DM).

Conclusion Utilization of QT within a high roughage diet greatly influenced primary gas fluxes from excreta. Although there were no differences for CH_4 the large difference in N_2O production, due to its greater global warming potential, inflated CO_2e values. The CO_2e trend demonstrates probable environmental impact. However, seasonal flux dynamics require further investigation.

References

Smith, P., Bustamante M, Ahammad H, Clark H, Dong H, Elsiddig EA, Haberl H, Harper R, House J, Jafari M, Masera O, Mbow C, Ravindranath NH, Rice CW, Abad CR, Romanovskaya A, Sperling F, and Tubiello F 2014. In: Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. pp. 7340–7349.

Estimating the part of residual energy intake associated with real differences in feed efficiency and not with errors in dairy cows

Amélie Fischer[1,2], Nicolas Friggens[3], Donagh Berry[4], Philippe Faverdin[2]

[1]Institut de l'élevage, Le Rheu, France, [2]INRA, Agrocampus-Ouest, Saint-Gilles, France, [3]INRA, AgroParisTech, Université Paris-Saclay, Paris, France, [4]Teagasc, Fermoy, Co. Cork, Ireland

E-mail: amelie.fischer67@gmail.com

Take home message When accurate high throughput monitoring and a steady environment are used, the variability of residual feed intake is only 8% of the variability of energy intake in Holstein cows over a full lactation. Even in these experimental conditions, only 59% out of these 8% are really associated with feed efficiency differences and not with errors.

Introduction Reducing the use of resources to maintain production and decrease dairy cow impact on the environment is a major challenge to tackle and is known as improving feed efficiency. Feed efficiency is often estimated with the residual intake defined as the difference between the actual feed intake and the feed intake predicted from the energy consumed or produced by the major biological functions (lactation, body reserves gain and loss and maintenance) (Berry and Crowley, 2013). As residuals of a statistical model, residual intake does not only include feed efficiency differences, but also the accumulated errors of model fitting and errors of measurement. Aggrey and Rekaya (2013) in broilers and Savietto *et al.,* (2014) in beef cattle used a mixed model with random animal effect to isolate the REI only associated with animal feed efficiency differences and not with errors. The objective of the present study was to estimate the part of residual intake variability in dairy cows really associated with feed efficiency differences and less with errors, thanks to a mixed model with random animal effect (Fischer *et al.,* 2017).

Material & methods Holstein cows were housed in a free-stall barn and fed with a single TMR based on corn silage and concentrates during the whole lactation. One hundred and nineteen cows were monitored throughout the first 238 days in milk for daily individual feed intake as the difference between offered dry matter and next morning refusals dry matter. All cows were also monitored for morning body weight after milking, daily milk yield, twice a week daily milk protein, fat and lactose, and monthly body condition score with a 0.25 increment scale going from 0 for an emaciated cow to 5 for a fat cow. Feed efficiency was estimated as the residual energy intake (REI): the residual of the linear regression of average net energy intake on average net energy in milk, average metabolic body weight and body condition score gain and loss, both multiplied by average body weight. To estimate the REI associated with feed efficiency and not with errors, the variables used to estimate REI - milk yield and composition, body condition score change, feed intake and body weight - were averaged per 2 week period to create repeated measures per cow during the 238 monitored days. A mixed model was then fitted with random cow-specific slopes for the variables and a random cow-specific intercept. The difference between the energy intake predicted with the cow-specific regression slopes and intercept and the energy intake predicted with the population average slopes and intercept was interpreted as animal variability in feed efficiency and not the error.

Results & discussion The variability of REI represented 8% of the variability of the actual net energy intake among the 119 Holstein cows over near the whole lactation. Of this 8% of REI variability, the cow-specific part isolated with the mixed model was only 59%. By definition, this 59% of REI variability is repeatable throughout time and associated with cows, and thus could be considered as feed efficiency differences. However, certain types of errors can be repeatable throughout time and associated with cows, such as sorting feed. The remaining 41% is by definition not repeatably specific to cows throughout time and therefore most probably associated with model fitting errors or measurement errors. However, when temporary events such as clinical mastitis, only affect intake or only affect a biological function, then it is cow-specific, but is not repeatable throughout time and will be included in the error of the mixed model and thus not considered as feed efficiency.

Conclusion When using high throughput monitoring of individual feed intake and of the major energy consuming functions in dairy cows fed with a single high energy diet during the whole lactation, residual energy intake differences among Holstein cows are only 8% of the total variability of feed intake. Thanks to a mixed model with cow-specific regression slopes and intercept, we were able to identify that of this 8%, only 59% may be associated with feed efficiency differences and not with model fitting errors or measurement errors.

Acknowledgements This study was supported by the national project Deffilait (ANR-15-CE20-0014) and APIS-GENE.

References

Aggrey SIE and Rekaya R 2013. Poultry Science. 92, 2600–2605.
Berry DP and Crowley JJ 2013. Journal of Animal Science 91, 1594-1613.
Fischer A, Friggens NC, Berry DC and Faverdin P 2017. Animal 12, 1396-1404
Savietto D, Berry DP and Friggens, NC 2014. Journal of Animal Science 92, 467–476.

Effect of basal diet on the methane mitigation effect from dietary fat supplementation

Pablo Alvarez[1], Richard Williams[2], Joe Jacobs[2], Murray Hannah[2], Karen Beauchemin[3], Richard Eckard[1], Peter Moate[1]

[1]The University of Melbourne, Melbourne, Victoria, Australia, [2]Agriculture Victoria, Department of Economic Development, Jobs, Transport and Resources, Ellinbank, Victoria, Australia, [3]Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada

E-mail: pabloah@student.unimelb.edu.au

Take home message The grain component in the basal diet does not affect the mitigating effects of dietary fat supplements on methane yield.

Introduction Methane is a potent greenhouse gas and it has previously been reported that it is possible to reduce enteric methane production by modifying ruminant diets (Pacheco *et al.,* 2014). Dietary fat supplementation mitigates methane emissions by reducing ruminal fibre digestion and by inhibiting ruminal microbes (Grainger & Beauchemin 2011). Chung *et al.,* (2011) reported the extent of methane mitigation due to dietary fat supplementation is dependent of the type of forage in the diet, but no research has yet reported the effect of type of concentrate. The aim of the research reported here was to study the effect of dietary fat supplementation with canola oil on enteric methane emissions and milk production from dairy cows fed diets containing corn or wheat.

Material & methods This experiment involved 32 Holstein-Friesian dairy cows that produced on average 24.9 ± 4.07 kg milk/day and had a bodyweight 635 ± 43.3 kg. The cows were allocated to one of four treatments: 8 kg DM/cow/d of either corn (CRN) or wheat (WHT), or these same treatments with the addition of 0.8 kg of canola oil (CPF and WPF). All animals were also offered 11.5 kg DM lucerne hay/d. There was a 1 week covariate period, followed by a 1 week adaptation period, after which cows were fed their full treatment diets for 3 weeks. Feed intake and milk production were measured daily. During the last week of the experiment, daily methane production was measured using the SF_6 method as described by Deighton *et al.,* (2014). Milk production data were analysed by ANCOVA and other data were analysed by ANOVA.

Results & discussion There was no overall effect of fat supplementation on energy corrected milk (ECM) production (Table 1). Cows fed the CRN and WHT diets had greater ($P < 0.05$) milk fat concentration than cows fed the CPF and the WPF diets (Table 1). Increasing the fat concentration in the diet reduced daily methane production of cows fed a wheat based diet ($P < 0.05$), but not a corn based diet ($P > 0.05$). However, daily mean methane emissions are primarily determined by DMI, and when correcting for DMI and expressing methane produced as methane yield (MY) there was no interaction between the effect of type of grain and fat supplementation. Increasing the fat concentration in the wheat based diet from 20 to 60 g/kg DMI reduced daily methane production by 10%, which is greater than the 8% reduction in daily methane production observed by *Moate et al.,* (2011) when feeding dairy cows with diets containing dietary fat at 51 to 65 g/kg DMI.

Table 1 Milk and methane production from cows offered each treatment.

Item	CRN	WHT	CPF	WPF	*P* (Grain)	*P* (Fat)	*P* (GrainxFat)
DMI kg/d	20.7	21.3	21.7	21.8	0.122	0.003	0.45
Milk (kg/d)	21.1^{a}	23.7^{ab}	26.1^{b}	24.9^{b}	0.57	0.002	0.05
ECM (kg/d)	23.2^{ab}	26.1^{b}	25.8^{b}	21.4^{a}	0.29	0.41	0.005
Milk Composition (g/kg)							
Fat	49.1^{c}	47.9^{c}	39.8^{b}	30.3^{a}	<0.001	<0.001	0.005
Protein	34.1	34.0	32.8	31.8	0.41	0.05	0.56
CH_4 (g/d)	524^{a}	637^{c}	523^{a}	569^{b}	<0.001	0.02	0.03
CH_4 (g/kg DMI)	25.5	29.9	24.1	26.2	<0.001	0.004	0.17

Means in the same row with different superscripts differ significantly ($P < 0.05$)

Conclusion Dietary supplementation with fat reduced methane yield when cows were fed a diet containing wheat and when the diet contained corn.

Acknowledgements This research was funded by the Victorian Department of Economic Development, Jobs, Transport and Resources; the University of Melbourne; Emissions Reduction Alberta; and Dairy Australia.

References

Chung YH, He ML, McGinn SM, McAllister TA and Beauchemin KA 2011. Animal Feed Science and Technology 166, 321-329.

Deighton MH, Williams SRO, Hannah MC, Eckard RJ, Boland TM, Wales WJ and Moate PJ 2014. Animal Feed Science and Technology 197, 47-63.

Grainger C and Beauchemin KA 2011. Animal Feed Science and Technology 166, 308-320.

Moate PJ, Williams SRO, Grainger C, Hannah MC, Ponnampalam EN and Eckard RJ 2011. Animal Feed Science and Technology 166, 254-264.

Pacheco D, Waghorn G and Janssen PH 2014. Animal Production Science 54, 1141-1154.

Application of partial least squares regression to predict feed efficiency based on feeding behaviour patterns in confined beef steers fed a concentrate diet

Ira Parsons, Gordon Carstens, William Kayser, Jocelyn Johnson

Texas A&M University, College Station, TX, USA

E-mail: ilparsons@tamu.edu

Take home message The use of partial least squares regression (PLSR) to predict residual feed intake (RFI) based on feeding behaviour traits has potential to improve selection for feed efficient beef cattle.

Introduction Previous research has shown that confined beef steers with feed-efficient (low RFI) phenotypes have lower ($P < 0.01$) frequency and duration of bunk visit (BV) and meal events (Lancaster *et al.,* 2009), and reduced ($P < 0.05$) day-to-day variation of feeding behaviour patterns (Parsons *et al.,* 2018) than high-RFI steers. Partial least squares regression is a statistical method designed to handle multivariate datasets comprised of variables with substantial collinearity. The objective of this study was to calibrate and validate PLSR-based models to predict feed efficiency of finishing beef steers using feeding behaviour traits as independent variables.

Material & methods Three trials were conducted with 508 crossbred steers (BW = 309 ± 56 kg) fed a high-grain diet in confined feedlot pens equipped with electronic feeders (GrowSafe System). Feed intake and feeding behaviour traits were measured for 70 d, and RFI computed as the residual from regression of DMI on ADG and $BW^{0.75}$, and residual gain as the residual from regression of ADG on DMI and $BW^{0.75}$. A total of 17 feeding behaviour traits were evaluated, including frequency and duration of bunk visit (BV) and meal events, head-down (HD) duration, time-to-bunk interval, maximum non-feeding interval and corresponding day-to-day variation (SD) of these traits. Additionally, 3 ratio traits were considered: BV frequency per meal event, HD duration per meal event and HD duration per BV event. Separate PLSR models were constructed for RFI and residual gain using cross-validation procedures (JMP; SAS Inst., Cary, SC). Feeding behaviour traits with variable of importance (VIP) scores < 0.8 (Wold *et al.,* 2001) were eliminated, and the optimal number of PLSR components used in each model based on the lowest predictive residual sum of squares. Model performance was evaluated using R^2 of cross-validation (R^2CV), mean bias, mean squared error of prediction (MSEP), and Spearman's rank correlation. Test-set validation of the RFI model was conducted by iteratively using 2 trials as training and the remaining trial as validation datasets, with evaluation based on the mean R^2 of calibration (R^2C) and validation (R^2V) for all 3 combinations of training and validation datasets, respectively.

Results The PLSR model for RFI used 9 principal components comprised of 9 feeding behaviour traits (Table 1), and explained 42% (R^2CV) of the variation in RFI. The mean bias, MSEP and Spearman's rank correlation for observed *vs* predicted RFI were 0.48, 0.36 and 0.59, respectively. Low-RFI steers exhibited 24 and 36% reductions in BV duration and HD duration, with corresponding 14 and 26% reductions in day-to-day variation of these traits compared to high-RFI steers (Parsons *et al.,* 2018). The high VIP scores of these 2 traits, as well as their corresponding day-to-day variances, indicate they were the most influential predictors of RFI. The residual gain model used 2 components comprised of 10 feeding behaviour traits but explained only 8% of the variation in residual gain. The mean bias, MSEP and Spearman's rank correlation for observed vs predicted residual gain were 0.13, 0.03, and 0.26, respectively. The test-set validation of the RFI model resulted in $R^2C = 0.40$ and $R^2V = 0.38$, with mean bias and Spearman's rank correlation being 0.53 and 0.59. Observed RFI classification (± 0.5 SD) was correctly predicted for 57% of steers, whereas, 38% were incorrectly predicted by one class different, and 5% by 2 classes (High vs Low RFI).

Table 1 Coefficients and VIP scores of selected feeding behaviour traits used in the RFI model.

Trait	Coefficient	VIP Score
Bunk visit (BV) duration	1.50	1.30
Head down (HD) duration	-1.18	1.30
HD duration SD†	-0.14	1.08
HD per BV duration	0.95	1.00
BV duration SD†	-0.12	0.96
HD per meal duration	-0.19	0.93
BV frequency	0.25	0.80
BV frequency SD†	0.06	0.72
Meal duration	-0.15	0.70

†SD indicates day-to-day variance of the feeding behaviour trait.

Conclusion Steers with divergent RFI phenotypes have distinctive differences in frequency and duration of BV and meal events, demonstrating their potential use as biomarkers for the prediction of feed efficiency in beef cattle. Results from this study revealed that PLSR models utilizing feeding behaviour traits as independent variables explained 42% of the variation in RFI, but only 8% of the variation in residual gain. Observed vs predicted Spearman's rank correlations were 0.59 for RFI and 0.26 for residual gain. Ongoing development of biosensor-based technologies to quantify feeding behaviour patterns will provide opportunities to indirectly measure feed efficiency via animal behaviour, reducing equipment and cost requirements over the 70 d trial period. Further research is warranted to further validate and evaluate the robustness of PLSR-based models to predict RFI in beef cattle.

References

Lancaster PA, Carstens GE, Ribeiro FRB, Tedeschi LO, and Crews DH 2009. Journal of Animal Science 87, 1528–1539
Parsons IL, Johnson JR, Kayser WC, Miller MD and Carstens GE 2018. Journal of Animal Science 95, (Abstr. #25)
Wold S, Sjostrom M and Eriksson L 2001. Chemometrics and Intelligent Laboratory Systems 58, 109-130

Metabolic responses to an epinephrine challenge during mid-lactation and the dry period in dairy cows classed as high or low RFI measured during growth

Kristy DiGiacomo[1], Emily Norris[1], Frank Dunshea[1], Ben Hayes[2], Leah Marett[3], William Wales[3], Brian Leury[1]

[1]The University of Melbourne, Parkville, Victoria, Australia, [2]Queensland Alliance for Agriculture & Food Innovation, The University of Queensland, Brisbane, Queensland, Australia, [3]Agriculture Victoria, The Department of Economic Development, Jobs, Transport and Resources, Ellinbank, Victoria, Australia

E-mail: kristyd@unimelb.edu.au

Take home message Lypolysis is reduced in low RFI dairy cattle in responses to a high dose of epinephrine

Introduction Residual feed intake (RFI) is a measure of feed efficiency, and a cow with a low RFI can obtain nutrients for equivalent maintenance, growth and production from a reduced amount of feed compared to a cow with a high RFI. Epinephrine (epi) is a catecholamine that mediates the rapid adjustment to the 'flight or fight' stress response via the sympathetic nervous system; and is a potent regulator of adipose tissue metabolism. Low RFI cows are hypothesised to be more responsive to the lipolytic functions of epi as reflected by an increased NEFA mobilisation post epi infusion.

Material & methods Dairy cattle previously assessed for RFI during growth (Williams *et al.,* 2011) and classified as either low (RFI -0.83 ± 0.294 DM kg/d; n=8) or high RFI (RFI 0.69 ± 0.187 DM kg/d; n=8) were utilized. An epi challenge was conducted during mid-lactation (ML, 122±23 DIM) and the dry period (Dry, 38d dry). Cows were fitted with an indwelling jugular catheter prior to the challenge, housed in metabolism stalls and fed a diet of lucerne cubes *ad libitum* then fasted for 12h prior to the challenge. The epi challenge was conducted using two doses (low dose of 0.1 µg/kg (low) and high dose of 1.6 µg/kg epi (high), given as adrenaline acid tartrate 1:1000), as per Sechen *et al.,* (1990). Blood samples were collected in lithium heparin collection tubes (BD) at regualar intervals pre- and post-infusion and centrifuged (1250g, 12min, 4°C) to isolate plasma. Plasma NEFA concentrations were measured using an enzyme assay (Wako); and plasma glucose concentrations were measured using a glucose oxidase enzymatic kit (Infinity, Thermo Scientific). Area under the curve (AUC) was calculated using a linear trapezoidal summation after correcting for baseline. Statistical analysis was conducted using the REML analysis function suitable for repeated measures in GenStat (15th edition) with the fixed factors of RFI, sample time and stage of lactation; and animal as the random factor. Parity was deemed to be non-significant and was not included in analysis. Data were log transformed prior to statistical analysis and reported as back transformed values.

Results & discussion Plasma metabolite responses to the high epi were greater in ML compared to Dry, and peak plasma NEFA concentrations were greater during ML in response to low epi (Table 1). Plasma NEFA $\text{AUC}_{0\text{-}30\text{min}}$ was greater for low compared to high RFI cows in response to high epi (P=0.02). A greater NEFA response to high epi suggests that low RFI cows have greater tissue responsiveness to epi, supporting the theory that high genetic merit cows prioritise the partitioning of energy for milk production rather than adipose tissue storage (Bauman *et al.,* 1985).

Table 1 Plasma responses to an epinephrine challenge in low or high RFI dairy cattle (G) during two stages of lactation (S).

			ML.		Dry			*P*-value		
Epi. Dose			Low	High	Low	High	SED	G	S	G x S
Low (0.1 µg/kg)	NEFA (µ*M*)	Peak	305	259	185	159	41.1	0.27	0.002	0.73
	NEFA (µ*M*.30min)	$\text{AUC}_{0\text{-}30\text{min}}$	1765	2125	1750	1496	505.1	0.88	0.39	0.41
	Glucose (m*M*)	Peak	5.1	4.8	4.6	4.6	0.24	0.37	0.10	0.29
	Glucose (m*M*.60min)	$\text{AUC}_{0\text{-}60\text{min}}$	16.3	7.33	11.01	9.69	13.34	0.62	0.87	0.68
High (1.6 µg/kg)	NEFA (µ*M*)	Peak	671	589	308	220	67.5	0.11	<0.001	0.96
	NEFA (µ*M*.30min)	$\text{AUC}_{0\text{-}30\text{min}}$	6864	5673	3015	2177	605.1	0.024	<0.001	0.70
	Glucose (m*M*)	Peak	8.1	8.3	6.2	6.2	0.37	0.68	<0.001	0.71
	Glucose (m*M*.60min)	$\text{AUC}_{0\text{-}60\text{min}}$	99	107	55	63	15.5	0.56	<0.001	0.95

Conclusion These results suggest that low RFI (more efficient) cows are more responsive to epi particularly during ML, and partition energy towards milk production rather than for adipose tissue accretion.

Acknowledgements This experiment was funded by the Dairy Futures CRC and supported by staff from Agriculture Vic.

References

Bauman DE, McCutcheon SN, Steinhour WD, Eppard PJ and Sechen SJ 1985. Journal of Animal Science 60, 583-592.
Sechen SJ, Dunshea FR and Bauman DE 1990. American Journal of Physiology, 258, E582-E588.
Williams YJ, Pryce JE, Grainger C, Wales WJ, Linden N, Porker M and Hayes BJ 2011. Journal of Dairy Science 94, 4715-4725

Genetic parameters and genome-wide association study for residual feed intake in pure breed Charolais young bulls

Sébastien Taussat[1], Romain Saintilan[1], Gilles Renand[2]

[1]*Allice, Jouy-en-Josas, France,* [2]*INRA, Jouy-en-Josas, France*

E-mail: sebastien.taussat@inra.fr

Take home message Genetic differences in residual feed intake exist among young Charolais bulls performance tested in station and several genome regions (QTL) were shown to be associated with this genetic variability.

Introduction In France, bulls used by artificial insemination are performance tested in station after weaning on growth, muscularity and feed efficiency ability. To have a selection criteria of feed efficiency that is independent of growth, the bulls are ranked for residual feed intake (RFI) that is the difference between the observed feed intake (FI) and the expected feed intake predicted using a multiple regression of FI on maintenance and production. The objectives of this study were to estimate genetic parameters of feed efficiency and to detect SNP associated with RFI in young French Charolais bulls.

Material & methods From 1980 to 2014, 4533 Charolais male calves were entered in performance test stations and fed *ad libitum* with a complete pellet diet. They were adapted over a minimum of 8 weeks and tested for 18 weeks. Individual FI and liveweight (double weighted at the start and the end of the test) were recorded. Average daily gain (ADG) was the final weight minus the start weight divided by length of test. Mean weight (MWT) was computed to obtain metabolic mean weight as $MWT^{0.75}$ (MMWT). RFI was obtained using Proc GLM on SAS by subtracting observed FI by expected FI, predicted with regression of FI by MMWT and ADG with year of test, station and control group as a class variable. Heritability and genetic correlations for RFI, FI and ADG were estimated by restricted maximum likelihood procedure using WOMBAT software (Meyer, 2007). An animal model was fitted which included combined fixed effect of year of test, station and control group, age at the start of test as a covariable, a random additive direct animal genetic effect and a random residual effect. Univariate and bivariate analyzes were computed to estimate heritability and genetic correlations, respectively. The genome-wide association study (GWAS) on RFI was obtained with GCTA software (Yang *et al.,* 2011). 1433 genotyped animals (HD chip or imputed from 50K to HD) were used for this analysis with 777K SNP. Quantitative trait loci (QTL) were then defined as the regions of DNA within 1Mb from the most significant SNP ($P\text{-value} < 0.0001$).

Results & discussion The RFI standard deviation (s.d.) is 0.47 kg/d while FI s.d. is 1.12 kg/d. The heritability of 0.26 for RFI shows that this trait can be genetically improved by selecting the best bulls. Berry and Crowley 2013 reported heritability of RFI from literature between 0.14 to 0.62. A selection on RFI will reduce the FI (Rg=0.58, table 1) without strong effect on ADG (Rg=0.15). For the GWAS analysis, 647,225 SNP remained after filtering. Several SNP are significantly associated with RFI (figure 1) and 16 QTL are defined. The strongest QTL is found on chromosome 14 with 105 markers inside, known to be associated with several milk or body traits but not with feed efficiency traits (AnimalQTLDatabase).

Table 1 Phenotypic and genetic parameters. Heritabilities are reported in diagonal, genetic correlations are above and phenotypic correlations below

	RFI	FI	ADG
RFI	0.26 (0.04)	0.58 (0.07)	0.15 (0.11)
FI	0.41 ***	0.36 (0.05)	0.66 (0.06)
ADG	0.000 (n.s.)	0.34 ***	0.27 (0.04)

*** represent $P < 0.0001$; n.s. is not significant

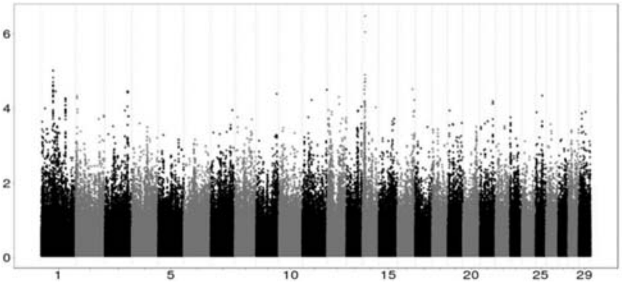


Figure 1 Manhattan plot of RFI. Each point is an SNP, chromosomes are in abscissa and –log P-value in ordinate

Conclusion As reported in other studies, genetic variability of RFI is moderate in young French Charolais bulls and a genetic improvement is possible through selection. The genetic relationship of RFI with other production traits needs to be studied before implementing that selection. The presence of several QTL means that some candidate genes may be involved in RFI. This analysis of feed efficiency of young bulls in performance testing stations will be completed with a study of feed efficiency and slaughter performance of male progeny.

Acknowledgements This study was granted fund by Allice, INRA and APIS-GENE.

References

Berry DP and Crowley JJ 2013. Journal of Animal Science 93, 1594-1613.
Meyer, K. 2007. Journal of Zhejiang University-Science B 8, 815–821.
Yang J, Lee SH, Goddard ME and Visscher PM 2011. The American Journal of Human Genetics 88, 76-82.

Effect of increasing levels of dietary fibre and fibre digestibility on methane production in dairy cattle

Mohammed Benaouda, Gilberto Apodaca-Martínez, Manuel González-Ronquillo, Octavio A. Castelán-Ortega

Universidad Autónoma del Estado de México, Toluca, Estado de México, Mexico

E-mail: oaco2002@yahoo.com.mx

Take home message In some livestock production systems in Mexico where cattle are fed low quality forages, methane emissions may be lower than previously reported in literature.

Introduction It is known that enteric methane (CH_4) emission by dairy cows increases as the fibre content of their diet increases (Knapp *et al*., 2014). However, this positive linear relationship may not work in tropical regions of Mexico and the world, where dietary fibre is high and has low quality in terms of its digestibility. The aim of this study was to evaluate the effect of increasing dietary fibre levels (more than 40% of neutral detergent fibre; NDF) and its digestibility on CH_4 methanogenesis, milk yield, milk composition and dry matter intake.

Material & methods Eight Holstein cows (112±38 days postpartum; mean±s.d.) were randomly assigned to four treatments of four dietary NDF levels (DM basis): 40.2 (diet A), 43.3 (diet B), 46.5 (diet C) and 50.5% (diet D), in a 4x4x2 Latin square experimental design. Cows were fed a TMR of corn-alfalfa silage and concentrate (60:40, forage:concentrate ratio) formulated to meet energy and protein requirements as recommended by NRC (2001). Concentrate comprised grounded corn, soybean meal, wheat bran and minerals/vitamins mixture. The only source of roughage in the diet A was corn-alfalfa silage, and to reach increasing contents of NDF in diets B, C and D, part of silage was replaced with chopped corn straw. Each experimental period lasted 21 days, during the first 14 days cows were adapted to diet in individual pens, and from day 15 to day 19 the digestibility trial was performed. On day 20 animals were moved to open-circuit respiration chambers where dry matter intake (DMI), milk production and CH_4 emission were measured.

Results No effect of increasing dietary fibre was detected on DMI ($P < 0.05$) likely because of the small particle size of the added corn straw. A linear decrease ($P < 0.0001$) in dry matter digestibility was found as NDF content increased in the diet (73.6, 66.4, 62.2 and 58.5%, for diets A, B, C and D respectively). CH_4 production and CH_4 yield (l/kg DMI) decreased ($P < 0.0001$) as dietary fibre content increased (421, 409, 362 and 336 l/day and 32.1, 28.1, 23.1 and 21.3 l/kg DMI, respectively). The percentage of gross energy intake lost as methane (Ym factor) decreased linearly from diets A to D (7.4, 6.6, 5.3 and 5.0%, respectively). Cows had similar milk production in all diets (mean = 17 l/day), however, fat content of milk was greater in cows fed higher fibre compared with lower fibre (3.95 *vs.* 3.65, respectively, $P < 0.05$). It was also observed that the higher inclusion of corn straw in the diet the larger amount of energy lost in faeces. This result was reflected in the ratio of metabolizable energy:Gross energy, which was reduced from 0.64 (diet A) to 0.50 (diet D). In terms of protein balance, cows showed similar nitrogen conversion despite the increasing fibre level in the diet. Using correlation analysis, the digestibility of the ADF (dADF) was found to explain better ($P < 0.0001$) the partitioning of energy to CH_4 production than DM digestibility (pearson coefficient = 0.89 *vs.* 0.54, respectively; Figure 1).

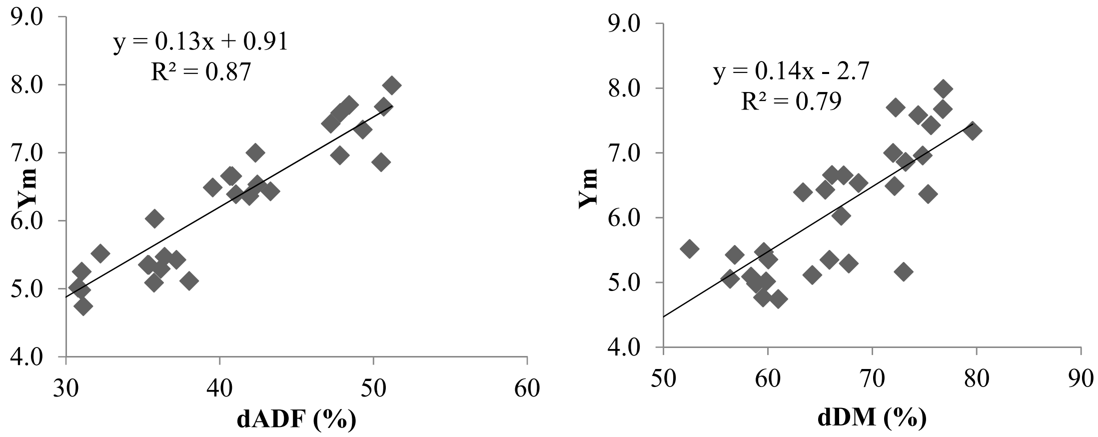


Figure 1. Linear relationship between Ym and ADF digestibility (dADF) and DM digestibility (dDM).

Conclusion It was concluded that increasing fibre content in dairy cows' diets in Mexico reduced DM and fibre digestibility, which resulted in less digestible substrate in rumen and hence CH_4 emission decreases.

References

Knapp JR, Laur GL, Vadas PA, Weiss WP and Tricarico JM 2014. Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. Journal of Dairy Science, 97, 3231–3261.
NRC. 2001. Nutrient Requirements of Dairy Cattle.

Multiplatform milk metabolomics highlights potential biomarkers related to methane emissions in dairy cows

Bénédict Yanibada[1], Diego Morgavi[1], Cécile Canlet[2], Mélanie pétéra[3], Maguy Eugène[1], Cécile Martin[1], Hamid Boudra[1]

[1]Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, Saint-Genès-Champanelle/Auvergne Rhone Alpes, France, [2]Toxalim, Research Centre in Food Toxicology, Université de Toulouse, INRA, ENVT, INP-Purpan, UPS,, Toulouse/Occitanie, France, [3]Université; Clermont Auvergne, INRA, UNH, Plateforme d'Exploration du métabolisme, MetaboHUB Clermont, CRNH Auvergne, Clermont-Ferrand/Auvergne Rhone Alpes, France

E-mail: benedict.yanibada@inra.fr

Take home message Metabolomics is a powerful tool for identifying potential markers of methanogenesis.

Introduction Enteric methane, a by-product of the ruminal fermentation process (Morgavi *et al.*, 2010) contributes significantly to greenhouse gas emissions (Baskaran *et al.*, 2009). For monitoring and adopting husbandry practices with a lower environmental footprint it is essential to develop non-invasive and easy to use methods at the herd level (Negussie *et al.*, 2017). Although fatty acids present in milk have been extensively studied as predictors of methane emissions, there is not much information on the usefulness of other metabolites present in milk as proxies of methanogenesis. In this study, we compared the Nuclear Magnetic Resonnance (NMR) and Liquid Chromatography coupled to Mass Spectrometry (LC-MS) milk metabolome of cows with reduced or normal methane emissions.

Material & methods Twenty-five primiparous Holstein cows received (treated n=12) or not (control n=13) an antimethanogenic treatment. Cows were fed a TMR containing corn silage (30%), grass hay (30%), and concentrate (40%). The treatment or control constituted less than 0.1% of the ration. The study lasted 6 weeks. At the end of the fifth week, milk samples were collected for metabolomics analysis. Milk metabolite fingerprinting was performed using NMR and LC-MS after protein precipitation. These analyses were combined with multivariate statistical analysis to highlight differences between groups and identify potential biomarkers. In week 6, methane measurements were carried out in open respiratory chambers on 16 cows (8 control; 8 treated) chosen at random.

Results & discussion Methane production was reduced by 23% in the treated group. No differences in dry matter intake and milk production was observed between groups. Multivariate metabolomics analysis showed a separation between control and treated groups for both NMR and MS techniques. Sixteen discriminant metabolites (Variables important in the projection, > 1.2) were identified (12 using NMR and 6 using LC-MS, Table 1).

Table 1 Discriminating metabolites identified using NMR and LC-MS.

Metabolites	*NMR	*MS
3-hydroxybutanoic acid	↓	-
Acetyl L Carnitine	-	↓
Citramalic acid	↑	-
Citrate	↑	-
Creatine /Phosphate	↑	-
Dimethylsulfone	↑	-
Dimethylsulfoxyde	-	↑
Glutamic acid	↓	-
Glutathione	↓	-
Leucine	↓	↓
Isoleucine	↓	↓
N acetyl glucosamine	↑	-
Phenylalanine	-	↓
Phosphocholine	↑	-
Pyrrolidine	-	↓
Valine	↓	-

(*) relative change treated compared to control
(-) not identified with the platform as a discriminant metabolite

Conclusion Combining MS and NMR allowed to obtain a better coverage of the milk metabolome than using a single technique. This complementarity was useful to identify potential biomarkers associated to methane-emission phenotypes.

Acknowledgements This research was supported by the Methane Consortium (Adisseo, Agrial, APIS-GENE, Deltavit, DSM, IDELE, Lallemand, MoyPark, Neovia, Techna, and Valorex) and the ANRT through a Cifre fellowship grant.

References

Baskaran R, Cullen R and Colombo S 2009. New Zealand Journal of Agricultural Research 52, 377-389.
Morgavi DP, Forano E, Martin C and Newbold CJ 2010. Animal 4, 1024-1036.
Negussie E, de Haas Y, Dehareng F, Dewhurst RJ, Dijkstra J, Gengler N, Morgavi DP, Soyeurt H, van Gastelen S, Yan T and Biscarini F 2017. Journal of Dairy Science 100, 2433-2453.

Impact of tannins on the net flux of ammonia, urea and glucose across the splanchnic tissues of sheep

Gilberto Vilmar Kozloski, Tiago Orlandi, Simone Stefanello, Diego Streit Zeni, Mariana Patricia Mezzomo

Universidade Federal de Santa Maria, Santa Maria, RS, Brazil

E-mail: gilberto.kozloski@ufsm.br

Take home message Low dietary tannin concentration did not affect urea or glucose production by liver of sheep

Introduction The impact of tannins on rumen fermentation and ammonia concentration has been consistently reported (Patra and Saxena, 2011) whereas, however, any preview study measured their effect on the amount of ammonia actually reaching the liver or the amount of urea produced by liver. Moreover, tannins increased the propionate concentration *in vitro* (Batha et. Al., 2009) and, thus, they could improve the hepatic gluconeogenesis *in vivo*. The objective of the present study was to evaluate the impact of a tannin extract on the net flux of ammonia, urea and glucose across the splanchnic tissues (ST) of sheep.

Material & methods The trial was conducted with five Texel male sheep (48 ± 3.2 kg body weight (BW)) surgically implanted with chronic indwelling catheters into the mesenteric, portal and hepatic veins, in two 21 days periods, in a cross-over design. Diet was oat/ryegrass (0.60) plus concentrate (0.40) offered twice daily. The concentrate was composed by soybean meal, cracked corn and wheat bran, added (Tannin) or not (Control) with 20 g/kg of tannin extract from *Acacia meanrsii*. Between days 15 to 21 of each experimental period, the plasma flow through portal-drained (PDV) and ST was measured thereafter the morning meal by downstream dilution of *p*-aminohippurate solution (15 g/L, pH 7.4) which was continuously infused (2.0 mL/min) into the mesenteric vein. Portal, hepatic and arterial blood samples were taken hourly during 4 hours. The net flux of metabolites across either PDV or ST was calculated by their venous-arterial concentration difference multiplied by plasma flow in portal or hepatic veins, respectively. The net flux across liver was calculated as ST minus PDV flux. Hourly results were averaged within animal/period for analysis. Data was analysed using the Mixed procedure of SAS including the random effect of periods and the fixed effect of treatments in the model.

Results & discussion The dry matter and N intakes were on average 1560 and 32 g/day, respectively, and were similar for both treatments. The net flux of glucose and ammonia N were similar for both treatments whereas the net flux of urea N across PDV, liver and ST tended to be lower ($p \leq 0.113$) in tannin treatment.

Table 1 Net flux (mg/h) of metabolites across portal-drained viscera, liver and total splanchnic tissues of sheep fed a diet without (Control) or with tannin extract from *Acacia meanrsii* (Tannin)

	Treatment			
Item	Control	Tannin	SEM	*p-value*
Portal-drained viscera:				
Glucose	-2942	-2066	433.3	0.160
Ammonia N	384	370	195.9	0.686
Urea N	-885	-621	253.0	0.101
Liver:				
Glucose	8627	7959	778.5	0.461
Ammonia N	-403	-378	204.5	0.490
Urea N	1462	1166	333.1	0.113
Total splanchnic tissues:				
Glucose	6102	6032	775.7	0.804
Ammonia N	-29	-27	14	0.868
Urea N	591	459	95.2	0.053

Conclusion The dietary inclusion of tannin extract from *Acacia meanrsii* at a rate of 8 g/kg DM did not clearly impact the glucose metabolism by ST whereas tended to decrease the urea production by liver of sheep. This effect, however, was not associated with decreased ammonia load from gut.

Acknowledgements The authors thank the CAPES and CNPq (Brazil) for scholarship support.

References

Batha R., Uyeno Y., Tajima K., Takenaka A., Yabumoto Y., Nonaka I., Enishi O., Kurihara M. 2009. Journal of Dairy Science 92, 5512-5522.

Patra A.K., Saxena J. 2011. Journal of the Science of Food and Agriculture 91, 24-37.

Insights in dry matter intake prediction in growing goats

Amelia Almeida[1], Luis Tedeschi[2], Kleber Resende[1], Bruno Biagioli[1], Antonello Cannas[3], Izabelle Teixeira[1]

[1]São Paulo State University (UNESP), Jaboticabal, Sao Paulo, Brazil, [2]Texas A&M University, College Station, Texas, USA, [3]University of Sassari, Sassari, Italy

E-mail: almeida.amelia@gmail.com

Take home message Goat size (*i.e.,* $BW^{0.68}$) is a potential reliable predictor of dry matter intake (DMI), besides NDF concentration in the diet should be considered as a predictor in future equations for predicting dry matter intake of growing goats.

Introduction Feed consumption by ruminants is one of the most complex processes to model due to the number of factors that influence hunger and satiety. Yet, modelling DMI in growing goats is critical for optimizing management decisions and the entire production system, because feed is the most expensive component of the productive system. In this sense, literature equations limitations and strengths were assessed (data not shown). The objective of this study was to provide insights for future DMI prediction equations for growing goats.

Material & methods We suggested allometric associations to explain DMI ($DMI=\beta_0 \times BW^{\beta 1}$). To parameterise the allometric equations, a summarised database with 66 DMI (g/d) records of intact male (n=24), female (n=23), and castrated male (n=19) growing Saanen goats was used (averaged DMI during each study period). The database used was pooled from four studies conducted at the Goat Laboratory of the Universidade Estadual Paulista. In these studies, goats weighing from 14.4 to 48.7 kg and with 2 to 17 months of age were fed *ad libitum* similar diets composed (DM basis) of dehydrated corn plant (46.2±0.7%) or Tifton hay (24.5±0.3%), corn grain (33.4±9.2%), soybean meal (17.6±2.9%) and oil (1.3±0.5%), limestone (1.1±0.3%), mineral supplement (2.2±0.9%), and ammonium chloride (0.7±0.4%). The formulated diets had 16.2±2.01% CP and 2.65±0.332 Mcal of ME/kg DM 32.1±2.01% FDN. The DMI and NDF intake in growing Saanen goats ranged from 478 to 1986 g DM and 0.774 to 1.32% BW, respectively. Equations were fitted using the NLMIXED procedure of SAS version 9.4 (SAS Systems Inc., Cary, NC), accounting for study random effect. We used *dummy* variables to test the effect of sex on parameter estimates; thus, CONTRAST statements were used for testing whether a regression parameter differed across sexes.

Results & discussion Sex affected the β_0 of the proposed Equation 1 (n=66, P<0.01, Root Mean Square Error - RMSE=88.9 g/d), where β_0 estimated for males (castrated did not differ from intact males, P>0.05) was different from that estimated for females ($P \leq 0.02$), whereas a common β_1 (allometric coefficient) for all sexes (P=0.33) in the allometric relationship between DMI (g/d) and BW (kg) was found. On the other hand, we did not detect sex effects for both parameter estimates (β_0, P=0.13; and β_1, P=0.12) when developing the proposed allometric equation associating NDF intake (%BW) and BW (kg), as shown in Equation 2 (n=66, P<0.01, RMSE=1.00 %BW).

$$DMI = \beta_0 \times BW^{0.672 \pm 0.158} \qquad (1); \qquad NDF_{intake} = 3.16 \pm 1.30 \times BW^{-0.319 \pm 0.078} \qquad (2)$$

where DMI is in g/d, β_0=93.9±46.3 for females and β_0=101±24.1 for intact and castrated males, and BW in kg. NDF_{intake} is the intake of NDF expressed as a proportion of BW.

In this regard, Kleiber (1961) stated that intake is proportional to maintenance requirement, which decreases per unit of BW as animal size increase. Therefore, feed intake relative to BW would decrease to the same extent (*i.e.,* $BW^{0.75}$). However, studies have shown that the scaling unit (*i.e.,* 0.75) is relevant to achieve comparability across species, which may lead to failure when modeling DMI between animals of different BW within species (Clauss *et al.*, 2007). In the face of the disagreement regarding the proper scaling unit to be used to scale DMI, we estimated that DMI has an allometric relationship with BW, and the allometric coefficient (*i.e.,* scaling unit) would be 0.672±0.158.

Conclusion The DMI should be a function of animal size (*i.e.,* $BW^{0.68}$) and consider some measurement of rumen fill (*i.e.,* we suggest NDF).

Acknowledgements This research was supported by grants from FAPESP/CAPES, 2014/14939-0, 2014/14734-9, and 2015/22600-5.

References

Clauss M, Schwarm A, Ortmann S, Streich WJ and Hummel J 2007. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 148, 249–265.
Kleiber M, 1961. The Fire of Life. An Introduction to Animal Energetics. John Wiley and Sons, Inc., New York.

Influence of chestnut tannins on *in vitro* crude protein rumen degradability kinetics of red clover silage

Sophie Herremans[1], Virginie Decruyenaere[1], Yves Beckers[2], Eric Froidmont[1]

[1]*Walloon Agricultural Research Centre, Gembloux, Belgium,* [2]*University of Liège - Gembloux Agro-Bio Tech, Gembloux, Belgium*

E-mail: s.herremans@cra.wallonie.be

Take home message Chestnut tannins in red clover silage reduce rumen dry matter and crude protein degradation. Adding tannins to silage could lead to better nitrogen use efficiency in ruminants.

Introduction In a context of environmental concern and feed cost reduction, the efficiency of inputs (especially feed) is a key point in farmers' strategies. In Western Europe's dairy sector, protein is one of the most expensive nutrients and therefore essential in the pursuit of efficiency. A way to improve nitrogen use efficiency may be a reduction of proteolysis in the rumen and an increased ratio of undegraded bypass proteins. This study aims at assessing the capacity of chestnut tannins to reduce nitrogen degradation in the rumen *in vitro*.

Material & methods Our experiment compared degradation of dry matter (DM) and crude protein (CP) from red clover laboratory-scale silages made without additive, with commercial acid (Sil70, Timac Agro) or chestnut tannins (0.08% MS, Silva Team). Oven-dried and ground silages were put in nylon bags (porosity 50µm; 3 to 6g per bag according to incubation time) immerged in jars containing rumen juice mixed with buffer and artificial saliva (Daisy I system, Ankom). For each time step (0; 0.5; 1; 3; 6; 12; 24; 72h) and additive, three replicates were immersed in rumen juice then washed in cold water after incubation time and wringed out. Sample at t0 was just washed and wringed out without incubation. Nylon bags were then oven-dried at 60°C and the residue was weighted before to be analyzed for CP content by NIR spectroscopy. Relative loss results are expressed as a ratio of remaining quantity in the bag after t0 wash. Results were then processed through the equation of Orskov & McDonald (1979). A general linear model was used to compare relative losses and degradation rates.

Results & discussion Regarding *in vitro* DM disappearance, both additives reduced silage DM degradability from 0.5 to 72h of incubation compared to the control silage (Figure 1; $P < 0.05$). After 72h, acid and tannin silages were respectively 20 and 25% less degraded than control. Tannin was even more efficient than acid for the first 12h ($P < 0.05$). CP degradation was slower and reached a plateau later for the tannin silage than for the other silages (Figure 2; $P < 0.05$ from 3 to 48h). Chestnut tannin was the only additive to reduce CP degradation rate ($P < 0.05$). Contrary to acid, tannins were thus able to reduce both DM and CP degradation in the rumen from the beginning and until at least 48h. According to several authors (Piluzza *et al.,* 2014), tannins form complexes with proteins making them less susceptible to lysis. This complex is thought to dissociate at low or high pH (Jones & Mangan, 1977), which can occur in the abomasum or in the duodenum and allow a higher absorption of proteins in the intestine.

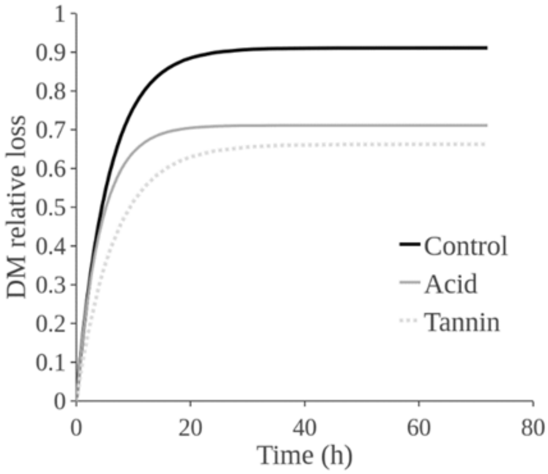


Figure 1 Dry matter relative loss from 0 to 72h.

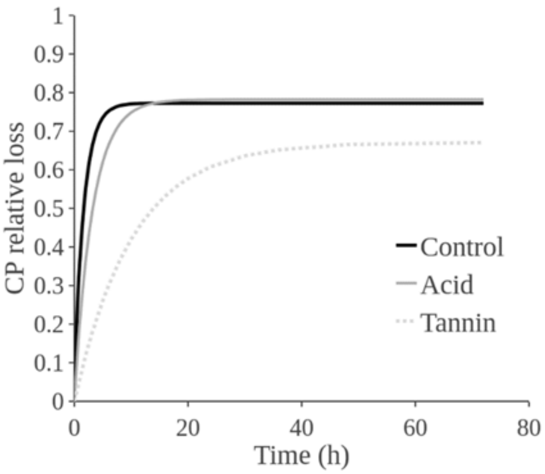


Figure 2 Crude protein relative loss from 0 to 72h.

Conclusion Our experiment showed the potential of chestnut tannins to decrease CP degradation in the rumen probably because tannin-protein complexes protected these molecules from lysis. By allowing more proteins to reach the intestine, chestnut tannins could improve feed nitrogen use efficiency in ruminants.

References

Jones WT and Mangan JL 1977. Journal of The Science of Food and Agriculture 28, 126–136.
Piluzza G, Sulas L and Bullitta S 2014. Grass and Forage Science 69, 32–48.
Orskov E and McDonald I 1979. Journal of Agricultural Science 92, 499-503.

Adding a native plant (*Allium mongolicum*) in the feed diet of cattle decreases methane emissions

Kaili Xie, Cheng Zhang, Zhaofeng Wang, Fujiang Hou

State Key Laboratory of Grassland Agro-ecosystems, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, China

E-mail: xiekl16@lzu.edu.cn

Take home message Native plant (*Allium mongolicum*) decreases livestock methane emissions.

Introduction Livestock GHG emissions account for 14.5% of global GHG emissions. As a highly palatable native plant for livestock, *Allium mongolicum* (AM) is widely distributed in the northern grasslands of China (Wingard *et al*., 2011). Feeding AM to livestock could improve their productivity and immune regulatory function. Besides, the rumen environment of livestock is changed through improving degradation rate and concentration of volatile fatty acids. To date, the effects of AM on methane emissions from ruminants has not been investigated *in vivo*. So, it is important to comprehensively understand whether feeding AM to livestock affects methane emissions. We set up a controlled trial consisting of adding AM in the diet of cattle at different proportions to test the above question.

Material & methods The study site (latitude 39.24°N, longitude 100.06°E, elevation about 1390 m above sea level) is located in Linze County, Gansu Province. The experiment used twelve 7 month old male cattle (175 ± 20 kg) (*Simmental*) which are widely distributed in this region. The experiment was based on a single factor random design, divided into three groups. There were four replicates per group. The basal diet was based on the Chinese recommended beef cattle breeding standards (NY / T815-2004). The basal diet included corn, soybean meal, bran, alfalfa and oats. Fresh AM was added to the basal diet with different proportions at 0mg/kg of BW (CK), 200 mg/kg of BW (Low AM, LA) and 400 mg/kg of BW (High AM, HA). The amount of AM was calculated based on dry matter. After 30 days of adaptation to the experimental diets, the cattle were housed in separate pens for 3 days and fed the experimental diets. Respiratory measurements were performed in a ventilated open-circuit respiration chamber for 36 hours. The methane emissions were measured by infrared absorption based gas analyser (CH_4) analyser. Statistical analyses was performed using SPSS 20. Differences in means among the four groups were tested by using analysis of variance (ANOVA) and Tukey's test.

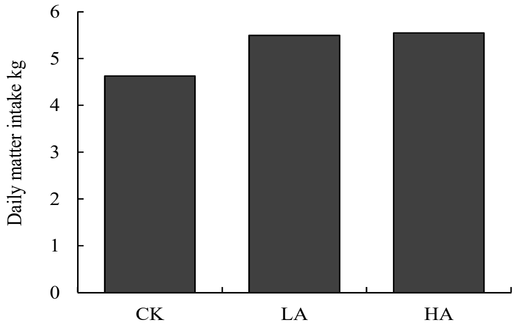


Figure 1: Effects of adding *Allium mongolicum* levels on average daily matter intake (DMI).

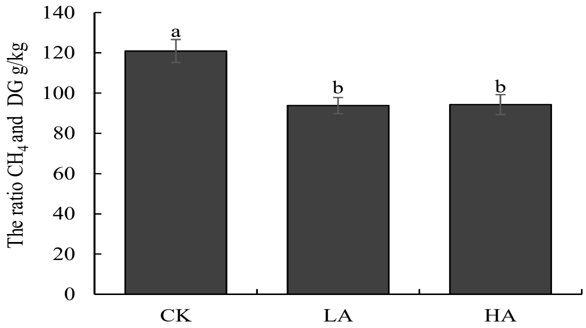


Figure 2: Effects of adding *Allium mongolicum* levels on the ratio methane emissions (CH_4) and average daily gain (ADG) *Different letters in the figure indicate significant differences ($p<0.05$).

Results Compared with the CK treatment, supplementation with AM can increase the DMI of cattle (Fig.1). The ratio of CH_4 and ADG in all AM treatments was shown to have a significant effect ($p=0.03$) compared to the CK treatments. With the increase in the amount of AM, the ratio CH_4 and ADG also decreases, and LA was lowest (Fig. 2).

Conclusion Adding a native plant (AM) in the diet of cattle decreased their methane emission. Dietary supplementation with AM significantly reduced the ratio of CH_4 and ADG (g/kg) and reduced environmental pollution. The supplementation of AM did not affect daily intake. Because AM is expensive, considering the economic benefit of material, dietary supplementation of AM at 200 mg/kg (BW) is preferable, and plant can be used as a feed additive to reduce methane emissions.

Acknowledgements This study was supported by the Program for National 973 Subject "The Biological-basic Study of Forage Varieties For Improving Pasture Productivity" (2014CB138706).

Reference

Wingard GJ, Harris RB, Pletscher DH, Bedunah DJ, Mandakh B, Amgalanbaatar S and Reading RP 2011. Journal of Arid Environments 75, 138-145.

In vitro investigation of the ruminal digestion kinetics of different N fractions of ^{15}N-labelled red clover silages and dried forage

Merko Vaga[2], Pekka Huhtanen[1]

[1]The Swedish University of Agricultural Sciences, Umeå, Sweden, [2]The Swedish University of Agricultural Sciences, Uppsala, Sweden

E-mail: merko.vaga@slu.se

Take home message The silage additives have little value on protein quality of red clover and *in vitro* techniques are good for evaluating soluble N fractions, but not yet good enough for insoluble.

Introduction Red clover is widely used as feed for dairy cattle, due to the higher protein concentration and lower need for fertilizers over grass. Furthermore, the presence of polyphenol oxidase (PPO) in red clover reduces protein breakdown during wilting and ensiling and can increase true protein content compared with grass silage. However, the efficiency of N utilisation for milk production with red clover-based diets has been lower than expected. As most current evaluation models use N fractionation of different degrees, to better understand ruminal N metabolism of red clover, protein degradation kinetics of different N fractions was studied.

Material & methods Red clover (*Trifolium pratense* cv. Betty) was grown on two replicate plots of 2 m^2 with one plot receiving labelled $^{15}NH_4{}^{15}NO_3$ fertiliser and the other unlabelled NH_4NO_3. The harvest was preserved as formic acid treated (FAS) and untreated silage (UTS) or as oven dried hay (DRC; 60°C). The freeze-dried forages were milled through 1 mm screen and then separated into three N fractions: soluble N (SN), insoluble N (ISN) and neutral detergent-insoluble N (NDIN). Isolated N fractions were incubated in pre-incubated rumen fluid as mixed diets, containing isolated NDF or soybean meal and a carbohydrate mixture (pectin, starch and maltose) to achieve a similar concentration of N and NDF as in the intact forage. The ammonia N (AN) in *in vitro* bottles containing unlabelled samples was labelled with ^{15}N with $(^{15}NH_4)_2SO_4$. Fluid samples were extracted at 0.25, 0.5, 1, 2, 3, 4, 6, 9, 20 and 28 h for determination of AN and soluble non-ammonia N (SNAN) concentration in the liquid phase and their ^{15}N enrichment. The size of the ^{15}N excess pools (^{15}NEP) of AN and SN was determined by multiplying pool sizes and the atom% ^{15}N excess (APE) in AN and SN. The disappearance of ^{15}N from labelled AN or SN and appearance of ^{15}N from labelled N fractions to AN pool were used to estimate fractional degradation rates of N fractions and microbial uptake. The treatment effect was analysed using mixed procedure in SAS by following model: $Yij = \mu + Ti + rj + eij$, where μ is overall mean, Ti is the treatment method, rj is the random effect of run and eij is the error.

Results & discussion On average, 53% of the initial dose of ^{15}N (195 µg), when administered as AN, disappeared within the first 15 min from AN ^{15}NEP. This was probably because the intracellular AN pool of microbes was deprived due to pre-incubation of the rumen fluid and rapid adsorption of AN by microbial cells. The SN consisted of 14-27% AN, being lower for DRC than for silages. Microbial synthesis from the SN fraction was highest for DRC and higher for UTS than for FAS (Figure 1). The greater utilisation of AN and SN by microbes ($P<0.01$) indicate higher microbial efficiency with DRC than with silages. Between 61-70% of the SN fractions disappeared within the first 15 min with some of it being released within the following 15 min. The fractional disappearance rates of SN fractions of FAS, UTS and DRC, were 0.273, 0.126 and 0.206 /h, respectively. By the end of incubation (28 h) only about 12% of ^{15}N from ISN fractions and only 3% from NDIS appeared in ammonia ^{15}NEP, with no significant differences between forages. The estimated microbial synthesis rate from AN was 0.279 and 0.329 /h with ISN fractions of FAS and UTS, respectively. The ^{15}NEP size in blank with ^{15}N-labelled AN decreased in the first nine hours, but slightly increased again during the last 19 hours indicating microbial lysis.

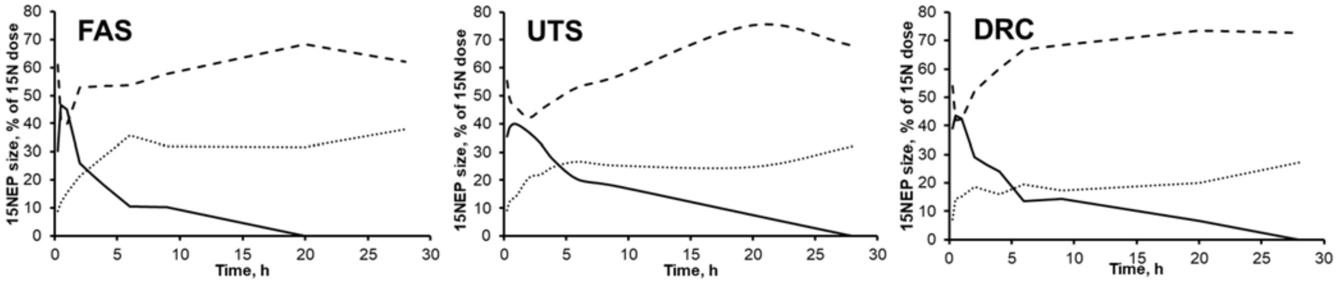


Figure 1: Observed ^{15}NEP size (% of ^{15}N dose) with ^{15}N-labelled SN fraction of FAS, UTS and DRC. Observed size of soluble non-ammonia ^{15}N pool (solid line), ammonia ^{15}N pool (dotted line) and microbial ^{15}N pool (dashed line).

Conclusion Rumen microbes seem to be capable of rapid uptake and release of AN and SN. The results here suggest higher microbial efficiency with DRC than with silage. The silage additives seem to have low effect on red clover N fractions as no significant differences in degradation parameters of ISN and NDIN between silage and hay was found. The degradation parameters estimated for the SN fraction were similar to those reported in other *in vitro* studies, but still lower than *in vivo* measurements, proving that the *in vitro* technique needs to be improved.

Acknowledgements This research was funded by The Swedish research council Formas.

In silico identification of low invasive biomarkers for feed efficiency in cattle

Isabelle Cassar-Malek, Muriel Bonnet

Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès- Champanelle, France

E-mail: isabelle.cassar-malek@inra.fr

Take home message A computational approach has identified a list of 69 candidate low invasive plasma biomarkers for feed efficiency in cattle as defined by residual feed intake metrics (RFI).

Introduction RFI measurement relies on an expensive and time-consuming method. Thus identification of low invasive RFI biomarkers is an issue. During this decade, the mechanisms of beef cattle feed efficiency have been examined through candidate gene expression or omic profiling in key organs/tissues. We made the hypothesis that computation of available omic data may enable the identification of plasma protein biomarkers for RFI in the prediction of tissue secretomes.

Material & methods Transcriptomic data from liver, muscle, adipose tissue, rumen, duodenum, and pituitary were collected from 5 publications on cattle (Chen *et al.,* 2011; Weber *et al.,* 2016; Tizioto *et al.,* 2015, 2016; Kong *et al.,* 2016) and 1 GEO dataset (GSE56705) (Table 1). Lists of unique Gene Names (GN) up- and down-regulated in low RFI groups were submitted to a custom analysis using ProteINSIDE. (http://www.proteinside.org/) to mine biological information and predict secreted proteins with Signal P sequence (Bonnet *et al.*, 2016). The lists of proteins secreted thanks to a SignalP were compared to a list of 1106 plasma bovine proteins from publications and proteome analysis. Gene Ontology (GO) enrichment tests (P value_Benjamini Hochberg < 0.05) were done with human orthologs to take advantage of the most complete annotation available.

Results & discussion We retrieved 663 up- and 621 down-regulated GN with a mapped identifier (ID). Among them, 145 and 128 were proteins predicted as secreted, of which 30 and 39 were detected in the plasma repertoire respectively (Table 1). These proteins are relevant candidate plasma proteins for RFI. Eight of them were detected in the plasma of efficient Charolais young bulls (communication Cassar-Malek *et al.*, this symposium).

Table 1 Computational prediction of plasma biomarkers for feed efficiency from omic published data.

	Up-regulation in low RFI			Down-regulation in low RFI		
Tissue	Unique GN	ID mapped	Secreted (signal P)	Unique GN	ID mapped	Secreted (signal P)
Liver	229	207	69	198	173	63
Muscle	193	182	18	166	149	18
Adipose	14	14	3	17	15	4
Pituitary	106	102	19	163	160	18
Duodenum	84	79	29	96	94	23
Rumen	80	79	7	32	30	2
Total	706	663	145	672	621	128
Plasma			30			39

Common GO (n=122) were detected between the lists of up- and down-regulated predicted plasma proteins. Biological processes (BP) included proteolysis, innate immune response, complement activation, blood coagulation, and lipid metabolic process. Molecular functions included serine-type endopeptidase activity and inhibitors, calcium binding, and receptor binding. Specific GO BP were detected for up- (*e.g.,* extracellular matrix disassembly, integrin-mediated signalling pathway, angiogenesis and lipid catabolic process) and down-regulated proteins (*e.g.,* immune response, blood coagulation, triglyceride metabolic process). Processes related to proteolysis or lipid metabolism may be linked to the body composition as one of the driver of RFI in cattle (Weber *et al*., 2016).

Conclusion This study provides candidate proteins for potential high throughput phenotyping of RFI using plasma. The next step is to validate their differential abundance in the plasma of high and low RFI cattle.

References

Bonnet M, Tournayre J and Cassar-Malek I 2016 Molecular BioSystems. 12, 2722-2734.
Chen Y, Gondro C, Quinn K, Herd RM, Parnell PF and Vanselow B. 2011 Animal Genetics 42, 475-490.
Kong RSG, Liang G, Chen Y, Stothard P and Guan LL. 2016. BMC Genomics 17, 592.
Tizioto PC, Coutinho LL, Decker JE, Schnabel RD, Rosa KO, Oliveira PSN Souza MM, Mourão GB, Tullio RR, Chaves AS, Lanna DPD, Zerlotini-Neto A, Mudadu MA, Taylor JF and Regitano LCA 2015 BMC Genomics 16, 242.
Tizioto PC, Coutinho LL, Oliveira PSN, Cesar ASM, Diniz WJS, Lima AO, Rocha MI, Decker JE, Schnabel RD, Mourão GB, Tullio RR, Zerlotini A, Tayloer JF and Regitano LCA Science Reports 6, 39493.
Weber KL, Welly BT, Van Eenennaam AL, Young AE, Porto-Neto LR, Reverter A and Rincon G PLOS ONE. 01611(3):e0152274.

Effect of inclusion of capulin leaf (*Prunus salicifolia*) in goats as a strategy for methane mitigation *in vitro*

Lizbeth Robles Jimenez[1], Jose Antonio Ruiz Perez[3], Nicolas DiLorenzo[2], Octavio Castelan Ortega[1], Jorge Osorio Avalos[1], Manuel Gonzalez Ronquillo[1]

[1]Universidad Autonoma de Estado de Mexico, Toluca, Estado de Mexico, Mexico, [2]University of Florida, Marianna, Florida, USA, [3]Centro de Bachillerato Tecnológico Agropecuario No. 150., Acambay, Estado de Mexico, Mexico

E-mail: mrg@uaemex.mx

Take home message The use of capulin leaf (*Prunus salicifolia*) in diets for goat kids reduces methane emissions.

Introduction The use of forages rich in condensed tannins is a strategy that has served to reduce methane (CH_4) emissions, however the way these compounds act in the rumen is not yet clear (Andrade-Rivero *et al*., 2012). The objective of this study was to evaluate the effect of different dietary levels of capulin leaves (*Prunus salicifolia*) in ruminal fermentation and digestibility through an *in vitro* study.

Material & methods *Prunus salicifolia* was included in the diet (on a dry matter [DM] basis) at two levels of 15% and 30% (P15 and P30) and compared to a control (P0) diet without leaves. Chemical composition (DM, Organic matter (AOAC,1991) NDF, ADF, Lignin (Van Soest *et al*. 1991) and *in vitro* gas production were determined (Theodorou *et al*., 1994). *In vitro* incubation (0.800 g DM) of the diet was incubated with the leaves of *Prunus salicifolia* at 0, 15 and 30% of inclusion.Three incubation periods were carried out, and in each one, samples were incubated in triplicate in a glass flask with 90 ml of buffer solution and 10 ml of ruminal fluid from 3 cannulated goats. Donor animals were fed a diet based on 83% forage and 17% concentrate (on a DM basis). Ruminal fluid was extracted and filtered through a triple layer of gauze, and homogenized with CO_2, then mixed and used as inoculum. The bottles were placed in a water bath at 39°C. The gas volume (ml gas/g DM) was recorded at 3, 6, 9, 12, 24, 36, 48, 72 and 96 hours of incubation using a Delta pressure transducer (Model 8804 HD). The accumulated gas volume of each sample was adjusted to the proposed model of Krishnamoorthy *et al*. (1991): $GP = B\ (1-e^{C\ (t-l)})$ where GP is the volume of total gas produced at time (t); B is the asymptotic GP (ml/g DM); C is the rate of GP (g/h), and l is the discrete lag time prior to gas production (h). After the *in vitro* incubation period the samples were filtered and dried (48 h, 60 °C) to measure the proportion of reduced DM (IVDTDM). The CH_4 was determined by gas chromatography and H_2S was determined by colorimetry. A completely randomized design was used, and a Tukey test was used when significant differences were observed between treatments ($P < 0.05$).

Results & discussion The production of CH_4/g DM initial and fermented was lower ($P < 0.05$) for P30 compared to the other dietary treatments, which indicates that the diets with greater amount of *P.salicifolia* produce less CH_4 without affecting ruminal fermentation. Treatments were similar ($P > 0.05$) in terms of *in vitro* gas production, IVDTDM and H_2S production.

Table 1 Effect of the inclusion level of *Prunus salicifolia* on fermentation parameters, *in vitro* gas production, and methane production.

Parameters	P0	P15	P30	SEM	P value
IVDTDM (% of DM)	49.58	48.65	47.28	4.56	0.8287
Parameters of gas production kinetics					
CH_4 total (mmol/g DM)	2.59	2.70	2.08	0.27	0.0635
CH_4 (ml/g DM initial)	0.64^a	0.67^a	0.46^b	0.08	0.0344
CH_4 (ml/g DM fermented)	1.29^a	1.37^a	0.99^b	0.13	0.0291
H_2S (mmol/g DM)	3.74	3.14	2.77	1.78	0.8024
B (ml gas/g DM)	349	334	347	6.31	0.053
C (g/h)	0.0408	0.0415	0.0400	0.0011	0.447
Lag time (h)	0.8656	0.9496	0.9773	0.096	0.502

IVTDMD, *In vitro* true dry matter digestibility

Conclusion Inclusion of *Prunus salicifolia* at 30% of inclusion in the diet reduces CH_4 emissions without changing DM digestibility under *in vitro* conditions. This feeding strategy could be used to reduce *in vivo* CH_4 emissions from goats, but this last point requires more investigation.

Acknowledgements This project was supported by UAEM 4335/2017.

References

Andrade-Rivero E, Martínez-Campos A, Castelán-Ortega O, Ríos-Quezada J. Pacheco-Ortege Y and Estrada-Flores J 2012. Tropical Subtropical Agroecosystems 15, 301-312.
Krishnamoorthy U, Soller H, Steingass H and Menke KH 1991. Journal of Animal Physiology and Animal Nutrition 65, 28-35
Theodorou MK, Williams BA, Dhanoa MS, McAllan AB and France J 1994. Animal Feed Science Technology 48, 185-197.

Effects of substrate on *in vitro* methane inhibition by 3-nitrooxypropanol

Pablo Alvarez[1], Peter Moate[2], Richard Williams[2], Joe Jacobs[2], Karen Beauchemin[3], Zoey Durmic[4], Joy Vadhanabhuti[4], Richard Eckard[1]

[1]The University of Melbourne, Melbourne, Victoria, Australia, [2]Agriculture Victoria, Department of Economic Development, Jobs, Transport and Resources, Ellinbank, Victoria, Australia, [3]Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada, [4]The University of Western Australia, Perth, Western Australia, Australia

E-mail: pabloah@student.unimelb.edu.au

Take home message These *in vitro* results suggest that 3-NOP is an effective methane mitigating agent across three different substrates.

Introduction Recent research has shown that dietary inclusion of the methyl-coenzyme M reductase inhibitor, 3-nitrooxypropanol (3-NOP; DSM Nutritional Products, Ltd, Kaiseraugst, Switzerland) can reduce ruminal methane production in dairy cows by between 30 and 60% (Haisan *et al*., 2014; Hristov *et al.,* 2015). However, it is not clear if the magnitude of methane mitigation depends upon the basal diet. The aim of the research reported here was to compare the inhibitory effect of 3-NOP on *in vitro* methane production from three different substrates, lucerne, corn and wheat.

Material & methods Fermentations were conducted using an Ankom GP *in vitro* system that consists of incubation bottles, pressure transducers with actuators to vent fermentation gas at a pre-set threshold, and gas bags to collect vented gas. This study consisted of six treatments: 1) lucerne hay, 2) corn grain, and 3) wheat grain all fed with and without addition of 3-NOP (0.08 mg/g DM). All substrates (1.0 g DM/bottle) were incubated separately in two different sources of pooled ruminal fluid. Each combination of substrate and ruminal fluid was incubated in three fermentation bottles. The experiment was replicated in two *in vitro* runs. After 24 hours of fermentation, total methane production (MPR), proportion (MPP), pseudo-yield (PMY), total gas production (GP), dry matter degradation (DMD), acetate to propionate ratio (A:P) and ruminal fluid pH were measured. Data were analysed by ANOVA with a factorial structure of substrate and 3-NOP and a blocking structure of run and ruminal fluid.

Results & discussion When combined with any of the three substrates, 3-NOP reduced MPR, MPP and PMY by up to 52%, and there was no interaction between substrate and 3-NOP. 3-NOP had no effect on ruminal fluid pH or dry matter degradation (Table 1). In our *in vitro* experiment, the reduction in methane production due to 3-NOP was within the range previously reported *in vivo* (Haisan *et al., 2*014; Hristov *et al.,* 2015).

Table 1 Total methane production (MPR; mL $24h^{-1}$), methane proportion (MPP mL L^{-1}), pseudo-methane yield (MY; mL g DMD ^{1}), gas production (GP; mL $24h^{-1}$), dry matter degraded (DMD; g kg^{-1} DM), acetate to propionate ratio (A:P) and pH resulting from the incubation of lucerne hay, corn and wheat with and without 3-nitrooxypropanol (3-NOP).

Treatment	MPR	MPP	PMY	GP	DMD	A:P	pH
Lucerne hay	77^{b}	25^{b}	201^{d}	304^{b}	392^{a}	2.4^{d}	6.3^{b}
Corn grain	86^{c}	27^{b}	151^{c}	323^{c}	587^{b}	2.1^{b}	6.0^{a}
Wheat grain	82^{bc}	26^{b}	130^{c}	320^{c}	631^{b}	2.1^{b}	6.0^{a}
Lucerne+3-NOP	37^{a}	14^{a}	100^{b}	276^{a}	390^{a}	2.3^{c}	6.3^{b}
Corn+3-NOP	41^{a}	13^{a}	70^{a}	315^{c}	587^{b}	1.9^{a}	6.0^{a}
Wheat+3-NOP	39^{a}	13^{a}	62^{a}	304^{b}	633^{b}	1.9^{a}	6.0^{a}
P (Substrate)	0.13	0.76	<0.001	<0.001	<0.001	<0.001	<0.001
P (NOP)	<0.001	<0.001	<0.001	<0.001	1.00	<0.001	0.99
P (Substrate × 3-NOP)	0.67	0.48	0.08	0.02	0.99	0.24	0.79
SED	4.27	1.39	10.31	4.78	22.7	0.05	0.04

Means in the same column with different superscripts differ significantly ($P < 0.05$)

Conclusion The magnitude of the methane mitigation effect of 3-NOP is independent of the type of substrate fermented.

Acknowledgements This research was funded by the Victorian Department of Economic Development, Jobs, Transport and Resources; the University of Melbourne; Emissions Reduction Alberta, Canada; and Dairy Australia.

References

Haisan J, Sun Y, Guan LL, Beauchemin KA, Iwaasa A, Duval S, Barreda DR and Oba M 2014. Journal of Dairy Science 97, 3110-3119.

Hristov AN, Oh J, Giallongo F, Frederick TW, Harper MT, Weeks HL, Branco AF, Moate PJ, Deighton MH, Williams SRO, Kindermann M and Duval S 2015. Proceedings of the National Academy of Sciences 112, 10663-10668.

A partial life cycle assessment of the greenhouse gas mitigation potential of feeding 3-nitrooxypropanol on two Australian dairy farms

Pablo Alvarez[1], Shannan Little[3], Peter Moate[2], Joe Jacobs[2], Karen Beauchemin[3], Richard Eckard[1]

[1]The University of Melbourne, Melbourne, Victoria, Australia, [2]Agriculture Victoria, Department of Economic Development, Jobs, Transport and Resources, Ellinbank, Victoria, Australia, [3]Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada

E-mail: pabloah@student.unimelb.edu.au

Take home message Feeding 3-nitrooxypropanol to dairy cows can reduce overall farm greenhouse gas (GHG) emissions by between 5% to 13%.

Introduction Research has shown dietary inclusion of 3-nitrooxypropanol (3-NOP) can reduce enteric methane production of dairy cows by between 30% and 60% without affecting dry matter intake (DMI), milk yield or milk composition (Hristov *et al.,* 2015); however, the net impact on whole farm GHG emissions is unclear. Therefore, the objective of this partial farm-gate life cycle assessment (LCA) was to quantify the effect of 3-NOP on whole farm GHG emissions of two Australian dairy farms.

Material & methods Two Australian dairy farms were modelled, one representing a standard producing farm (SPF) and one representing a high producing farm (HPF). The farms were modelled over a six-year period, covering the period from birth to slaughter of a group of dairy cows including 4 lactation cycles. The SPF farm had 340 cows, a stocking rate of 1.7 cows/ha and milk production of 20 kg/hd/d, while the HPF farm had 500 cows, a stocking rate of 2.5 cows/ha and milk production of 25kg/hd/d. There were two 3-NOP feeding scenarios; 3-NOP fed to the milking cows during the first 120 days of lactation (scenario 1) and 3-NOP fed to the milking cows during the entire 300-day lactation (scenario 2). Farm GHG emissions (carbon dioxide equivalent (CO_2-e)) were calculated based on the Australian National Greenhouse Gas Inventory method, using the Greenhouse Accounting Framework calculator for dairy, accounting for production and transport of major farm inputs such as fuel, gas, fertilizers and grains (Browne *et al.,* 2011). The system boundary of the partial LCA was defined as the farm gate, including emissions associated with on-farm activities and emissions associated with pre-farm production and transport of major pre-farm production inputs including NOP and purchased feeds. Based on the findings of Hristov *et al.,* (2015), it was assumed that feeding 3-NOP had no effect on milk yield, but that daily methane production decreased by 32%.

Results & discussion Over the entire six-year period, for the SPF farm, scenario 1 reduced GHG emissions by 5.6% and GHG intensity by 5.5%, while scenario 2, reduced GHG emissions by 13.1% and GHG intensity by 12.8% (Table 1). For the HPF farm, scenario 1 reduced GHG emissions by 5.7% and GHG intensity by 5.1%, while scenario 2, reduced GHG emissions by 13.1% and GHG intensity by 12.2%.

Table 1 Whole farm GHG emissions and intensity for two Australian dairy farms, a standard producing farm (SPF) and a high producing farm (HPF), where lactating cows were fed diets supplemented with 3-nitrooxypropanol at 200 mg/kg DMI, during the first 120 days of lactation (Scenario 1) or during 300 days of lactation (Scenario 2)

	SPF			HPF		
	Control	Scenario 1 (120 d)	Scenario 2 (300 d)	Control	Scenario 1 (120 d)	Scenario 2 (300 d)
Emissions (t CO_2e/year)						
Enteric methane	1,390	1,261	1,087	2,264	2,041	1,752
Manure methane	199	199	199	327	327	327
Direct nitrous oxide	204	204	204	315	315	315
Indirect nitrous oxide	99	99	99	185	185	185
Energy carbon dioxide	263	263	263	440	440	440
Pre-farm emissions	107	110	114	303	307	314
Total GHG	2,262	2,135	1,965	3,834	3,615	3,332
GHG intensity (kg CO_2e/kg FPCM[1])	1.09	1.03	0.95	0.98	0.93	0.86

[1]FPCM; Fat and protein corrected milk

Conclusion Feeding 3-NOP to either farm reduced overall farm GHG emissions by between 5.6% and 13.1% and GHG intensity between 5.1% and 12.8%. Thus, feeding 3-NOP has potential to generate a net reduction in whole farm GHG emissions.

Acknowledgements This research was funded by the Victorian Department of Economic Development, Jobs, Transport and Resources; University of Melbourne; Emissions Reduction Alberta, and Dairy Australia.

References

Browne NA, Eckard RJ, Behrendt R and Kingwell RS 2011. Animal Feed Science and Technology 166, 641-652.

Hristov AN, Oh J, Giallongo F, Frederick TW, Harper MT, Weeks HL, Branco AF, Moate PJ, Deighton MH, Williams SRO, Kindermann M and Duval S 2015. Proceedings of the National Academy of Sciences 112, 10663-10668.

Effects of dietary neutral detergent fibre and non-fibre carbohydrates ratio on methanogenesis, rumen fermentation and rumen microbial flora in sheep

Yunlong Liu[1], Jingmei Ding[2], Shuru Cheng[2], Kaidong Deng[3], Qiyu Diao[1], Kai Cui[1], Yan Tu[1]

[1]Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China, [2]Gansu Agricultural University, Lanzhou, China, [3]Jinling Institute of Technology, Nanjing, China

E-mail: tuyan@caas.cn

Take home message Dietary neutral detergent fibre and non-fibre carbohydrates ratio can affect methane production by modifying rumen fermentation mode and by changing rumen microbial flora.

Introduction Plant fibres produce methane through rumen fermentation, which not only reduces the utilization of feed energy, but also contributes to the greenhouse effect and causes global warming. In ruminants, approximately 95.5% of the methane is produced by feed fermentation in the rumen (AGO 2003). At present, the research on methane emission reduction measures mainly focuses on the additives. However, these additives are either toxic to the hosts or only have a transient effect. Some research shows that properly raising the concentrate level can improve feed utilization and reduce methane emission, but the effect of concentrate level on methane emission from lamb is poorly reported. The present study was to investigate the effects of dietary neutral detergent fibre and non-fibre carbohydrates ratio on methanogenesis, rumen fermentation and rumen microbial flora in sheep, and provide a theoretical basis for the formulation of reasonable diets and methane emission reduction for sheep.

Material & methods A total of twelve Dorper × Small Tailed Han sheep with permanent rumen fistula were randomly divided into 4 groups and fed with neutral detergent fibre (NDF) and non-fibre carbohydrates (NFC) ratio of 3.60 (Diet 1), 2.70 (Diet 2), 1.77 (Diet 3), and 1.04 (Diet 4) at maintenance level. The trial was conducted according to a 4×4 Latin square design and the duration of each period was 23d including 10d for the adaptation period. This work was carried out while maintaining body weight. Feed was supplied 2 times daily at 0730 and 1630h for *ad libitum* intake and water was freely accessible. The methane emissions were determined using an open-circuit respirometry system, rumen fluids were obtained to determine the fermentation parameters, and microbial assessment was done by qPCR. Data referring to rumen fermentation parameters and microbial flora measured at each sampling time were analysed using repeated measures ANOVA of SPSS software (SPSS Inc., Chicago. IL, USA) and significance was declared at $P<0.05$.

Results & discussion The results showed that the molar proportion of propionate of rumen fluid increased from 15.85 to 18.52% ($P=0.006$) with decreasing of dietary NDF and NFC ratio, while that of the ratio acetate to propionate decreased from 4.32 to 3.67 ($P=0.006$). Populations of *R. flavefaciens* ($P=0.034$) and *F. succinogenes* ($P=0.023$) were significantly decreased, while no difference was observed in the population of methanogens ($P=0.348$) and protozoa ($P=0.530$). Daily methane output decreased from 36.22 to 33.1 L/d ($P=0.040$) (Table 1).

Table 1 Effects of dietary neutral detergent fibre and non-fibre carbohydrates ratio on the parameters of rumen fermentation, rumen microbial population and daily methane production.

Items	Diets				SEM	*P* -value
	1	2	3	4		
Acetate (%Total VFA)	70.44	67.01	65.01	64.45	1.02	0.070
Propionate (%Total VFA)	15.85^{c}	16.97^{cb}	17.58^{b}	18.52^{a}	0.15	0.006
Acetate:Propionate	4.32^{a}	4.28^{ab}	4.12^{b}	3.67^{c}	0.04	0.006
R. flavefaciens (10^8)	1.40^{b}	2.28^{a}	1.00^{bc}	0.84^{c}	0.10	0.034
F. succinogenes (10^7)	1.35^{b}	2.50^{a}	0.47^{c}	0.50^{c}	0.20	0.023
Methanogens (10^7)	4.89	5.12	6.90	5.28	2.70	0.348
Protozoa (10^5)	1.89	2.12	2.38	1.98	0.10	0.530
CH_4 daily production/(L/d)	36.22^{a}	37.3^{a}	35.1^{ab}	33.1^{b}	1.10	0.040

^{a,b,c} Mean values within a row with different superscripts differ significantly ($P < 0.05$).

Conclusion In conclusion, under the maintenance level, as the dietary NDF and NFC ratio decreased, daily methane emissions (L/d) of sheep reduced. These effects were probably due to modify rumen fermentation mode by changing the microbial population or the rate of passage of digesta through the rumen.

Acknowledgements This work was supported by the [National Natural Science Foundation of China] under Grant [No: 41475126] and the [Ministry of Science and Technology of China] under Grant [No: 2012BAD39B05-3].

References

AGO (Australian Greenhouse Office): National Greenhouse Gas Inventory 2001 with Methodology Supplements. AGO, Canberra, Australia, 2003.

In vitro effect of heat processed flaxseed on ruminal fermentation and methane emission response

Farzaneh Mohammadi[1], Mohsen Danesh Mesgaran[1], Alireza Vakili[1], Mohammadreza Dehghani[2]

[1]*Ferdowsi University of Mashhad, Mashhad, Iran,* [2]*Zabol University, zabol, Iran*

E-mail: f_mohamady66@yahoo.com

Take home message It has been demonstrated that physically processed flaxseed may alter rumen fermentation.

Introduction Flaxseed is an annual or biannual plant that has been cultivated as a commercial plant in over thirty countries all over the world. It yields seeds that mature 30-60 days after flowering and are a rich source of both edible and non-edible oils (34 - 40% DM). The seeds have long been used in animal diets (Matheson, 1976). The objective of the current research was to investigate the effect of different heat processing including roasting, water boiling and oven drying of whole flaxseed on *in vitro* ruminal fermentation, digestibility parameters and methane emission using gas production technique**.**

Material & methods Samples of the whole flaxseed were obtained from several local markets. Samples were composited and any external weeds and dust were removed manually. Physical heat processing including roasting, boiling and oven heating was applied. A sub-section of the samples were ground through a 2 mm screen using CyclotecTM milling machine (Foss.1093, Denmark). Whole flaxseed was roasted by indirect heating on fire using a pan as the container for 2, 4, 6, 8 and 10 min. Whole flaxseed was boiled for 0.5, 1, 2 and 4 h by indirect heating of a boiler containing flaxseed and water at the ratio of 1 to 3 wt/vl. All boiled samples were dried at room temperature. The oven heating of the seeds was also carried out using air forced oven drying (Behdad Co., BC Oven 70, 3493, Iran) at 65, 95 and or 125 °C for 4, 8 and 12 h. After each heat processing, all the samples were milled through a 2 mm screen using CyclotecTM milling machine and kept in refrigerator at 4 °C for subsequent gas production procedure. The gas production procedure was performed as described by Theodorou *et al.,* (1994), using 500 mg DM of each samples in 4 replicates and 3 runs. Methane emission was determined using gas detector (SR2-BIO System, Sewerin, Germany).

Results & discussion Dry matter disappearance (DMD) was significantly decreased from 1 to 2 hours of boiling and milled flaxseed had the water boiled flaxseed reduced significantly ($P< 0.01$) as the time of boiling increased (Table 1). Results of the present study showed that heat processing of flaxseed is capable of manipulating the extent of emitted methane in the medium. Roasting, boiling and oven drying efficiently decreased methane emissions by approximately, 25, 40 and 60 %, respectively. Addition of oilseed supplements to ruminant diets has been shown to decrease methane output in ruminants fed conserved forages (Sejian *et al.,* 2011).

Table 1 Dry matter disappearance (DMD %), methane emission (ml) and methane emission per DMD (ml/mg) of raw whole flaxseed and the physically heat treated whole flaxseed.

		Time of roasting (min)		Time of boiling (h)		oven drying temperature (°C)			SEM	P-value of contrasts	
parameters	Raw flaxseed	2	6	1	2	65	95	125		1	2
DMD	52^{c}	56^{b}	50^{c}	42^{d}	37^{e}	60^{a}	60^{a}	39^{e}	1.50	0.01	0.01
Methane production	5.3^{a}	4.7^{b}	4.2^{c}	2.9^{c}	2.4	1.6^{c}	1.7^{c}	1.0^{d}	0.04	0.01	0.01
Methane emission per DMD	0.021^{a}	0.016^{b}	0.017^{b}	0.014^{c}	0.013^{c}	0.005^{b}	0.005^{b}	0.005^{b}	0.00	0.01	0.01

SEM: Standard error of mean; ^{a,b,c,d,e} Rows with different superscripts are statistically different ($P< 0.05$)
1: Raw flaxseed *vs.* Heat processing; 2: Roasting *vs.* Boiling and Oven Drying

Conclusion It has been concluded both *in vitro* rumen fermentation and methane emissions of flaxseed were impacted by physically heat processing the seeds. In addition, the gas production constant rate of milled flaxseed was higher than those of the roasted flaxseed at 6 and 8 minutes. Rumen fermentation also significantly decreased as the time of boiling increased, while roasting did not make any significant decrease. Oven drying caused a reduction in dry matter disappearance of flaxseed. Methane emissions decreased in the boiled and oven dried seeds.

References

Matheson EM 1976. Flaxseed. In: EM Matheson (ed.) Vegetable Oil Seed Crops in Australia, Holt, Rinehart and Winston, Sydney, Australia, 111-121.
Sejian V, Larkitz J, Ezeji T and Lal R 2011. Research Veterinary Science 4, 1–8.
Theodorou MK, Williams BA, Dhanoa MS, McAllan AB, and France J 1994. Animal Feed Science and Technology 48, 185-197.

An antimethanogenic index for meadow plants consumed by ruminants

Didier Macheboeuf[1], Agnès Cornu[1], Sylvain Kerros[2], François Recoquillay[2]

[1]*INRA, UR1213 Herbivores, Centre Auvergne-Rhone-Alpes, Saint-Genès-Champanelle, France,* [2]*Phytosynthese, 57 avenue Jean Jaurès, Mozac, France*

E-mail: didier.macheboeuf@inra.fr

Take home message An antimethanogenic index could complete information on the multiple services provided by a meadow and is an opportunity to re-introduce plants rejected in borders as a consequence of grassland management in the previous decades.

Introduction Permanent meadows, especially in mountainous areas, have numerous advantages over temporary meadows in terms of ecosystem services as they help protect biodiversity, confer resilience to climate stresses (*e.g.,* drought), and meet rising societal demand (*e.g.,* high-quality meat and cheese). However, the past decades have seen big changes in grassland management (*e.g.,* unbalanced cutting frequencies, fertilizer doses) to achieve higher productivity, but with a concomitant decline in botanical composition and biodiversity (Pierik *et al.* 2017). Here we develop a new antimethanogenic index (AMI) that could characterize the plants growing not just in the meadows but also in neighbouring environments like hedges, borders, under-brush, and ditches. The purpose of the AMI is to evaluate the potential of a plant species to reduce methane emissions by grazing ruminants, while taking into account its energy value. The AMI could add new information about the multiple services provided by pasture, and thus rehabilitate some plants that had become marginalized.

Material & methods The AMI was built from *in vitro* rumen fermentation data. A set of 212 plants was collected in the French Massif Central area, at the flowering stage in the vast majority of cases. Samples were frozen in liquid nitrogen, freeze-dried, ground, and kept out of the light. The fermentation profiles (gas and volatile fatty acids (VFA) produced) were determined by incubating 600-mg pure plant substrates in 40-mL rumen mixed bacterial cultures (buffer solution:rumen fluid at 2:1, v/v) for 24h at 39°C. All incubations were repeated 3 times. Each run period included 4 incubations of perennial ryegrass (PRG*)* used as controls for a total of 48 runs. Methane and VFA productions were normalized and expressed as a ratio of mean PRG values for each period to eliminate inter-period drift. A plant was declared antimethanogenic ($p<0.01$) when its methane production was lower than the value fitted to the methane=f(VFA) linear regression, minus 2.58 times the standard deviation (s.d.) of PRG (s.d.), with s.d. assumed as uniform on the experimental domain. The index was calculated as: AMI=(Af-Am)/Amax, where Af is the CH_4 value fitted to the CH_4 =f(VFA) linear regression minus 2.58 times the PRG s.d., Am is measured CH_4 value, and Amax is maximum (Af-Am) value observed among the 212 plant samples.

Results & discussion Outliers, namely 16 very particular plants that had a big effect on methane (1 methanogenic activator; 15 antimethanogenic) were discarded to calculate the regression ($CH4=1.06 (+-0.04) VFA-0.12 (+-0.03)$, $R^2=0.80$, RSE=0.08). The plants were then classified into three groups: 44 plants that activated methanogenesis (AMI < -0.20, over the grey zone, figure 1), 106 plants with normal stoichiometry (Demeyer 1981) of fermentation (-0.20<= AMI <= 0, grey zone), and 64 plants with antimethanogenic effect (AMI > 0) below the grey zone. The strongest effect was observed with *Bidens tripartita* (AMI=1), thereby confirming our previous observations (Macheboeuf *et al.* 2014), but numerous other plants had a promising positive AMI, including *Origanum vulgare* (0.60), *Scrophularia nodosa* (0.55), *Serratula tinctoria* (0.49), *Succisa pratensis* (0.19), *Polygonum bistorta* (0.17) and *Hypericum perforatum* (0.16).

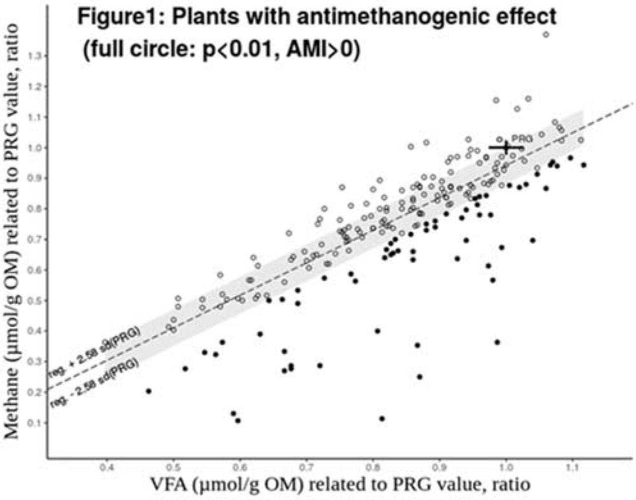


Figure1: Plants with antimethanogenic effect (full circle: p<0.01, AMI>0)

Conclusion AMI was able to classify plants according to their antimethanogenic potential while taking into account their VFA production. AMI findings also highlight the importance of keeping certain species in the meadow. Once a meadow's botanical composition is known, the AMI index could be included in the criteria used to evaluate its environmental potential.

Acknowledgements We thank ethnobotanist G. Lalière and the Conservatoire Botanique National du Massif Central for plant collection.

References

Demeyer DI 1981 Agriculture and Environment 6, 295-337
Macheboeuf D, Coudert L, Bergeault R, Laliere G and Niderkorn V 2014. Animal 8, 1797-1806.
Pierik ME, Gusmeroli F, Marianna GD, Tamburini A and Bocchi S 2017. Agriculture, Ecosystems and Environment 244, 14–21

Kinetics of nitrogen secretion in milk and excretion in urine and faeces of four common dairy feeds intrinsically enriched with ^{15}N

Kristan F. Reed[1], Tiago Barros[2], J. Mark Powell[1], Michel A. Wattiaux[2]

[1]USDA-Agricultural Research Service, U.S. Dairy Forage Research Center, 1925 Linden Drive, Madison, WI, USA, [2]Department of Dairy Science, University of Wisconsin-Madison, 1675 Observatory Drive, Madison, WI, USA

E-mail: wattiaux@wisc.edu

Take home message N from four feed sources exhibited the same fractional rate of secretion in milk and excretion in urine, but not in faeces.

Introduction Most ruminant nutrition models aggregate protein from individual feeds into composite pools such as rumen degradable or metabolizable protein. However, this aggregation assumes additivity and relies on the assumption that composition and structure of individual feeds' N compounds do not influence digestion, metabolism, and subsequent secretion and excretion. Feeds intrinsically labelled with the stable isotope ^{15}N can be used to test these assumptions and determine if N fractions from different feeds do in fact behave similarly during ruminant digestion and metabolism.

Material & methods Alfalfa silage (AS), corn silage (CS), corn grain (CG) and soybean meal (SBM) were labelled with ^{15}N enriched fertilizer as described by Powell *et al.,* (2017) and fed to 12 pregnant multiparous lactating cows (3 per treatment) in four diets. Diets were identical other than replacement of the non-labelled AS, CS, CG, or SBM with the labelled feed as described by Barros *et al.,* (2017). Enrichment of ^{15}N in milk was measured every 12 hours and in faeces and urine every 6 hours during the 96-hour labelled feeding period and for an additional 96 hours after cessation of labelled feeding. A piece-wise single pool first-order exponential model was developed to describe the rise and fall of ^{15}N enrichment in milk, urine, and faeces from each of the feeds during the entire 176-hour collection period assuming the rates of excretion/secretion are equivalent in the rising and decaying periods (Eq. 1).

$$y_{ij} = \begin{cases} 0 & \mathrm{t}_j < \mathrm{L}_{1i} \\ \mathrm{b}_i - \mathrm{b}_i e^{-\mathrm{k}_i(\mathrm{t}_j - \mathrm{L}_{1i})} + \mathrm{e}_{ij} & \mathrm{L}_{1i} < \mathrm{t}_j < \mathrm{L}_{1i} + \mathrm{L}_2 \\ \mathrm{b}_i e^{-\mathrm{k}_i(\mathrm{t}_j - (\mathrm{L}_{1i} + \mathrm{L}_2))} + \mathrm{e}_{ij} & \mathrm{L}_{1i} + \mathrm{L}_2 < \mathrm{t}_j \end{cases} \quad \mathrm{e}_{ij} \sim \mathrm{N}\left(0, \sigma^2/\overline{y_{\imath\jmath}}\right) \quad \text{[Eq.1.]}$$

In Eq. 1, y_{ij} is the atom percent excess (APE) of milk (MN), urine (UN), or faecal N (FN) from diet i (i = AS, CS, CG, SBM) for the t_j^{th} time point where t =[0, 176]; b_i is the asymptotic enrichment of the ith diet; k_i is the fractional rate of N secretion in milk and excretion in urine and faeces for the ith diet; L_{1i} is the delay in appearance of labelled N in a pool in relation to the initial time of feeding the labelled diet; L_2 is the time from L_1 to the time when APE begins to decay from the maximum APE for MN, UN, or FN after cessation of labelled feeding. Model variances (e^2_{ij}) were weighted by the mean observed APE of the ith diet at the jth time point for each excretion fraction ($\sigma^2/\overline{y_{\imath\jmath}}$). Models were fitted in R statistical software using the nlme package to accommodate the heteroscedastic error structure. Pre-planned linear contrasts were conducted with the multcomp package to test for significant differences between diet specific parameters while reducing the possibility of Type I errors.

Results Estimates for b_i, the asymptotic maximum APE for each pool and diet (data not shown) were in general agreement with results reported by Barros *et al*. (2017) indicating that the ratio of FN:MN and UN:MN was greater for the silage feeds than for the grains. The asymptotic estimates of maximum APE from our work further suggest that the ratio of UN:MN was higher for AS (1.41) than for CS (1.26) but lower for SBM (0.967) than for CG (1.10). Kinetic parameter estimates (Table 1) indicate that the delay in appearance of the label (L_1) varies between diets for MN, UN and FN. Furthermore, the fractional rate of FN excretion (k) is diet specific. Hence, N transformation and transactions along the gastro-intestinal tract vary with feed ingredient but the fractional rate of MN secretion and UN excretion is independent of feed ingredient.

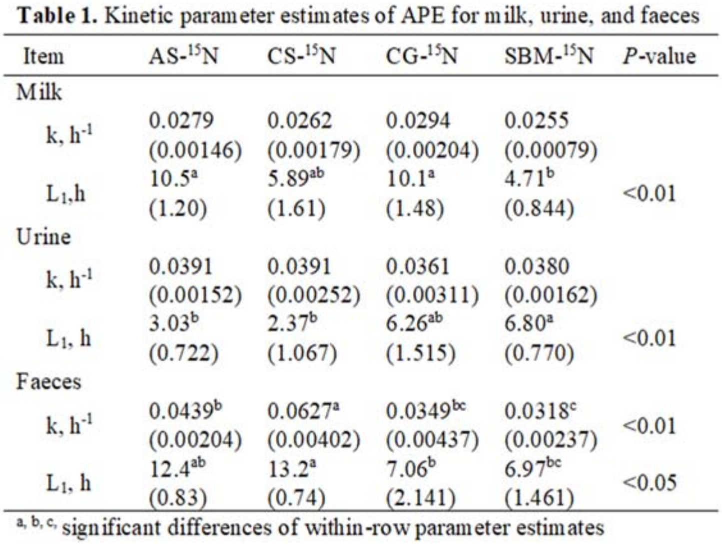


Table 1. Kinetic parameter estimates of APE for milk, urine, and faeces

Item	AS-^{15}N	CS-^{15}N	CG-^{15}N	SBM-^{15}N	*P*-value
Milk					
k, h^{-1}	0.0279 (0.00146)	0.0262 (0.00179)	0.0294 (0.00204)	0.0255 (0.00079)	
L_1,h	10.5^a (1.20)	5.89^{ab} (1.61)	10.1^a (1.48)	4.71^b (0.844)	<0.01
Urine					
k, h^{-1}	0.0391 (0.00152)	0.0391 (0.00252)	0.0361 (0.00311)	0.0380 (0.00162)	
L_1, h	3.03^b (0.722)	2.37^b (1.067)	6.26^{ab} (1.515)	6.80^a (0.770)	<0.01
Faeces					
k, h^{-1}	0.0439^b (0.00204)	0.0627^a (0.00402)	0.0349^{bc} (0.00437)	0.0318^c (0.00237)	<0.01
L_1, h	12.4^{ab} (0.83)	13.2^a (0.74)	7.06^b (2.141)	6.97^{bc} (1.461)	<0.05

$^{a, b, c,}$ significant differences of within-row parameter estimates

Conclusion Lag time and rate of N excretion in faeces varies with feed ingredient revealing unique digestive and metabolic behaviours.

Acknowledgements USDA Hatch Multi-State Research Formula Funds # WIS01724 and WIS01892 and USDA Agricultural Research Service award # 58-3655-4-04.

References

Barros, T, Powell JM, Danes MAC, Aguerre MJ, and Wattiaux MA 2017. Animal Feed Science and Technology 229, 91-96.
Powell JM, Barros T, Danes MAC, Aguerre MJ, Wattiaux MA and Reed KF 2017. Agriculture, Ecosystems & Environment 239, 274-282.

Association rule mining to help detect plant phenolic compounds putatively involved in decreased ruminal methane production *in vitro*

Didier Macheboeuf[1], Sylvie Guillaume[2], Clara Leguay[1,3], Sylvain Kerros[3], Agnès Cornu[3]

[1]INRA, UR1213 Herbivores, Centre Auvergne-Rhone-Alpes, Saint-Genès-Champanelle, France, [2]Auvergne University, LIMOS - UMR CNRS 6158, Clermont-Ferrand, France, [3]Phytosynthese, 57 avenue Jean Jaurès, Mozac, France

E-mail: didier.macheboeuf@inra.fr

Take home message In the virgin forest of unknown bioactive plant phenolic compounds, association rules work like bushcutters clearing pathways to help discover active compounds.

Introduction In the search for natural alternatives to synthetic chemicals able to mitigate methane emission by ruminants, bioactive plant secondary metabolites are valuable candidates. However, these phytochemicals come in a myriad of chemical structures, and any one plant may contain hundreds of them. Even in plant extracts containing tannins, saponins or essential oils, it is difficult to link the presence of a compound or combination to the plant's activity. Here we focus on low-molecular-weight (<1000 Da) phenolic compounds from hydroalcoholic plant extracts, using association rules mining as a tool to emerge compounds involved in the antimethanogenic activity of plants during ruminal fermentation.

Material & methods A set of 208 plants was collected from the French Massif Central area. Samples were frozen in liquid nitrogen, freeze-dried, ground, and kept out of the light to protect the integrity of the phenolic compounds. Fermentation profiles (gas production and composition, and volatile fatty acids (VFA) produced) were determined *in vitro* by incubating 600-mg pure plant substrates in rumen mixed bacterial cultures (buffer solution:rumen fluid at 2:1, v/v) for 24h at 39°C. All incubations were repeated 3 times. Each run period included 4 incubations of perennial ryegrass (PRG*)* used as the control for a total of 48 runs. Plant production of methane and VFA were normalized and expressed as a ratio of mean PRG values for each period to eliminate inter-period drift. A plant was declared antimethanogenic ($p<0.01$) when its methane production was lower than the value fitted to the methane=f (VFA) linear regression, minus 2.58 times the standard deviation (s.d.) of PRG.
Phenolic compound profiles were obtained using an Agilent 1100 HPLC system. After ethanol:water (80:20) extraction, chromatographic separation was performed with a water:methanol gradient for 90 min on an Agilent C18 column and recorded at 280 and 320 nm with a diode array detector. A mix of 21 pure phenolic compounds was systematically injected in each analytical sequence (comprising about 10 plant extracts), in which flavone was taken as reference to calculate relative retention times (RRT). Sequence alignment was carried out by repositioning the standard RRTs. The area values were binarized (absence/presence). The resulting pair of matrices, *i.e.,* 280 and 320nm, comprised 208 plants × 1092 peaks.
Association rules were created for frequent antecedent-consequent patterns using *ARA* software (*Association Rules Analyzer*, Papon 2016). The consequent was tied to the antimethanogenic item and antecedents were itemsets from peak matrices. Support(S) and confidence(C) criteria were used to identify important relationships. S indicated how frequently the itemset appeared in the database. C is a measure of how often the rule was found to be true.

Results & discussion The consequent item included 64 antimethanogenic plants (Macheboeuf *et al.,* 2014), of which 15 had strong effect (outliers of the methane=f (VFA) linear regression). The analysis for these plants generated from 29 to 161 peaks, with an average of 100. The very low filling ratios of the two peak matrices (0.10 and 0.09) justified the use of association rules rather than multivariate analysis. The very frequent peaks (>0.5) were discarded before data mining to avoid false-positives. With the minimum thresholds of 5 for S and 0.5 for C, there were 205 candidate peaks. In a first strategy, results were filtered via the constraints of a) co-occurrence of the peak in the 280 and 320 nm matrices and b) $C > 0.65$, which narrowed the candidate peaks down to 28. In a second strategy, the constraints were that the peaks had to be major (*i.e.,* more than 10 times the area of the median peak) and present in the plants that showed high antimethanogenic effect (outliers), which narrowed the candidate peaks down to 24. Combining the two strategies resulted in 7 candidate peaks. One peak was easily identified as gallic acid. Based on absorbance spectrum between 200 and 400 nm, three others were cinnamic acid derivatives and two were flavonols.

Conclusion Association rules mining was able to select a compact number of peaks making identification feasible. The effect of these pure compounds now has to be verified for proof of the concept. While the algorithm works with qualitative data, using strategy which selects among the major peaks of the profiles serves to integrate the quantitative aspect.

Acknowledgements We thank ethnobotanist G. Lalière and the *Conservatoire Botanique National du Massif Central for plan*t collection.

References

Macheboeuf D, Coudert L, Bergeault R, Laliere G and Niderkorn V 2014. Animal*,* 8 1797-1806.
Papon P A 2016. Thesis, https://tel.archives-ouvertes.fr/tel-01412054 .

Daily methane emissions and yields in dairy cows are predicted from methane to carbon dioxide ratio in breath

Peter Moate[1], Matthew Deighton[2], Murray Hannah[1], Joe Jacobs[1], Bill Wales[1], Sean Richard Williams[1]

[1]DEDJTR, Ellinbank, Victoria, Australia, [2]CropMark, Christ Church, New Zealand

E-mail: peter.moate@ecodev.vic.gov.au

Take home message Measurement of methane and carbon dioxide in breath exhalations of dairy cows offers potential as an indirect method to measure their methane emissions and yields.

Introduction Enteric methane emissions from ruminant animals and their contribution to greenhouse gases are responsible for global warming. There are a number of direct methods (respiration chambers, SF_6, C-Lock system) for measuring enteric methane emissions, but these either involve expensive equipment or are technically challenging. Recently, Madsen *et al.,* (2010) proposed that the methane:carbon dioxide ratio (CH_4/CO_2) in exhaled breath could be used to quantify methane emissions from ruminants. The aim of the current research was to assess the potential of this technique using data from a large number of dairy cows fed on a wide range of diets.

Material & methods Our analysis used data from 105 lactating dairy cows involved in 9 experiments where each experiment had 3 or 4 dietary treatments with between 8 and 12 cows per treatment. There were 24 diverse dietary treatments resulting in 312 cow-diet combinations. Measurements on individual cows of dry matter intake (kg/d), and emissions (g/d) of methane and carbon dioxide were generally made over either 2 or 3 days in respiration chambers using methods described by Moate *et al.,* (2011). Individual methane emissions (g/day) and yields (g/kg DMI) were regressed against CH_4/CO_2 using a mixed model with nested random effects for Day within Cow within Treatment within Experiment, and fitted using ReML in GenStat 19.1.

Results & discussion Our data support the proposal of Madsen in that there was a strong ($P < 0.001$) linear relationship between methane emissions and the CH_4/CO_2 ratio in exhaled gases. A novel and important finding of this research is the strong ($P < 0.001$) linear relationship between methane yield and CH_4/CO_2. Although in this experiment, both emissions of methane and carbon dioxide were measured by respiration chambers, it is feasible to obtain a representative sample of the daily emissions of both methane and carbon dioxide using the breath sampling equipment used for the SF_6 technique. Alternately CH_4 and CO_2 in breath could be measured by electronic sensors mounted on animals. This approach may provide an inexpensive method to allow large numbers of animals to be evaluated for their methane emissions.

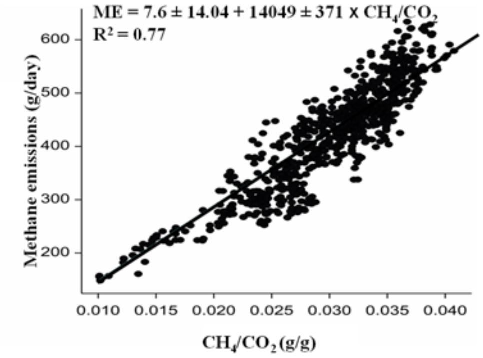


Figure 1 Linear relationship between methane emissions (ME) and CH_4/CO_2 in dairy cows.

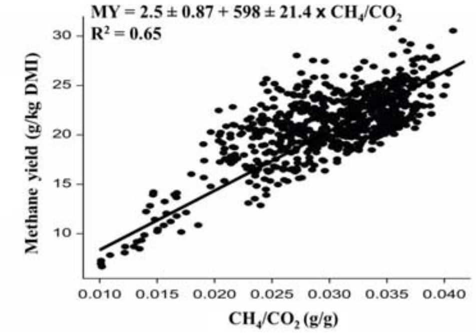


Figure 2 Linear relationship between methane yield (MY) and CH_4/CO_2 in dairy cows.

Conclusion The strong linear relationships between ruminal methane emissions and CH_4/CO_2 ratio and between methane yield and CH_4/CO_2 ratio may have application as indirect methods for predicting methane emissions and yields.

Acknowledgements This research was funded by Agriculture Victoria, Meat and Livestock Australia and Dairy Australia.

References

Madsen J, Bjerg B S, Hvelplund T, Weisbjerg M R and Lund P 2010. Livestock Science 129, 223-227.
Moate PJ, Williams SRO, Grainger G, Hannah MC, Ponnampalam EN and Eckard RJ 2011. Animal Feed Science and Technology 166-167, 254-264.

Effects of dietary protein level and lysine/methionine ratio on performance, nutrient digestibility for weaned calves

Fanlin Kong, Qiang Yun, Yan Tu, Kai Cui, Hui Tao, Qiyu Diao

Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China

E-mail: tuyan@caas.cn

Take home message Appropriate dietary lysine (Lys) to methionine (Met) ratio in starter feeds with low protein levels can lead to the same production performance and lower N emission compared with the high protein level starter.

Introduction Lys and Met are the main limiting amino acids (Murphy & O'Mara, 1993) in cattle feed. Suitable protein levels and Lys/Met ratio insure nutritional sufficiency and good body condition. The cultivation of weaned calves plays an important role in prospective milk production. So, weaned calves were used to study the effect of protein levels and Lys/Met ratios in starter feeds on growth performance, nutrient digestibility and nitrogen metabolism.

Material & methods Twenty four weaned calves were randomly divided into four groups and fed a control starter diet with a crude protein (CP) level of 19.64%DM (control group) a Lys/Met ratio of 3.35:1, or a test diet (CP:15.22% DM) with a Lys/Met ratio of 2.5:1, 3.1:1 or 3.7:1 for 8 weeks. The different ratios were achieved by adding rumen by-pass amino acid. The grass hay and the starter feeds fed to the weaned calves were used as the basal diet in a proportion of 60:40 of dry matter. The calves were weighed and blood samples were collected on the initial and final day of the trial. Four calves were selected from each group and digestion trials were conducted from the third week of the trial by total feces collection method. A one-way ANOVA was used to assess the effect of the treatments by SAS statistical software, version 8.2. Treatment differences with $P<0.05$ were considered statistically significant, and $0.05 \leq P < 0.10$ was designated as a tendency.

Results & discussion The result indicated that the average daily gain of calves in group 3.1:1 was 834 g/d, which was 10.21% higher than that of calves in control group numerically ($p>0.05$, Table 1). Serum arginine and valine concentrations in group 3.7:1 were lower than those in the control calves ($P<0.05$, Table 2), but it did not differ among test groups. The Lys/Met ratio had no influence on DW, CP, EE, NDF and ADF digestibility ($p>0.05$). However, the utilization and apparent biological value of nitrogen in group 3.1:1 and group 3.7:1 were higher than group 2.5:1 and the control group ($p>0.05$, Table 1).

Table 1 Effects of dietary Lys/Met ratios on the growth performance and nitrogen utilization of calves.

Items	Groups			
	Control	2.5:1	3.1:1	3.7:1
Body weight gain (kg)	42.4±11.4	41.8±16.9	46.7±8.0	43.8±6.5
Average daily gain(g/d)	757.1±203.4	746.4±301.3	834.5±143.6	782.1±115.4
N intake	108.01±0^{a}	97.59±8.13^{ab}	95.66±7.05^{b}	97.12±12.37^{ab}
Fecal N(g/d)	31.4±3.3	30.8±8.7	26.5±6.3	26.9±5.4
Urinary N(g/d)	34.2±10.6	25.5±6.8	24.2±2.9	24.6±2.6
N utilization (%)	39.2±7.7	41.6±15.5	46.4±12.1	46.1±11.5
N apparent biological value (%)	55.6±12.2	59.8±16.5	63.7±9.5	63.4±10.8

N utilization = (N intake- Fecal N- Urinary N)/N intake
N apparent biological value = (N intake- Fecal N-Urinary N)/(N intake-Fecal N). ^{a,b} Mean values within a row with different superscripts differ significantly ($P < 0.05$).

Table 2 Effects of dietary Lys/Met ratios on plasma concentrations of free amino acid (μmol/dL) of calves.

Items	Groups			
	Control	2.5:1	3.1:1	3.7:1
Lys	7.9±2.6	6.1±3.2	6.7±4.6	6.8±3.1
Met	1.4±0.5	1.5±0.8	1.5±1.0	1.9±1.2
Thr	3.9±1.4	3.5±2.2	3.7±2.0	4.0±2.3
Gly	29.5±11.1	21.1±8.6	27.1±13.7	24.1±9. 7
Ser	7.8±2.9	5.1±2.1	6.6±3.4	6.1±2.8
Arg	16.7±4.2^{a}	8.9±4.9^{b}	13.0±8.7^{ab}	10.3±3.6^{b}
Ile	9.1±2.4	7.2±3.8	7.7±4.8	6.9±3.8
Leu	10.6±2.9	9.2±4.7	9.3±5.9	9.9±4.4
Val	18.4±5.3^{a}	14.0±6.6^{ab}	13.6±6.9^{ab}	11.1±3.3^{b}
Phe	4.2±1.7	3.1±2.0	3.2±2.5	2.7±1.4
His	4.0±1.7	3.3±2.0	3.8±1.9	3.8±2.2
Tyr	4.0±1.2	3.2±1.8	3.3±2.4	3.8±2.1

^{a,b} Mean values within a row with different superscripts differ significantly ($P < 0.05$).

Conclusion The possibility of optimizing the lysine to methionine ratio at low protein levels was 3.1:1 and the calves fed the low protein diet with a lysine to methionine ratio of 3.1:1 had improved growth performance, and increased deposition of protein.

Acknowledgements This research was supported by grants from ruminant feed innovation research group in Feed Research Institute of Chinese Academy of Agricultural Sciences.

References

Murphy JJ and Mara FO, 1993. Journal of Livestock Science 35,117-134.

Are dietary strategies to mitigate enteric methane emission effective across ruminant species?

Sanne van Gastelen[1,2], Jan Dijkstra[2], André Bannink[1]

[1]*Wageningen UR Livestock Research, Wageningen, The Netherlands,* [2]*Wageningen University & Research, Wageningen, The Netherlands*

E-mail: sanne.vangastelen@wur.nl

Take home message The effectiveness of forage-related CH_4 mitigating strategies differs across ruminant species, whereas the effectiveness of other dietary mitigation strategies appears to be less species-specific.

Introduction Results of various dietary strategies to mitigate enteric methane (CH_4) emissions in ruminants have been reported in literature. However, the level of feed intake, the type of diet, and the rumen morphology and functionality differs between ruminant species. Consequently, the effectiveness of these dietary measures to mitigate CH_4 emission might also differ between species despite the similarity of CH_4 forming pathways in the rumen. The goal of the present study was to evaluate whether dietary strategies to mitigate CH_4 emissions in ruminants are equally effective in sheep, beef cattle and dairy cattle.

Material & methods Scopus was used to perform a literature survey, with the focus on forage quality, forage type/replacement, forage to concentrate ratio, and feed additives that have been tested in multiple ruminant species. Scientific papers had to fulfil each of the following criteria: (i) experiment conducted *in vivo*, (ii) CH_4 production measured directly (*i.e.,* not indirectly estimated), (iii) composition of the basal diet described, (iv) results available on dry matter intake (DMI), and on CH_4 production (g/d) or CH_4 yield (g/kg DMI or % GEI; gross energy intake), and (v) statistical analysis performed. Although preferred, studies did not have to apply the dietary strategies on multiple ruminant species simultaneously. Per study (n = 75), the effect size of the dietary strategies was expressed as a proportion (in %) of the control CH_4 emission, enabling comparison between ruminant species.

Results & discussion Forage quality, usually reflected in organic matter digestibility, directly influences rumen CH_4 production. Improved digestibility (+25%) of grass herbage or grass silage for dairy cattle increased DMI (+14%) and CH_4 production (g/d; +8%), whereas CH_4 yield (g/kg DMI or as % of GEI) and, if available, CH_4 intensity (g/kg milk) decreased by 10% and 19%, respectively. Similar to dairy cattle, DMI and CH_4 production increased for beef cattle (+10% and +7%, respectively), but CH_4 yield (both in g/kg DMI and as % of GEI) were unaffected with improved digestibility (+33%). For sheep, DMI was unaffected, whereas CH_4 yield (% of GEI) increased (+7%) with improved digestibility (+17%).

Replacing one type of forage by another may also reduce CH_4 emission. Increased levels of corn silage at the expense of grass pasture, grass silage, or alfalfa silage, for dairy cattle resulted in an increased DMI and CH_4 production (+9% and +8%, respectively), whereas CH_4 emissions generally decreased [CH_4 yield (g/kg DMI) −4%, CH_4 yield (% of GEI) −6%, and CH_4 intensity (g/kg milk) −8%]. This strategy did not affect CH_4 emission to the same extent in beef cattle and sheep; DMI generally decreased (−9% for beef and −19% for sheep) with CH_4 production (g/d) following the same trend, and with CH_4 yield (g/kg DMI) increased by 5% for beef cattle and unaffected for sheep.

Another CH_4 mitigation strategy is an increased proportion of concentrates at the expense of forage. The results were consistent for all ruminant species. Increased intake of concentrates increased DMI in cattle (+19% for dairy and +23% for beef) and sheep (+4%), with CH_4 production (g/d) generally following the same trend as DMI, and with decreased CH_4 yield (g/kg DMI; ranging from −7% for sheep to −26% for beef) and CH_4 intensity [g/kg product (*i.e.,* milk yield for dairy, average daily gain for beef and sheep); ranging from −10% for sheep to −31% for beef). The use of feed additives appears to have potential to mitigate enteric CH_4 emission. The results of the application of 3-nitrooxypropanol were consistent across ruminant species. The DMI was unaffected and CH_4 emission (all units) decreased across all ruminant species [for both CH_4 (g/d) and CH_4 (g/kg DMI): −35% for dairy, −50% for beef, −16% for sheep]. Similarly, nitrate appears to be an effective feed additive with all ruminant species. The DMI was generally unaffected and CH_4 yield (g/kg DMI; −22% for dairy, −12% for beef, −15% for sheep) decreased upon feeding nitrate.

Conclusion The effectiveness of forage-related CH_4 mitigating strategies, including increases in grass (herbage or silage) digestibility or replacement of different forage types by corn silage, differs across ruminant species. These strategies are most effective for dairy cattle with decreased CH_4 yield, had no effect in beef cattle or resulted in a minor increase in CH_4 yield, and resulted in inconsistent findings for sheep (*i.e.,* both increased and decreased CH_4 yield). This is most likely due to differences in DMI and apparent total tract digestibility responses between ruminant species. In general, the effectiveness of other dietary mitigation strategies, including increased concentrate feeding, or adding 3-nitrooxypropanol or nitrate, appears not to be species-specific. This illustrates that the modes of action of latter strategies are not affected by differences in feed intake, rumen physiology and characteristics, and basal diet composition between the different ruminant species.

Acknowledgements The authors acknowledge the project “Low Emission Animal Feed”, which received financial support of the Dutch Ministry of Economic Affairs (The Hague, the Netherlands), Product Board Animal Feed (Zoetermeer, the Netherlands), and the Dutch Dairy Board (Zoetermeer, the Netherlands).

Responses to incomplete essential amino acid profiles at the same metabolizable protein supply in lactating dairy cows

Kelly Nichols[1,2], André Bannink[2], Jan Dijkstra[1]

[1]*Animal Nutrition Group, Wageningen University and Research, Wageningen, The Netherlands,* [2]*Wageningen Livestock Research, Wageningen University and Research, Wageningen, The Netherlands*

E-mail: kelly.nichols@wur.nl

Take home message Incomplete essential amino acid profiles within the same metabolizable protein supply can achieve similar milk protein yield as a complete profile, except when branched-chain amino acids are infused alone.

Introduction Supplementing specific essential amino acids (EAA) as a portion of metabolizable protein (MP) in low (<14% on a DM basis) CP dairy cow diets could allow desirable levels of milk production and improved N-use efficiency. Mammary flexibility for amino acid (AA) use in response to whole-body AA partitioning and endocrine signals suggest particular AA groups may stimulate milk protein synthesis and minimize AA catabolism (Haque *et al.,* 2012). Thus, we investigated the effect of supplying different EAA profiles within a constant level of MP on lactation performance and concentration of plasma metabolites and insulin.

Material & methods Five early-lactation, rumen-fistulated dairy cows (81 ± 10.9 DIM; 2nd lactation n = 1, 3rd lactation n = 4) fed a total mixed ration consisting of 58% corn silage, 16% alfalfa hay, and 26% concentrate containing 6.80 MJ/kg NE_L and 13.0% CP on a DM basis were abomasally infused for 5 d with EAA mixtures in a 5 x 5 Latin square design. All EAA mixtures delivered 562 g/d of AA in proportions relative to their content in casein. The infusion treatments were 1) saline (SAL), 2) all EAA in the profile of casein (EAA), 3) Ile, Leu, Val (BCAA), 4) Met, Phe, Thr, Trp, Arg, His, Lys (EAA–), and 5) Ile, Leu, Met, Phe, Trp, Val, His (Gr 1+BCAA). Cows were fed every 2 h using an automated feeder and were milked twice daily. Arterial blood samples were collected over 5 time points on d 4 of infusion and analyzed for plasma metabolites. Milk composition and yield from the final 3 d of infusion and mean plasma metabolite concentrations were subjected to ANOVA using PROC MIXED of SAS, where treatment and period were fixed effects and cow was a random effect. Multiple comparisons between treatment means were made using the Tukey-Kramer method.

Results & discussion Infusion of EAA, EAA–, and Gr 1+BCAA stimulated similar yields of total milk, protein, and lactose (Table 1). Infusion of the complete EAA profile resulted in the lowest plasma urea level compared with other EAA profiles. Arterial glucose concentration was unaffected by treatment, but infusion of incomplete EAA profiles affected plasma insulin concentrations. Arterial BHBA, NEFA and TAG concentrations were unaffected by treatment (results not shown). Infusion of BCAA stimulated the same level of milk protein yield as SAL despite a 1.3-kg lower DMI.

Table 1 Performance of dairy cows receiving 5-d abomasal infusions of saline or 562 g/d EAA in different profiles

	Treatment						
	SAL	EAA	BCAA	EAA–	Gr 1+BCAA	SEM	*P*
DMI, kg/d	19.1	19.7	17.8	19.9	19.8	1.01	0.06
Milk, kg/d	29.4^{a}	34.4^{b}	30.8^{ab}	33.3^{ab}	34.4^{b}	2.00	0.01
Fat, g/d	1247	1281	1315	1273	1321	62.3	0.52
Protein, g/d	902^{a}	1126^{b}	907^{a}	1034^{ab}	1089^{b}	51.9	<0.01
Lactose, g/d	1385^{a}	1581^{b}	1463^{ab}	1563^{ab}	1575^{b}	89.2	0.03
Urea, mg/dL	11	11	11	14	13	2.1	0.41
Arterial concentration							
Glucose, m*M*	3.64	3.64	3.58	3.71	3.36	0.140	0.49
Urea, m*M*	2.26^{a}	2.50^{a}	2.78^{ab}	3.12^{b}	2.80^{ab}	0.239	0.01
Insulin, mIU/L	20.5^{ab}	16.3^{bc}	13.9^{c}	16.9^{abc}	20.6^{a}	2.98	<0.01

$^{a-b}$Means within a row with no common superscripts differ ($P < 0.05$)

Conclusion With the exception of BCAA infusion, supplying the same amount of MP from different EAA profiles can achieve similar total milk and milk protein yield as a complete profile. The presence of group 1 AA appears to be important for maintaining a similar level of milk protein synthesis when imbalanced EAA profiles are infused. Changes in AA catabolism and endocrine responses may have facilitated mammary gland flexibility for AA-use when imbalanced AA profiles are infused within a constant supply.

Acknowledgements The work is part of the Feed4Foodure program supported by the Vereniging Diervoederonderzoek Nederland and the Dutch Ministry of Economic Affairs.

References

Haque MN, Rulquin H, Andrade A, Faverdin P, Peyraud JL and Lemosquet S 2012. Journal of Dairy Science 95, 5876-5887.

The combined effects of supplementing monensin and 3-nitrooxypropanol on methane emissions, growth rate, and feed conversion efficiency in beef cattle fed high forage and high grain diets

Diwakar Vyas[4,1], Aklilu Alemu[1], Sean McGinn[1], Stephane Duval[2], Maik Kindermann[3], Karen Beauchemin[1]

[1]Lethbridge Research and Development Center, Agriculture and Agri-Food Canada, Lethbridge, Canada, [2]DSM Nutritional Products France, Research Center for Animal Nutrition, Saint Louis Cedex, France, [3]DSM Nutritional Products, Basel, Switzerland, [4]University of Florida, Department of Animal Science, Gainesville, USA

E-mail: karen.beauchemin@agr.gc.ca

Take home message 3-Nitrooxypropanol (NOP) decreased enteric methane (CH_4) emissions and improved feed conversion efficiency in beef cattle fed high forage and high grain diets regardless of whether monensin (MON) was included in the diets.

Introduction Strategies to reduce enteric CH_4 production from beef cattle may help lessen environmental concerns and improve feed efficiency (Vyas *et al.,* 2016). Previous studies have shown that NOP decreased CH_4 yield (g/kg DM) by about 30% in beef cattle fed high-forage diets (Romero-Perez *et al.,* 2014; Vyas *et al.,* 2016) and up to 80% in cattle fed high-grain diets (Vyas *et al.,* 2016), with the response dose dependent. The ionophore MON is routinely used in commercial feedlot diets in many countries to improve feed efficiency. Thus, the current study explored the effects of NOP, when supplemented alone or in combination with MON, on CH_4 emissions, growth rate and feed conversion efficiency in growing and finishing cattle.

Material & methods Crossbred yearling steers (n=240) were used in a 238-d feeding trial. They were fed a high-forage diet based on barley silage for the first 105 d (backgrounding phase), transitioned for 28 d, and then fed a high-grain diet (*i.e.,* finishing phase) based on barley grain for the last 105 d. Steers were stratified by weight (heavy, light) and blocked into 12 heavy and 12 light pens at the main feedlot (8 cattle/pen) and 4 pens each for heavy and light weight groups at a secondary feedlot (6 cattle/pen). The experiment was conducted as a randomized block design with 4 treatments arranged in a 2 × 2 factorial layout; 2 levels of NOP (with, without) were combined with 2 levels of MON (with, without). Treatments were: 1) Control (no additive); 2) MON (monensin supplemented at 33 mg/kg DM); 3) NOP (3-nitrooxypropanol supplemented at 200 mg/kg DM for backgrounding or 125 mg/kg DM for finishing phase); 4) MONOP (33 mg/kg DM MON supplemented with either 200 mg/kg DM or 125 mg/kg DM NOP). Five animals per treatment were moved to chambers for methane measurements during both phases. The data were subsequently analyzed as a 2 × 2 factorial design using a MIXED procedure of SAS. Statistical significance was declared at $P \leq 0.05$ and a tendency to significance was declared at $0.05 < P \leq 0.10$.

Results & discussion No interaction was observed between NOP and MON for dry matter intake (DMI), average daily gain (ADG), and gain-to-feed ratio (G:F) for cattle fed high-forage and high-grain diets, indicating that the effects of NOP and MON were independent when combined. During the backgrounding phase, DMI was decreased by 7% with NOP ($P < 0.01$) while no effects were observed with MON ($P = 0.12$). Compared with control, NOP and MON improved ($P < 0.01$) G:F by 5 and 4%, respectively, while NOP+MON improved G:F by 10%. NOP decreased CH_4 yield (g/kg DMI) by 42%, while there was no effect of MON on CH_4 yield, and NOP+MON decreased CH_4 yield by 39%. During the finishing phase, NOP and MON individually tended to reduce DMI ($P = 0.06$) by 5% compared with control. G:F ratio was improved by 3% with NOP ($P < 0.01$), no effects were observed with MON ($P = 0.58$), while NOP+MON improved G:F by 6%. CH_4 yield was decreased by 37% with NOP, tended to increase ($P = 0.09$) with MON, while it decreased by 39% with NOP+MON.

Conclusion Overall, the results demonstrate efficacy of NOP in reducing enteric CH_4 emissions and improving feed conversion in cattle fed backgrounding and finishing diets. Both NOP and MON improved feed conversion efficiency in the backgrounding phase, but only NOP improved feed conversion in the finishing phase. We conclude that NOP is an effective CH_4 inhibitor that can be added to conventional beef cattle diets containing MON without incurring negative effects on performance. Furthermore, the study suggests a possible link between sustained reduction in CH_4 and improved feed conversion efficiency in beef cattle, which may encourage beef producers to adopt this CH_4 mitigation approach if the product becomes commercially available.

Acknowledgements This project was supported by Emissions Reduction Alberta, Agriculture and Agri-Food Canada and DSM Nutritional Products.

References

Romero-Perez A, Okine E, McGinn S, Guan L, Oba M, Duval S, Kindermann M and Beauchemin, K 2014. Journal of Animal Science 92, 4682-4693.
Vyas D, McGinn S, Duval S, Kindermann M, and Beauchemin K 2016. Journal of Animal Science 94, 2024-2034.

The effect of γ-aminobutyric acid addition on *in vitro* ruminal fermentation characteristics and methane production of a high concentration ration

Yan-Lu Wang, Zhi-Hui Zhang, Ya Wen, Hong-Jian Yang, Sheng-Li Li

State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing, China

E-mail: yanluwang@yeah.net

Take home message Dietary addition of GABA enhanced total VFA production and shifted rumen fermentation towards more acetate and less propionate and butyrate production and methane emissions.

Introduction γ-Aminobutyric acid (GABA) is an amino acid found in plants and bacteria and known as an inhibitory neurotransmitter in the mammalian brain (Watanabe *et al.,* 2002). Recently, GABA was tested as a new feed additive to improve growth performance, reduce disease occurrence in calves (Matsumoto *et al.,* 2009) and alleviate heat stress of dairy cows (Cheng *et al*., 2014). However, it is not clear how GABA would affect rumen fermentation and methane production. This study aimed to determine the effect of GABA addition on *in vitro* ruminal fermentation and methane production.

Material & methods A total mixed ration (500 mg) containing 30% whole corn silage and 70% concentrate was weighed into 120 mL glass bottles containing 0 (Control), 10, 20, 30, 40 and 50 mg GABA per bottle following the addition of 50 mL medium (pH 6.85; Menke and Steingass, 1988) and 25 mL filtrated rumen fluid collected from three rumen-fistulated Holstein cows. The bottles were then connected to an automated gas recording system (AGRS-III, China Agricultural University) to record gas production (GP) and airbags to collect fermentation gases. Five bottles per treatment in a run were incubated at 39°C for 72 h, and the incubation was repeated for three runs. Variables for gas and VFA concentration and composition) were analysed using the GLM procedure of SAS (1999). Orthogonal polynomial contrasts were performed to determine linear and quadratic dosage effect of GABA addition, and significance $P<0.05$ was declared unless others noted.

Results & discussion After 72 h incubation, the GABA addition in comparison with the control did not alter GP, but the GABA addition linearly increased the total VFA production.

Table 1 Effect of GABA addition on in vitro rumen gas, VFA and CH_4 yield (ml/g DM)

		GABA addition (mg/bottle)						*P* value	
Items	Control	10	20	30	40	50	S.E.M	Linear	Quadratic
GP (ml/g DM)	112.1	103.3	115.2	117.2	130.9	116.7	9.59	NS	NS
tVFA (mM)	116.2	116.4	119.3	128.1	131.8	139.4	2.36	<0.01	NS
VFA pattern (%, molar)									
Acetate	68.6	74.2	73.9	70.6	71.6	70.8	0.72	NS	<0.01
Propionate	13.5	11.9	12.0	12.1	12.6	12.7	0.48	NS	0.02
Butyrate	7.9	7.3	7.2	8.3	8.6	9.0	0.27	<0.01	0.01
Valerate	3.01	1.38	1.49	1.44	1.33	1.83	0.223	NS	NS
BCVFA	6.83	5.08	5.25	7.49	5.78	5.56	0.478	NS	0.01
NGR	5.30	6.8	6.77	6.61	6.46	6.21	0.261	NS	<0.01
CH_4 yield (ml/g DM)	16.6	15.0	17.7	18.1	19.7	19.7	0.32	<0.01	NS

Regarding the VFA pattern in molar proportion, the GABA addition in comparison with the control altered VFA producing pattern. Increasing GABA addition linearly increased butyrate production, quadratically decreased acetate production and quadratically increased the production of propionate and branch-chained VFA (BCVFA) including iso-butyrate and iso-valerate. Consequently, the ratio of non-glucogenic acids to glucogenic acids (NGR) was quadratically decreased by the GABA addition. Increasing GABA addition linearly increased the methane production. VFAs are the main end products of rumen fermentation and represent the main supply of metabolisable energy for ruminants, and the addition of GABA promoted VFA production, suggesting that GABA could enhance the nutrient digestion in the rumen to provide more fermentable energy for host ruminants, implicating dietary addition of GABA my promote milk yield in lactating cows.

Conclusion Increasing GABA addition promoted total VFA production and shifted rumen fermentation towards more acetate and less propionate and butyrate production. Meanwhile, methane production was increased by the GABA addition. Potential increase of milk yield and methane emission by increasing GABA addition should be given further investigation when it is applied as a feed additive in lactating cows.

Acknowledgements The study was funded by the National Key Basic Research Program (Project no. 2011CB100801) of Ministry of Science and Technology of the People's Republic of China.

References

Cheng JB, Bu DP, Wang JQ, Sun XZ, Pan L, Zhou LY and Liu W 2014. Journal of Dairy Science 97, 5599-5607.
Menke KH and Steingass H 1988. Animal Research and Development 28, 7–55.
Matsumoto D, Takagi M, Fushimi Y, Okamoto K, Kido M, Ryuno M, Imura Y, Matsunaga M, Inokoshi K, Shahada F, Deguchi E 2009. Journal of Veterinary Medical Science 71, 651-656.
Watanabe M, Maemura K, Kanbara K, Tamayama T and Hayasaki H 2002. International Review of Cytology. K.W. Jeon, Academic Press. 213, 2-47.

The consistency of feed efficiency ranking and the mechanism explaining efficiency variation among growing calves

Aviv Asher[1], Shabtay Ariel[2], Cohen-Zinder Miri[2], Aharoni Yoav[2], Miron Joshuah[2], Halachmi Ilan[4], Haim Abraham[3], Tedeschi Louis[5], Carstens Gordon[5], Johnson Kristen[6], Brosh Arieh[2]

[1]*Northern R&D, Migal – Galilee Technology Center, The city of Kiryat Shmona, Israel,* [2]*Institute of Animal Science, ARO, Newe Yaar Resarch Center, Ramat Yishay, Israel,* [3]*University of Haifa, Haifa, Israel,* [4]*Institute of Agricultural Engineering, Bet-Dagan, Israel,* [5]*Texas A&M University, Texas, USA,* [6]*Washington State University, Washington, USA*

E-mail: avivasher78@gmail.com

Take home message: Residual Feed Intake (RFI) ranking is repeatable across diet qualities and ages. The main mechanism characterized efficient compared to inefficient RFI calves is the greater protein-to-fat ratio in the deposited tissues of the efficient calves and lower than expected heat production (HP) of the efficient calves.

Introduction Despite of the highly economic importance of increasing cattle efficiency, the biological mechanisms controlling efficiency is not fully clear. Residual feed intake (RFI) has been used to estimate feed efficiency (FE) in beef cattle, as it is moderately heritable and genetically independent of average daily gain (ADG) and body weight (BW) (Crowley *et al*., 2010). The current study investigated the possible mechanism for explaining individual FE variation in growing calves and the consistency of individual calves' RFI ranking across different diet qualities and physiological age.

Material & methods Twenty-six Holstein calves were housed for 405 days in a shaded open feedlot containing individual feed troughs. Calves were tested during three periods (P1, P2 and P3). During P1 and P3, calves were fed a high-quality diet (ME =11.8 MJ/kg DM) and a low-quality diet (ME = 7.69 MJ/kg DM) during P2. Periods lasted 84, 119, 127 days, respectively. Individual dry matter intake (DMI), ADG, body condition score (BCS), diet *in vivo* digestibility, metabolizable energy intake (MEI), HP and retained energy (RE), were measured in all periods. The RE/ADG ratio was calculated for representing fat to protein ratio in gain. In each period the RFI was calculated for all animals as the residuals from a multiple regression model regressing DMI on ADG and mid test $BW^{0.75}$. Animals were grouped into high RFI (H-RFI > 0.5 SD above the mean), medium RFI (M-RFI 0.5 SD above and below the mean), and low RFI (L-RFI < 0.5 SD below the mean). The repeatability of each calves' RFI ranking group at the 3 periods was tested by total agreement procedure using Kappa index P statistic value. At the end of P3 the animals were slaughtered and carcass composition and slaughter traits were collected and analysed.

Results & discussion In all period's calves' RFI trait was not correlated with diets digestibility, calves' age, BW, BCS and ADG ($P < 0.05$). In all periods, L-RFI group had lowest DMI, MEI, HP, RE and RE/ADG. Longissimus dorsal muscle of the L-RFI group had a significant higher proportion of protein to fat compared to the H-RFI group ($P < 0.05$). It was in consistence with the RE/ADG ratio difference between RFI groups that was found in all tested periods. We suggested that the main mechanism separating L-from H-RFI calves is the protein-to-fat ratio in the deposited tissues and consequently in the entire body. When efficiency was related to kg/day (DMI and ADG) and not to daily retained energy, the selected efficient L-RFI calves deposited more protein and less fat per daily gain than less efficient H-RFI calves. However, when the significant greater heat increment and maintenance energy requirement of protein compared to fat deposition in tissue were considered, we could not exclude the hypothesis that variation in efficiency is partly explained by efficient energy utilization. Based on the Kappa index, the ranking classification of calves to groups according to their RFI efficiency was repeatable between P1 and P2, P2 and P3, and P1 and P3 ($P < 0.001$, 0.05, 0.001, respectively).

Conclusion The greater protein-to-fat ratio in tissue gain was the major factor that characterized the more efficient calves as classified by RFI. The present study supports the hypothesis that more efficient energy utilization contributes significantly to the more efficient maintenance and production, which characterizes the calves classified as L-RFI compared to the calves classified as H-RFI. In the present study, classifying growing Holstein bull calves to their RFI ranking group was consistent across a wide range of diets and ages. This novel repeatability of RFI ranking throughout different growing periods and also when tested under close to maintenance conditions highlight the RFI index as stable candidate for marker-assist selection for feed efficiency.

Acknowledgements This study was supported by grant IS-39988-07 from BARD, the United States Israel Binational Agricultural Research and Development Fund.

References

Crowley JJ, McGee M, Kenny DA, Crews DH Jr, Evans RD and Berry DP 2010. Journal of Animal Science 88, 885-894.

A candidate proteomic signature from the plasma of Charolais bulls to phenotype feed efficiency

Isabelle Cassar-Malek, Gonzalo Cantalapiedra-Hijar, Arnaud Delavaud, Muriel Bonnet

Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès- Champanelle, France

E-mail: isabelle.cassar-malek@inra.fr

Take home message We have identified a set of candidate biomarkers for feed efficiency in the plasma proteome of young Charolais bulls underlying the contribution of non-productive functions such as immunity.

Introduction The measurement of the feed efficiency (FE) requires an individual and strict control of the ingestion and performances of animals at least over a period of 70 days. We aimed at developing biomarkers from low invasive samples the combination of which will predict FE individual variations.

Material & methods We examined the plasma proteome of two groups of Charolais young bulls according to their residual feed intake (RFI) and feed conversion efficiency (FCE) metrics (Meale *et al.*, 2017). The study included 17 extremes animals: n=9 positive RFI vs n= 8 low RFI negative (0.69 vs -0.75 kg/j). Prior to nano LC-MS/MS analysis, the plasma samples were depleted of high-abundance proteins using the Proteominer technology (Cassar-Malek *et al*, 2015). Statistical analysis of data included ANOVA, Principal Components Analysis (PCA) and correlation analysis (Pearson). Lists of proteins were analysed using ProteINSIDE (http://www.proteinside.org/) to mine biological information. Gene Ontology (GO) enrichment tests (P value_Benjamini Hochberg < 0.05) were done with human orthologs to take advantage of the most complete annotation available for molecular functions and biological processes. A similar workflow was applied to the same animals ranked according to their FCE (0.17 [low FCE] vs 0.23 [high FCE] kg/kg).

Results & discussion A PCA analysis of all data was applied to remove outliers (n=2/per RFI group) from further analysis. Then a differential abundance according to RFI was revealed for 51 proteins (P <0.1) including 4 uncharacterised proteins. A correlation between plasma abundance and RFI values was detected for 24 proteins out of the 51 proteins (-0.795 <r2 <+0.806). Nine of the 51 differential proteins- 5 of the proteins correlated to RFI- were detected in a repertoire of secreted proteins identified by computation of published RFI omic datasets (Cassar-Malek and Bonnet, this symposium). These proteins are candidate biomarkers for RFI. The top GO Molecular Functions of the differential proteins were: calcium ion binding, serine-type endopeptidase inhibitor activity, lipid binding, carbohydrate binding, growth factor activity, regulation of insulin-like growth factor receptor signaling pathway. GO Biological Processes were mainly related to immunity (immune response, innate immune response), inflammatory response, complement and coagulation cascades, protein modification (protein folding, proteolysis, negative regulation of endopeptidase activity) and lipid metabolism (lipid transport, triglyceride and cholesterol homeostasis). These findings agree with the recent report that non-productive functions including immunity may contribute to inter-individual variations in feed efficiency in pig (Gondret *et al.*, 2017) and cattle (Weber *et al.*, 2016; Alexandre *et al*, 2015).

Examination of proteomic data according to the FCE index revealed 21 differentially abundant proteins and 7 correlated proteins (-0.599< r2 < +0,637). Comparison of FCE and RFI datasets showed that 6 proteins out of the 9 identified above as candidate RFI biomarkers are specific for RFI while 3 of them are common to both RFI and FCE.

Conclusion This is the first study identifying candidate biomarkers for feed efficiency in the plasma of cattle. It opens perspectives for biomarkers validation and high-throughput evaluation of this trait in ruminants.

Acknowledgements The authors acknowledge APIS-GENE for funding the study**.**

References

Alexandre PA, Kogelman LJA, Santana MHA, Passarelli D, Pulz LH, Fantinato-Neto P, Silva PL, Leme PR, Strefezzi RF, Coutinho LL, Ferraz JB, Eler JP, Kadarmideen HN and Fukumasu H 2015. BMC Genomics 16, 1073
Cassar-Malek I, Delavaud A, Barboiron C, Chambon C and Picard B 2015. In: ICAP 2015 (p. 211). 4th International Congress on Analytical Proteomics, Lisbon, PRT (2015-09-07 - 2015-09-09).
Gondret F, Vincent A, Houée-Bigot M, Siegel A, Lagarrigue S, Causeur D, Gilbert H and Louveau I 2017 BMC Genomics 18, 244.
Meale SJ, Morgavi DP, Cassar-Malek I, Andueza D, Ortigues-Marty I, Robins RJ, Schiphorst AM, Laverroux S, Graulet B, Boudra H, Cantalapiedra-Hijar G 2017. Journal of Agricultural and Food Chemistry 65, 9817-9827.
Weber KL, Welly BT, Van Eenennaam AL, Young AE, Porto-Neto LR, Reverter A and Rincon G PLOS ONE. 01611(3):e0152274

Effect of brewer's grains and rapeseed meal on methane emissions and milk production of dairy cows

Dorien Van Wesemael[1], Leen Vandaele[1], Sam De Campeneere[1], Veerle Fievez[2], Nico Peiren[1]

[1]ILVO (Flemish research institute for agriculture, fisheries and food) - Animal Sciences Unit, Melle, Belgium, [2]Ghent University - Laboratory for Animal Nutrition and Animal Product Quality, Ghent, Belgium

E-mail: dorien.vanwesemael@ilvo.vlaanderen.be

Take home message Combining brewer's grains and rapeseed meal to increase the dietary fat content, lowers enteric methane emissions and increases milk production of dairy cows.

Introduction According to Grainger and Beauchemin (2011) fat supplementation is the most promising dietary strategy to reduce enteric methane (CH_4) emissions from ruminants. They also propose the use of by-products which contain fat to mitigate not only enteric CH_4 emissions, but total greenhouse gas emissions. One of the possible by-products, rapeseed meal (RSM), is found to be at least as good as soybean meal as protein supplement for lactating dairy cows (Huhtanen *et al.*, 2011). Another fat-rich by-product is brewer's grains (BG), a suitable protein source that balances intake of high starch diets (Dhiman *et al.*, 2003). In an *in vivo* trial we assessed the combined effect of BG and RSM on CH_4 emissions and milk production of dairy cattle.

Material & methods The reference roughage diet consisted of 65% maize silage and 35% grass silage (on dry matter basis), with soybean meal as protein supplement. The treatment roughage diet consisted of 53% maize silage, 31% grass silage and 16% BG, with RSM as protein supplement. The amount of concentrates, a balanced compound feed and the protein supplement, was calculated for each cow individually. We used 12 high-productive (33.2±4.5 kg milk/day and 170±12 days in milk) Holstein Friesian cows, divided into two uniform groups (reference and treatment). In a six weeks pre-treatment period all cows received the reference diet (167 g crude protein (CP)/kg DM; 28 g crude fat (CF)/kg DM). In the subsequent treatment period of six weeks, the treated cows received the treatment diet (161 g CP/kg DM; 39 g CF/kg DM) and the reference cows still got the reference diet. At the end of both periods, the CH_4 emissions of all cows were measured in open-circuit chambers. Data were analysed by using a linear mixed model with group, period and their interaction as fixed effects and cow as random effect. A significant interaction effect indicates a significant treatment effect. One cow from the control group was ill during the pre-treatment period and was omitted from the analyses.

Results & discussion Milk production of the treated cows increased during the treatment period, whereas the milk production of the reference cows decreased, and dry matter intake was not affected by diet (Table 1). CH_4 emissions decreased for the treated cows and were constant or increased for the reference cows (Table 1). The reduction in CH_4 emissions we observed in our treated cows (-17%; -15%; -19%) was at least twice the reduction (-8%; -5%; -9%) obtained in Moate *et al.* (2011), although the difference in CF content of the reference and treatment diet in our trial was smaller than for their control (26 g CF/kg DM) and BG (51 g CF/kg DM) diet.

Table 1 Least squares means of dry matter intake (DMI), milk production and CH_4 emissions for both groups (reference and treated) in both experimental periods (pre-treatment and treatment)

	Reference cows		Treated cows		SE[1]	p-value
	pre-treatment	treatment	pre-treatment	treatment		group*period
DMI (kg/day)	22.3	21.7	22.5	22.1	1.06	0.53
Milk (kg/day)	30.8	27.8	30.5	31.1	1.8	< 0.01
CH_4 (g/day)	452	445	509	422	35	< 0.001
g CH_4/kg DMI	20.2	20.5	22.5	19.1	1.0	< 0.001
g CH_4/kg milk	15.0	16.5	16.7	13.5	1.3	< 0.001

[1] Due to an unbalanced design SE differs between groups; largest SE is published

Conclusion Increasing the fat content of dairy cattle diets by using a combination of fat-rich by-products, such as brewer's grains and rapeseed meal, was an effective way of decreasing the enteric CH_4 emissions and increasing milk production.

Acknowledgements This research was funded by the government agency Flanders Innovation and Entrepreneurship (VLAIO, former IWT – Belgium; LA135081).

References

Dhiman TR, Bingham HR and Radloff HD 2003. Journal of Dairy Science 86, 2914–2921.
Grainger C and Beauchemin KA 2011. Animal Feed Science and Technology 166-167, 308-320.
Huhtanen P, Hetta M and Swensson C 2011. Canadian Journal of Animal Science 91, 529-543.
Moate PJ, Williams SRO, Grainger C, Hannah MC, Ponnampalam EN and Eckard RJ 2011. Animal Feed Science and Technology 166-167, 254-264.

The combined effects of supplementing 3-nitrooxypropanol and lipids on emissions of methane and hydrogen, digestibility, and rumen fermentation in beef cattle fed a forage based diet

Megan Smith[1,2], Diwakar Vyas[2,5], Limin Kung[1], Stephane Duval[3], Maik Kindermann[4], Karen Beauchemin[2]

[1]*Department of Animal and Food Sciences, University of Delaware, Newark, Delaware, USA,* [2]*Lethbridge Research and Development Centre, Agiculture and Agri-Food Canada, Lethbridge, Alberta, Canada,* [3]*DSM Nutritional Products France, Saint Louis Cedex, France,* [4]*DSM Nutritional Products, Basel, Switzerland,* [5]*University of Florida, Department of Animal Science, Gainesville, Florida, USA*

E-mail: karen.beauchemin@agr.gc.ca

Take home message Feeding 3-nitrooxypropanol (NOP) to beef cattle fed a forage based diet lowered enteric methane (CH_4) yield by 32%, and by 52% when NOP was combined with polyunsaturated lipids. However, unlike feeding NOP, feeding lipids to reduce CH_4 lowered organic matter digestibility.

Introduction NOP has been shown to lower CH_4 yield (g/kg dry matter [DM] intake) by 30 to 80% in beef cattle (Romero-Perez *et al.,* 2014; Vyas *et al.,* 2016), depending upon dose and diet. However, NOP also increases gaseous hydrogen (gH_2) losses (Hristov *et al.,* 2016), which represents an inefficiency in energy capture. Lipid supplementation has also been shown to lower CH_4 yield, with lipids serving as an alternative H_2 sink that competes with methanogens for available substrate. We hypothesized that feeding NOP with unsaturated lipids would lower CH_4 emissions, while reducing gH_2 losses due to increased utilization of reducing equivalents through biohydrogenation.

Material & methods Eight Angus crossbred heifers (732 ± 43 kg) were used in a double 4 × 4 Latin square design with four 28-d periods and four dietary treatments (DM basis): 1) control, 2) canola oil (5% added fat, 8.3% total dietary fat), 3) NOP (200 mg/kg; DSM Nutritional Products Ltd., Kaiseraugst, Switzerland), and 4) NOP (200 mg/kg) and canola oil (5% added fat) combined. The basal diet (DM basis) consisted of 90% forage (barley silage) and 10% supplement. Methane and gH_2 production were measured using respiratory chambers over 3 d each period. Digestibility was measured by total collection of feces and urine. Rumen content samples were obtained at 0, 3, 6, 9, and 12 h after feeding on d 14 and d 17 of every period and analysed for volatile fatty acid concentration and dissolved hydrogen in rumen fluid (dH_2, digital microsensor amplifier with automatic sensor detection). The data were analyzed using a MIXED procedure of SAS. Statistical significance was declared at $P \leq 0.05$ and a tendency to significance was declared at $0.05 < P \leq 0.10$.

Results & discussion Feeding NOP alone lowered CH_4 yield by 32%, but dH_2 and gH_2 were increased ($P < 0.01$) compared with control although diet digestibility was not affected. Acetate proportion decreased while propionate proportion increased ($P < 0.01$) as expected with NOP, indicating a shift in H_2 sinks in the rumen. Feeding lipids alone decreased CH_4 yield by 25% ($P < 0.01$); however, organic matter digestibility decreased by 6 percentage units. Lipids also decreased the acetate:propionate ratio and increased dH_2 and gH_2 concentrations compared with the control ($P < 0.001$), although to a lesser extent than NOP. Combining NOP and lipids lowered CH_4 yield by 51% compared with the control, decreased dH_2 and gH_2 compared with NOP alone ($P < 0.001$), and decreased acetate proportion and increased butyrate compared with oil alone. However, the decrease in digestibility caused by feeding lipids remained.

Conclusion The results demonstrate efficacy of both NOP and lipids in reducing enteric CH_4 emissions, but unlike NOP, lipids caused a decline in diet digestibility. The reduction in CH_4 production with NOP was associated with an increase in both dissolved and gaseous forms of H_2, critical intermediates of the fermentation process. Feeding polyunsaturated lipids together with NOP decreased the loss of gH_2 associated with feeding NOP, but the decrease in diet digestibility due to lipid feeding precludes it from being recommended as a CH_4 mitigant for cattle fed high forage diets, at least at the high level used in this study.

Acknowledgements This project was supported by Emissions Reduction Alberta, Agriculture and Agri-Food Canada and DSM Nutritional Products.

References

Romero-Perez A, Okine EK, McGinn SM, Guan LL, Oba M, Duval SM, Kindermann M and Beauchemin KA 2014. Journal of Animal Science 92, 4682-4693.

Vyas D, McGinn SM, Duval SM, Kindermann M and Beauchemin KA 2016 Journal of Animal Science 94, 2024-2034.

Hristov AN, Oh J, Giallongo F, Frederick TW, Harper MT, Weeks HL, Branco AF, Moate PJ, Deighton MH, Williams SR, Kindermann M and Duval S 2015. Proceedings of the National Academy of Sciences of the United States of America 112, 10663-8

Dry matter intake and ruminal environment of lambs fed a total mixed ration (amylaceous or fibrous) supplemented with fresh forage

Gonzalo Fernandez Turren[1], José María Arroyo[2], Agustina Fontes[1], Soledad Grignola[1], Analia Perez-Ruchel[2], José Luis Repetto[1], Cecilia Cajarville[2]

[1]Departamento de Bovinos, Insituto de Producción Animal, Facultad de Veterinaria, Universidad de la República, Ruta 1 km 42.5, 80100, San José, Uruguay, [2]Departamento de Nutrición Animal, Insituto de Producción Animal, Facultad de Veterinaria, Universidad de la República, Ruta 1 km 42.5, 80100, San José, Uruguay

E-mail: gonzalofernandezt@gmail.com

Take home message The replacement of cereal grains by fibrous by-products as energy sources in combined diets (TMR and pasture) did not affect the dry matter intake but affected the digestibility and rumen environment of lambs fed mixed diets.

Introduction The combination of grazing pasture and TMR (combined diets) is increasingly used in temperate regions. Our group has information regarding the inclusion of different levels of pasture to a total mixed ration (TMR) (Pérez-Ruchel *et al.,* 2017), but the effect of the replacement of cereal grains by fibrous by-products as sources of energy in mixed diets has been scarcely studied in lambs. The objective of this study was to evaluate different energy sources included in the TMR on the dry matter intake (DMI), digestibility and ruminal environment of lambs fed with mixed diets (pasture + TMR).

Table 1 Ingredients of total mixed rations (TMR) and chemical composition of feeds.

	Mix_A	Mix_F
Ingredients (g/kg DM)		
Raygrass hay	97.0	97.0
Ground corn	272	-
Wheat grain	202	-
Soybean meal	152	-
Soybean hulls	218	194
Defattened corn germ	-	650
Mineral-vitamin premix	59.0	59.0
Chemical composition (g/kg DM)		
Crude proteinCrude protein	156	161
Neutral detergent fiber	284	437
Starch	326	130

Mix_A: TMR amylaceous; Mix_F: TMR fibrous

Material & methods Eighteen lambs Corriedale×Ile de France (32.2±1.82 kg) fitted with permanent rumen catheters, were individually housed in metabolism cages and randomly assigned to the treatments: alfalfa pasture *ad libitum* "ALF", total mixed ration using amylaceous "Mix_A" or fibrous "Mix_F" energy source offered at 75% of the potential intake in two equal meals/day (08:00 and 18:00h) complemented with fresh alfalfa cut daily (CP = 226, and NDF = 366 g/kg DM) from 10:00 to 18:00 h. Daily intake of TMR and forage was measured for 10 days, weighing the amount of feed offered and the orts. Dry matter digestibility was determined measuring intake and fecal excretion. Ruminal fluid samples were collected hourly from hour 08:00 to 20:00 and at 0:00 and 04:00 h to determine pH, volatile fatty acids (VFA) and NH_3-N. The data were studied by analysis of variance with a model that included the fixed effect of treatment on n=6 replicates of animals. The pH, VFA and NH_3-N concentrations were analyzed as repeated measures.

Results & discussion Data are shown in Table 2 The DMI was higher for mixed diets ($P<0.01$), while the DM digestibility of Mix_A was greater ($P<0.05$). The ruminal pH was lower for Mix_F. Surprisingly propionate concentration was higher for Mix_F ($P < 0.05$) while butyrate was higher for mixed diets ($P < 0.05$). NH_3-N ($P < 0.01$) was higher for ALF.

Table 2 Dry matter intake (DMI), apparent digestibility, and ruminal parameters of lambs fed only alfalfa (ALF) and TMR Amylaceous (Mix_A) or Fibrous (Mix_F) combined with fresh forage.

	ALF	Mix_A	Mix_F	SEM[1]	P^2		
					T	H	T×H
DMI (g/d)	1164^{b}	1472^{a}	1476^{a}	27.1	<0.01	-	-
DM digestibility	0.688^{b}	0.753^{a}	0.690^{b}	0.0147	<0.01	-	-
pH	6.49^{a}	6.30^{ab}	6.14^{b}	0.072	<0.01	<0.01	0.267
Acetate (mM)	100	95.3	85.4	5.32	0.137	<0.01	<0.05
Propionate (mM)	31.8^{b}	38.9^{b}	47.0^{a}	3.49	<0.05	<0.01	<0.01
Butyrate (mM)	14.3^{b}	20.1^{a}	19.3^{a}	1.65	<0.05	<0.01	<0.01
NH_3-N (mg/dl)	28.4^{a}	21.8^{b}	16.1^{c}	1.31	<0.01	<0.01	<0.01

[1]Standard error of means (n=6); [2] Significance level of treatment (T), time (H), interaction treatment × time (T × H); $^{a\text{-}c}$Means within a row with different superscripts differ ($P < 0.05$).

Conclusion The inclusion of fibrous by-products in the TMR did not affect the dry matter intake but affected the digestibility and rumen environment the lambs fed with mixed diets.

Acknowledgements This work was supported by the ANII funded project Innovagro FSA_1_2013_1_12561.

References

Pérez-Ruchel A, Repetto JL and Cajarville C 2017. Animal 11, 2175-2183.

SmartCow: an integrated infrastructure for increased research capability and innovation in the European cattle sector

René Baumont[1], Richard Dewhurst[2], Björn Kuhla[3], Cécile Martin[1], Lene Munksgaard[4], Chris Reynolds[5], Mike O'Donovan[6], Andrea Rosati[7], Léa Tourneur[8]

[1]INRA, UMR Herbivores, Saint-Genès-Champanelle, France, [2]SRUC, Edinburgh, UK, [3]Leibniz-Intitut fur Nutztierbiologie, Dummerstorf, Germany, [4]AARHUS Universitet, Tjele, Denmark, [5]University of Reading, Reading, UK, [6]Teagasc, Fermoy, Cork, Ireland, [7]EAAP, Roma, Italy, [8]INRA Transfert, Paris, France

E-mail: rene.baumont@inra.fr

Take home message The SmartCow project integrates European cattle research infrastructures, to promote their coordinated use and development, and thereby help the cattle sector face the challenge of sustainable production.

Introduction The future sustainability of cattle production will require improved resource use efficiency, reduced GHG emissions, and improved animal health and welfare (*e.g.,* Animal Task Force 2016). Moreover, the European Strategy Forum on Research Infrastructures (ESFRI) roadmap (2016) clearly identifies a gap for farm animal research infrastructures (RIs) and states that new RI efforts at EU-level are needed to face the challenges of developing sustainable livestock systems that address the dual challenges of the need for increased protein production and climate change. In this context, coordination, harmonisation and access to European research infrastructures are essential to support research and innovation for cattle production, and to support a sustainable, smart and competitive cattle sector in Europe.

Material & methods The SmartCow project mobilises 10 research institutions (INRA-France, SRUC-UK, WU and WUR-DLO-Netherlands, UREAD-UK, FBN-Germany, Teagasc-Ireland, AU-Denmark, IRTA-Spain, CRA-W-Belgium), 1 international organization promoting knowledge exchange for animal science (EAAP), 1 technical institute working on livestock farming (IDELE), 1 SME specialised in big data for the Agri-food industry (AgriMetrics) and 1 company specialised in project management and technology transfer (INRA Transfert). Scientific and technical skills in animal nutrition, genetics, health and welfare, data management and knowledge transfer will be combined to i) develop RIs and stakeholder networks in the cattle sector to improve research practice and foster innovation; ii) offer transnational access to the most advanced research facilities and equipment for the cattle sector; and iii) improve quality and ethics of cattle research through identification and promotion of best practices, new measurements techniques, and smart technologies.

Results & discussion The SmartCow project answering the call H2020-INFRAIA2016-2017 was selected by the European Commission for 4 years funding starting from 1st February 2018. Three types of activities will be developed (Figure 1).

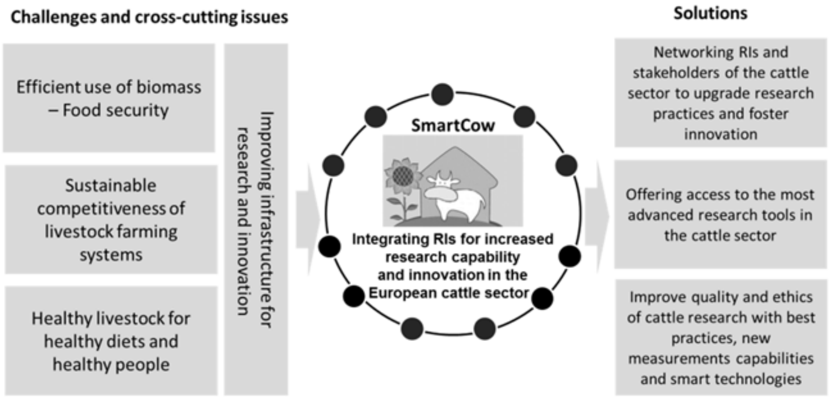


Figure 1 Overall concept of the SmartCow project.

Networking activities will: i) develop an inventory of animals, RIs and capabilities in European countries, ii) harmonise and standardise procedures in animal care and measurements, design of experiments, and data recording, and iii) disseminate project outcomes and involve stakeholders in the discussion of research priorities.

Transnational access to ten major RIs (INRA, SRUC, WU, WUR/DLO, UREAD, FBN, Teagasc, AU and IRTA) will provide access to around 2500 dairy and 1000 beef cattle and facilitate up to 30 research projects (60 users) to be financed by the SmartCow project after selection through specific calls.

Joint research activities will improve the quality and ethics of research in SmartCow RIs by: i) refining *in vivo* methods to evaluate feed efficiency and emissions, ii) developing proxies of feed efficiency and emissions, and iii) advancing analysis of data from sensors used to monitor animal behaviour. SmartCow considers the welfare of experimental animals and the implementation of the 3R principles (Replace, Refine and Reduce) as a crosscutting issue.

Conclusion The SmartCow consortium includes RIs in the cattle sector with a strong interest in the field of sustainable dairy and beef production, drawing on a wide range of animal sciences. It is a first step towards the integration of RIs for dairy and beef cattle which are of European and global interest.

Acknowledgements This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 730924.

References

Animal Task Force (ATF), 2016. A strategic research and innovation agenda for a sustainable livestock sector in Europe. http://animaltaskforce.eu/Portals/0/2nd%20White%20Paper/ATF-2nd%20whitepaper_final.pdf
EFSRI Roadmap (2016). Strategy report on research infrastructures. http://ec.europa.eu/research/infrastructures

Prediction of individual dairy cow performance using LC-MS metabolome spectra

M. Larsen, A.J. Buitenhuis, M.S. Hedemann, J. Sehested

Aarhus University - Foulum, Tjele, Denmark

E-mail: mogens.larsen@anis.au.dk

Take home message These preliminary data indicate a possibility to predict individual feed intake and milk production in later lactation from the plasma metabolome in week 2 after calving.

Introduction Dairy farmers need to increase the efficiency of conversion of feed into milk from both economic and environmental standpoints. The objective was to investigate the potential of untargeted metabolomics analysis of blood plasma to predict performance in the individual cow level with the future prospect of developing a new selection criterion.

Material & methods The current study is based on performance data and blood plasma samples from 53 Holstein cows used in an experiment investigating extended lactation with two feeding strategies (Gaillard *et al.,* 2015). Plasma was sampled at 1, 2, 4, 8, and 30 weeks in milk (WIM) and spectra of the metabolome were obtained by LC-MS as described by (Hedemann and Damgaard, 2012) modified to be performed using 96-wells plates.To predict dry matter intake (DMI), milk yield (MY) and feed conversion efficiency (MY:DMI ratio) from the LC-MS spectra, a partial least square regression was performed using the pls package (Mevik and Wehrens, 2007) in R (version 3.4.2). The prediction equation was based on either 1, 2, 4, 8, or 30 WIM LC-MS spectra data by using the leave one out method across diets, and subsequently used to predict the measured values at each WIM separately.

Results & discussion Prediction equations could be generated for DMI at 1, 2, 4, and 8 WIM whereas DMI at 30 WIM could not be predicted based on the LC-MS spectra. Prediction equations could be generated for MY at 1, 2, 4, 8, and 30 WIM, whereas feed conversion efficiency at all time points could not be predicted based on the LC-MS spectra.

At 2 and 4 WIM, DMI and MY could be predicted reasonably accurate (Table 1). Correlations across time were in the range of 0.35 to 0.69 depending on the WIM the prediction equation was build. In the range of 2 to 8 WIM, the correlations between predicted and observed DMI was over 0.60. This correlation drops under 0.50 when DMI at 1 and 30 WIM was predicted based on the equations for 2, 4, and 8 WIM respectively. A similar pattern was shown for MY (Table1). The predicted MY versus the observed MY has a high phenotypic correlation when the same WIM (*e.g.,* predicted MY at 4 WIM versus observed MY at 4 WIM).

The explanatory principal components (PCs) in the prediction equations for the three phenotypes needs further investigation to elucidate which metabolites that contribute significant to the predictions.

Table 1 Phenotypic correlations of predicted *vs.* measured dry matter intake and milk yield for each week in milk (WIM)[1].

Predicted/measured	Dry matter intake					Milk yield				
WIM	1	2	4	8	30[2]	1	2	4	8	30
1	0.69	0.60	0.44	0.50	-	0.83	0.58	0.63	0.58	0.53
2	0.46	0.84	0.61	0.63	-	0.60	0.97	0.62	0.66	0.35
4	0.48	0.69	0.83	0.64	-	0.47	0.57	0.96	0.53	0.43
8	0.42	0.60	0.62	0.71	-	0.53	0.57	0.63	0.69	0.45
30	0.35	0.44	0.42	0.41	-	0.38	0.36	0.33	0.43	0.82

[1] A prediction model based on the LC-MS data was made for each WIM separately, and subsequently used to predict the measured values at each WIM separately. [2] A prediction equation for dry matter intake at 30 WIM could not be estimated.

Conclusion The preliminary data indicate that LC-MS data could be used to predict DMI and MY of individual cows at 2 and 4 WIM reasonably accurately, with correlations between observed and predicted DMI >80%, and for MY >90%. This correlation drops fast when the prediction equations are used to predict DMI and MY at other time-points. Based on the preliminary data, feed conversion efficiency could not be predicted based on the LC-MS spectra.

Acknowledgements The Danish AgriFish Agency (GUDP #34009-14-0848) is acknowledged for funding the work.

References

Gaillard C, Friggens NC, Taghipoor M, Weisbjerg MR, Lehmann JO, Sehested J 2015. Journal of Dairy Science 99, 2221-2236.
Hedemann MS and Damgaard BM. 2012. Comparative Biochemistry and Physiology D7, 322-327.
Mevik BH and Wehrens R 2007. Journal of Statistical Software 18, 1-24.

Effect of rumen protected methionine on urinary nitrogen excretion in beef cattle

Yuchao Zhao, Md Sazzadur Rahman, Yu Bao, Ke Zhou, Guangyong Zhao

China Agricultural University, Beijing, China

E-mail: zhaogy@cau.edu.cn

Take home message Dietary supplementation of rumen protected methionine decreases urinary nitrogen excretion from beef cattle.

Introduction The nitrogen (N) use efficiency of beef cattle is lower than other species of livestock. The N excretion, particularly the urinary N excretion, from beef cattle production systems, not only contributes to the ground and surface water pollution but also leads to the nitrous oxide (N_2O) emission (Reed *et al.,* 2015). Most of the urinary N of cattle is present in the form of urea (52.1-93.5%) (Dijkstra *et al.,* 2013), which is much more vulnerable to loss than the faecal N. Methionine is typically considered to be the first limiting amino acid for beef cattle (Klemesrud *et al.,* 2000). Supplementing rumen protected methionine (RPM) would balance the amino acid profiles in duodenum and is expected to improve the N use efficiency. The aim of the trial was to study the effect of dietary supplementation of RPM on the urinary N excretion of beef cattle.

Material & methods Eight castrated growing Simmental cattle, 24-month-old, with an initial live weight of 494±28 kg, were used as the experimental animals. Four levels of RPM (DL-Methionine, purity ≥ 85.0%, Evonik Industries, Essen, Germany), *i.e.,* 0, 10, 20 and 30 g/cattle/d (equivalent to 0, 1.54, 3.08, 4.62 g/kg DM of diet), were added to the basal ration in a replicated 4 × 4 Latin square design. The basal ration (CP=128.7 and NDF=397.3 g/kg DM) consisted of 462.6g corn silage, 369.8g corn, 42.5g corn gluten meal, 68.2g soybean meal, 43.5g wheat bran, 6.7 g sodium chloride and 6.7 g sodium bicarbonate per kg DM. RPM was added to the ration and well mixed before feeding. The ration was divided into two equal meals, which were fed at 7:00 h and 17:00 h, respectively. The cattle were housed in individual pens and had free access to drinking water. Each experimental period lasted 19 d, consisting of 14 d for adaptation and 5 d for sampling. During each period, a consistent DMI of 6.49 kg for each cattle was maintained and no orts were observed. During each sampling phase, the urine was completely collected using a rubber funnel connected to a plastic tube and a plastic bucket surrounded with ice packs. The total volume of urine from each cow was measured and 1% of the urine was sampled and added with H_2SO_4 (10%, v/v). The N of urine and feed samples was analysed using the Kjeldahl method. The urinary urea was analysed using the kits (Nanjing *Jiancheng* Bioengineering Institute, Nanjing, China) on a spectrophotometer. The data were analysed using the general linear model procedure of SAS 9.4. Linear and quadratic effects (no significance was detected) due to RPM addition were determined using polynomial contrasts.

Results & discussion Supplementing RPM decreased the excretions of the urinary N ($p<0.01$), urea ($p<0.05$), the ratios of urinary N/N intake ($p<0.01$) and urea N/N intake ($p<0.05$). Supplementing RPM did not affect the ratio of urea N/ total urinary N ($p>0.05$). The decreased urinary N and urea could be effective for mitigating N_2O emission from the urine patches.

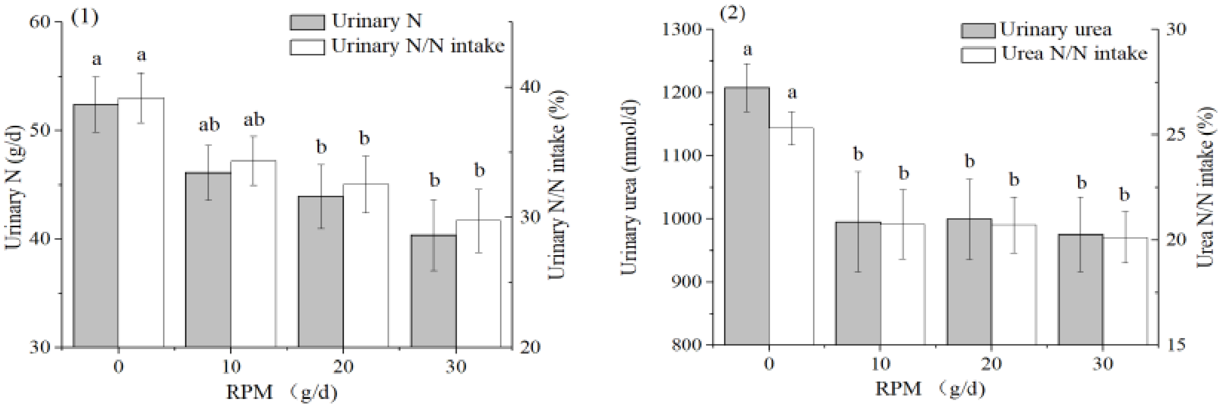


Figure 1 Effects of adding RPM on the urinary N excretion and the ratio of urinary N/N intake (1); and the urinary urea excretion and the ratio of urea-N/N intake (2). Means with no common superscripts within the same bars differ significantly ($p<0.05$).

Conclusion Dietary supplementation of RPM decreased the excretions of the urinary N and the urea. It is needed to study the effects of dietary supplementation of RPM on the faecal N excretion and other urinary nitrogenous components of beef cattle.

Acknowledgements The authors thank Evonik Greater China for providing RPM product.

References

Dijkstra J, Oenema O, van Groenigen JW, Spek JW, van Vuuren AM and Bannink A 2013. Animal 7, Suppl. 2(s2), 292-302.
Klemesrud MJ, Klopfenstein TJ and Lewis AJ 2000. Journal of Animal Science 78, 199-206.
Reed KF, Moraes LE, Casper DP and Kebreab E 2015. Journal of Dairy Science 98, 3025-35.

Prediction of methane emissions from Holstein dairy cows based on milk fatty acid profile

Adeline Bougouin[1], J. A. D. Ranga Niroshan Appuhamy[2], Anne Ferlay[1], Ermias Kebreab[3], Cécile Martin[1], Peter Moate[4], Chaouki Benchaar[5], Peter Lund[6], Maguy Eugène[1]

[1]UCA, INRA, VetAgro Sup, UMR Herbivores, Saint-Genès-Champanelle, France, [2]Iowa State University, Ames IA 50014, USA, [3]UCD, Davis CA 95616, USA, [4]Victoria State Government, Ellinbank, Victoria 3821, Australia, [5]Sherbrooke Research and Development Centre, Sherbrooke QC J1M 0C8, Canada, [6]Aarhus Universitet, Tjele, DK-8830, Denmark

E-mail: adeline.bougouin@inra.fr

Take home message Five milk fatty acids have the potential to predict methane emissions from Holstein dairy cows.

Introduction For the past decade, attention has been directed towards the use of milk fatty acids (MFA) as biomarkers for prediction of CH_4 emissions from dairy cows because of potential common biochemical pathways in the rumen (Van Gastelen and Dijkstra, 2016). However, equations have been developed based on a narrow range of diets and with limited number of data. The aim of this study was to describe the more general potential of individual MFA as predictors of CH_4 emissions, based on individual data of cows fed a wide range of diets.

Material & methods A total of 825 CH_4 emission measurements (g/day) from individual lactating Holstein dairy cows linked to milk samples characterized by fatty acid composition (% of total FA), were obtained from 34 *in vivo* experiments (Australia, Canada, Denmark, France). Dry matter intake, diet chemical composition (OM, NDF, starch, EE), milk yield, fat, protein and lactose contents, and animal characteristics (DIM, BW) were also gathered in the database. Outliers were removed (n=1) from the dataset and missing values on diet chemical compositions were estimated according to ruminant feeding tables (INRA, 2007). The diets were mainly based on maize silage (297), grass silage (157), legume hay (157), or a mixture of forages (215). The database contained control diets (198) and CH_4 mitigating treatments including lipid supplementation (198), different forage or concentrate types (149; 140), probiotics (58), plant extracts (33), saponins (8), or tannins (4). Methane emissions were measured using open-circuit respiration chambers (462) and the SF_6 method (363), while MFA were analysed by gas chromatography. Pearson's pairwise correlation coefficients were obtained between the 46 initially collected individual MFA with CH_4 emissions to determine the most correlated individual MFA per MFA family (saturated FA, branched FA, *cis* monounsaturated FA, *trans* monounsaturated FA and polyunsaturated FA). Pearson's pairwise correlation coefficients were also obtained for each pair of MFA. Principal component analysis (PCA) was performed, using the FactoMinR package in R, on the MFA significantly correlated to CH_4 production ($r \geq |0.3|$) along with other variables in order to identify potential predictors of CH_4 emissions. Missing values for individual MFA were estimated and replaced with plausible values using the MissMDA package in R.

Results & discussion Six individual MFA were significantly correlated with CH_4 emissions (Table 1), with *t*10-C18:1 being the most negatively correlated. The first two PCA axes (Figure 1) explain together 39% of the total variance. The PCA1 discriminates C10:0, C8:0, CH_4, milk fat, and milk protein (positively), *t*10-C18:1, *t*11,*c*15-C18:2, *iso*-C17:0 (+*t*9-C16:1), *c*11-C18:1, milk lactose and NDF (negatively). Only C8:0 was strongly correlated to other FA (C10:0, and *t*10-C18:1, $r \geq |0.5|$; data not shown). Thus, C10:0 was preferred to represent the saturated FA family. Results were comparable to previous studies showing relationships between CH_4 emissions *c*11-C18:1, *t*10-C18:1, *t*11*c*15-C18:2 (negative), and C10:0 (positive) (Van Gastelen and Dijkstra, 2016). The shift to more milk *t*10-C18:1 production is known to be related to changes in rumen biohydrogenation process, low pH and subsequent low CH_4 emissions (Van Lingen *et al.,* 2014). The milk C10:0, synthesised *de novo* from rumen acetate, is related to fiber intake and ultimately CH_4 emissions.

Table 1 Summary statistics of some variables included in the database and their correlation[1] with CH_4 emissions.

Variables	Mean	SD	Min	Max	r
DMI (kg/d)	20.5	3.42	10.8	32.2	0.57
CH_4 (g/d)	414	105	84	708	-
Milk (kg/d)	28.6	7.36	6.5	50.7	0.24
Milk fat (%)	3.8	0.70	1.3	6.5	0.21
C8:0	1.2	0.32	0	2.0	0.35
C10:0	2.8	0.80	0.6	4.9	0.34
iso C17:0[2]	0.4	0.18	0.0	1.1	-0.31
*c*11-C18:1	0.8	0.49	0.3	4.7	-0.34
*t*10-C18:1	0.9	1.48	0.0	11.3	-0.45
*t*11*c*15-C18:2	0.3	0.52	0.0	3.6	-0.29

(g/d)[1]Correlation Pearson on the training dataset (n=825)
[2]Coelution with *t*9-C16:1. Named isoC17.0.coel in PCA.

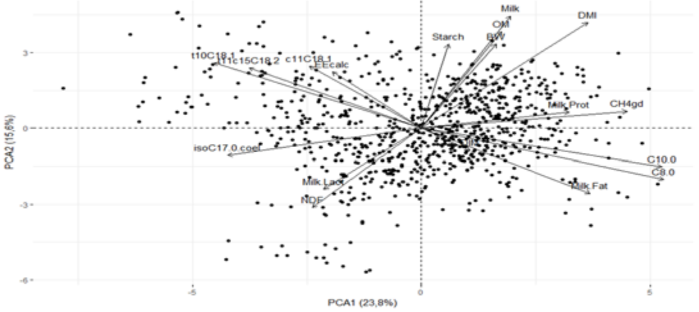


Figure 1 PCA score plot for the individual data with the first (PCA1) and second (PCA2) dimension.

Conclusion Five MFA (C10:0, *iso*-C17:0 (+*t*9-C16:1), *c*11-C18:1, *t*10-C18:1, *t*11,*c*15-C18:2), are potential predictors of the daily CH_4 emissions from dairy cows. Furthermore, they are not correlated, thus suitable for inclusion in more complex prediction models.

Acknowledgements This study was supported by Adisseo France SAS, Agrial, APIS-GENE, Deltavit, DSM Nutritional Products AG, IDELE, INRA, Lallemand, Moy Park Beef Orléans, Neovia, Techna France Nutrition, Valorex, and ANRT with a Cifre grant.

References

INRA. 2007. Edition Quae, Versailles, Paris.
Van Gastelen S and Dijkstra J 2015. Journal of the Science of Food and Agriculture 96, 3963-3968.
Van Lingen H, Crompton LA, Hendriks WH, Reynolds CK and Dijkstra J 2014. Journal of Dairy Science 97, 1–18.

The nitrogen use efficiency in growing lambs is negatively impacted by both the dietary protein content and the age of animals: towards a phase-feeding approach

Mickaël Bernard[1], Aurélien Jeanleboeuf[5], Laurence Sagot[4], Paul Cheng[3], Angélique Quereuil[2], Gonzalo Cantalapiedra-Hijar[2]

[1]INRA, Herbipôle, Saint-Genès-Champanelle, France, [2]INRA, Université Clermont Auvergne, VetAgro Sup, UMR Herbivores, Saint-Genès-Champanelle, France, [3]Faculty of Veterinary and Agricultural Sciences, Dookie Campus, The University of Melbourne, Victoria, Australia, [4]Institut de l'Elevage - Ciirpo, Saint Priest Ligoure, France, [5]lycée agricole de Radinghem, Radinghem, France

E-mail: mickael.bernard@inra.fr

Take home message Nitrogen use efficiency decreases as growing lambs become older. Animal performances may benefit from phase-feeding programs based on reducing dietary protein levels as lambs become older.

Introduction The decline in the efficiency of protein use as animals become older has been reported in cattle (INRA, 2018) and pig production (NRC, 2012), and suggest the potential benefit to adapt the dietary protein content to the animal growth phase. In growing lambs, although different studies have shown that the dietary protein content may impact the N use efficiency, there is limited data confirming a decline in this efficiency as growth progresses. Thus, this study aimed at assessing how the N use efficiency and performances are impacted by the dietary protein content when a N balance trial was performed on the same animal at two different growth phases (early *vs* late fattening period).

Material & methods Twenty-four growing male lambs were assigned to one of three experimental concentrates (n=8/treatment) differing in crude protein (CP) content. Concentrates were formulated at 20%CP (dry matter basis) to mimic feeding practices in French conditions and at 17%CP and 15%CP to evaluate lower CP content. Concentrates were offered with wheat straw, both ingredients were fed *ad libitum* (>10% of refusals). Animals received their respective experimental concentrate from three weeks old (before weaning) to slaughter (fixed at a 48 kg of body weight). At weaning, lambs were housed in individual pens allowing daily feed intake recording. The N use efficiency was assessed through an N balance trial carried out for all animals at two different periods, at 30 and 60 days post-weaning, respectively. Each N balance trial lasted 4 days for animal adaptation to cages and 6 days during which a total collection of faeces and urine was conducted. Nitrogen determination was performed on feed, refusals, faeces and urines pooled by period and using the Kjeldahl method (ISO 5983.1997). The overall average daily gain (ADG; g/d) was calculated as the slope from the regression of body weight on time for the whole period (from 3 weeks old to slaughter, that is 120 days) as well as for two equal sub-periods around the two N balance trials of 45 days each. Statistical analysis were performed in R software. Linear and quadratic effects of dietary protein content were tested. Because no significant quadratic effects were found, only linear effects as well as its interaction with period will be presented here.

Results The overall ADG increased linearly (P = 0.01) as the dietary protein content increased (317, 355 and 372 g/d for 15%, 17% and 20%CP diets, respectively) leading to a linear decrease (P = 0.02) in the fattening period length (130, 118, and 113 days for 15%, 17% and 20%CP diets, respectively). However, this linear effect on growth was only observed at early fattening but not later (Table 1; Diet x Period, P = 0.008). Likewise, a linear decline in the N use efficiency as the dietary CP content increased was noted at early fattening but not at late fattening (Diet x Period, P = *0.04)*. On average, nitrogen use efficiency (NUE) strongly decreased from early (average 35.0%) to late (average 27.6%) fattening period.

Table 1 Nitrogen balance (g/d), average daily gain (ADG; g/d) in growing lambs fed increasing protein levels at early (30 days post-weaning) and late (60 days post-weaning) fattening.

	Early fattening			Late fattening				P-value		
	15%CP	17%CP	20%CP	15%CP	17%CP	20%CP	SEM	Linear	Period	D x P
N intake	26.0	31.4	40.2	33.3	42.1	52.8	1.42	<0.001	<0.001	0.23
Fecal N	10.5	10.9	12.6	12.2	14.7	15.3	0.60	0.14	<0.001	0.24
Urinary N	5.47	8.99	15.8	11.3	16.3	23.0	0.42	<0.001	<0.001	0.19
N balance	10.1	11.6	11.8	9.95	11.0	14.5	0.99	0.33	0.45	0.26
ADG	281^{a}	349^{ab}	383^{b}	349^{ab}	340^{ab}	308^{ab}	19.2	0.006	0.85	0.008
NUE, %	38.8^{a}	36.9^{ab}	29.3^{b}	29.6^{b}	25.8^{c}	27.4^{bc}	1.71	0.001	<0.001	0.04

Conclusion The positive effect of dietary protein content on lamb performances was only observed at early fattening, while its negative impact on urinary N excretion was noted at both early and late fattening periods. Because the N use efficiency decreased as animals become older, our results justify the need to evaluate in future experiments a phase-feeding approach in lambs consisting in lowering the dietary protein content diets as animals reach progressively their adult body weight.

Acknowledgements This project was supported by the National Establishment of products of agriculture and sea. The authors are grateful to the staff of INRA UE 1414 Herbipôle and UMR 1213 Herbivores.

References

INRA, 2018. INRA feedings system for ruminants. Wageningen Academic Publishers, The Netherlands, 640pp
National Research Council. (2012). Nutrient requirements of swine. National Academies Press.

627

Digestibility, digesta passage and methane emission of Holstein x Boran heifers supplemented with sweet potato vine silage

Asep Ali[1], Daniel Korir[2], Shimels Wassie[3], John Goopy[2], Lutz Merbold[2], Klaus Butterbach-Bahl[2,4], Uta Dickhoefer[3], Eva Schlecht[1]

[1]Animal Husbandry in the Tropics and Subtropics, University of Kassel and University of Göttingen, Witzenhausen, Germany, [2]International Livestock Research Institute, Nairobi, Kenya, [3]Animal Nutrition and Rangeland Management in the Tropics and Subtropics, University of Hohenheim, Stuttgart-Hohenheim, Germany, [4]Institute of Meteorology and Climate Research, Atmospheric Environmental Research, Karlsruhe Institute of Technology, Garmisch-Partenkirchen, Germany

E-mail: schlecht@uni-kassel.de

Take home message Upgrading poor quality roughage diets through moderate supplementation with sweet potato vine silage offers an effective approach to mitigate methane emission intensities from enteric fermentation in tropical dairy production systems.

Introduction About 80% of Kenya's population live of agriculture, which also is the country's dominant sector of greenhouse gas (GHG) emissions. Domestic livestock contribute at least 30% to the national GHG emissions. A major reason for this is the poor quality of forage consumed by the vast ruminant population. Supplementing diets of productive animals with locally available feeds of higher nutritional quality, such as sweet potato vine silage (SPVS), can improve nutrient digestibility and may thus reduce enteric methane (CH_4) production intensities. Our study aimed at verifying this relationship for a typical smallholder dairy system in Kenya.

Material & methods We used a 2 (diets) by 2 (periods) design, feeding a roughage diet (R; 61.4% wheat straw plus 38.6% Rhodes grass hay) and a supplement diet SPVS (80.4% R plus 19.6% SPVS; all on dry matter (DM) basis). During 21 d adaptation and 14 d measurement per period, diets were offered at 2.5% live weight (LW) to 4 Holstein × Boran heifers (148 ±9.75 kg LW). Quantitative and qualitative data on feed intake and faecal excretion were collected during 7 d, whereby on day 1 heifers were fed a pulse dose of ytterbium (Yb)-marked fibre particles (3-5 cm long; 560 mg Yb kg^{-1} LW). Yb-containing faeces were collected every 4 to 12 h during the subsequent week. In sampling week 2, CH_4 production was determined in open circuit respiration chambers (22.5 h d^{-1} per animal) with 3 measurements taken per animal per diet. Samples of feed offered, refused and of faeces were analysed for proximate composition and Yb-content (faeces) following standard protocols. Time of first marker appearance (TT), ruminal passage rate (λ) and rumen mean retention time (RMRT) were determined with SAS 9.1 NLIN procedure (Richter & Schlecht, 2006). PROC MIXED was used to conduct ANOVA with diet and period as fixed and animal as random effect. Simple linear regression analysis was performed using REG procedure.

Results Dry matter digestibility (DMD) was higher and CH_4 production per unit of digested DM intake (DDMI) lower in heifers supplemented with SPVS (Table 1). Methane yield (g kg^{-1} DMI) and CH_4 production per unit DDMI decreased with increasing ruminal passage rate and increased with prolonged rumen mean retention time of feed particles (Table 2).

Table 1 Dry matter intake (DMI), particle passage para-meters (see Table 2), DM digestibility (DMD), CH_4 per unit DMI and per unit digested DMI (DDMI) in heifers offered roughage (R) alone or with sweet potato vine silage (R+SPVS).

Variable	R	R+SPVS	SEM	P-value
DMI (g $kg^{-0.75}$ LW)	70.9	76.0	4.22	0.480
Particle passage				
λ (% h^{-1})	3.5	3.8	0.19	0.340
TT (h)	18.9	16.8	0.85	0.320
RMRT (h)	56.8	54.4	2.61	0.436
DMD (g/kg)	474^a	510^b	9.19	0.023
CH_4 production				
g kg^{-1} DMI	30.6	28.1	1.24	0.251
g kg^{-1} DDMI	64.7^b	55.2^a	3.31	0.048

Within rows, means with different superscripts differ at $P \leq 0.05$ (Tukey post-hoc test). LW: live weight.

Table 2 Correlation coefficients (r) and significance[1] of the relationship between ruminal passage rate (λ), post ruminal transit time (TT), and rumen mean retention time (RMRT) of particles and CH_4 production in heifers offered roughage alone or with sweet potato vine silage.

	Methane Production					
	g d^{-1}		g kg^{-1} DMI		g kg^{-1} DDMI	
λ (% h^{-1})	0.45	n.s	-0.93	***	-0.85	**
TT (h)	-0.53	n.s.	0.00	n.s.	0.17	n.s.
RMRT (h)	-0.50	n.s	0.92	**	0.85	**

[1]Significance levels: n.s. not significant; (*) $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Conclusion Supplementation with SPVS improved DM digestibility and decreased CH_4 per DDMI. These phenomena can be explained by a faster rumen passage of digesta particles. Offering about 20% SPVS to an otherwise poor-quality diet thus seems to be a promising strategy to improve the production and decrease the environmental impact of tropical dairy cattle.

Acknowledgement The project was funded by Federal Ministry for Economic Cooperation and Development, Grant No. 5521914.

Reference

Richter H and Schlecht E 2006. Animal Feed Science & Technology 128, 135-154.

Intake, live weight gain and feed efficiency of Charolais and Holstein-Friesian steers offered zero-grazed grass

Mark McGee, David Kenny, Claire Fitzsimons

Teagasc, AGRIC, Grange, Dunsany, Co. Meath, Ireland

E-mail: mark.mcgee@teagasc.ie

Take home message Holstein-Friesian (HF) steers consumed 0.22 more grass relative to weight and had a 0.10 poorer FCE compared to Charolais (CH) steers.

Introduction Feed efficient cattle are central to the economic and environmental sustainability of beef production enterprises. Due to the abolition of milk quotas in the European Union, the subsequent expansion of the Irish dairy herd has meant that proportionately more beef is derived from dairy-bred compared to suckler-bred animals. In temperate climates, such as Ireland, dairy-beef and suckler-beef production systems are largely based on grazed grass herbage (McGee *et al.*, 2005; Drennan and McGee, 2009). There is relatively little published information comparing grass herbage intake of beef cattle genotypes. The objective of this study was to accurately quantify intake, growth and feed efficiency of HF steers compared to suckler-bred CH steers offered zero-grazed grass.

Material & methods One hundred and ninety-six spring-born steers comprising of 99 CH and 97 HF were used. Steers were individually offered zero-grazed grass herbage (dry matter (DM), 196 g/kg; organic matter digestibility, 803 g/kg) *ad libitum* in three daily meals at 0600 h, 1030 h and 1330 h. The grass was harvested, twice daily (0530 h and 1000 h) from *Lolium perenne* dominant swards using a 'zero-grazer'. Mean pre- and post-mowing compressed sward heights were 11.0 cm (SD = 2.5) and 4 cm (set height of zero-grazer), respectively, and mean pre-mowing herbage mass was 1936 (SD = 711) kg DM/ha. The herbage was supplemented with 50 g minerals and vitamins/head/day, thrice weekly. Following a dietary adaption period of 14 days, individual dry matter intake (DMI) and growth was measured over a 70-day period. Average daily live weight gain (ADG) of each steer was computed as the coefficient of the linear regression of live weight (kg) on time (days). The residuals of the regression of DMI on ADG and mid-test metabolic live weight were calculated and used to compute individual residual feed intake (RFI) coefficients for each animal. Data were statistically analysed using the GLM procedure of SAS; the model contained the fixed effect of breed.

Results & discussion HF steers were 24 days older ($P<0.001$) than CH (Table 1), which reflects the mean calving dates of the national dairy and suckler cow herds in Ireland. Despite their older age, HF were 80 kg lighter ($P<0.001$), and had a lower ADG ($P<0.05$), compared to CH. Grass DMI, on an absolute basis and especially when expressed relative to live weight, was higher ($P<0.001$) for HF than CH. In contrast, Clarke *et al.* (2009) comparing late-maturing beef breeds with Friesian steers reported no difference ($P>0.05$) in grazed grass DMI (measured using n-alkanes) between genotypes, both absolutely and relative to weight; in that study the beef breeds were also heavier (0.15) than the dairy breed. The FCE and RFI of CH steers was superior ($P<0.001$) to HF, which concurs with high-concentrate finishing studies (Clarke *et al.*, 2009).

Table 1 Age, dry matter intake (DMI), live weight gain (ADG), feed conversion efficiency (FCE) and residual feed intake (RFI) of Charolais and Holstein-Friesian steers offered zero-grazed grass.

		Charolais	Holstein-Friesian	s.e.m.	P-value
Age – initial (days)		465	489	1.4	***
Daily grass DMI	(kg)	9.07	9.47	0.058	***
	(g/kg live weight)	16.2	19.7	0.12	***
Live weight, mid-test (kg)		562	482	4.0	***
ADG (kg)		1.34	1.27	0.022	*
FCE (kg live weight/kg DMI)		0.147	0.134	0.0023	***
RFI (kg DM/d)		-0.27	0.29	0.051	***

Conclusion The proportionately 0.14 lighter and 0.05 slower-growing HF steers consumed 0.04 more grass DM resulting in an inferior FCE and RFI compared to CH. This inefficiency has implications for beef farm carrying capacity and economics.

Acknowledgements Irish DAFM, Research Stimulus Fund - Project IdentiFEED-13/S/519.

References

Clarke AM, Drennan MJ, McGee M, Kenny DA, Evans RD and Berry DP 2009. Livestock Science 126, 57-68.
Drennan MJ and McGee M 2009. Livestock Science 120, 1-12.
McGee M, Keane MG, Neilan R, Moloney AP and Caffrey PJ 2005. Irish Journal of Agricultural and Food Research 44, 215-231.

Relationship between residual feed intake and methane production in dairy heifers

Holly Flay, Barbara Kuhn-Sherlock, Kevin Macdonald, Mark Camara, John Roche

DairyNZ, Hamilton, New Zealand

E-mail: john.roche@dairynz.co.nz

Take home message Dairy heifers categorised as low residual feed intake (RFI*; i.e.,* high feed efficiency) have lower dry matter intake (DMI) for the same live weight gain (Lwtg), the same daily methane (CH_4) production (g/d), and higher CH_4 yield (g/kg DMI) than heifers categorised as high RFI (*i.e.,* low feed efficiency).

Introduction An increasing demand for animal products has increased interest in improving feed conversion efficiency through reducing residual feed intake (RFI; Macdonald *et al.,* 2014). Further, reducing the contribution of agricultural methane (CH_4) production to climate change requires understanding the cow-level factors that contribute to CH_4 emissions. Considering that CH_4 is a major source of digestible energy loss in ruminant animals, it is plausible that these traits are favourably correlated and that selection for reduced RFI would also reduce CH_4 emissions. Our objective was to compare the CH_4 production between dairy cow breeds and RFI categories (*i.e.,* high or low) to elucidate this relationship.

Material & methods We estimated CH_4 production (g/d) and yield (g/kg dry matter intake; DMI) for 20-22-month-old heifers in a 2 x 2 factorial arrangement including two breeds of dairy cattle (Jersey: Jer and Holstein-Friesian: HF) previously designated as either high RFI (+2.0 kg DM) or low RFI (-2.1 kg DM; n = 14/treatment). The heifers (split into two periods) were housed in a free stall facility for approximately 30 d with unrestricted access to dried lucerne cubes from feeding stations. The stations electronically monitored feed disappearance in real-time and extracted all exhaled gas for real-time CH_4 analysis (DMI-CH_4 stations; C-Lock Inc., Rapid City, SD, USA). Individual animal CH_4 and DMI were measured for the duration of both periods and heifers were weighed thrice weekly. Mean daily CH_4 emissions were calculated for each animal. We estimated the fixed effects of breed, RFI category, the interaction between breed and RFI category, and the random effect of animal using a linear model that tested for significant differences between the fixed effect means.

Results & discussion The interactions between breed and RFI category were not statistically significant; therefore, only means of main effects are presented in Table 1 HF heifers were significantly heavier and consumed more than Jer, but there were no significant effects of breed on DMI/kg Lwt or Lwtg. RFI category had no significant effect on Lwt or Lwtg, but DMI and DMI/kg Lwt were 9.2% and 10.6% less in low RFI heifers. Because of the lower DMI, Jer heifers produced less CH_4/d, but not per kg DMI or per kg Lwtg. RFI category had no significant effect on daily CH_4 production (g/d) or CH_4/kg Lwtg; but, CH_4/kg DMI was greater in low RFI heifers because of their lower DMI. The results probably reflect more complete digestion of ingested feed in low RFI heifers, consistent with previously reported greater physical breakdown of feed (Gregorini *et al*., 2015) and a higher apparent digestibility of organic matter (Rius *et al*., 2012) in low RFI animals.

Table 1 Least square means (± SEM) for Lwt, DMI, Lwtg and CH_4 in Jer and HF heifers from low or high RFI categories.

	n	Lwt (kg)	DMI (kg DM/d)	Lwt gain (kg/d)	CH_4 (g/d)	CH_4/DMI (g/kg DMI)	CH_4/Lwtg (g/kg Lwtg)
Jer	28	408 ± 7.0	11.3 ± 0.29	1.2 ± 0.06	242 ± 4.3	21.6 ± 0.51	219 ± 9.6
HF	28	479 ± 7.0	12.4 ± 0.29	1.3 ± 0.06	267 ± 4.3	21.9 ± 0.51	211 ± 9.6
P-value		<0.001	0.01	0.09	<0.001	0.65	0.57
Low RFI	28	448 ± 9.8	11.3 ± 0.29	1.2 ± 0.06	253 ± 4.9	22.7 ± 0.47	222 ± 9.5
High RFI	28	439 ± 9.8	12.4 ± 0.29	1.3 ± 0.06	256 ± 4.9	20.7 ± 0.47	208 ± 9.5
P-value		0.50	<0.01	0.31	0.63	<0.01	0.30

Conclusion Selecting dairy heifers for low RFI is unlikely to affect daily CH_4 production (g/d) but could increase CH_4 yield (g/kg DMI).

Acknowledgements Funded by the New Zealand Government to support the objectives of the Livestock Research Group of the Global Research Alliance on Agricultural Greenhouse Gases. The authors gratefully acknowledge the assistance of DairyNZ technical and farm staff.

References

Gregorini P, Waghorn GC, Kuhn-Sherlock B, Romera AJ, Macdonald KA 2015. Journal of Dairy Science 98, 6486–6491.
Macdonald KA, Pryce JE, Spelman RJ, Davis SR, Wales WJ, Waghorn GC, Williams YJ, Marett LC and Hayes BJ 2014. J Journal of Dairy Science 97, 1427–35.
Rius AG, Kittelmann S, Macdonald KA, Waghorn GC, Janssen PH and Sikkema E 2012. Journal of Dairy Science 95, 5024–5034.

Faecal natural ^{15}N abundance may sign the between-animal variation in diet digestibility of beef cattle

Gonzalo Cantalapiedra-Hijar[1], Anne De la Torre[1], Isabelle Constant[1], Lucette Genestoux[1], Mathilde Grand[2], Céline Chantelauze[1], Donato Andueza[1], Pierre Noziere[1]

[1]Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès-Champanelle, France, [2]Elucidation of Biosynthesis by Isotopic Spectrometry Group, CEISAM, CNRS−University of Nantes UMR6230, B.P. 92208, F-44322 Nantes, France

E-mail: gonzalo.cantalapiedra@inra.fr

Take home message The faecal natural ^{15}N abundance is positively correlated to the apparent organic matter digestibility in beef cattle fed the same diet.

Introduction Energy excreted in faeces represent the most important feed energy loss under certain dietary conditions, and thus markedly limit the efficiency of feed conversion into animal products. Huge progress has been achieved to understand dietary factors affecting the diet digestibility (INRA, 2018). However, there exists a considerable animal variability in the apparent organic matter digestibility (**OMD**) between individuals reared in similar conditions, which should be known if we wish to adapt feed formulation to the animals' genetic potential. The ^{15}N abundance of faeces is naturally enriched over the diet consumed (Cantalapiedra-Hijar *et al.,* 2015). This is manly the consequence of the contribution of the naturally ^{15}N enriched animal protein (*i.e.,* endogenous faecal N) to faecal N excretion. We hypothesize that individual variability in OMD is impacted by individual differences in endogenous faecal protein. Thus, this study aimed at testing whether faecal natural ^{15}N abundance (**faecal δ^{15}N**) could be negatively correlated to the digestibility of beef cows consuming the same diet.

Material & methods Sixteen non-pregnant non-lactating Charolais cows (708±87kg) were used to measure OMD (De La Torre *et al.,* 2016). Briefly, four periods of digestibility trials were carried out successively using either high- (100% hay, periods 1 and 2) or low-fiber diet (maize silage/concentrate, 67/33, periods 3 and 4). Each period lasted 2-3 weeks *ad libitum* followed by 7 days of total faeces collection at 95% ad libitum. Spot fecal samples were obtained in the last day of each of the four experimental periods both in the morning (0800 before feeding) and in evening (1600). Spot fecal samples (n = 128) were dried and ground at 1mm and analyzed for natural ^{15}N abundance as previously described (Cantalapiedra-Hijar *et al.,* 2015). Isotopic values from the two faecal spot samples collected the same day were averaged and regressed on individual OMD measured for each period and diet (n = 64). Data were analyzed as repeated measures (package nlme in the R software) and considering the cow as a random effect and diet, period nested within diet and the interaction between faecal δ^{15}N and diet as fixed effects.

Results No effect of period within diet nor of the interaction faecal δ^{15}N × diet ($P > 0.10$) were observed on OMD. Consequently, these effects were removed in the final statistical model. As expected, the effect of diet on the OMD was highly significant ($P < 0.001$) with a difference of 11.7 points (%) on average between the high- and low-fiber diet. The faecal δ^{15}N values were positively correlated ($P < 0.001$) to OMD of beef cows consuming strictly the same diet (Figure 1), each increment (‰) of faecal δ^{15}N was associated to an increase of 1.38 points (%) in OMD. Because faecal δ^{15}N has been negatively correlated to the N use efficiency (Cantalapiedra-Hijar *et al.,* 2015) present results may suggest a negative relationship between apparent diet digestibility and N use efficiency at the individual level.

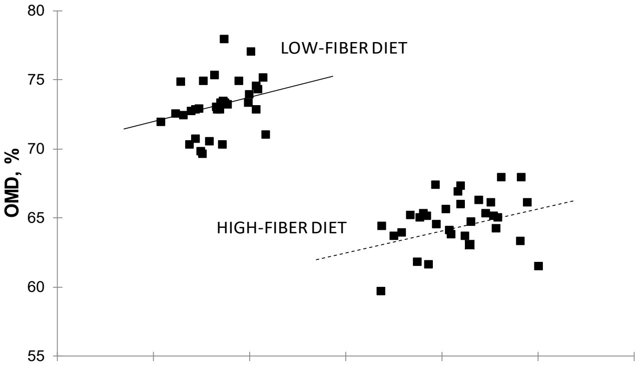


Figure 1 Relationship between faecal natural ^{15}N abundance and apparent dry matter digestibility in beef cows fed both low- and high-fiber diets. Equation Low-Fiber diet: OMD = 66.6 + 1.38 × faecal δ^{15}N. Equation High-Fiber diet: OMD = 54.9 + 1.38 × faecal δ^{15}N.

Conclusion Our hypothesis is rejected: faecal δ^{15}N is not negatively but rather positively correlated to the OMD in animals consuming the same diet. Present results could indicate a trade-off at the individual level between digestibility and N use efficiency.

Acknowledgements This project was supported by APIS-GENE.

References

Cantalapiedra-Hijar G, Ortigues-Marty I., Sepchat B, Agabriel J, Huneau JF and Fouillet H 2015. British Journal of Nutrition 113,1158-1169

INRA, 2018. INRA feedings system for ruminants. Wageningen Academic Publishers, The Netherlands, 640pp

De La Torre A, Andueza D, Beaumont R, Renand G, Rudel S, Genestoux L, Cantalapiedra-Hijar G and Nozière P 2016. In 67th Annual Meeting of the European of Animal Science

Estimation of methane production by lactating cows using spot air samples with different estimating equations

Taketo Obitsu[1], Fuminori Terada[2], Itoko Nonaka[3], Kouji Higuchi[3], Masayuki Hayashi[3], Tomoyuki Suzuki[4], Toshihisa Sugino[1], Yuzo Kurokawa[1]

[1]Hiroshima University, Higashihiroshima-shi, Japan, [2]Tohoku University, Sendai-shi, Japan, [3]National Agriculture and Food Research Organization, Tsukuba-shi, Japan, [4]National Agriculture and Food Research Organization, Nasushiobara-shi, Japan

E-mail: tobitsu@hiroshima-u.ac.jp

Take home message Methane production by individual cows in a practical barn can be quantified using spot air samples from AMS with estimating equations developed using theoretical and empirical approaches.

Introduction Dairy cows constitute a major source of enteric methane (CH_4) emissions in the livestock sector. To assess individual differences in CH_4 emission among dairy cows, a technique using the CH_4:CO_2 concentration ratio for spot air samples from around the cow's head in an automatic milking system (AMS) stall was developed (Madsen *et al*., 2010). To validate this approach, we evaluated variations in CH_4 production related to diurnal fluctuation and estimating equations.

Material & methods In experiment 1, 21 milking Holstein cows reared in a free-stall barn with AMS were used. The milk yield of cows ranged from 18 to 54 kg/day. The cows were provided a partial mixed ration 5 times per day from 0930 to 1630 h. Spot air samples were collected from the inlet attached to the AMS feed box at each milking for four consecutive days. CH_4 and CO_2 concentrations in the collected air samples was analysed using a gas analyser (MGGA, LGR, CA, USA) at 1-second intervals. The concentration ratio of CH_4:CO_2 in air samples at each milking was averaged for each cow. Using the average ratio, 4% fat-corrected milk (FCM, kg/day) yield and body weight (BW, kg) of each cow, daily CH_4 production (L/day) was estimated using the following two equations. Eq. 1 was based on theoretical heat production (HP, kcal/day) of dairy cows (NARO, 2017) and O_2 consumption derived from McLean's equation (McLean, 1986). Eq. 2 was the multiple variable equation established using the data set (n = 121) of CH_4 production by lactating cows obtained using respiration chambers in the Research Center of National Agriculture and Food Research Organization (NARO), Japan.

CH_4 production = HP/a × CH_4:CO_2 (Eq. 1)
a: theoretical conversion factor (20.5/4.184 = 4.90) (McLean, 1986),
$HP = NE_m \times BW^{0.75} + E_{milk}/k_L \times (1 - k_L) \times FCM$ yield, NE_m: net energy for maintenance (116.3 kcal/$BW^{0.75}$),
E_{milk}: milk energy (733 kcal/kg FCM), k_L: efficiency of utilization of metabolisable energy (0.62)
CH_4 production = $-526 + 0.564 \times BW + 8.38 \times FCM$ yield $+ 51.5 \times CH_4:CO_2$ (Eq.2)

The effect of cows and milking time on the CH_4:CO_2 ratio was analysed statistically using a mixed model with JMP, with time as fixed effect and cow and day as the random effects. CH_4 production estimated using the different equations was compared using a paired t-test. Spearman rank correlation was used to examine the persistency of ranking for CH_4 production of individual cows between the two equations.

In experiment 2, 4 lactating Holstein cows (average milk yield±SD, 25±3.6 kg/day) were kept in the respiration chambers after 2 days of adaptation and provided a TMR at 1000 and 1900 h. CH_4 production was calculated using CH_4 concentration in outgoing air and ventilation volume or adapting CH_4:CO_2 ratio for the Eqs. 1 and 2 described above. Additionally, respiratory quotient (RQ) was calculated. CH_4 estimated using the different methods was compared using a paired t-test.

Results In experiment 1, hourly means of the CH_4:CO_2 ratio was higher ($P < 0.05$) during the feeding period (from 1000 to 1700 h) than before start feeding. The range of CH_4:CO_2 ratio for individual cows was 0.079–0.097 (mean, 0.087; CV, 5.6%; cow effect, $P = 0.06$). Although CH_4 production had a positive linear correlation with FCM yield, the mean CH_4 production estimated using Eq. 1 (565 L/day; CV, 14.6%) was slightly (9%) lower ($P < 0.05$) than that estimated using Eq. 2 (616 L/day; CV, 15.0%). However, the ranking of CH_4 production for individual cows was similar ($r_s = 0.975$, $P < 0.01$) between two equations. In experiment 2, CH_4 production by cows (mean±SD) estimated using the CH_4:CO_2 ratio with Eq. 1 (460±63 L/day) or Eq. 2 (488±56 L/day) was slightly (10-15%) lower ($P < 0.05$) than that estimated using CH_4 concentration and ventilation rate (543±64 L/day). However, the corrected values (530±95 L/day) of CH_4 production by Eq. 1 with RQ (1.15±0.054) were close to those obtained using ventilation rate.

Conclusion Similar individual variation in CH_4 production of lactating cows was observed between the theoretical and empirical estimating equations. Inclusion of RQ in the theoretical equation would improve the accuracy of the estimation.

Acknowledgements This study was supported by the Ministry of Agriculture, Forestry and Fisheries of Japan.

References

Madsen J, Bjerg BS, Hvelplund T, Weisbjerg MR and Lund P 2010. Livestock Science 129, 223–227.
McLean JA 1986. British Journal of Nutrition 55, 631-633.
NARO 2017. Japanese Feeding Standard for Dairy Cattle. Japan Livestock Industry Association, Tokyo. Japan.

Timing of grazing in alfalfa: what impact does it have in nutrient intake, rumen environment and digestibility of dairy cows in mid lactation?

Alvaro Santana, Cinthia Fernández, Victoria Zerbino, Eduardo Morales, Micaela García, German Antúnez, Cecilia Cajarville, José Luis Repetto

Facultad de Veterinaria, UdelaR, Montevideo, Uruguay

E-mail: bobasantana@gmail.com

Take home message Fractionating the grazing session lead to a higher intake of alfalfa, allowing dairy cows to maintain DMI and CP intake in respect to TMR diet.

Introduction The aim of this study was to evaluate the effects of inclusion and fractionating of alfalfa grazing sessions in the rumen environment, nutrient (DM, NDF, ADF, CP) intake and digestibility (DM, NDF) in mid lactation dairy cows.

Material & methods Twelve mid-lactation multiparous Holstein cows were assigned to the following treatments in a quadruplicated 3 × 3 Latin square design: 1) total mixed ration (TMR) during 22 h (TMR + 0h), 2) TMR during 14 h plus 8 h of grazing alfalfa in one session after afternoon milking (TMR + 8h) or 3) TMR during 14 h plus two 4 h grazing sessions after each milking (TMR+4+4h). Each period lasted 19d with 12d for adaption and 7d of measurements. Alfalfa (18.3% DM, 35.5% NDF, 20.6% CP) or TMR (36.6% DM, 32.2% NDF, 21.1% CP) intake of DM, NDF, ADF and total N was individually measured every day. Total-tract nutrient digestibility was determined following Huhtanen *et al.,* (1994) using indigestible NDF as an internal marker. The pH was measured with a digital pH meter and NH_3–N concentration was analyzed by spectrophotometry according to Weatherburn (1967). The VFA were analyzed using HPLC (Dionex Ultimate 3000, Sunnyvale, CA). Milk production was individually recorded for 5 d and expressed as kg/d of FCM 3.5%. All data were analyzed using the PROC MIXED procedure of SAS software version 9.1 (SAS Institute Inc., Cary, NC).

Results & discussion The results are shown in Table 1 The inclusion of alfalfa did not affect either fiber intake or digestion depressed DMI and CP intake in respect to TMR diet. Nevertheless, fractionating the grazing session lead to a higher intake of alfalfa, with a similar DMI and CP intake. The high fermentation rate of alfalfa could be responsible for the higher concentration of ruminal NH_3-N for TMR+ 4+4h treatment, and the lower ruminal pH observed for TMR+ 4+4h and TMR+8h.

Table 1 Milk production, nutrient intake and digestibility, ruminal pH, VFA, and NH_3-N of dairy cows in mid-lactation with different allocation time at grazing alfalfa.

Item	TMR+ 0h	TMR+ 4+4h	TMR+ 8h	SEM	Treatment (T)	Hours (H)	T × H
3.5% FCM, kg/d[1]	27.4	28.7	27.6	2.15	0.70	–	–
DMI – Total, kg/d	23.3_{a}	21.7_{ab}	19.4_{b}	0.84	0.03	–	–
DMI –Alfalfa, kg/d	0.0_{c}	8.2_{a}	7.0_{b}	0.26	<.01	–	–
Nutrient intake, kg/d							
CP	5.13_{a}	4.68_{ab}	4.21_{b}	0.171	0.02	–	–
NDF	7.48	7.32	6.55	0.301	0.11	–	–
ADF	4.85	5.11	4.54	0.205	0.20	–	–
Digestibility (%)							
DM	68.8	64.7	65.5	1.65	0.22	–	–
NDF	51.0	46.5	51.0	2.66	0.43	–	–
NH_3-N, mg/100mL[2]	12.3_{b}	16.0_{a}	13.5_{b}	0.61	<.01	<.01	0.01
Ruminal, pH	6.31_{a}	6.14_{b}	6.19_{b}	0.051	<.01	<.01	0.07
VFA Total, mM	107.3	113.2	107.8	6.45	0.67	0.06	0.16
VFA, mol/100 mol							
Acetic acid	71.1	71.8	69.1	1.15	0.20	0.00	0.18
Propionic acid	20.7	20.2	22.2	0.94	0.25	0.01	0.20
Butyric acid	8.2	8.0	8.7	0.32	0.25	<.01	0.00

a,b,c – Within a row means with different subcripts are different ($P < 0.05$). [1]3.5% Fat corrected milk (kg/d).

Conclusion It was possible to include more than 8 kg of alfalfa in the diet of dairy cows without affecting intake, digestibility and milk production. The fractionation of the grazing session contributed to increase intake of alfalfa, maintaining total DMI and CP intake.

References

Huhtanen P, Kaustell K, and Jaakkola S 1994. Animal Feed Science and Technology 48, 211–227.
Weatherburn MW 1967. Analytical Chemistry 39, 971–974.

Effect of *Pithecellobium dulce, Tagetes erecta* and *Cosmos bipinnatus* in reducing rumen methanogenesis in dairy cows

Gloria S. Hernández-Pineda[1], Paulina E. Pedraza-Beltrán[1], Luisa T. Molina[2], Francisca Avilés-Nova[1], Mohammed Benaouda[1], Octavio A. Castelán-Ortega[1]

[1]Universidad Autónoma del Estado de México, Estado de México, Mexico, [2]Molina Center for Energy and the Environment, California, USA

E-mail: oaco2002@yahoo.com.mx

Take home message Supplementation of low doses of *Cosmos bipinnatus* decreases methane emissions by cattle.

Introduction Abundant *in vitro* experiments on the effect of tanniferous plants (TP) on reducing rumen CH_4 production have been conducted in order to find natural alternatives to decrease cattle CH_4 emissions. However, the results on the use of TP in literature are contrasting because in some cases a small CH_4 reduction was observed whereas in others a large reduction was identified. On the contrary, there are few studies where the effect of TP plants on rumen methanogenesis and animal performance have been evaluated *in vivo* (Patra and Saxena 2010). Our group conducted several *in vitro* studies to evaluate the potential of *Tagetes erecta* (Te) and *Cosmos bipinnatus* (Cb) in reducing methanogenesis, observing a maximum reduction of up to 39% and 11% respectively (Gomaa *et al.*, 2017). It is hypothesised in the present work that *Te*, Cb and *Pithecellobium dulce* (Pd) supplemented at low doses can reduce the *in vivo* methane production similarly as they do *in vitro*. Thus, the objective of the present study is to evaluate the effect of these plants in reducing rumen methanogenesis, without affecting milk production and dry matter intake in dairy cattle.

Material & methods A 4 × 4 Latin square experimental design was used, in which four multiparous Holstein cows of 553±72.4 kg live weigh, at their mid lactation and average milk yield of 18±3 kg/day were used. The experiment lasted 92 days divided into four experimental periods of 23 days each. All cows had free access to maize-alfalfa silage in a proportion 50:50, 4 kg of concentrate/day and *ad libitum* access to water. The four treatments consisted of the supplementation of 0.5 kg DM/day/cow of each of the three experimental plants plus the control, which was the di*et al*one with no experimental plant added. The CH_4 production was measured with a respiration chamber of the head-box type. The CH_4 analyser used provides a maximum resolution of 0.0001% to 0.01%. Dry matter intake (DMI) was determined by weighing the diet offered in the morning and collecting and weighing the orts the next morning. The digestibility of the dry matter (DMD) was calculated by subtracting the dry matter weight of the faeces produced daily from the DMI.

Results Table 1 shows the effect of the experimental plants on DMI, DMD, milk yield and CH_4 production. Results show that no significant differences ($p>0.05$) were observed between the control and the treatments for all the productive variables except for CH_4 production (g/d) with Cb. The treatment with Cb reduced CH_4 production in 70.4 g/d (<16%) in relation with the control diet ($p<0.05$). The percentage of CH_4 reduction achieved due to the inclusion of Cb is comparable to the value reported *in vitro* by Gomaa *et al.* (2017) even though the percentage of the TP in the experimental diet was lower *in vivo* than in the *in vitro* trial (3.5% *vs.* 10% of dietary DM), which confirms our hypothesis that Cb reduces methanogenesis when supplement at low doses. Results for Ym suggest a trend towards reducing gross energy conversion to CH_4 production by the inclusion of *Cb* since the DM and GE intakes are almost the same for cows in the Control and Cb treatments. However, more research is needed before conclusive results.

Table 1. Effect of the tanniferous plants on methanogenesis and animal performance

		Treatment		
Item	Control	*P. dulce*	*C. bipinnatus*	*T. erecta*
Live weight, Kg	568 ± 63^{a}	583 ± 57^{a}	576 ± 68^{a}	569 ± 69^{a}
DMI, kg DM/d	14.7 ± 3^{a}	19.7 ± 6^{a}	14.5 ± 3^{a}	16.8 ± 4^{a}
Milk yield, kg/d	17.3 ± 3^{a}	17.4 ± 3^{a}	16.9 ± 3^{a}	15.7 ± 3^{a}
DMD, %	55.6 ± 6^{a}	62.8 ± 9^{a}	58.8 ± 16^{a}	62.5 ± 12^{a}
CH_4, g/d	439.8 ± 69.5^{a}	455.6 ± 75.2^{a}	369.4 ± 29.3^{b}	388.2 ± 35^{a}
CH_4 , g/kg of DMI	30.8 ± 7.8^{a}	24.3 ± 5.7^{a}	26.3 ± 5^{a}	23.8 ± 5^{a}
CH_4, g/kg of DMD	65.3 ± 17.2^{a}	51.9 ± 12.1^{a}	55.9 ± 11.4^{a}	53 ± 12.9^{a}
CH_4, g/kg of milk	26.4 ± 6.4^{a}	27.2 ± 8.6^{a}	22 ± 2.1^{a}	25 ± 3.5^{a}
GE intake, MJ/d	229.0 ± 46.8^{a}	311.7 ± 94.5^{a}	233.2 ± 55.6^{a}	268.4 ± 62.9^{a}
Ym, %	10.9 ± 2.9^{a}	8.5 ± 1.9^{a}	9.0 ± 1.7^{a}	8.3 ± 1.7^{a}

Ym= methane conversion factor, values in brackets are standard deviation. ^{ab} significant differences between treatments (P<0.05).

Conclusion Results show that supplementation of low doses of Cb has potential to reduce ruminal methanogenesis in dairy cattle without affecting productive variables. No effect was observed with the other TP tested in this work.

Acknowledgements This research was supported by the Molina Center for Energy and the Environment under UNEP Contract GFL-4C58, and the Universidad Autónoma del Estado de México grant UAEM 3474/2013CHT.

References

Gomaa MR, González-Ronquillo M, Arredondo-Ramos J, Molina LT and Castelán-Ortega OA 2017. Ecosistemas y Recursos Agropecuarios 4, 371-380.
Patra A K and Saxena J 2010. A. Phytochemistry 71, 1198–1222.

Potential new indices of phosphorus status in growing beef steers

Stephen Anderson[1], Simon Quigley[2], Natasha Steiger[1], Dennis Poppi[2]

[1]University of Queensland, St Lucia, Queensland, Australia, [2]University of Queensland, Gatton, Queensland, Australia

E-mail: stephen.anderson@uq.edu.au

Take home message Plasma Ca and vitamin D concentrations may help define the phosphorus status of growing steers.

Introduction Phosphorus (P) is a key limiting nutrient during the wet season in many extensive beef cattle production systems in northern Australia, South America and South Africa. In contrast, excess dietary P is a major issue in intensive dairy cattle production systems in many countries. To determine optimal P nutrient intake in cattle it is necessary to define the P status of animals, the P content of the basal diet and the amount of P required to achieve production targets. Inorganic P concentration in the circulation is commonly used as the best index of P status, but it exhibits acute responses to changes in dietary P intake and is therefore more indicative of dietary P content rather than P status of the animal. Other markers that reflect physiological and/or biochemical processes, in particular gut uptake or bone metabolism, over the long-term may better define P deficiency or P excess. Here we examined new biomarkers of P status in growing beef steers.

Material & methods Thirty *Bos indicus* crossbred steers (228±2 kg) were fed *ad libitum* treatment diets of increasing P content (0.9, 1.3, 1.8, 2.1 and 2.4 g/kg DM) for a 6-month period. Dry matter intake and liveweight were recorded, and indicators of P status were measured in plasma, faeces and bone samples obtained throughout the experimental period. Full details of methods and production responses are published elsewhere (Quigley *et al*. 2014). Plasma concentrations of inorganic P and total calcium (Ca) were determined by standard biochemistry. Plasma 1,25-dihydroxy vitamin D (calcitriol) concentrations were measured by radioimmunoassay (Immunodiagnostic Systems). Results were analysed by ANOVA, with post-hoc Tukey test for comparison between treatments.

Results & discussion Plasma P concentrations of steers offered the 1.8 g P/kg DM diet were intermediate between low P diets (0.9P and 1.3P) and high P diets (2.0P and 2.4P), and had a concentration of 1.6 mM that could be considered as adequate P status. Low P diets were associated with increased plasma Ca concentration, with hypercalcemia (>2.8 mM) being observed in all steers on the 0.9P diet. Such an inverse relationship between plasma Ca and P in steers is similar to that previously described in growing sheep with low P diets (Breves *et al*., 1985), and thought to be the result of increased bone resorption to maintain P homeostasis. Indeed the plasma Ca to P concentration ratio may be a simple index of bone mineral mobilisation in cattle with P deficiency (Anderson *et al*., 2017), and in the current study steers consuming low P diets exhibited high Ca to P ratios (Table 1). However low P diets in growing steers were also associated with increased plasma calcitriol concentrations indicating that, at least in long-term P deficiency, increased gut uptake of Ca and P is a normal physiological response. Further steers on the highest P diet (2.4P) had markedly reduced plasma calcitriol concentrations. Such differences in plasma calcitriol concentrations challenge the notion that P does not regulate vitamin D in ruminants. Ongoing analyses of markers over time will provide better insight into physiological mechanisms that respond to changes in diet P intake, and the validity of potential new markers of P status.

Table 1 Plasma mineral and vitamin D concentrations in steers 4 months after commencement of diets.

Treatment P g/kg DM	0.9	1.3	1.8	2.1	2.4
P mM	1.01 ± 0.06^{a}	1.09 ± 0.11^{a}	1.60 ± 0.01^{b}	2.18 ± 0.17^{c}	2.19 ± 0.13^{c}
Total Ca mM	3.17 ± 0.10^{a}	2.84 ± 0.01^{b}	2.69 ± 0.03^{bc}	2.62 ± 0.03^{c}	2.52 ± 0.03^{c}
Ca to P ratio	3.2 ± 0.3^{a}	2.7 ± 0.3^{a}	1.7 ± 0.1^{b}	1.2 ± 0.1^{c}	1.2 ± 0.1^{c}
Calcitriol pg/mL	199 ± 36^{a}	195 ± 51^{a}	97 ± 16^{b}	50 ± 10^{bc}	31 ± 4^{c}

Values are mean ± SEM. Superscripts denote a significant (P<0.05) difference, Tukey's post-hoc test.

Conclusion Long-term P deficiency in steers is associated with hypercalcemia and increased calcitriol, whilst high P diets suppressed calcitriol. Plasma calcitriol and plasma Ca to P concentration ratio may indicate the P status of growing steers.

Acknowledgements This study was funded by Meat and Livestock Australia.

References

Anderson ST, Kidd LJ, Benvenutti MA, Fletcher MT and Dixon RM 2017. Animal Production Science 57, 2291-2303.
Breves G, Ross R and Höller H 1985. Journal of Agricultural Science (Cambridge) 105, 623-629.
Quigley SP, Poppi DP and Shatz TJ 2014. Meat and Livestock Australia Report B.NBP.0537. ISBN 9781740362627

Effect of rumen-protected methionine supplementation on feed efficiency and performance of post-weaning growing Charolais bulls fed a high-forage diet

Lahlou Bahloul[1], Evan Titgemeyer[2], Bernard Sepchat[3], Isabelle Ortigues-Marty[4], Gonzalo Cantalapiedra-Hijar[4]

[1]Adisseo France SAS, Antony, France, [2]Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS 66506-1600, USA, [3]Herbipôle, INRA, Saint-Genès-Champanelle, 63122, France, [4]Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores,, Saint-Genès-Champanelle, 63122, France

E-mail: lahlou.bahloul@adisseo.com

Take home message The principle of limiting amino acids (AA) as observed in dairy cows and swine appears to apply to growing beef cattle as well.

Introduction Improvements of gain in response to supplementation with rumen undegradable protein in growing cattle fed high-forage diets indicate that rumen-bypass protein may correct for AA deficiency and increase protein deposition (Titgemeyer and Löest, 2001). Because methionine (Met) has been identified as the first limiting AA for ruminant growth from microbial protein (Storm and Ørskov, 1984), which is the predominant metabolizable protein source in forage-fed ruminants, Met can be expected to be a limiting AA in growing beef cattle fed high-forage diets (Titgemeyer and Merchen, 1990). The objective was to evaluate the gain in feed efficiency (FE) and beef cattle performance when high-forage diets formulated at two protein levels (High *vs* Normal) were supplemented with rumen-protected Met (RP-Met) in post-weaning growing Charolais bulls.

Material & methods Thirty-six growing-fattening Charolais bulls (age 286±21d; weight 336±7.2 kg) were randomly assigned to a 2 × 2 factorial design. During 7 months of a feed efficiency test, bulls were fed 60:40 grass silage:concentrate diets. Iso-net energy diets were formulated for two metabolizable protein levels [120% (High) *vs* 100% (Normal) of INRA PDI requirement] through the inclusion of variable proportions of tanned soybean meal. Two levels of dietary Met were formulated within each protein level: control (1.9% PDIE) *vs* balanced (2.4% PDIE) profile using RP-Met (Smartamine M). Measures of individual dry matter intake (DMI; kg/d) and body weight (BW, kg) were conducted daily and fortnightly, respectively. Feed was sampled weekly and pooled throughout the experiment. Average daily gain (ADG; kg/d) was determined individually as the difference between final and starting BW divided by the feeding period in days. FE (%) was calculated as ADG divided by DMI. Carcass composition (fat and muscle in kg and %) was estimated from sixth rib dissection. Blood samples were collected on days 50, 99 and 150 and analysed for urea-N.

Results Compared to the control, RP-Met supplementation increased ADG of young bulls (P = 0.02) with a trend for a greater effect at High *vs* Normal protein level (Protein x Met interaction P = 0.10). Although FE and DMI were not significantly affected by RP-Met supplementation, this improvement in ADG was due to the combination of nonsignificant increases in FE (+5%; P = 0.11) and DMI (+3%; P = 0.26) in response to balancing Met. Blood urea concentration tended to be lower (P = 0.09) with RP-Met supplementation (-28%), suggesting greater N efficiency for this group. Final BW at slaughter was greater (+4%; P = 0.005) for balanced Met *vs* control group, and this was independent of dietary protein content. Fat carcass content, expressed in %, increased with RP-Met supplementation (P = 0.03) in agreement with the exponential response of body lipid content to body weight gain assumed by ruminant feeding systems.

Table 1 Responses to supplemental protein and RP-Met.

Item	Diets Normal	Normal + Met	High	High + Met	SEM	P-Value Prot	Met	ProtxMet
DMI, kg/d	8.25	8.41	8.21	8.57	0.23	0.80	0.26	0.68
Final BW, kg	659	673	678	720	9.63	0.001	0.005	0.14
ADG, kg/d	1.53	1.57	1.56	1.78	0.06	0.03	0.02	0.10
FE, %	18.6	18.7	19.0	20.6	0.68	0.06	0.11	0.21
Urea, g/l	0.10	0.11	0.22	0.16	0.02	0.001	0.09	0.09
Muscle, kg	292	296	305	312	6.18	0.02	0.39	0.77
Muscle, %	73.4	73.1	73.5	72.3	0.40	0.32	0.05	0.30
Fat, kg	52.3	54.7	54.9	62.5	1.76	0.005	0.005	0.14
Fat, %	13.1	13.5	13.2	14.4	0.35	0.13	0.03	0.24

Conclusion Supplementation with rumen-protected methionine had a positive and significant effect on the growth rate of fattening young bulls fed high-forage diets.

Acknowledgements The project was funded by Adisseo S.A.S. France and conducted at INRA-VetAgroSup, UMRH-Theix.

References

Titgemeyer EC and Löest CA 2001Journal of Animal Science 79(E-Suppl), E180-E189.
Titgemeyer EC and Merchen NR (1990). Journal of Animal Science, 68, 750-757.
Storm E and Orskov ER 1984. The British Journal of Nutrition, 52, 613-620.

Chitin as an indigestible marker could be used to measure fecal output in dairy cows by NIRS

Seppo Ahvenjärvi[1], Esa Mäntysaari[1], Martin Lidauer[1], Juha Nousiainen[2], Laura Nyholm[2]

[1]*Natural Resources Institute Finland, Jokioinen, Finland,* [2]*Valio Oy, Helsinki, Finland*

E-mail: seppo.ahvenjarvi@luke.fi

Take home message Chitin, determined by near-Infrared reflectance spectroscopy (NIRS), can be used to measure fecal output in dairy cows.

Introduction Measuring fecal output is an elemental part of animal nutrition studies. Firstly, nutrient digestibility is determined based on fecal output combined with feed intake measurements. Secondly, indirect methods to determine feed intake often rely on fecal output measurements using external markers coupled with diet digestibility based on internal markers. The objective of this study was to evaluate chitin as a marker to measure fecal output in dairy cows and predict their daily feed intake using digestibility estimated by an internal marker indigestible NDF.

Material & methods An experiment was conducted to measure fecal output in rumen-cannulated dairy cows using chitin as an external marker. Chitin was a commercial product isolated from crab shells. The experiment consisted of two periods (period 1 and 2), each that lasted for 26 days. Six lactating Nordic Red dairy cows were divided into two groups of three cows. Each group was fed a total mixed ration consisting of grass silage and concentrate mixture. The proportion of concentrates in the diet was either 40 or 60% of dry matter (DM). Diets offered in period 1 were changed between cow groups at the beginning of period 2. Feed intake of each cow was measured daily as a difference between DM offered in feed and recovered in refusals. Fecal DM output was measured by 1) total collection feces and 2) by reference to chitin intake and concentration in feces. Chitin (279 g/d) was administered directly into the rumen via rumen fistula twice daily at 0600 and 1800 h. Chitin concentrations in feces were determined by NIRS. To calibrate the NIRS analysis for determination of chitin, 96 fecal samples with known chitin concentration were prepared.

Results Chitin was completely recovered in feces (99.5%). This finding was consistent with similar fecal DM output measured using total collection method or estimated using chitin (7.98 *vs* 7.99 kg/d). However, the relationship between fecal DM output estimated using chitin or measured by total collection indicated substantial variation between individual observations (Figure 1, standard error of prediction 0.67 kg/d). The relationship between measured DM intake and that estimated based on fecal output estimated using chitin and using the same digestibility for all cows (Figure 2, standard error of prediction 2.0 kg/d).

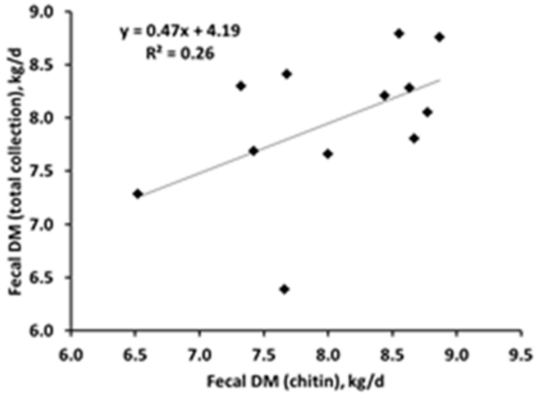


Figure 1 The relationship between fecal DM outputs estimated using chitin or measured by total collection.

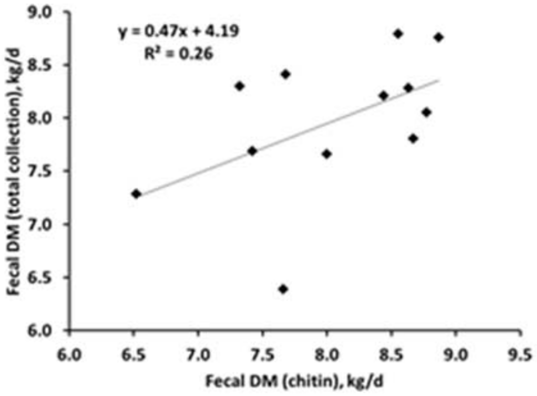


Figure 2 The relationship between measured dry matter intake and that estimated using chitin and indigestible NDF.

Conclusion Complete recovery of chitin in feces and accurate fecal output measurements indicate that chitin is a promising marker that can be analyzed using NIRS. However, further work is needed to improve the precision of chitin concentrations measurements in feces. Owing to low precision of estimated fecal DM output measurements using chitin as a marker, the precision of feed intake estimates was compromised.

Prediction of methane emissions from dairy cows by near infra-red spectroscopy of faeces using a portable instrument

Giulia Ferronato[1], Ali R. Bayat[2], Johanna Vilkki[2], Luigi Calamari[1], Paolo Bani[1]

[1]*Università Cattolica del Sacro Cuore, Piacenza, Italy,* [2]*Natural Resources Institute Finland (Luke), Jokioinen, Finland*

E-mail: paolo.bani@unicatt.it

Take home message Predicting methane emissions from dairy cows using near infrared (NIR) spectroscopy of faecal samples provided promising results which must be confirmed.

Introduction Knowledge on methane (CH_4) emissions represents a crucial point to establish nutritional and genetic mitigation strategies, particularly in dairy cows. As technical and economic problems hinder the possibility of large scale direct measurements of CH_4 emissions, there is a need for proxies that allow indirect measurements (Negussie *et al.,* 2017). The aim of this study was to verify preliminarily the possibility of developing calibration curves for the CH_4 emissions phenotypes from NIR spectra of dried faeces samples acquired by a portable instrument.

Material & methods The study was conducted using 34 Nordic Red dairy cows from calving to 112 days after calving. The animals were fed three diets including a diet based on grass silage and concentrate (55:45 on DM basis), the same diet supplemented with rumen-protected conjugated linoleic acid or with a concentrate high in starch and containing a mixture of sunflower and fish oil. Ruminal CH_4 emission was measured using respiratory chambers during 4 days after 1 day of acclimatization in each of four measurement periods (3, 7, 11 and 15 weeks of lactation), together with dry matter intake (DMI) and milk yield and composition. Total faecal collection was conducted in the same time with CH_4 measurement. Representative samples of faeces were oven dried at 55°C and ground to pass a 1 mm sieve. A total number of 115 faecal samples were used. Near infra-red (NIR) reflectance measurements were performed with a portable Micro-NIR spectrometer (VIAVI Solutions Inc., San Jose, CA, USA). The spectra acquired were processed using partial least square (PLS) method (SAS 9.4 Statistical Analysis System 9.4, SAS Institute Inc., Cary, USA). The validation was assessed using cross validation method by the leave-one-out sample procedure with number of leaving samples equal to 1. The accuracy of the validation curves was assessed considering the coefficient of determination (R^2_{val}), the root mean square error of cross-validation (RMSECV) and the residual prediction deviation (RPD_{val}).

Results & discussion Table 1 shows the results obtained with the validation of the prediction model of four CH_4 phenotypes. Best results were obtained for CH_4 production expressed as g/day (R^2_{val}=0.58, RPD_{val}=1.55) (Figure 1). Methane yield (g/ kg DMI) displayed a R^2_{val}=0.49. Methane intensity expressed as g/kg of milk (R^2_{val} =0.46, RPD_{val}=1.36) showed better results compared to when it was reported as g/kg of energy corrected milk (ECM; R^2_{val} =0.30, RPD_{val}=1.19). The predictive potential was comparable to other proxies summarized by Negussie *et al.,* (2017).

Table 1. Validation of the prediction models.

Parameter	Frequency regions (nm)	Extracted factors	R^2_{val}	RMSECV	RPD_{val}
CH_4, g/day	1602-1370.7; 1297.7-1139.5; 1066.4-914.3	9	0.58	65.5	1.55
CH_4, g/kg DMI	1675-1522.9; 1449.8-1291.6; 1218.6-1066.4	10	0.49	1.68	1.40
CH_4, g/kg milk	4675-1522.9; 1370.7-1218.6; 1066.4-914.3	10	0.46	1.41	1.36
CH_4, g/kg ECM	1602-1443.7; 1297.7-1139.5	8	0.30	1.34	1.19

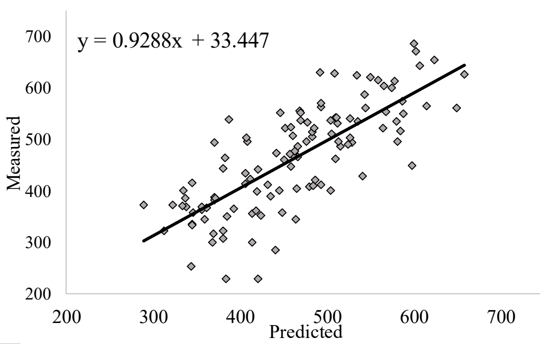


Figure 1 Linear regression of measured versus predicted CH4 (g/day); y = 0.9288x + 33.447.

Conclusion The results obtained in this study suggest the possible interest of NIR spectroscopy applied on dried faecal samples as a proxy to predict CH_4 emissions from lactating dairy cows fed different diets. The possibility to analyse fresh faeces should be also investigated. These preliminary results could be improved by including a greater number of animals kept under different management and dietary conditions.

References

E. Negussie, de Haas Y, Dehareng F, Dewhurst RJ, Dijkstra J, Gengler N, Morgavi DP, Soyeurt H, van Gastelen S, Yan T, Biscarini F 2017. Journa/ of Dairy Science 100, 2433-2453.

In vitro methane and ammoniacal nitrogen production of Marandu grass supplemented with lipid sources

Rayanne Costa[3], Luciano Cabral[1], Leni Lima[1], Bruno Pedreira[2], Mirceia Mombach[1], Nelcino de Paula[1], Karine Camargo[3], Juliam Kely Rocha[1]

[1]UFMT, Cuiabá - MT, Brazil, [2]Embrapa, Sinop - MT, Brazil, [3]FCAV/UNESP, Jaboticabal - SP, Brazil

E-mail: rayannevianacosta@gmail.com

Take home message The corn oil appears to be efficient in mitigating methane production in 24 hours.

Introduction Ruminal fermentation is a process resulting from the set of microbial activities (metabolism) that transform dietary constituents into products considered useful to animals, such as volatile fatty acids, or useless as methane gas (CH_4) which represents a consequent energy loss, resulting in lower animal performance (Valadares Filho and Pina, 2006), besides being considered as one of those responsible for the greenhouse effect. Aiming at efficient production, ruminal fermentation modulation strategies have been used to maximize the fermentation of cellulosic compounds, to reduce fermentation losses (methane and ammonia) and to optimize the synthesis of microbial protein. One of these is the inclusion of lipids in the diet. However, little is known about its effect on tropical forages. The objective of this work was to evaluate the isolated effect of lipid sources from corn and soybean on the production of methane and ammoniacal nitrogen using the *in vitro* gas production technique.

Material & methods Two lipid sources (corn and soybean oil) and their inclusion levels (0, 3, 6 and 9% DM) were evaluated in a tropical forage diet (*Brachiaria brizantha* cv. Marandu), divided into a 2×4 factorial by means of the *in vitro* gas production technique. Ruminal fluid (inoculum) from 2 adult donor cattle, provided with permanent ruminal cannulae. The inoculum samples were collected and filtered in a cloth with a porosity of 250 μm and packed in preheated thermos flasks with water at 39 ° C. The pH was measured and the CO_2 kept constant, until the moment of inoculation. In the 120 ml amber flasks, 500 mg of forage (CP = 99.5 g, NDF = 640 g / kg DM) or the association thereof with the treatment levels. 40 mL of McDougal's buffer (1949) duly reduced with addition of reducing solution were added and inoculated with 10 mL of ruminal liquid, under a constant CO_2 sparge. The vials were sealed and placed in a water bath at 39 ° C under constant stirring. At 24 and 48 hours incubation, a pressure gas volume and pressure (Data Logger GN200) and a gas sample (10 mL) of each vial were collected using a graduated syringe and samples of its contents and were used for the determination of methane. The determination of methane concentration was carried out by means of a Gas Chromatography (GC-2014 Shimadzu Corporation, Kyoto, Japan). The NH_3-N was determined according to the methodology described by AOAC (1990). The datas of the factorial design 2×4 (two sources of oils and four levels of inclusion) were analyzed using the MIXED procedure of SAS (version 9.3) The model includes fixed source effect, oil inclusion level and oil source interaction × inclusion level. Differences between treatments were declared significant at $P<0.05$.

Results & discussion Methane concentration was higher in treatments supplemented with soybean oil (4.09 mg/mL) when compared to corn oil (2.00 mg/mL) ($P <0.05$) at the 24 hours, however, there was no effect after 48 hours of fermentation. The level of oil had no effect on methane or ammonia production (Table 1). However, there was an interaction between lipid source and inclusion level at 48 hours ($P <0.05$) for NH_3-N. This indicates that the concentration of NH_3-N produced probably differ in the presence of different sources of lipids in the rumen (Villaça *et al.,* 1999). As a consequence, as concentrations of NH_3-N, independently of the lipid source, can be strongly related to the high degree of unsaturation of the same, which may contribute to a reduction of the protozoan population (Ivan *et al.,* 2001).

Table 1 *In vitro* concentration of methane and NH_3-N as a function of the inclusion of sources and levels of oil under fermentation of Marandu grass.

Parameters	Corn oil [a]				Soybean oil [a]				SE	P-value		
	0	3	6	9	0	3	6	9		Oil	Level	O*L
24 hours												
Methane [b]	1.76	2.87	2.16	1.21	3.42	2.90	5.54	4.51	2.38	0.031	0.778	0.530
NH_3-N [c]	3.49	2.57	2.47	3.37	2.05	2.29	2.82	2.28	0.64	0.186	0.930	0.513
48 hours												
Methane [b]	6.16	5.58	5.74	8.88	4.71	6.69	13.62	5.89	2.62	0.530	0.374	0.174
NH_3-N [c]	2.51	1.96	3.92	1.75	1.56	4.56	1.60	2.04	0.61	0.835	0.119	0.004

[a]: % DM; [b]: mg/dL; [c]: mL/L; SE: standard error.

Conclusion In our experimental conditions, corn oil appears to be efficient in mitigating methane production in 24 hours and is an efficient short-term strategy for animals fed with tropical forage.

References

Ivan M, Mir PS and Koenng KM 2001. Small Ruminant Reseach 41, 215-227.
Valadares Filho SC and Pina DS 2006. Nutrition of Ruminants 151-182.
Villaça M, Ezequiel JMB and Kronka SDN 1999. Brazilian Journal of Animal Science 654-659.

Mean retention time of particles and solutes on the digestive tracts of growing Saanen goats: an exploratory approach

Marcelo Gindri, Rafael Leite, Antonio Ferraudo, Izabelle Teixeira

São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal, São Paulo, Brazil

E-mail: marcelo_gindri@yahoo.com.br

Take home message Body weight, fibre intake, and fibre digestibility play an important role in predicting solute mean retention time in goat reticulorumens. Further, particle mean retention time in reticulorumen and cecum are related in goats.

Introduction Digesta mean retention time (MRT) plays an important role on digestive efficiency in ruminants (Ellis, 1978). However, studies of factors influencing MRT have been poorly investigated in growing goats, especially at the level of total gastrointestinal tract (GIT). Few studies have evaluated MRT in goats by adopting the multivariate approach, neglecting the possible relationship between factors influencing MRT. Exploratory multivariate factor analysis (MFA) focuses on understanding the interrelationship of the measured variables. Moreover, MFA may be useful for developing biological models. Thus, the objective of this study was to identify variables related to the MRT of particles and solutes of growing Saanen goats, using MFA.

Material & methods Our dataset involved 103 individual records of castrated males (n = 36), females (n = 34), and intact males (n=33) growing Saanen goats slaughtered at 15, 22, 30, 37, and 45 kg BW. All animals were fed *ad libitum* with a similar diet (45% of forage in offered diet, dry matter (DM) basis; 191 ± 25.5 and 379 ± 44.7 g/kg of DM of crude protein and neutral detergent fibre (NDF), respectively). Variables evaluated in our dataset were BW (kg), DM intake (DMI, kg/day), NDF intake (NDFI, kg/day), MRT of particles (MRT_p, h) and solutes (MRT_s, h) in GIT segments, ratio of indigestible NDF (iNDF) and NDF (iNDF:NDF ratio) of ingested diet, total NDF digestibility (1 – [(iNDF:NDF ratio of ingested diet)/(iNDF:NDF ratio of colon-rectum (CR) digesta)]; NDF_d), wet weight of GIT tissues segments (kg), wet weight of digesta pool size of GIT segments (kg), and digesta DM concentration of GIT segments (g/kg of wet digesta). The MRT was assessed by using the method of emptying the GIT compartments in slaughtered animals, adopting iNDF and Cr-EDTA as makers for particles and solutes, respectively. The evaluated GIT segments were reticulorumen (RR), omasum (O), abomasum (Ab), small intestine (Si), cecum (Ce), and CR. The statistical analysis was performed with the psych package (Revelle, 2014) in R version 3.4.2 (R Core Team). First, a Pearson correlation matrix was computed. Bartlett's test and Kaiser–Meyer–Olkin (KMO) tests were applied on the correlation matrix to assess the database adequacy to MFA (*i.e.,* identify if there is a latent structure of dependency among our dataset variables). Parallel analysis was used to choose the number of factors. Finally, the MFA was computed. Varimax as orthogonal rotation and maximum likelihood as factoring method were used in the parallel analysis and MFA. To describe factors, we considered factor loadings > |0.50|.

Results Bartlett's test ($P < 0.01$) and KMO value (0.85) indicated that there is a latent structure of dependency among the variables of our dataset. According to parallel analysis, three mutual orthogonal factors retained a considerable amount of the original variability of the dataset variables (65%). The first factor retained 31% of the original variability of the dataset, and it was positively related to BW, DMI, NDFI, wet weight of all GIT tissues segments, and wet weight of digesta pool size of RR, O, Si, and CR. The second factor retained 24% of the original variability of the dataset, and it was positively related to iNDF:NDF ratio of ingested diet, wet weight tissues of Ab, RR, and O, MRT of solutes in RR, O and CR, and BW, and negatively related to NDF_d, and NDFI. Finally, the third factor retained 10% of the original variability of the dataset, and it was positively related to wet weight of Ce digesta pool size, MRT_p in RR and Ce, and MRT_s in Ce. Furthermore, DM concentration of digesta of all GIT segments, wet weight of Ab digesta pool size, MRT_p in CR, and MRT_s in Ab and Si were not related to any factor.

Conclusion The MRT_s in RR, O, and CR, wet weight of RR, O, and Ab tissues, BW, iNDF:NDF ratio of ingested diet, NDFI and NDF_d are related in growing Saanen goats. Among those, we suggest that BW, iNDF:NDF ratio of ingested diet, NDF_d and NDFI are candidate variables to be predictors of MRT_s in RR of growing Saanen goats because they can conveniently be recorded *in vivo*. Furthermore, MRT_p in RR and Ce, MRT_s in Ce, wet weight of Ce digesta pool size are related in growing Saanen goats. Our findings may improve our understanding about digestive physiology of goats and help the development of nutrition models for goats.

Acknowledgements National Council for Scientific and Technological Development, Brazil (CNPq, scholarship #141615/2017-6) and São Paulo Research Foundation (FAPESP, grant #2008/57302-0).

References

Ellis WC 1978. Journal of Dairy Science 61, 1828-1840.
Revelle W 2014. Psych: procedures for personality and psychological research. R package version, 1(1). Northwestern University, Evanston.

Application of NX-RH-201 reduces methane production in a rumen simulation technique (Rusitec) experiment

Melanie Eger, Susanne Riede, Gerhard Breves

Department of Physiology, University of Veterinary Medicine Hannover, Hannover, Germany

E-mail: melanie.eger@tiho-hannover.de

Take home message NX-RH-201, a combination of garlic and citrus compounds (Mootral™), is effective in reducing ruminal methane production in an *ex vivo* system (Rusitec) without negative effects on rumen fermentation.

Introduction Methane emissions by livestock contributes to global warming and results in a loss of digestible energy for the ruminant. Therefore, numerous approaches to reduce methane emissions have been investigated (Riede *et al.*, 2013, Duarte *et al.,* 2017). However, results are often varying and some compounds exhibit negative effects on fermentation parameters. Organosulphur compounds may reduce methane production without impairing organic matter degradation (Soliva *et al.,* 2011). Here, NX-RH-201 (Mootral™), a feed additive containing organosulphur compounds from garlic combined with citrus polyphenols was applied in the rumen simulation technique to investigate its effects on rumen fermentation patterns and methane production.

Material & methods The rumen simulation technique (Czerkawski and Breckenridge, 1977) was used to test the effects of two different dosages of Mootral™. Rumen contents of two German Holstein cattle were used to start the experiment. The experiment consisted of an equilibration phase of 7 days, an experimental phase of 7 days and a withdrawal phase of 4 days. Twelve fermentation vessels were used for the experiment. Each fermentation vessel received a daily substrate of 7 g hay and 3 g concentrate. During the experimental phase four different treatments were applied: three vessels each were used as controls (CON), received 1g of Mootral™ per day (LD), 2 g Mootral™ (HD) or 4.1 mg of monensin (MON). In the withdrawal phase, no additions were made. During the whole experiment, pH and redox potential were measured daily. The concentration of short chain fatty acids (SCFA) was assessed by gas chromatography and the concentration of NH_3-N was measured photometrically from daily effluent samples during experimental and withdrawal phase. Fermentation gas was collected in gas bags and the percentages of methane and carbon dioxide were quantified by gas chromatography. Statistical analysis of the last days of experimental period (day 14) and withdrawal period (day 18) was performed using GraphPad Prism 7.02. Data were assessed for normal distribution of residuals by applying the Kolmogorov-Smirnov test. Two-way ANOVA for repeated measurements was applied to detect significant effects of the factors Time, Treatment or Interactions of Time x Treatment, in case of significances Sidak post-test was applied.

Results Several parameters of ruminal fermentation were affected by treatment. During application of the compound methane production was reduced to 4.8% of the production in CON fermenters by LD treatment, to 0.3% by HD treatment and to 29.7% in MON treated fermenters. At day 18, methane production in LD, HD and MON group accounted for 53.7%, 6.6% and 37.9% of CON values. Application of LD or HD resulted in a significant lower pH at day 14 and day 18 compared to MON treatment (Treatment: $p < 0.01$). However, the effect was less pronounced at day 18 (Time $p < 0.001$, Interaction, $p = 0.07$). Redox potential was more negative for HD fermenters compared to MON fermenters only at day 14 (Time $p < 0.05$, Interaction $p < 0.01$). The concentration of NH_3-N tended to be elevated at day 14 in HD fermenters (Interaction: $p = 0.08$). The total production rate of SCFA was significantly increased in HD fermenters compared with CON and MON fermenters and in LD fermenters compared with MON fermenters; this effect was more pronounced on day 14 compared to day 18 (Treatment $p < 0.001$, Time $p < 0.01$).

Conclusion The mixture of organosulphur compounds from garlic combined with citrus polyphenols effectively reduced methane production in the rumen simulation technique, while exhibiting positive effects on SCFA production. Most effects were still present after 4 days of withdrawal indicating a slow reduction of the effects.

Acknowledgments We thank Zaluvida Corporate AG for providing Mootral™ for this study.

References

Czerkawski JW and Breckenridge G 1977. British Journal of Nutrition 38, 371-384.
Duarte AC, Holman DB, Alexander TW, Kiri K, Breves G and Chaves AV 2017. Frontiers in Microbiology 8, 1076.
Riede S, Boguhn J and Breves G 2013. Archives of Animal Nutrition 67, 368-380.
Soliva CR, Amelchanika SL, Duval SM and Kreuzer M 2011. British Journal of Nutrition 106, 114-122

Complete nitrogen balance in dairy cows: does every drop of sweat count?

Nadège Edouard[1], Angèle Suzanne[1,2], Philippe Lamberton[1], Benoît Rouillé[2], Philippe Faverdin[1]

[1]PEGASE, INRA, Agrocampus-Ouest, Rennes, France, [2]Institut de l'élevage Service Productions laitières, Paris, France

E-mail: nadege.edouard@inra.fr

Take home message Complete N balance measurements should consider N flows through cows' sweat as a potential contribution to pollutant emissions, in relation with feeding and climatic conditions.

Introduction Estimation of nitrogen use efficiency and losses towards the environment are often based on nitrogen balance measurements at the animal level. However, these N balances are most of the time positive in the literature, unaccounted N representing between 2 and 8% of the nitrogen ingested and being positively linked with the diet N level (Spanghero and Kowalski, 1997). If this unaccounted N probably has minor consequences on efficiency calculation (N intake and N in milk measurements being reliable), it could have strong impacts whether these losses contribute or not to the pollutant emissions from the system (*e.g.,* NH_3, N_2O). Urea is the major N metabolic waste and is mostly excreted in the urine. Urea is also know to diffuse passively and should therefore be found in all aqueous body compartments. In addition, Cutullic *et al.* (2013) suggested that the unaccounted N could be positively related to the animal water balance (evapotranspiration) above thermoneutrality. We therefore tested the hypothesis that sweat contains nitrogen in the form of urea, all the more that diet N level is high, and that the consideration of this flow could contribute to reduce unaccounted N levels.

Material & methods As a preliminary step, we elaborated a device for the recovering of sweat on the animal skin surface, composed of an acidified compress placed under a plastic capsule, maintained on the cow hip (one per hip) for 24h. The experiment was performed in closed and controlled experimental rooms. It followed a double Latin 4 x 4 square design, using 8 Prim'Holstein dairy cows, during 4 periods of 3 weeks. We compared 2 diet N levels (N-: diet poor in degradable nitrogen, 13.5%CP; N+: diet rich in degradable nitrogen, 17.5%CP), 2 ambient temperatures (T-: 18°C during the whole day; T+: diurnal peak of 28°C for 7 hours, 20°C during the night) and their interaction. The first week was used as an adaptation period to the diet (N- or N+). The temperature factor (T- or T+) was applied during the second and third weeks. Complete N balance measurements were performed including N losses through the cows' sweat (for half of the cows).

Results & discussion The diet N level greatly affected N intake and N excretion in urine (Table 1). N in milk was also 10 g/cow/day higher for N+ because of a higher milk production (+1 kg/cow/day). Temperature did not seem to influence N balance parameters even if a tendency ($p<0.1$) could be observed for N secreted in urine and sweat. Sweat urea content was on the average comprised between 150 and 300 mg/L, tending to be higher for T+ ($p<0.1$). These levels, believed to be minimums considering potential losses during sampling analyses, were quite close to plasma urea contents. However, inter and intra-cow variations were very elevated.

Table 1 Effects of nitrogen level and temperature and on N balance parameters (g/cow/day).

		N-		N+		RMSE	N effect[1]	T effect[1]	N×T effect[1]
	n	T-	T+	T-	T+				
N intake	31	402	393	539	534	25	***	ns	ns
N in milk	31	144	141	156	152	9	***	ns	ns
N in urine	31	121	109	220	208	18	***	t	ns
N in faeces	31	119	115	117	115	10	ns	ns	ns
N in sweat	15	3	5	4	6	2	ns	t	ns

[1] Results from the ANOVA model, ns: non-significant, t: $p<0.1$, ***: $p<0.001$

Conclusion This study showed that sweat contained urea, leading to N losses between 8 and 21% of unaccounted N at the animal level. At the skin surface, this urea could volatilize and contribute to gas emissions at the barn level. More research is now needed to refine the sweat recovering device to decrease inter and intra-cows variations and ensure that urea is not lost during sampling. Considering this flow should contribute to better evaluate complete N balance at the animal level in contrasted feeding and climatic situations.

Acknowledgements This work was supported by Ademe.

References

Spanghero M and Kowalski ZM 1997. Livestock Production Science 52, 113-122.
Cutullic E, Faverdin P, Edouard N and Peyraud, JL 2013. Report of the FP7 EU RedNex project.

Effect of two herbage allowances on dry matter intake and methane emissions of primiparous beef cows in continuous grazing

M. Soledad Orcasberro[2], Cecilia Loza[2], José Gere[4], Pablo Soca[3], Laura Astigarraga[1]

[1]*Facultad de Agronomia-UDELAR, Montevideo, Uruguay,* [2]*Facultad de Agronomia-UDELAR, Cerro Largo, Uruguay,* [3]*Facultad de Agronomia-UDELAR, Paysandú, Uruguay,* [4]*Unidad Tecnológica Nacional-Facultad regional de Buenos Aires (UTN-FRBA), CONICET, Buenos Aires, Argentina*

E-mail: sorcasberro@fagro.edu.uy

Take home message Forage management could optimize DMI, and possibly reduce CH_4 emissions.

Introduction The use of different management practices has shown that there is great potential to reduce greenhouse gases (GHG) emissions produced by livestock, through improved grazing management (Becoña *et al.,* 2014). Recently, new management technologies of forage allowance in rangeland have shown promising results in beef production per unit area (Soca *et al.,* 2013), but the impact on relevant environmental variables such as methane emissions (CH_4) has not been quantified yet. Besides, there is no further information on CH_4 emissions from pregnant heifers grazing natural grassland in Uruguay. Therefore, the aim of the study was to evaluate the effect of two native pasture allowances on CH_4 emissions and dry matter intake (DMI) in primiparous cows kept on native pastures in Uruguay.

Material & methods The trial included 20 heifers (10 per treatment, Hereford and Aberdeen Angus), from the second third of gestation (May 2015) until weaning (April 2016), with two treatments of herbage allowance (8 and 5 kg DM/kgLW, annual average) under continuous grazing. Herbage allowance (HA) was adjusted monthly, through the appraisal of available forage and the "put and take" method to adjust animal live weight (LW) to the intended HA. Every month, experimental cows were weighted and body condition score (BCS, 1-8 scale) was determined. During May (second third of gestation), August (late gestation) and November (lactation), CH_4 emissions were determined according to Johnson *et al.,* (1994) adapted by Gere and Gratton (2010) and DMI was estimated using chromium oxide as an indigestible marker. The experiment was a completely random design. Data was analysed with the Mixed procedure of SAS (2001), as repeated measures. Since there was no interaction between the treatment and the sampling period ($P > 0.05$), this data is not shown in the results.

Results Both daily methane emission (HA8: 214±13.9 *vs.* HA5: 203±13.9 g/d, P=0.58) and DMI (HA8: 9.2±0.41vs HA5:8.6±0.41 kg DM/d, P=0.27), did not differ according to the herbage allowance, which resulted in similar CH_4 emission per kg of DMI (23.9±1.94 *vs* 24.0±1.94 g CH_4/kg DMI, respectively, P=0.98) and methane yield Ym (7.8±0.65 *vs* 7.9±0.65, P=0.87). In the same way, LW adjusted for uterine weight (342±12.1 *vs* 344±12.1 kg, P=0.91) and BCS (4.0±0.11 *vs* 3.9±0.11 P=0.57) were similar between treatments. However, all variables were significantly affected by the period of measurement ($P \leq 0.0001$), with a marked increase in November (spring), except for CH_4 per kg of DMI (P=0.76) and Ym (P=0.76) that remained similar to the values observed in the previous periods of measurements.

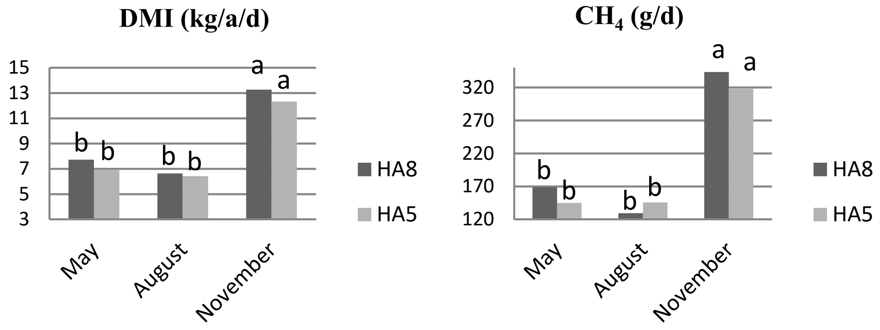


Figure 1 Effect of two herbage allowances on dry matter intake and methane emissions of primiparous beef cows in continuous grazing in three periods of measurements (means with a common letter are not significantly different, $P > 0.05$).

Conclusion The results show that the CH_4 emissions and DMI were significantly affected by the period of measurement, but not by the level of forage allowances used in this study, while CH_4 per kg of DMI remained unchanged. Besides, they are considered relevant since they are the first data obtained on methane emissions in pregnant heifers grazing natural grasslands in Uruguay.

Acknowledgements This research was supported by grants from the National Agency for Research and Innovation (ANII) and the Faculty of Agronomy-UDELAR.

References

Becoña G, Astigarraga L and Picasso V 2014. Sustainable Agriculture Research 3, 89-105.
Gere J and Gratton R 2010. Latin American Applied Research 40, 377-382
Johnson K, Huyler M, Westberg H, Lamb B and Zimmerman P 1994. Environmental Science Technology 28, 359–362.
Soca P, Carriquiry M, Do Carmo M, Scarlato S, Astessiano AL, Genro C, Claramunt M and Espasandín AC. 2013d. Seminario de actualización técnica: Cría Vacuna. Montevideo: INIA. 97-118 p.

Between-cow variation in rumen fermentation and omasal and milk fatty acids associated with methane production

Jonas de Souza[1], Heidi Leskinen[2], Kevin Shingfield[4], Adam Lock[1], Pekka Huhtanen[3]

[1]Department of Animal Science, Michigan State University, East Lansing, MI, USA, [2]Animal Genomics, Green Technology, Natural Resources Institute Finland (Luke), Jokioinen, Finland, [3]Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, Umeå, Sweden, [4]Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, UK

E-mail: desouzaj@msu.edu

Take home message Our results demonstrate that while some milk fatty acids (FA) are positively or negatively associated with methane predictions, these estimates are also influenced by energy balance and endogenous synthesis of FA.

Introduction Since direct measurements of methane (CH_4) are difficult to perform under regular farm conditions, the development of prediction equations to estimate CH_4 output has gained considerable interest (Ramin and Huhtanen, 2013). In this regard, milk FA profile can reflect changes in absorbed FA composition, which in turn is affected by ruminal metabolism of FA and microbial synthesis (Fievez *et al.,* 2012), and may therefore predict important changes in the ruminal fermentation. The potential utilization of milk FA to predict CH_4 has been extensively studied and models have selected several different FA as potential CH_4 predictors, which suggests an influence of other dietary and animal factors affecting these estimates. Therefore, the examination of the between-animal differences in a large data set originating from variations in digestion physiology and rumen microbial ecology may help elucidate this variation. The objective of our meta-analysis was to evaluate between-cow variation and repeatability in fermentation variables and omasal and milk FA contributing to methane emission. We hypothesized that some milk FA can be related with estimates of CH_4 output, but only a small portion of the variation is attributed to between-cow differences.

Material & methods Data were taken from 9 studies with rumen-cannulated dairy cows, conducted using either a Latin square or switch-back design, in the Nordic countries. Digesta flow measurements were conducted using omasal sampling with a triple-marker system. Production of CH_4 and volatile FA (VFA) were calculated based on VFA stoichiometry (CH_4VFA). Cows in this data set had a wide range of milk production (5.0 to 41.8 kg/d), BW (490 to 770) and ME balance (-62.3 to 71.2 MJ/d). The major FA flowing to omasum was C18:0 with a wide range of omasal flow (7.2 to 72.6 g/100 g FA). There was a large variation in the summation of de novo (14.2 to 34.9 g/100 g FA), mixed (19.6 to 43.5 g/100 g FA) and preformed milk FA (24.8 to 64.2 g/100 g FA). Variance components of the selected variables were calculated by taking into account experiment, diet within experiment, period within experiment, and cow within experiment as random factors. Empirical models were developed between the variables of interest regarding their biological value by univariate and bivariate mixed model regression analysis.

Results & discussion The omasal flow of odd- and branched-chain FA (OBCFA) had higher variation associated with diet than between-cow variation with very low repeatability (<0.15). For *de novo* milk FA, the variation associated with diet was ~ 3-fold higher than the between-cow variation; repeatability for these FA was moderate (0.34–0.58). For milk OBCFA, the variation associated with diet was more than double the between-cow variation with low repeatability (0.15-0.18). Also, for both C18:1 *cis*-9 and C18:3 *cis*-9 *cis*-12 *cis*-15 (preformed milk FA) diet variation was more than double the between-cow variation, but repeatability was moderate (0.45). Among the *de novo* milk FA, C4:0 ($p < 0.01$) was positively related with stoichiometric CH_4VFA, while for OBCFA, C15:0 anteiso ($p < 0.01$) and C15:0 ($p < 0.01$) were negatively related with stoichiometric CH_4VFA. Importantly, for all the OBCFA when evaluating the relationship between omasal FA and milk FA we observed positive intercept estimates (all $p < 0.01$), which may indicate endogenous post-ruminal synthesis of these FA. In order to evaluate whether energy balance and FA omasal flow would affect milk FA profile, we evaluated the relationship among these variables. Interestingly, energy balance affected the concentration of several milk FA. For milk OBCFA, C13:0 iso, C15:0 iso, C15:0 anteiso, and C15:0 were positively influenced by omasal flow of their respective FA (all $p < 0.01$) and by energy balance ($p < 0.05$). In contrast, the concentration of milk C17:0, C18:0 iso, C18:0, C18:1 *cis*-11, and C18:3 *cis*-9 *cis*-12 *cis*-15 were positively influenced by omasal flow of their respective FA (all $p < 0.01$) but negatively associated with energy balance ($p < 0.05$).

Conclusion Our results indicate that for most omasal and milk FA examined, a larger variation is attributed to diet than between-cow differences with low to moderate repeatability. Although some milk FA were positively or negatively associated with CH_4VFA, there was a pronounced effect of energy balance on these estimates. Additionally, although OBCFA have been indicated as markers of rumen function, our results indicate that endogenous synthesis of these FA may occur. Therefore, this likely limits the utilization of milk FA as a proxy for methane predictions.

References

Fievez V, Colman E, Castro-Montoya JM, Stefanov I and Vlaeminck B 2012. Animal Feed Science and Technology 172, 51–65.

Ramin M and Huhtanen P 2013. Journal of Dairy Science 96, 2476– 2493.

Modelling variations in partition of carbon balance in lactating ruminants

Daniel Sauvant, Sylvie Giger-Reverdin

AgroParisTech-INRA, Paris, France

E-mail: sauvant@agroparistech.fr

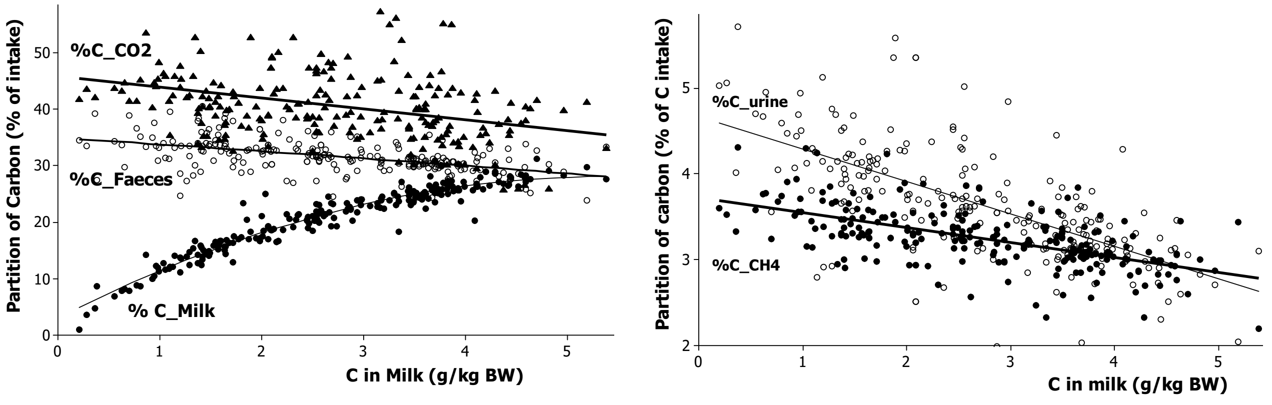
Take home message Carbon partition in lactating ruminants provides C flows of various significations in terms of environmental impact. Its major factor of variation is milk production level: when it increases, partition of C in faeces, CH$_4$, urine and CO$_2$ decrease.

Introduction Knowledge on the variations of carbon (C) balance in dairy ruminants is necessary to accurately quantify C flows in farming systems, especially in life cycle analyses. Therefore, this study was performed to quantify the various inflows and outflows of C and their major variations in lactating cows and goats.

Material & methods For the current purpose, a database pooling results of calorimetric studies carried out on lactating ruminants (80 experiments, 227 treatments, trt, 180 on lactating cows, 47 on lactating goats) was developed. Calculations of the flows of C from the various flows of organic matter (diet, faeces, milk) consider that the proportions of C is, as classically assumed, of 0.45, 0.52 and 0.76 in carbohydrates, proteins and lipids respectively. The measured energy losses as CH$_4$ (75 g C/Mcal) and urine (105 g C/Mcal) were translated in terms of matter and then flows of C. Data on heat production (HP) were used to evaluate expired CO$_2$ (111 mgC/Mcal HP; Pedersen *et al.*, 2008).

Results& Discussion The dietary C content was of 446.2 ± 13.9 g/kg dry matter, for milk it was of 92.6±0.78 g C/Mcal. There was no significant difference between dairy cows and goats when C flows were expressed on a Body Weight (BW) basis. In these lactating ruminants, the flows of C expressed on a BW basis (g C/kg BW) associated with intake, faeces, CH$_4$, urine, CO$_2$, milk were respectively of 13.13 ± 3.43, 4.23 ± 1.33, 0.43 ± 0.13, 0.47 ± 0.19, 5.35 ± 1.35 and 2.70 ± 1.15 g/kg BW. Resulting body C balance is of 0.06 ± 0.82 g/kg BW. When reported on the basis of 100 for C intake, the partition of ingested C between %C_faeces, %C_CH$_4$, %C_urine, %C_CO$_2$, % C_milk were respectively of 31.7 ± 6.12, 3.28 ± 0.80, 3.65 ± 1.42, 40.69 ± 6.52 and 19.71± 6.32 %.

Milk production level is the 1st factor of variations in C partition. Figures 1 and 2 present the major intra-experiment trends. When C produced in milk increased, its coefficient of C partition increased curvilinearly (%C_Milk = 32.0 (1-exp$^{(-0.41\ C)}$) with a maximum asymptotic value of 32%. For the 4 other variables the trends were linear and they decrease at respective rates of -1.26, -0.17, -0.41 and -1.70 %/g C in milk/kg BW for %C_faeces, %C_CH$_4$, %C_urine and %C_CO$_2$. Beyond this factor, the residual variations around these relationships are partly caused by feeding factors.



Figures 1 and 2 Intra-experiment trends in partition of C intake in function of level of C produced in milk.

Conclusion Partitions of C are different according to the considered flows, in lactating ruminants partitions vary mainly in function of the level of milk production with a decrease of % of C lost in faeces, CH$_4$, urine and CO$_2$ when C in milk increases.

References

Pedersen S, Blanes-Vidal V, Joergensen H, Chwalibog A, Haeussermann A, Heetkamp MJW and Aarnink AJA 2008. Agricultural Engineering International 10, 1-19.

Protein supply of grazing dairy cows supplemented with concentrates compared to dairy cows feda total mixed ration

Dirk von Soosten, Ulrich Meyer, Julia Hartwieger, Sven Dänicke

Institute of Animal Nutrition, Friedrich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health, Braunschweig, Germany

E-mail: ulrich.meyer@fli.de

Take home message Concentrate supplementation to grazing dairy cows is a feeding strategy to overcome the discrepancy between nitrogen excess and energy availability for rumen microbes.

Introduction Microbial efficiency is a result of availability of nitrogen and degradability of carbohydrates in the feedstuffs. Due to an unfavourable relationship of nitrogen (excess) to energy (limited) for rumen microbes on full pasture, efficiency of ruminal mCP synthesis is diminished. Therefore, the objective of the present study was to investigate the effect of a concentrate supplementation during grazing on efficiency of mCP synthesis in dairy cows.

Material & methods Nine pluriparous lactating German Holstein cows fitted with rumen and duodenum cannulae were used in an experiment lasting for 12 weeks (wk). Animals were divided in two groups. Four animals of a confinement group (CG) received a total mixed ration (TMR) composed of 35% maize silage, 35% grass silage and 30% concentrate (on a dry matter (DM) basis) for *ad libitum* consumption during the trial and were housed in a freestall barn. The remaining five animals were allocated to a pasture group (PG) and were slowly introduced to pasture [wk0,1: TMR CG, wk2: 3 h pasture access/d + TMR CG, wk3,4: 12 h pasture access/d + TMR CG, wk5-11: pasture-only + 4.5 kg concentrate (DM)/d (day)]. In week 9 samples of duodenal chyme were taken immediately after milking times (twice a day) for five consecutive days and pooled over the sampling period according to von Soosten *et al.,* (2016). For the calculation of the daily duodenal dry matter flow (DMF) chromium oxide (Cr_2O_3) was used as a marker. The microbial N fraction of the duodenal non-ammonia N was estimated by near infrared spectroscopy (NIRS; Lebzien and Paul, 1997) to calculate daily duodenal mCP flow. The fermented organic matter (FOM) in the rumen, the utilizable crude protein (uCP) and the undegradable crude protein (UDP) were calculated according to equations presented by Aschemann *et al.,* (2012). Data were analysed by using STATISTICA software version 12.

Results The mCP flow at the duodenum was lower in the PG compared to the CG. The FOM (% of OM intake) in the rumen was similar in both groups. The microbial efficiency expressed as mCP per kg FOM remained unchanged. The amount of UDP at the duodenum related to crude protein intake was not influenced, while uCP flow at the duodenum was higher in the CG. All data are presented in Table 1

Table 1 Influence of concentrate supplementation to grazing dairy cows (PG) compared to animals receiving a total mixed ration in confinement (CG) on FOM, mCP, protein degradation and uCP (Values presented as means ± standard deviation).

	CG (n = 4)	PG (n = 5)	p-value
mCP (g/day)	2291 ± 430	1376 ± 198	0.007
FOM (g/kg of OM intake)	550 ± 40	590 ± 80	0.529
mCP/FOM (g/kg)	184 ± 7	173 ± 65	0.772
mCP/ME intake (g/MJ)	8.6 ± 0.5	8.3 ± 1.9	0.786
UDP (g/kg of CP)	270 ± 70	320 ± 20	0.258
uCP (g/d)	3284 ± 368	2229 ± 189	0.002

mCP=microbial crude protein; FOM=fermented organic matter; ME=metabolizable energy; UDP=undegradable crude protein; uCP=utilizable crude protein.

Conclusion In the present study grazing animals with concentrate supplementation synthesized less mCP at comparable microbial efficiency to the CG.

Acknowledgements This research was supported by grants from the Ministry for Science and Culture, Hannover, Germany.

References

Aschemann M, Lebzien P, Hüther L, Südekum KH and Dänicke S 2012. Archives of Animal Nutrition 66, 303-318
Lebzien P and Paul C 1997. Animal Feed Science and Technology, 68, 225-233.
von Soosten D, Meyer U, Hüthere L and Dänicke S 2016. Proceedings of the Society of Nutrition Physiology 25, 50.

Meta-analysis of relationships between mastication time, particles retention time and dry matter intake in cattle

Daniel Sauvant[1], Pierre Nozière[2], René Baumont[2]

[1]*AgroParisTech-INRA, 75231 Paris Cedex 05, France,* [2]*UMR Herbivores INRA-VetagroSup, 63122 Saint Genes-Champanelle, France*

E-mail: sauvant@agroparistech.fr

Take home message Mastication time has curvilinear relationships with retention time of particles and intake in cattle with opposite influences around approximatively 10 h of mastication. The two involved fill factors seem to be rumen motricity and particles comminution.

Introduction Level of dry matter intake (DMI%Body Weight), mean residence time of particles in the rumen (MRT, d) and, more rarely, mastication time (Mast, h) are key parameters of the recent feed unit systems. Sauvant *et al.* (2014) observed a negative curvilinear relationship between DMI%BW and MRT (DMI%BW = 1.25 + 3.79 exp(-0.83 MRT), n=122, nexp=53, RMSE=0.19), the purpose of this study is to look more precisely the simultaneous relationships between Mast and both MRT and DMI%BW.

Material & methods A selected subset of the database "Bovidig" concerned only 50 experiments with simultaneous measurements of DMI%BW, MRT and Mast. MRT is the result of the ratio Rumen DM load/fecal outflow of DM. The interpretation was performed by a meta-analysis to focuse on the 2 by 2 intra-experiment regressions.

Results & discussion There is a curvilinear intra-experiment relationship between Mast and MRT (Figure 1):

MRT = 2.62 – 0.202 Mast + 0.0105 Mast² (n=115, nexp=50, RMSE=0.16) [1]

Figure 1 shows that there is a minimum value of MRT when Mast = 9.62 h. Moreover, there is no difference between dairy cows (open squares) and growing cattle (black circles) in this relationship. Moreover, there is a curvilinear intra-experiment relationship between DMI%BW and Mast, but with an inverse curvature of the equation [1] (Figure 2):

DMI%BW = 1.50 + 0.264 Mast - 0.0112 Mast² (n=113, nexp=50, RMSE=0.25) [2]

The maximum value of DMI%BW is achieved for Mast = 11.00h which is close to the value corresponding to the minimum of MRT. Again there is also no difference in the intra-experiment response of dairy cows and growing cattle. For diets poorly masticated (Mast=5.58±2.25 h for diets with Mast<8h) which have a low content of NDF from forage (20.2±11.8%DM), the longer transit time is likely the outcome of the simultaneous decrease of the rumen motricity (Sauvant *et al.,* 2018) and thus an increased rumen fill which could contribute to reduced DMI%BW. For more masticated diets (Mast=13.82±1.14 h for diets with Mast>12h) which have a higher NDF from forage (34.1±16.6%DM) the increased fill is likely due to the limitation of the efficiency of the comminution work. Both effects are quite consistent with the negative relationship between MRT and DMI%BW (Sauvant *et al.*, 2014).

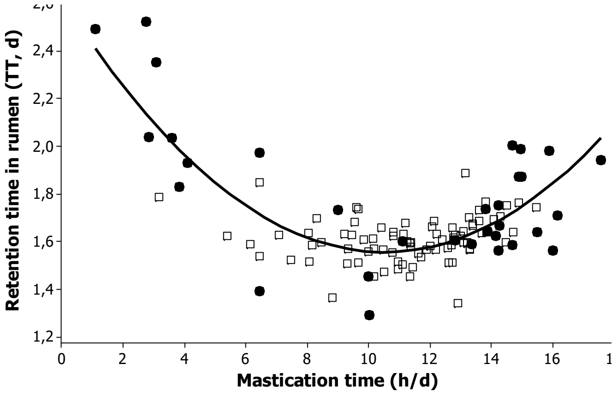


Figure 1 Influence of mastication on particles MRT.

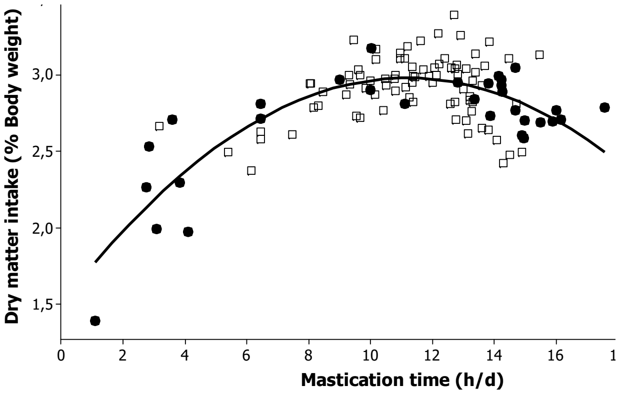


Figure 2 Relationship between mastication and DMI%BW.

Conclusion Mastication time presents a curvilinear relationship with MRT in cattle stressing two major reasons of fill effect, the lack of motricity for low masticated diets and the limited capacity of comminution for highly masticated diets. These simultaneous variations in Mast and MRT explained at least partly the intra-experiment variations of DMI.

References

Sauvant D, Nozière P and Baumont R 2014, Animal Production Science 54, 2012-2020.

Sauvant D, Giger-Reverdin S and Peyraud JL, 2018. Digestive welfare and rumen acidosis. Chapter 15 in INRA 2018, "Inra Feeding System for Ruminants" Ed Wageningen Academic Publishers, Wagenigen, The Netherlands, pp. 213-218.

Hepatic mitochondrial density of mature beef cows grazing different herbage allowances of native pastures

Alberto Casal[1], Adriana Cassina[3], Mercedes Garcia-Roche[2,3], Pablo Soca[1], Mariana Carriquiry[2]

[1]Departamento de Produccion Animal y Pasturas- Facultad de Agronomia EEMAC – Universidad de la Republica, Paysandu, Uruguay, [2]Departamento de Produccion Animal y Pasturas- Facultad de Agronomia – Universidad de la Republica, Montevideo, Uruguay, [3]Center for Free Radical and Biomedical Research (CEINBIO) and Departamento de Bioquímica, Facultad de Medicina – Universidad de la Republica, Montevideo, Uruguay

E-mail: alcas@adinet.com.uy

Take home message Hepatic mitochondrial density increased with energy intake in cows grazing native pastures

Introduction Mitochondria are recognized to be central for meeting energy demands and maintaining metabolic homeostasis (Rolfe and Brand, 1997). Their abundance and activity would reflect the capacity of cells for energy generation (Harper *et al.,* 2002). Do Carmo *et al.*, (2016) showed increased productivity (productive/reproductive response; *i.e.,* +19% and +15% of kg of weaned calf) in cows grazing high *vs.* low herbage allowances of native pastures as well as in crossbred *vs.* purebred cows. Thus, we hypothesized that greater cow productivity would be associated with improved efficiency in energy metabolism. The aim of this study was to evaluate the effect of controlling the intensity of grazing native pasture (bioma Campos), through control of herbage allowance, on hepatic mitochondrial density of beef cows of different genotypes.

Material & methods Mature beef cows (n=32) were used in a complete randomized block design with a factorial arrangement of herbage allowance (2.5 *vs.* 4 kg DM/d; LO *vs.* HI) of native pastures (52% DM, 8.4% CP 39.7% NDF), and cow genotype (Angus-Hereford *vs.* F1 reciprocal crosses; PU *vs.* CR). The experiment was conducted during three years and at the end of the third year at 192± 10 days postpartum cows were slaughtered, and hepatic samples were collected, stored at -80 °C and total DNA and RNA isolated. The mitochondrial density was determined by mtDNA:nuclear DNA ratio [NDUFV1 (nDNA) and CO1 (mtDNA)], mRNA expression of citrate synthase (CS) and CS activity. Relative mRNA expression of genes encoding for *CS* and mitochondrial respiratory chain proteins (*NDUFC1, SDHA, UQCRH, COX19,* A*TP5E)* were determined using SYBR-Green real time qPCR. Data were analyzed using a mixed model that included herbage allowance, cow genotype and their interaction as fixed effects and block as a random effect.

Results & discussion The hepatic mtDNA:nDNA ratio, *CS* mRNA and activity were greater ($p \leq 0.04$) in HI than LO cows (Figure 1) but were not affected ($p \geq 0.46$) by cow genotype or their interaction. (239.5 *vs.* 234.5 ± 12 arbitrary units (AU), 0.79 *vs.* 0.74 ± 0.04 AU; 46 vs 42 ± 2 mU/mg of liver protein for hepatic mtDNA:nDNA ratio, *CS* mRNA and activity for HI *vs.* LO respectively). The hepatic gene expression of mitochondrial respiratory chain proteins *NDUFC1*, *SDHA*, *UQCRH*, *COX19* and ATP5E mRNA were not affected ($p \geq 0.25$) by herbage allowance, cow genotype or their interaction. The liver is a highly active metabolic tissue and is the central organ of metabolism in ruminants. The mitochondrial pathways are not only where main ATP synthesis occurs but also constitutes one of the fundamental pathways in the utilization of propionate (generated at ruminal level) for hepatic neoglucogenesis. Indeed, as Do Carmo *et al.,* (2016) estimated the energy consumption was 10-12% higher in cows grazing high *vs.* low herbage allowances of native pastures, the increased mitochondria density would suggest a hepatocyte adaptation to nutrient utilization.

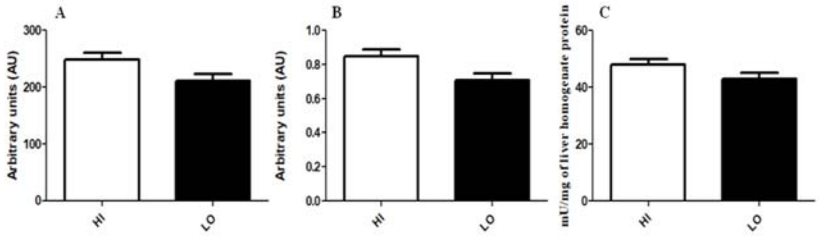


Figure 1 Hepatic mitochondrial density markers, mtDNA:nDNA ratio (**A**), Citrate synthase mRNA expression (**B**) and Citrate synthase activity (**C**), of beef cows grazing high (HI) and low (LO) herbage allowances of native grasslands (means ± se; $p > 0.05$).

Conclusion Cows grazing high herbage allowance would present a greater density of mitochondria in the liver tissue than cows grazing a low herbage allowance, and this effect was independent of the genotype of the animals. This could be associated to an increased metabolic activity associated with greater cow energy intake and productivity.

Acknowledgements This research was supported by grants from research projects INIA-FPTA2007 #262 and CSIC Grupos I+D awarded to M. Carriquiry and graduate fellowship ANII #POS_2011_1_3328 awarded to A. Casal.

References

Do Carmo M, Claramunt M, Carriquiry M and Soca P 2016. Journal of Animal Science 94, 84-92.
Harper ME, Antoniou A, Bevilacqua L, Bezaire V, and Monemdjou S 2002. Journal of Animal Science 80, E90-E97.
Rolfe DFS and Brand MD 1997. Bioscience reports 17, 9–16.

Nitrogen isotopic signatures in mixed rumen cellulolytic bacteria differ depending on the nitrogen source

Gonzalo Martinez-Fernandez[1], Gonzalo Cantalapiedra-Hijar[2], Diego Morgavi[2], Chris S. McSweeney[1]

[1]*CSIRO, Agriculture & Food, Queensland Bioscience Precinct, St Lucia, QLD, Australia,* [2]*INRA, Université Clermont Auvergne, VetAgro Sup, UMR Herbivores, St Genès Champanelle, France*

E-mail: mar778@csiro.au

Take home message Nitrogen isotopic signatures in rumen bacteria could reflect the efficiency of N utilization in the rumen.

Introduction Nitrogen exists in the environment as two stable isotopes forms: ^{14}N and ^{15}N (less abundant and heavier). A promising biomarker for N use efficiency in ruminants is based on the N isotopic discrimination ($\Delta^{15}N_{animal-diet}$; the difference between animals and their diet in natural abundance of ^{15}N). The more efficiently animals assimilate dietary N, the closer are δ ^{15}N values in animal proteins and diet. Therefore, this approach may have potential to discriminate individuals with different N partitioning when fed the same diet. However, little is known about the contribution of rumen bacteria to the isotopic N discrimination and published studies (Wattiaux *et al.,* 1995) have not simulated the low N intake levels prevailing in some farming systems, such as in northern Australia. The natural ^{15}N enrichment of rumen bacteria over the diet previously reported (Cantalapiedra-Hijar *et al.,* 2016) could stem from an imbalance between two N isotope discriminating pathways in the rumen: ammonia uptake by bacteria (mainly through glutamate dehydrogenase favoring ^{14}N) *vs* ammonia release from bacteria (amino acid deaminase favoring ^{14}N). The objective of this experiment was to study the influence of different levels and sources of N on the N isotopic signatures of rumen fibrolytic bacterial communities from northern Australia cattle rumen inoculum.

Material & methods An *in vitro* experiment was conducted in batch cultures to assess the effects of two sources of N supplied at 5 levels. Organic (peptone) and inorganic (ammonium chloride) N were compared as N sources. The five levels of N used were: 1.5, 3, 4.5, 6 and 12 mM N (final concentration in the media) plus a baseline level (0.5 mM ammonia-N) without an extra N source added. The inoculum source was obtained from cattle grazing in northern Australia during the dry season. A selective media for cellulolytic bacteria with cotton thread and acid swollen filter paper as cellulose sources was used (adapted from McSweeney *et al.,* (2005)). The media was adjusted to have a maximum ammonia-N concentration of 0.5 mM. A commercial wireless system (AnkomRF Gas Production, Ankom Technology, NY, USA) consisting of bottles equipped with pressure sensor modules and a reception base station connected to a computer was used to release pressure. After 120 h of incubation at 39 ºC, the fermentation was stopped by placing the bottles in ice and the content collected for VFA, ammonia and N isotopic analyses from the bacterial pellet. The effect of level, N source and their interaction were analysed for the variables as a univariate analysis of variance using the GLM procedure of SPSS (IBM, version 21.0), with the bottle as the experimental unit. Effects were considered significant at $P \leq 0.05$. When significant differences were detected, differences among means were tested by pairwise comparisons (LSD test).

Results & discussion The bacteria totally degraded the cotton cellulose source by all the treatments and levels after 120 h of incubation. A significant ($P < 0.001$) interaction by N source and levels was observed for ammonia-N, VFAs and ^{15}N. When bacteria were cultured with inorganic N (ammonium chloride), the bacterial pellet was increasingly depleted in ^{15}N compared to the baseline level (from -2.1 to -5.50; $P < 0.001$). In contrast, when the N source was organic N (peptone) the bacterial pellet was increasingly enriched in ^{15}N compared to the baseline level (from -2.1 to 0.58; $P < 0.001$). Ammonia-N concentration, was not significantly different between the two N treatments but a significant effect was observed by level of N used, increasing from 26 to 172 and 151 mg ammonia-N/L for baseline and level 5 of ammonium chloride and peptone respectively. Total VFAs concentration and profile were significantly different by level and nitrogen source. This suggest that the bacterial ammonia uptake:release ratio was highly different between the two N sources, with higher values obtained when ammonium chloride was used.

Conclusion The results indicate that N isotopic signature in rumen bacteria may change according to the balance between synthesis of microbial protein from ammonia versus non-ammonia N sources. Further studies should determine whether natural ^{15}N abundances in rumen bacteria could also reflect the efficiency of N utilization for bacterial protein synthesis in the rumen.

Acknowledgements This research was supported by the INRA-CSIRO linkage program.

References

Cantalapiedra-Hijar G, Fouillet H, Huneau JF, Fanchone A, Doreau M, Noziere P and Ortigues-Marty I 2016. Animal 10, 221-229.
McSweeney CS, Denman SE and Mackie RI 2005. In Methods in gut microbial ecology for ruminants, 23-37
Wattiaux MA and Reed JD 1995. Journal of Animal Science 73, 257-266

Effect of a saponins-containing additive on the digestive and productive performance of dairy goats.

A. Ignacio Martín-García[1], Alejandro Belanche[1], Pierre Chicoteau[2], Pierre Caillis[2], David R. Yáñez-Ruiz[1]

[1]Estación Experimental del Zaidín (CSIC), Granada, Spain, [2]Nor-Feed, Beaucouzé, France

E-mail: ignacio.martin@eez.csic.es

Take home message An additive with saponins improves the N balance in goats after 6 weeks of use.

Introduction Increasing attention has been placed on the use of natural products such as saponins-containing plants extracts with proven antimicrobial properties that can improve the overall performance of ruminants by slowing down the release of N, enhance fermentation and microbial protein synthesis (MPS) and reduce urinary excretion of N (Santoso *et al.,* 2006). Saponins can reduce the rumen protozoa and increase volatile fatty acid (VFA) production, suggesting that it may be effective in shifting the rumen carbon balance to increase carbohydrate digestion (Belanche *et al.,* 2015). However, the literature also consistently describes a gradual decrease of effectiveness of saponins after 7-10 days of administration, mainly by the activity of some rumen bacteria. Alternatively, it could be hypothesized that using a blend of several types of saponins (such as the commercial saponin plants blend additive SPB) could minimize this microbial adaptation. The objective of this study was to investigate the long-term effects of including SPB in the diet of dairy goats on body weight (BW), *in vivo* digestibility, milk yield and composition and rumen fermentation characteristics.

Material & methods The study was conducted using three groups of Murciano-granadina goats (n=6) receiving, respectively, 0, 1 and 3.3 g/goat/day of SPB during six weeks in early lactation. Animals were offered a 40:60 (F:C) diet with a 21.8% CP content. The milk yield was recorded daily and milk samples were collected weekly for analysis. The BW was noted weekly. After six weeks goats were housed in individual pens for 5 days to monitor daily intake, and afterwards, before the morning feeding, a 50 ml sample of rumen content was obtained via oral probe to study the fermentation profile. Then goats were allocated in metabolic cages to perform a digestibility trial. The BW, diet intake and milk yield and composition data were analysed by GLM repeated measures procedure, including as covariable the corresponding values for each parameter obtained before the SPB administration. The digestibility and rumen fermentation data were analysed by ANOVA using SPSS.

Table 1 Effect of a saponin plants blend additive (SPB) on the digestive and productive performance of dairy goats.

SPB dosage (g/d)	0	1	3.3	SEM	P	SPB dosage (g/d)	0	1	3.3	SEM	P
Body weight, kg	43.3	43.2	43.2	0.6	ns	DM digestibility, g/kg	708	704	676	23.7	ns
Total intake, kg/d	2.00	1.97	2.01	0.11	ns	OM digestibility, g/kg	736	733	704	7.3	†
FPCM[1] yield, kg/d	2.26	2.33	2.16	0.50	ns	CP digestibility, g/kg	798	770	782	7.13	ns
Lactose, %	5.05^{a}	4.94^{b}	5.00^{ab}	0.03	*	PD[2], µmol/kg $BW^{0.75}$	110^{b}	126^{b}	296^{a}	36.9	*
Milk total solids, %	13.9	13.5	13.6	0.3	ns	Urine N, g/kg $BW^{0.75}$	1.44	1.02	1.41	0.072	†
Total rumenVFA, mM	96.6	86.5	91.9	4.1	ns	N bal.[3], g/kg $BW^{0.75}$	1.56	2.26	2.08	0.160	†
Ac/Prop in the rumen	2.99	3.58	3.05	0.21	ns	Ret. N[4], g/kg $BW^{0.75}$	1.00	1.53	1.32	0.142	†
Rumen lactate, mg/L	110	96.3	162	14.8	†	Feed eficiency[5], %	22.7	20.0	18.5	1.24	ns
Total rumen N, mg/100 mL	148	149	165	5.32	ns	Entodiniinae[6], %	88.9^{b}	89.9^{ab}	91.0^{a}	0.41	*
Peptide N, %	50.8	51.4	50.4	2.40	ns	Diplodiinae[6], %	0.513	0.230	0.147	0.081	†
Amino acid N, %	24.5	25.3	25.3	1.75	ns	Ophryscolecinae[6], %	2.92^{a}	1.19^{b}	0.030^{b}	0.407	**
Ammonia N, %	24.9	23.1	24.4	1.45	ns	Isotricha spp., %	7.69	8.69	8.75	0.362	ns

[1]fat and protein corrected milk; [2]purine derivatives in urine; [3] intake N - fecal N - urine N; [4]N balance – milk total N; [5](milk energy/energy intake) × 100; [6] main rumen protozoa subfamilies observed; within a row, means not bearing a common superscript letter differ (P < 0.05); ** P<0.01, * P<0.05, † P<0.1, ns not significant (*n*=6).

Results & discussion There were no statistical differences in the BW during the course of the trial, or the individual feed intake nor milk production and composition. The DM digestibility and the rumen fermentation parameters remained unchanged among treatments although the lactate tended to be higher in the rumens of animals with dosage 3.3 g/d. Nevertheless, OMD tended to be lower for 3.3 g/d, indicating an impact on fibrolytic activity by decreasing the digestibility of NDF and ADF, despite no obvious inhibiting effect on protozoal numbers remained after 6 weeks of treatment (data not shown). Although the different nitrogen fractions in the rumen were similar among doses, the estimated MPS was substantially higher for 3.3 g/d dose. In accordance with Santoso *et al.,* (2006) the dose 1.0 g/d promoted a trend to lower urine N and increase N balance and retention N values while the feed efficiency remained unaltered.

Conclusion The use of SPB did not clearly increase the nutritive value of the diet under the assay conditions described, but led to a decline in urinary N, and a concomitant increase in N balance, being of particular relevance to promote a more environmentally sustainable agriculture.

Acknowledgements This study was funded by NOR-FEED (Plants and plant extracts for animal nutrition).

References

Belanche A, Pinloche E, Preskett D and Newbold CJ 2015. FEMS Microbiology Ecology 92.
Santoso B, Mwenya B, Sar C and Takahashi J 2006. Animal Feed Science and Technology 129,187-195

Evaluation of organic matter digestibility on individual faeces samples on beef cattle in the tropics

Michel Naves[1], Farant Alain[3], Godard Xavier[3], Nepos Angebert[3], Mulciba Pierre[3], Marine Leprince[3], Tatiana Silou-Etienne[1], Boval Maryline[2]

[1]INRA, URZ, Petit Bourg, Guadeloupe, France, [2]INRA, AgroParisTech, UMR Mosar, Paris, France, [3]INRA, PTEA, Le Moule, Guadeloupe, France

E-mail: michel.naves@inra.fr

Take home message Prediction of organic matter digestibility by NIRS on individual dung or rectal samples may be a good indicator of feed efficiency in beef cattle in the tropics.

Introduction The improvement of feed efficiency in ruminants is a challenge, for its possible benefits on the economic results and on the environmental impacts. Moreover, it may have great implications in the tropics, where grass and forage feeding systems are predominant. However, the individual variations of feeding efficiency are difficult to assess, especially at pasture. Prediction of OMd by the mean of NIRS maybe a useful tool to evaluate this parameter (Boval *et al*., 2004).

Material & methods Our experiment involved 8 Creole steers, 15 months old, maintained in individual feeding cages during the 8 week trial. They were fed with *Digitaria decumbens* grass, cut on the same forage plot. A first cut was performed three weeks before the beginning of the experiment, in order to allow a homogenous regrowth of the grass in the plot. The animals were fed *ad libitum* daily with the same forage, so that the age of the proposed grass varies from 24 days to 55 days.

Each day (Monday to Thursday), the individual amount of forage offered was weighed, and distributed in two meals (at 7 and 12 h); a unique forage sample for the group of steers was collected. The forage refusal was also weighed daily (Tuesday to Friday) for each steer, before the first meal, and individual samples of the forage refusal were collected. Three types of faecal samples were collected daily: Tuesday to Friday, after the first meal, an individual sample was collected from a dung pat, for all the steers (sample 1); after the second meal, a second sample was collected either from a dung pat or from the rectum (sample 2), on 4 steers each type of sample; finally, Wednesday to Saturday, the total amount of faeces was weighted and homogenized, and a mean sample was collected. These daily mean samples were pooled weekly for each steer.

The laboratory analysis consisted of a standard determination of organic matter (OM) of the pooled faeces and of the forage samples, as well as an NIRS measurement of each faecal sample (samples 1 and 2, and pooled mean sample). The apparent mean OM digestibility was calculated weekly, as the ratio of OM retained (proposed – refused - excreted) on the ingested OM (proposed – refused); these values served for the calibration of the NIRS spectrum. The NIRS spectrum of samples 1 and 2 were used to predict the OMd on each sample. A total of 45 weekly measurement of apparent digestibility, and 371 predictions of OMd on individual samples were collected. The statistical analysis consisted of a correlation assessment of the individual measures of OMd among them or with the weekly means.

Results As expected, the apparent digestibility varied throughout the experiment, from 81.3 % at about 27 days of age to 61.6 % at about 53 days of age, according to the individual and the week. NIRS predictions of OMd on individual samples reflected the variability of the apparent mean digestibility (Figure 1) observed. NIRS predictions made on independent sub-samples collected daily were highly correlated (R^2=0.91) (Figure 2). The correlation of subsequent NIRS predictions made during the week varied from 0.87 to 0.66 according to the time interval between the samples.

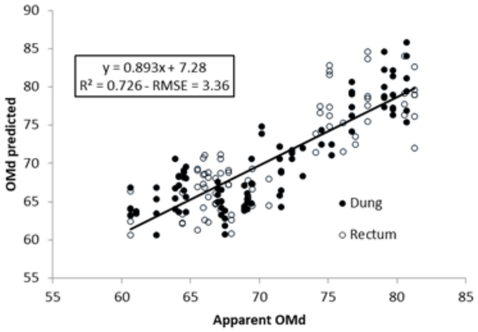


Figure 1 Relationship between apparent OMd and NIRS prediction on individual dung or rectum samples (sample 2).

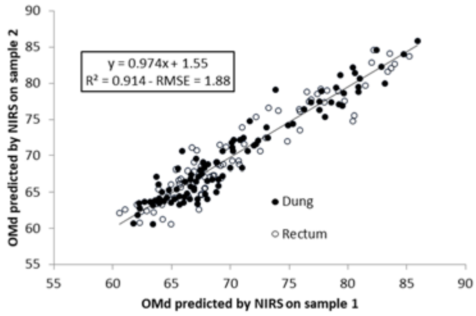


Figure 2 Relationship between OMd predicted by NIRS on samples 1 and 2.

Conclusion This trial confirms that OMd predicted by NIRS in single samples may be useful to evaluate feed efficiency. These results may have practical applications in the field, for instance for the evaluation of individual variations. They have to be tested on more experimental and field trials, on different feeding regimes and type of animals, with more individuals.

Acknowledgements This research was supported by the Region and FEDER fund, in AgroEcoDiv project

References

Boval M, Coates DB, Lecomte P, Decruyenaere V and Archimede H 2004. Animal Feed Science and Technology 114, 19-29.

Effects of feeding tropical herbal fortified Urea Molasses Block (UMB) on feed consumption, digestibility and daily weight gain of beef cattle in West Sumatra – Indonesia

Rusmana Ningrat, Montesqrit Montesqrit, Erpomen Erpomen

University of Andalas, Padang, Indonesia

E-mail: oz.ningrat@gmail.com

Take home message To accelerate the Beef Self-Sufficiency Programmes in Indonesia requires the development of livestock feed which is directed at the use of locally available feedstuffs derived from agricultural and industrial waste. The use of herbal fortified Urea Molasses Block (UMB) in rations of local beef cattle can increase feed consumption, digestibility of dry matter, organic matter, crude protein and daily weight gain.

Introduction West Sumatra Province has been designated as one of 20 priority provinces for Beef Self-Sufficiency Programmes in Indonesia. Our previous in *vitro* studies showed that some tropical herbal plants could potentially have other benefits not just limited to the content of nutritional substances, but also as a compound to manipulate rumen microbes, stimulating growth and at the same time used for the maintenance of livestock health. The results showed that tropical herbal plants affect the rumen microbes and provide a positive effect on the fermentation rumen (the concentration of NH_3 and VFA) and gas production *in vitro*. This research aimed to study the effects of feeding tropical herbal fortified UMB on feed consumption, digestibility and body weight gain of beef cattle.

Material & methods Research material used was 10 local beef cattle in Pauh Sub-District of West Sumatra. The experimental design used in this study was a completely randomized design (CRD) with 5 treatments and 2 replications., treatment A (fresh grass + UMB as Control), B (fresh grass + white turmeric (*Curcuma zedoaria*) enriched UMB, C (fresh grass + Green chiretta (*Andrographis paniculata*) enriched UMB, D (fresh grass + Brotawali (*Tinospora crispa)* enriched UMB), E (fresh grass + Java ginger (*Curcuma xanthorrhiza* Roxb) enriched UMB. Ingredients used in making UMB were: molasses, urea, rice bran, mineral mixture, salt, tapioca and cement as binding agents. The parameters observed are digestability of dry matter, organic matter, crude protein and body weight gain.

Results & discussion The results of the analysis of variance showed highly significant effects ($P<0.01$) of all parameters measured compared to the control diet, however, treatment B (UMB fortified with *C. zedoria* showed the best results on all parameters measured (Table 1). The provision of antioxidants of *C. zedoria* (Jayaprakasha *et al*, 2006) fortified to UMB significantly effects the productivity of beef cattle.

Table 1 Effects of feeding tropical herbal fortified UMB on feed consumption, digestibility and daily weight gain of beef cattle.

Treatments	Feed Consumption (g/h/day)			Feed Digestibility (%)			ADG
	DMI	OMI	CPI	DMI	OMI	CPI	(g/h/d)
A	5630^{e}	5217^{e}	480.26^{d}	61.49^{b}	65.58^{b}	5576^{b}	230.18^{d}
B	6340^{a}	5865^{a}	549.83^{a}	76.46^{a}	82.68^{a}	8041^{a}	539.58^{a}
C	6070^{b}	5651^{b}	529.11^{b}	78.13^{a}	80.99^{a}	8085^{a}	352.62^{b}
D	5890^{cd}	5457^{cd}	509.50^{c}	76.51^{a}	76.70^{a}	7654^{a}	304.21^{c}
E	5950^{c}	5503^{c}	513.97^{bc}	76.67^{a}	76.67^{a}	7893^{a}	328.92^{bc}
SE	0.074	0.061	10.679	13.12	17.39	30.39	48.817

Conclusion The conclusion of this study is the use of white turmeric herbal fortified UMB increase dry matter intake (6340 g/h/d), organic matter (5865 g/h/d), crude protein (549.58 g/h/d) and increased the digestibility of dry matter from 61.49 % to 76.94%, organic matter from 65.58 % to 79.26%., crude protein from 55.76 % to 79.18% and body weight gain from 230.18 to 539.58 g/h/d.

Acknowledgements We would like to thank the Ministry of Research and Technology and Higher Education of the Republic of Indonesia for the research funding under the scheme of University Excellence Research. The authors wish to thank Jasma, Rika and Tika at the University of Andalas for their careful technical assistance.

References

Jayaprakasha GK, Jaganmohan RL and Sakariah KK 2006. Food Chemistry 98, 720-24.

How do animals differing in experience and genetic merit behave and perform when grazing biodiverse mountainous pastures?

Madeline Koczura[1], Matthieu Bouchon[3], Germano Turille[4], Joel Berard[1], Michael Kreuzer[1], Anne Farruggia[2], Bruno Martin[2]

[1]ETH Zürich, Institute for Agricultural Science, Animal Nutrition, CH-8092 Zurich, Switzerland, [2]Université Clermont Auvergne, INRA, VetAgro Sup, UMR1213 Herbivores, F-63122 Saint Genès Champanelle, France, [3]Université Clermont Auvergne, INRA, UE Herbipôle, F-15190 Marcenat, France, [4]Institut Agricole Régional d'Aoste, Zootecnia, I-11100 Aosta, Italy

E-mail: madeline.koczura@usys.ethz.ch

Take home message The performances of low-yielding dairy cows experienced in highland grazing does not differ from that of less experienced higher-yielding cows when grazing biodiverse mountainous pastures.

Introduction Previous experience and genetic merit affect dairy cows' feeding behaviour and performance. When grazing biodiverse and heterogeneous mountain pastures, appropriate feeding choices can help cows maintaining performance when the nutritional value of the grass is declining. Our aim was to experimentally determine how animals with different combinations of experience and genetic merit behave and perform when grazing such pastures with different slopes compared to less diverse pastures. The research shall clarify whether or not animals with lower genetic merit and more experience on mountainous pastures are more resilient to these conditions than less experienced high genetic merit cows.

Material & methods Three groups of twelve late-lactating cows were composed of four Holstein (Ho), four Montbéliarde (Mo) and four Aosta Red Pied (Rp), the latter with lower milk production potential. Cows were monitored during 2 weeks in June 2017 (weeks 24 and 26). All animals had grazing experience but for Ho and Mo this experience was on less biodiverse pastures and at lower altitudes than that of Rp. No concentrate was fed. Pastures (0.3 ha/cow) were 1) a grass-dominated pasture with low diversity (L) and 2) two mountainous pastures both about 23 species/m² but with different slopes (A1: 22°; A2: 7°). Milk yield (MY) was recorded daily and averaged per week. Potential milk yield (MY pot) was calculated for each cow using the model of Coulon and Pérochon (2000) and the difference between MY and MY pot was calculated (ΔMY). Body weight (BW) of the cows was measured twice a day and averaged per week. During 2 successive days in each week, the behaviour of two animals per type per pasture was observed by scan-sampling every 5 min, and Jacob's selectivity indexes (SI) were calculated according to Dumont *et al.*, (2007). On the same dates, individual milk samples were collected and analysed for fat and protein content (NIRS). The MIXED procedure of SAS with repeated statement was used to test the effects of animal-type, pasture type, week and their interactions. Data obtained in the week before the experiment were used as covariates.

Results & discussion Most effects observed in this experiment were inherent to the animal types: as expected the Ho and Mo had a higher MY and BW than Rp cows whose milk was the richest in protein and the poorest in fat (Table 1). Surprisingly, the gap to the potential MY did not significantly differ between cow types. The Mo cows avoided the mature grasses, unlike Rp cows. On average, MY was higher on L pasture than A2, with an intermediate level in A1. From week 24 to 26, MY from the cows on L-pasture dropped to A-pasture level (interaction pasture × week; $P < 0.05$, data not shown) which is coherent with the faster loss of nutritional value in productive pastures due to the drought encountered at that time of the season. Indeed, on L-pasture, during week 26, cows selected more mature herbs and less short vegetative grasses than during week 24 (data not shown). Other than assumed, all three animal types had similar MY and ΔMY on the three pastures, on average and during week 24 and 26 (interaction not significant).

Table 1 Performance and selection indices measured in the three dairy cow types on each pasture.

	Animal type			Pasture type				*P*-value		
	Ho	Mo	Rp	L	A1	A2	SEM[1]	Animal	Pasture	A × P
Milk yield (kg/d)	16.2^{a}	17.4^{a}	12.1^{b}	16.3^{a}	15.2^{ab}	14.1^{b}	0.62	<0.001	<0.001	0.651
Δ milk yield (kg/d)	-2.6	-1.4	-2.7	-1.7	-2.1	-3.0	0.87	0.104	0.146	0.648
Milk fat (g/kg)	37.3^{ab}	39.5^{a}	36.6^{b}	38.2	38.3	36.9	1.04	0.004	0.242	0.736
Milk protein (g/kg)	30.1^{b}	31.8^{a}	32.6^{a}	31.5	31.1	32.0	0.43	<0.001	0.350	0.013
Body weight (kg)	657^{b}	685^{a}	510^{c}	617	614	621	6.3	<0.001	0.751	0.997
SI short grasses	0.24	0.42	0.32	0.22^{b}	0.29^{ab}	0.47^{a}	0.180	0.224	0.036	0.120
SI mature grasses	-0.20^{ab}	-0.31^{b}	0.13^{a}	0.48^{a}	-0.42^{b}	-0.45^{b}	0.159	<0.001	<0.001	0.131

[ab] Within the same trait, values without common superscripts differ ($P < 0.05$) ; [1]Standard Error of the Mean.

Conclusion This result does not indicate that Aosta Red Pied cows are more resilient to biodiverse mountain pastures than Holstein or Montbéliarde whatever the type of grassland, at least under the present conditions where the three types of animals were grazing together.

Acknowledgements This research was funded by the French government IDEX-ISITE initiative 16-IDEX-0001.

References

Coulon JB and Pérochon L 2000. INRA Productions Animales 13, 349-360
Dumont B, Rook AJ, Coran Ch and Röver KU. 2007. Grass and Forage Science 62, 159-171.

Genetic markers are associated with the ruminal microbiome and metabolome in grain and sugar challenged dairy heifers

Helen Golder[1,2], Jennifer Thomson[3], Stuart Denman[4], Chris McSweeney[4], Ian Lean[1,2]

[1]*Dairy Science Group, School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Camden, NSW, Australia,* [2]*Scibus, Camden, NSW, Australia,* [3]*Department of Animal and Range Sciences, Montana State University, Bozeman, MT, USA,* [4]*CSIRO Animal, Food and Health Services, Queensland Bioscience Precinct, St. Lucia, QLD, Australia*

E-mail: heleng@scibus.com.au

Take home message Inter-animal variation is partially explained by host genetics.

Introduction There is interest in understanding the links between the mammalian genotype and the closely associated enteric biome. These associations influence diseases including ruminal acidosis (RA), a complex nutritional disorder of worldwide interest. It is difficult to achieve a balance between feeding ruminants readily fermentable carbohydrates to meet the energy demands required for productivity and to minimise health risks that result from starch and sugars. Studies note considerable variation in responses among cattle fed a common diet designed to induce RA (Penner *et al.,* 2009; Golder *et al.,* 2014). Control strategies are unlikely to manage RA in the entire herd due to this variation (Golder *et al.,* 2014). Firkins and Yu (2015) note that differences in the microbiome structure among animals within the same diet group often exceeds those among different diet groups. These differences reflect different host genetics and interactions with the rumen microbiome (Hernandez-Sanabria *et al.,* 2013). Bovines appear to have a core microbiome that differs among individuals (Jami and Mizrahi, 2012). It may be the result of genetics, environment, or their interaction, or how this may influence susceptibility to RA or other disorders. A mechanistic understanding of gut function and microbiota is relevant to understanding RA as well as other disease conditions and production outcomes. The aim of this work was to examine associations between the genome, metabolome, and microbiome, hence to investigate the among animal variation in cattle fed feed additives and subjected to a non-life-threatening, but substantial, starch and fructose challenge (Golder *et al.,* 2014). The objective is to use this exploratory work to provide a rationale for a larger field study where markers for the susceptibility of RA may be identified for possible use in breeding decisions. We hypothesised that associations would occur between the bovine genome, metabolome, and microbiome and quantitative trait loci (QTL) would be identified.

Material & methods Initially, heifers (n = 40) allocated to five feed additive groups were fed 20-days pre-challenge with a total mixed ration and additives. Fructose [0.1% of bodyweight (BW)/day] was added for the last 10 days pre-challenge. On day-21 heifers were challenged with 1.0% of BW dry matter wheat + 0.2% of BW fructose + additives. Rumen samples were collected *via* stomach tubes weekly (day-0, 7, and 14) and at five times over 3.6 hours after challenge and analysed for pH and volatile fatty acid (VFA), ammonia, D- and L-lactate concentrations. Relative abundance of bacteria and archaea were determined using Illumina MiSeq. Genotyping was undertaken using a 150K Illumina SNPchip. Genome-wide association was performed for metabolite and microbiome measures on individual phenotypes (n = 33) using an additive model and linear regression. Bonferroni adjustment was applied, and population stratification corrected. Positional candidate genes were identified in genomic regions with multiple significant associations or around markers that were significantly associated to a phenotype over multiple measurement periods.

Results Few genome associations occurred with rumen pH, concentration of acetate, propionate, total VFA, or ammonia, or the relative abundance of the Firmicutes, Bacteroidetes, and Spirochaetes phyla. Metabolites and microbial phyla that had markers associated and QTL were: acetate to propionate ratio (A:P), D-, L-, and total lactate, butyrate, acidosis eigenvalue, Actinobacteria, Chloroflexi, Euryarchaeota, Fibrobacteres, Planctomycetes, Proteobacteria, and Tenericutes. A putative genomic region overlapped for Actinobacteria, Euryarchaeota, and Fibrobacteres and covered a region that codes for matrix extracellular phosphoglycoprotein. Other overlapping regions were: (1) Chloroflexi, Tenericutes, and A:P, (2) L- and total lactate and Actinobacteria, and (3) Actinobacteria, Euryarchaeota, Fibrobacteres, and A:P.

Conclusion Notwithstanding the small population size, genome-wide associations with the metabolome and microbiome were identified supporting our hypothesis and suggest that markers for RA susceptibility exist. These findings assist in understanding the occurrence of inter-animal variation in response to physiological challenge both in the field and experimentally. These findings also provide a rationale for a larger study with a population that has variation in acidosis. In the future rumen function markers may aid in identification and management of animals predisposed to rumen issues.

Acknowledgements Support from *Scibus*, The University of Sydney, DairyNSW, and Lallemand Animal Nutrition.

References

Firkins J and Yu Z 2015. Journal of Animal Science 93, 1450-1470.
Golder HM, Celi P, Rabiee AR and Lean IJ 2014. Journal of Dairy Science 97, 985-1004.
Hernandez-Sanabria E, Goonewardene LA, Wang Z, Zhou M and Moore SS 2013. PLoS ONE 8, e58461-e58461.
Jami E and Mizrahi I 2012. Anaerobe 18, 338-343.
Penner GB, Taniguchi M, Guan LL, Beauchemin KA and Oba M 2009. Journal of Dairy Science 92, 2767-2781.

Serotonin regulates maternal calcium homeostasis during the transition period of cashmere goat

Lu Jin, Haizhou Sun, Riga Namei, Dan Sang, Shengli Li, Chongzhi Zhang, Chunhua Zhang, Ting Ru

Inner Mongolia Academy of Agriculture and Animal Sciences, Hohhot, China

E-mail: happyjinlu@163.com

Take home message Serotonin induces parathyroid hormone–related protein for increasing calcium mobilization from bone during the transition from pregnancy to lactation in goats.

Introduction An increasing demand for calcium during pregnancy and lactation can result in both clinical and subclinical hypocalcemia during the early lactation period in several mammalian species. Serotonin (5-HT) was recently identified as a regulator of lactation and bone turnover (Hernandez *et al.,* 2012). Calcium mobilization from bone is stimulated by mammary secretion of parathyroid hormone-related protein (PTHrP), which is present in human, rat, goat, and bovine milk and in the serum of the lactating mother (Law *et al.,* 1999). The purpose of this study was to determine whether supplementation of the maternal diet with a 5-HT precursor (5 hydroxy-l-tryptophan, 5-HTP) or tryptophan (TRP) would increase maternal bone turnover and calcium mobilization to maintain appropriate circulating maternal concentrations of ionized calcium during lactation.

Material & methods Thirty pregnant cashmere goats were randomly assigned to 3 experimental groups. All groups received a daily *i.v.* infusions of saline (Control group; n = 10), saline containing 0.178 mg of TRP/kg of BW (TRP group, n = 10) or 0.178 mg of 5-HTP/kg of BW (5-HTP group, n = 10). Cashmere goats were intravenously infused daily for approximately 7 days prepartum. Blood samples were collected on the morning d 3 before the first infusion, then after parturition blood samples were taken every three days until d 15, and a final sample was collected on d 30. On d 9 of lactation goats were slaughtered, and femur and mammary gland tissue were obtained for RNA isolation. Relative levels of specific gene mRNA were quantified using SYBR® Prime ScriptTM RT-PCR Kit following the manufacturer's instructions. Data (mRNA expression) were calculated by the $2^{-\triangle\triangle CT}$ method and were evaluated using analysis of variance and Duncan's multiple range test using SAS. Differences with a $p<0.05$ were considered significant, while differences with $0.05 < P < 0.10$ were regarded to be a statistical trend.

Results Compared with the control, infusion of TRP or 5-HTP up-regulated PTHrP, NCX, PMCA2, SPCA1, CaSR and RANK-L mRNA expression in mammary glands but had no effect on RANK and OPG mRNA expression in femurs (Table 1) . Cashmere goats infused with TRP or 5-HTP had higher circulating concentrations of serotonin and calcium during the whole transition period. The highest serum concentrations in calcium were observed on the day 30 after parturition.

Table 1 mRNA expression of calcium transporters in mammary glands and bone resorption markers in femurs on day 9 of lactation period in cashmere goats.

Group^{1}	Mammary gland						Femur	
	PTHrP	NCX1	PMCA2	SPCA1	CaSR	RANK-L	RANK	OPG
Control	1.008^{b}	1.017^{b}	1.922^{c}	1.048^{c}	1.186^{b}	1.019^{b}	1.001	1.255
TRP	1.708^{ab}	1.351^{ab}	4.938^{b}	5.077^{b}	2.210^{a}	2.279^{a}	0.752	0.798
5-HTP	2.082^{b}	1.969^{a}	8.161^{a}	6.774^{a}	2.248^{a}	2.520^{a}	0.999	0.774
SEM	0.152	0.251	0.681	0.775	0.294	0.276	0.129	0.245
P-value	0.028	0.035	0.011	0.034	0.045	0.040	0.697	0.574

^{1}SEM means standard error of the mean, a, b: means within the same column without the same superscripts differ significantly (P<0.05).

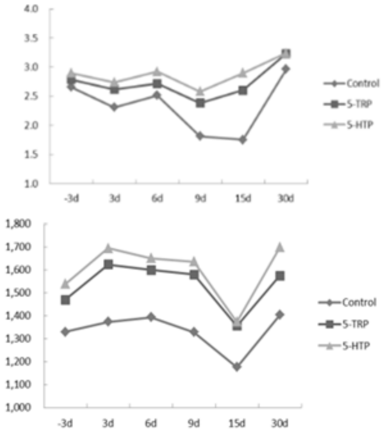


Figure 1 Mean concentrations in serum serotonin (ng/L) (the upper) and calcium (mmol/L) (the lower) in cashmere goats receiving infusion of saline (control) TRP or 5HTP.

Conclusion We propose that treatment of cashmere goats with 5-HTP or TRP during the prepartum period may provide beneficial for postpartum calcium homeostasis, although through different mechanisms. Serotonin activates expression of various calcium pumps and transporters in the mammary gland to stimulate transport of Ca from blood to milk during mouse lactation. PTHrP is secreted into the circulation and will bind its receptor PTH1R on the osteoblast cell in the bone increasing production of receptor activated nuclear factor kappa B (RANKL), which binds its receptor (RANK) on the osteoclast cell in the bone tissue, activating Ca liberation from bone. Further studies focused on hypocalcemia should pay attention to the breed, as our data demonstrates that even untreated cashmere goats have dramatically different mechanisms of calcium homeostasis around the time of parturition. We can state confidently, however, that 5-HTP warrants further investigation as a strategy for the prevention of subclinical and clinical hypocalcemia in cashmere goats.

Acknowledgements The authors thank Namei Riga for her help with the laboratory analyses and the crew of the Wulateqianqi livestock improvement workstation for their help with animal care.

References

Hernandez LL, Gregerson KA and Horseman ND. 2012. American Journal of Physiology, Endocrinology and Metabolism. 302, E1009–1015.

Law FM, Moate PJ, Leaver DD, Diefenbach-Jagger H, Grill V, Ho PW and Martin TJ 1999. Journal of Endocrinology 128, 21–26.

655

Relationship between the expressions of volatile fatty acid absorption genes and circadian clock factor genes in ruminal epithelium

Mengzhi Wang[1], Jian Gao[1], Jialiang Ouyang[1], Beibei Jiang[1], Juan-Jose Loor[2]

[1]*Yangzhou University, Yangzhou, Jiangsu Province, China,* [2]*University of Illinois, Urbana, Illinois, USA*

E-mail: mengzhiwangyz@126.com

Take home message Knowing the mechanism of circadian rhythm on volatile fatty acid absorption is more important for ruminants in their rumen metabolism regulation.

Introduction Circadian clock widely exists in mammalian cells and its genes in gastrointestinal tract could affect the absorption and transport of macronutritions and the expression of related transporters (Pacha and Sumova, 2013). In the intestine of nocturnal rodents, circadian clock regulates the mRNA expression of ion transporter proteins, including DRA, AE1, NHE3, etc. (Soták *et al.*, 2011). As the main product of rumen fermentation and principal energy source for ruminants, volatile fatty acids (VFA) are absorbed by rumen epithelium (Bilk *et al.*, 2005). Thus, our hypothesis is that an internal relationship exists between the gene expression of VFA transporters and circadian clock in ruminal epithelial cells.

Material & methods Primary goat ruminal epithelial cells were extracted from 3 Boer goats at 1.5 months of age. When the cultures reached 70% confluence, about 2 days, the cells were respectively treated with 15 mmol/L of acetate, propionate or butyrate in medium for 24 h. The control was set as 5% glucose in medium to balance energy supply. The expressions of circadian gene and VFA transporter genes were determined. Values were analysed by SPSS software (Version 22.0, IBM Corp); Correlation analysis was conducted with Pearson correlation analysis by the R programming language. *ACTB and GAPDH* were used as internal control genes, the relative gene expression was calculated with the $2^{-\Delta\Delta Ct}$ method.

Results & discussion VFAs decreased *Clock* but increased *Bmal1*, compared with the control. Butyrate increased *Per2* and *Per3* compared with other groups. *Cry1* in response to butyrate was greater than the control, while *Cry2* was greater with propionate and butyrate compared with acetate and the control. Furthermore, VFAs upregulated *PAT1*, but downregulated *MCT1* and *MCT4* and had no difference for *NHE1* (Table 1). Besides, positive correlations existed between *clock* and *MCT1*, *clock* and *MCT4*, *Per2* and *AE2*, *Per2* and *vH+ ATPase*, *Per3* and *vH+ ATPase*. Negative correlations were found between *Bmal1* and *MCT1*, *Bmal1* and *MCT4*, *Per2* and *MCT1*, *Per2* and *MCT4*, *Per3* and *MCT4*, and *Cry2* and *MCT4* (Table 2).

Table 1 Effects of volatile fatty acids on gene expression of circadian clock in ruminal epithelial cells[1].

	Control	Acetate	Propionate	Butyrate	SEM	*p*-value
Clock	1.03	0.53	0.29	0.44	0.095	<0.00
Bmal1	1.01	1.71	3.95	2.81	0.251	<0.00
Per2	1.01	1.34	1.46	2.17	0.142	<0.00
Per3	1.01	0.84	1.12	6.91	0.190	<0.00
Cry1	1.01	0.65	0.60	1.59	0.069	<0.00
Cry2	1.00	0.99	1.73	1.91	0.110	<0.00
PAT1	1.01	2.56	2.52	2.22	0.196	<0.00
AE2	1.02	1.48	0.77	1.07	0.088	<0.00
MCT1	1.02	0.66	0.41	0.23	0.079	<0.00
MCT4	1.02	0.62	0.44	0.18	0.089	<0.00
vH^+	1.01	0.93	0.97	2.44	0.273	<0.00
NHE1	1.02	1.16	0.99	1.38	0.219	0.263

[1] Values with different superscripts in the same row differ significantly ($p < 0.05$).

Table 2 Correlationship between circadian clock genes and SCFA transporter genes in ruminal epithelial cells.

	Clock	*Bmal1*	*Per2*	*Per3*	*Cry1*	*Cry2*
PAT1	-0.30	0.36	0.24	-0.15	-0.33	0.32
AE2	0.03	-0.24	0.54^{**}	0.27	-0.02	-0.31
MCT1	0.80^{**}	-0.48^{**}	-0.42^{*}	-0.37	0.31	-0.31
MCT4	0.80^{**}	-0.60^{**}	-0.61^{**}	-0.50^{**}	-0.02	-0.54^{**}
vH^+ *ATPase*	-0.17	-0.14	0.80^{**}	0.91^{**}	0.25	-0.15
NHE1	-0.27	0.36	-0.08	0.16	-0.01	0.20

Note: * meant $0.01 < p < 0.05$, ** meant $p < 0.01$. Sample number was 36.

Conclusion This *in vitro* VFAs treatment affect the gene expression of both circadian factor and VFA transporter, and the negative correlations existed in gene expression between *MCTs* and most circadian factors except for *Clock*. The precise regulatory mechanisms involving circadian factors on VFA absorption in the rumen epithelium merit further research.

Acknowledgements The research was supported by grants from National Natural Science Foundation of China (31672446).

References

Bilk S, Huhn K, Honscha K, Pfannkuche H and Gäbel G 2005. Journal of Comparative Physiology B 175, 365-374.
Pácha J and Sumová A 2013. Acta Physiologica 208,11-24.
Soták M, Polidarová L, Musílková J, Hock M, Sumová A and Pácha J 2011. American Journal of Physiology-Gastrointestinal and Liver Physiology 301, G1066-1074.

Dietary nitrogen influences the hepatic signalling pathway of the somatotropic axis in young goats

Caroline Firmenich, Nadine Schnepel, Alexandra Muscher-Banse

Physiology, University of Veterinary Medicine Hannover, Hannover, Germany

E-mail: alexandra.muscher@tiho-hannover.de

Take home message An adequate dietary nitrogen supply is essential for young goats to ensure the appropriate physiologic functionality of the somatotropic axis in the liver.

Introduction Ruminants are thought to cope easily with a reduced nitrogen (N) intake due to efficient rumino-hepatic circulation of urea. Dietary crude protein (CP) concentrations of 11 – 12% were recommended to meet the requirements of growing goats Society of Nutrition Physiology (2003). However, in previous studies it was found that decreased dietary N intake caused significant changes in mineral homeostasis in young goats (Muscher AS and Huber K 2010, Elfers *et al.*, 2015) . During an N reduced diet, blood calcium (Ca), calcitriol and insulin-like growth factor 1 (IGF-1) concentrations were diminished. The liver synthesised IGF-1 after pulsatile stimulation by growth hormone (GH) from the pituitary gland. In blood IGF-1 is bound to IGF-1 binding proteins (IGFBP) and coupled with the acid labile subunit (ALS); a ternary complex is formed. IGFBPs and ALS help to prolongate the half-life of circulating IGF-1. The GH binds to the hepatic GH receptor (GHR) and initiates the Janus kinase-signal transductors and activators of transcription (JAK-STAT) pathway leading to IGF-1 secretion. The JAK-STAT signalling pathway is controlled via negative feedback by suppressor of cytokine signalling (SOCS) proteins. Therefore, it was hypothesised that components of the somatotropic axis were modulated by an N reduced diet in young goats.

Material & methods Seventeen male goats were divided in two groups (n = 8 and n = 9), receiving either an elevated (CP = 182 g/kg DM) or a reduced N supply (CP = 79 g/kg DM). The diet of the control group was enriched with urea as an N source, whereas the N reduced diet were fortified with Sipernat 22S to adjust the weight. The diets were isoenergetic and the individual feeding was carried out two times a day. Chopped wheat straw was offered at 25% of the concentrate weight. The goats had free access to water. After six weeks of feeding, blood samples were taken shortly before slaughtering. The animals were sacrificed by exsanguination after captive bolt stunning. The liver tissue samples were taken, frozen in liquid N_2 and stored at -80 °C until further preparation. Serum IGF-1 concentrations were analysed by RIA and concentrations of plasma GH were analysed by ELISA. The mRNA expression of hepatic GHR, IGF-1, ALS, STAT5B, JAK2, SOCS1, SOCS2 and SOCS3 was determined by *q*PCR with SYBR Green PCR assays. The primers used for production of recombinant DNA were derived from caprine ovine or bovine sequences. Absolute copy numbers were determined using calibration curves generated with cloned PCR fragment standards. The expression of genes of interest was normalized to 18S rRNA as stable expressed reference gene. The protein expression of IGFBP2, IGFBP3, IGFBP4 and IGFBP5 was determined by quantitative Western ligand blot analysis as described previously (4). Data were analysed by unpaired Student's t-test.

Results & discussion All animals were growing and gained weight during the experimental feeding. The feed intake and the feed efficiency were not affected by any dietary treatment. Serum IGF-1 levels were decreased due to the N reduced feeding although plasma GH levels were not affected. The mRNA expression of hepatic IGF-1, ALS and GHR was significantly diminished in the N reduced fed animals. The mRNA expression levels of hepatic JAK2, STAT5B and SOCS1 were not modulated. By contrast, mRNA expression of SOCS2 and SOCS3 increased significantly due to the N reduced feeding. Protein expression levels of IGFBP2 and IGFBP3 increased in the goats receiving an N reduced diet. The decline in IGF-1 concentrations during dietary N reduction could be mediated by reduced levels of hepatic GHR. Increased expression levels of SOCS2 and SOCS3 might contribute to diminished levels of IGF-1.

Conclusion Components of the somatotropic axis are modulated by an N reduced diet in young goats. Due to the non-physiological relationship between constant levels of plasma GH and diminished IGF-1 concentrations, a decoupling of the somatotropic axis in response to an N reduced feeding seems possible in young goats.

Acknowledgements The authors wish to thank Prof. Dr. G. Breves and K. Elfers, PhD for their support. This research project was supported by the Deutsche Forschungsgemeinschaft (DFG, Mu 3585/1-3).

References

Elfers K, Wilkens MR, Breves G and Muscher-Banse AS 2015. British Journal of Nutrition 114, 1949-1964.
Laeger T, Wirthgen E, Piechotta M, Metzger F, Metges C, Kuhla B and Hoeflich A 2014. Journal of Dairy Science 97, 2876-2885.
Muscher AS and Huber K 2010. The Journal of Steroid Biochemistry and Molecular Biology 121, 304-307.
Society of Nutrition Physiology (2003) Recommendations for the Supply of Energy and Nutrients to Goats. The Committee for Requirement Standards of the Society of Nutrient Physiology Report, no. 9. Frankfurt am Main: DLG-Verlag.

Effects of dietary protein level of lactating dairy cows on epigenetic changes in their offspring

Alex Bach[1,2], Less Crompton[3], Francesc Fàbregas[2], Anna Arís[2], Chris Reynolds[3]

[1]ICREA, Institució Catalana de Recerca i Estudis Avançats, Barcelona, Catalonia, Spain, [2]Department of Ruminant Production, IRTA, Barcelona, Catalonia, Spain, [3]School of Agriculture, Policy and Developmen, University of Reading, Reading, UK

E-mail: alex.bach@icrea.cat

Take home message The epigenome of calves born to cows either fed low (~14%) or high (~18%) CP diets differ substantially.

Introduction Nutritional status of the pregnant cow is an important factor exerting epigenetic regulations of the offspring. In adult dairy cows, close to 70% of the gestation coincides with lactation, when the placenta must compete for nutrients with the mammary gland (Bach, 2012). However, macro-nutrient (mainly energy and protein) needs of dairy cows for pregnancy are typically ignored until cows are 190 d pregnant. González-Recio *et al.,* (2012) described an impact on milk production, health, and longevity when pregnancy and lactation coincided, precluding the offspring born to the most productive cows to fully express their potential additive genetic merit during their adult life, and hypothesized that differences in nutrient supply could be one of the reasons behind these observations. Dietary protein is used inefficiently for milk protein production by dairy cows, with approximately 75% of N intake excreted in manure (Bach *et al.,* 2006). Reducing dietary protein concentration should have positive effects on the environment, but the long-term effects of under or over feeding dietary protein relative to metabolizable protein requirement are not certain, including effects on the foetus. We hypothesized that feeding different amounts of dietary protein to pregnant dairy cows during lactation could also have an effect on the performance of the offspring through epigenetic mechanisms. Thus, the objective of this study was to evaluate potential epigenetic changes of calves born to lactating cows fed three levels of dietary crude protein (CP) concentrations for at least one entire lactation.

Material & methods Fifteen female Holstein calves born to multiparous (second and third lactation) dams were randomly selected from a larger group of cows (215 in total) that at the onset of their first lactation were fed a TMR formulated to contain 14, 16, or 18% CP (n=5 per diet), and thus provided and amount of metabolizable protein below (**LOW**), at (**Control**), or above (**HIGH**) predicted metabolizable protein requirements, respectively. Differences in CP were obtained by using incremental substitution of wheat, wheat feed, and soy-hulls with soybean and rapeseed meal. During the dry period, dams were fed a common diet to meet requirements. Details of the larger experiment involving all cows can be found in Reynolds *et al.,* (2016). After birth (between 1 and 7 d of life) a blood sample was obtained from the jugular vein to isolate peripheral blood mononuclear cells and their DNA was submitted to next-generation sequencing on an Illumina HiSeq 4000. Samples were clustered based on the similarity of their methylation profiles using the Ward distance method using the R package MethylKit. Sites that were differentially methylated were grouped into functional clusters using DAVID Bioinformatic Resources.

Results & discussion The cluster analysis separated in two distinct groups samples from calves born to LOW from those born to HIGH dams, with the exception of 1 calf born to HIGH that was grouped with LOW calves (Figure 1). However, CONTROL calves could not be grouped separately from HIGH calves, and LOW calves did not cluster differently from CONTROL calves. CpG sites that were differentially methylated could be group into five main clusters according to their functionality: calcium and mineral transport, regulation of cell proliferation, cellular membrane integrity and cell migration, cellular protein production, and a group of genes strongly associated with body weight and height (*WNT7A*) and energy metabolism (*IGFBP2*, *IGFBP5*, *OPRK1*, *ASGR1*, and *GLRX*).

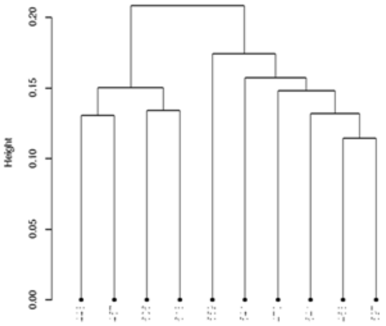


Figure 1. Cluster of LOW (Blue) or HIGH (Red) calves based on their methylation pattern.

Conclusion The epigenome of calves born to cows underfed or overfed CP differs noticeably.

References

Bach A 2012. Journal of Animal Science 90, 1835–1845.

Bach A, Raeth-Knight ML, Stern, MD and Linn JG 2006. Protein, amino acid, and peptide requirements for proper rumen function and milk production. MN Nutrition Conference. Pages 67-79. September 20, St. Paul, MN, USA.

González-Recio O, Ugarte E and Bach A 2012. PLoS ONE 7, e51816.

Reynolds CK, Crompton LA, Humphries DJ and Jones, AK, 2016**.** Long term implications of feeding low protein diets to first lactation dairy cows. *In* J. Skomial, and H. Lapierre (eds.) *Proceedings of the 5th International Symposium on Energy and Protein Metabolism*, EAAP Publ. No. 137. Wageningen Academic Publishers, The Netherlands, 263-264.

Feed restriction modifies liver transcriptomic profile of suckling Assaf lambs

Alba Santos[1], F. Javier Giráldez[1], David Kenny[2], Javier Frutos[1], Sonia Andrés[1]

[1]*Instituto de Ganadería de Montaña, CSIC-Universidad de León, Grulleros, León, Spain,* [2]*TEAGASC, Grange, Dunsany, Ireland*

E-mail: sonia.andres@eae.csic.es

Take home message Feed restriction during the suckling period impacts lipid and protein metabolism of suckling lambs.

Introduction Early feed restriction during the suckling period may impact feed efficiency traits during post-weaning phases (Santos *et al*., 2018). In this sense, differential gene expression between individuals is a key source of variation that can be used to identify candidate genes and pathways involved in metabolic programming (Ruchat *et al*., 2014). Hence, the aim of this work was to identify differentially expressed (DE) genes (RNA-seq) in the liver tissues of suckling female lambs milk restricted during the suckling period.

Material & methods Eight new born female Assaf lambs were penned individually, four of them were fed milk replacer (200 g dry matter/L) *ad libitum* (ADL; 192 mL/kg LBW) whereas the other group (restricted, RES) only received 120 mL/kg LBW. All the lambs were weighed twice a week throughout the suckling period until they were 35.-days-old, when all of them were slaughtered and a piece of liver was excised (left lobe) for transcriptomic analysis (RNA-seq) using an Illumina HiSeq 2500 sequencer. Differential expression analysis was performed with the Bioconductor package DESeq2 (version 1.12.4) and statistical analyses of DE genes were performed in R (version 3.3.1), according to Conesa *et al.* (2016). Genes were considered to be differentially expressed if their *P*-value, adjusted for multiple testing using the false discovery rate method (FDR), was less than 0.05. Moreover, Kyoto Encyclopedia Genes and Genomes (KEGG) pathways enrichment analysis was carried out with the kegga function of the edgeR package.

Results & discussion The characteristics of the pre-weaned lambs are summarised in Table 1 Differential gene expression analysis identified 386 DE genes, 198 of them being annotated genes in the KEGG pathway, with 118 genes being up-regulated and 80 down-regulated in the RES group relative to the ADL group. Moreover, KEGG annotation and enrichment analysis allowed identification of those enriched pathways in which DE genes were involved, mainly related to the cytochrome P450, steroid hormone biosynthesis, amino acids biosynthesis and drug metabolism, among others. The expression of genes related to the synthesis of glycerolipids (*e.g., MOGAT1*) and long chain fatty acids (*e.g., ELOVL4*, *ELOVL7*) was decreased, whereas that relative to ω-oxidation (*e.g., ADH1C*) and β-oxidation of fatty acids (*e.g., ECI1* and *ECHS1*) was increased in the RES lambs. On the other hand, the down-regulation of genes related to protein processing in endoplasmic reticulum (*e.g., YOD1*, *CRYAB*, *SEL1L*) might have altered protein degradation through the proteasome, which also presented genes modified by feed restriction (*e.g., PSMB9* and *PSMD6*). Moreover, several genes involved in amino acid metabolism were altered in the RES lambs (*e.g., AGXT2*, *ACMSD*, *ACY1*). This fact, together with the increased expression of genes involved in protein synthesis like those related to ribosomal proteins (*e.g., RPL22L1*, *LOC101102787* and *LOC101104725*) or ribosome biogenesis (*e.g., POP7*) might be indicative of an attempt of the growing organism for maintaining normal physiological functions for growth under feed restriction conditions. Finally, other genes related to steroid hormone biosynthesis, belonging predominantly to the cytochrome P450 family (*e.g., CYP2C19*, *CYP2D14*) were over-expressed in these animals.

Conclusion A restricted milk replacer intake during the suckling period of pre-weaned lambs reduces both, the live body weight at slaughter and carcass performance. Moreover, the liver transcriptome was deeply modified by early feed restriction to increase β-oxidation of fatty acids as a source of energy for the early feed restricted lambs.

Table 1 Performance traits of lambs fed *ad libitum* (ADL) or restricted (RES) during the artificial rearing phase.

	ADL	RES	RSD	*P*-value
Birth weight, kg	3.99	4.12	0.192	0.409
Slaughter weight, kg	11.6	7.90	0.739	< 0.001
Age at slaughter, days	35.0	34.3	1.50	0.494
Hot carcass weight, kg	5.65	3.57	0.407	< 0.001
Cold carcass weight, kg	5.44	3.40	0.396	< 0.001
Perirenal fat, g	104	34.6	19.99	0.002

Acknowledgements This work was funded by the Spanish Ministry of Economy, Industry and Competitiveness (MINECO, AGL2014-54124R). Alba Santos gratefully acknowledges receipt of a predoctoral grant from the Spanish Ministry of Education, Culture and Sport (ref: FPU15/01630).

References

Conesa A, Madrigal P, Tarazona S, Gomez-Cabrero D, Cervera A, McPherson A, Szcześniak MW, Gaffney DJ, Elo LL, Zhang X and Mortazavi A 2016. Genome Biology 17, 13.
Ruchat S-M, Bouchard L and Hivert MF 2014. Current Nutrition Reports 3, 281–288.
Santos A, Giráldez F J, Trevisi E, Lucini L, Frutos J and Andrés S 2018. Journal of Animal Science [In press].

Effects of birth weight on meat fatty acid profile of fattening lambs

Carmen Valdés, Alba Santos, Javier Frutos, Francisco Javier Giráldez, Sonia Andrés

Instituto de Ganadería de Montaña (CSIC-Universidad de León)., León, Spain

E-mail: cvals@unileon.es

Take home message Meat from lambs slaughtered at the same weight (27 kg) contains more saturated fatty acids when animals are born with a low body weight regardless the daily dry mater intake during the fattening period.

Introduction Early nutritional programming during pregnancy is considered a key factor for feed efficiency and product quality, since it deeply impacts skeletal muscle and adipose tissue development (Bell and Greenwood, 2016). The objective of this work was to study the effect of body weight (BW) at birth on the growth performance and content of fatty acids of meat from lambs slaughtered with 27 kg and being fed the same amount of feedstuff during the fattening period.

Material & methods Two experimental groups (6 animals in each group, matched by litter size) of male Merino lambs with different BW at birth [low BW (LW; 3.88±0.281 kg) and high BW (HW; 5.80±0.647 kg)] were used in this work. Lambs were penned individually with their corresponding dam during the suckling period and were weaned when they had reached 15 kg of BW; then, they were penned individually and offered daily 35 g/kg BW of a complete pelleted diet (43% barley, 15% corn, 24% soybean meal 44, 15% barley straw, 2% sodium chloride and 1% sodium bicarbonate) to ensure no differences in dry mater intake during the fattening period. Lambs were weighed twice weekly throughout the experiment and were slaughtered when they reached 27 kg of BW. The *longissimus thoracis* (LT) muscle was removed from the carcass at 24 h post mortem and used to measure the fatty acid (FA) content (Moran *et al*., 2013). Growth performance and FA content of meat samples were analysed by one-way ANOVA using GLM procedure of SAS.

Results & discussion During both the suckling and fattening periods, average daily gain was smaller ($P<0.001$) in LW lambs (186 and 141 g/d) than in HW lambs (253 and 190 g/d). As a consequence, LW lambs were older ($P<0.01$) than HW lambs at weaning (53 *vs*. 32 days) and required longer ($P<0.05$) fattening periods (88 days) than HW lambs (64 days) to reach the slaughter BW (27 kg). Total FA content of meat tended to be higher ($P=0.057$) in LW lambs and showed some differences between both groups (Table 1). Thus, there was a greater amount of saturated and monounsaturated FA whereas polyunsaturated FA content was unaffected by birth BW. As a consequence, the nutritional interesting indexes of the meat from LW lambs were worse when compared to those of the HW group.

Table 1 Fatty acid (FA) content (mg/100 g fresh matter) of meat from fattening lambs (27 kg of live body weigh) of low birth (LW) or high (HW) body weight at birth.

	LW	HW	RSD	*P*-value
Total FA	1985	1409	465.5	0.057
Saturated FA (SFA)	886	580	225.2	0.041
Monounsaturated FA (MUFA)	788	569	187.7	0.071
cis–MUFA	694	517	168.1	0.090
trans–MUFA	93.7	52.3	31.80	0.047
Polyunsaturated FA (PUFA)	219	203	35.5	0.443
Total CLA	11.2	7.78	4.43	0.216
Nutritional interesting indexes				
n–6/n–3	14.4	14.1	3.21	0.853
11*t*/10*t*	0.063	0.128	0.05	0.049
PUFA/SFA	0.250	0.355	0.0671	0.022

Conclusion Low body weight at birth reduces the animal growth during both milk fed and fattening periods and increases the amount of saturated fatty acids in the meat, thus worsening the nutritional indexes of this product.

Acknowledgements This research was supported by the Spanish Ministry of Economy, Industry and Competiveness (MINECO, AGL2014-54124R) and by the Spanish Ministry of Education, Culture and Sport (MECD, FPU15/01630).

References

Bell AW and Greenwood PL 2016. Animal Production Science 56, 1217-1232.
Morán L Giráldez FJ, Panseri S, Aldai N, Jordán MJ, Chiesa LM and Andrés S 2013. Food Chemistry 138, 2407-2414.

Regression analysis of body measurements to estimate body weight in Ghezel sheep breed

Marziyeh Ebrahimi, Gholamali Moghaddam, Seyed Abbas Rafat, Sadegh Alijani

Department of Animal Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

E-mail: marzebrahimi@tabrizu.ac.ir

Take home message Body measurements including heart girth, withers height, and croup height can be used to estimate body weight of Ghezel ewes and ewe lambs.

Introduction It is essential for sheep farmers to know the weight of their animals for adjusting feed supply, predicting medication doses, and more importantly allow reproduction of ewe lambs with proper body weights. Though, in nomadic and rural breeding systems, livestock farmers which do not have access to weighing scales, because of the high expense or remoteness (Hosseinzadeh Shirzeyli *et al*., 2013). Therefore, prediction of body weight, especially for ewes and ewe lambs through measuring body measurements is a practical alternative for the farmers. Accordingly, the present study was designed to determine regression equations for predicting body weight through body measurements in ewes and ewe lambs of Iranian fat-tailed Ghezel sheep.

Material & methods During the breeding seasons of 2016 and 2017, Ghezel ewe lambs (n=43; one year old) and ewes (n= 56; two years old) were weighed, and body measurements were taken, including: hock height (HH), body depth (BD), body length (BL), heart girth (HG), elbow height (ELH), croup height (CRH), ischium width (IW), hip length (HIL), pelvic width (PW), shank width (SHW), shoulder width (SHW), body barrel (BB), and withers height (WH). All measurements were in cm. For analysis, REG procedure of SAS software (SAS Studio) was used based on linear, polynomial and stepwise multiple regressions to estimate body weight equations. Body measurement variables were used in a step-wise regression method to develop body weight equations. Finally, estimated full model equations were reported in Table 1 Then, practical equations were developed by selecting two traits with the highest correlation coefficient with body weight (Table 1).

Results & discussion Results of the linear regression is presented for Ghezel ewe lambs and ewes in Table 1 Results of the present study indicated that HG and WH are variables with a high correlation for estimating the body weight of ewes, while HG and CRH have a high correlation with ewe lambs' body weight (Table 1). In another study on several Iranian sheep breeds (Mehrabani, Zandi, Shaal, and Makoui), their regression equations for estimating body weight included body length, chest girth, withers height, and hip width (Hosseinzadeh Shirzeyli *et al*., 2013). Yilmaz *et al.* (2013) reported a high correlation of body length and chest girth with the body weight of Karya sheep. Musa *et al.* (2012) found heart girth, withers height, and hip height as the most appropriate measurements for predicting body weight in Sudanese Shugor sheep. Also, Afolayan *et al.* (2006) found chest girth to be the variable with the highest accuracy for predicting body weight in Yankasa sheep. Similarly, in the practical model equations of the present study, a high correlation coefficient of heart girth (chest girth) with live body weight was observed.

Table 1 Full and practical regression equations for predicting body weight of ewe and ewe lambs from body measurements.

Animals	Model types	Equations*	Adjusted R^2	P-Value
Ewe (two years old)	Full model	BW= -80.08+ 0.49 WH+0.36 HG + 0.29 BB+ 1.01 IW+ 2.03 SHW	0.96	<0.0001
	Practical model	BW= -62.93+0.55 WH+0.78 HG	0.92	<0.0001 <0.0001
Ewe lamb (one year old)	Full model	BW= -57.14+0.06 BL+0.30 BB+0.52 HIL+0.40 CRH +0.90 SHW+0.39 BD	0.88	<0.0001
	Practical model	BW= -25.84+0.22 CRH+0.55 HG	0.64	<0.0001

* WH: withers height, HG: heart girth, BB: body barrel, IW: ischium width, SHW: shank width, BL: body length, HIL: hip length, CRH: croup height, BD: body depth.

Conclusion It is concluded that HG and WH are more useful indicators for estimating the live body weight of ewes (two years old), while HG and CRH are better candidates for estimating the live body weight of Ghezel ewe lambs (one year old). As adjusted R^2 is higher in the practical model for predicting body weight of two year old ewes compared with ewe lambs, body weight of two year old ewes can be predicted more accurately than ewe lambs. Also, as we obtained two different equations for two different ages, it means that the relationship among body morphometric traits and body weight are different and we cannot use one model for all ages in Ghezel ewes.

References

Afolayan RA, Adeyinka IA and Lakpini CAM 2006. Czech Journal of Animal Science 51, 343.
Hosseinzadeh Shirzeyli FH, Lavvaf A and Asadi A 2013. Songklanakarin Journal of Science & Technology 35, 507-511.
Musa AM, Idam NZ., and Elamin KM 2012. Journal of Animal and Feed Research 2, 27-29.
Yilmaz O, Cemal I and Karaca O. 2013. Tropical Animal Health and Production 45, 397-403.

Effect of α-lipoic acid on oxidative status, lipid metaboic parameters and liver enzyme activities in transition dairy cows

Heng Wang, Jianji Li

College of Veterinary Medicine, Yangzhou University, Yangzhou, China

E-mail: sdaulellow@163.com

Take home message α-lipoic acid supplementation can improve antioxidant capacity, lipid metabolism and protect liver function in transition dairy cows.

Introduction Periparturient dairy cows inevitably experience metabolic challenges that result in a negative energy balance (EB), and passively mobilize fatty acids from adipose tissues, which can lead to fatty liver disease, and to be in an oxidative stress state. Alpha-lipoic acid, a potent antioxidant and essential member of mitochondrial dehydrogenases, has shown potent reactive oxygen species-scavenging capabilities along with a proven clinical safety record, and thus this study was conducted to determine whether α-lipoic acid has beneficial effects on EB, lipid metabolism, and hepatoprotective effects in periparturient dairy cows.

Material & methods Forty-eight Holstein dairy cows at 20d before parturition were randomly allocated into four groups (12 in each group). Each group was fed with different doses (0, 3, 5 or 8 g/d evenly mixed with the diet) of α-lipoic acid for a consecutive 50 days, respectively. The diets for all animals consisted of a base ration fed as daily total mixed ration comprising 10 kg corn silage, 13 kg grass silage, 3 kg dehydrated alfalfa and 3 kg of a commercial concentrate composed of cereal grains and oilseeds. All animals were kept under identical conditions. Blood samples were collected at the beginning and at intervals of 10 days after supplementation. Glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) were measured on UV/VIS spectrophotomerer by the commercial ELISA kits. Meanwhile, the following parameters indicative of lipid metabolism and serum enzyme activities were measured with an automatic biochemical analyzer: triglyceride (TG), cholesterol (Chol), nonesterified fatty acid (NEFA), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and alkaline phosphatase (AKP), γ-glutamyl transferase (γ-GT), lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Results & discussion The GSH-Px and SOD, as the indicators of oxidative status, were significantly higher in the group with 5 g/d supplementation after 20 days and in the group with 8 g/d supplementation after 40 days than that in the control group (0 g/d), respectively ($P<0.05$ or $P<0.01$). Serum CAT activities were markedly higher in all supplementation groups after 30 days than that in the control group ($P<0.05$). Whereas supplementation with 5 g/d after 40 days or with 8 g/d after 50 days significantly reduced the serum MDA contents, compared with control group ($P<0.05$). The data of lipid metabolic parameters were shown as a general declining trend without significant differences in the groups with α-lipoic acid supplementation. Only serum NEFA concentrations were markedly reduced after supplementation with 8 g/d for 30 days, compared with control group ($P<0.05$). Serum liver enzyme tests showed that AST activities were significantly lower in all supplementation groups after 30 days than that in control group ($P<0.05$ or $P<0.01$). While no significant differences were found in other parameters between the treated groups and the control.

Conclusion These findings indicate that α-lipoic acid supplementation can improve antioxidant capacity, lipid metabolism and protect liver function in transition dairy cows.

Acknowledgements This research was supported by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD) in China.

References

Fiore E, Perillo L, Piccione G, Gianesella M, Bedin S, Armato L, Giudice E and Morgante M. 2016. Journal of Dairy Research 83, 438-441.
Ghinis-Hozumi Y, González-Dávalos L, Antaramian A, Villarroya F, Piña E, Shimada A, Varela-Echavarría A and Mora O 2015. Journal of Animal Science 93, 3820-31.

Body weight parameters and progesterone concentration of Ghezel ewe lambs taken over two years

Marziyeh Ebrahimi, Banafshe Fardoost, Gholamali Moghaddam

[1]*Department of Animal Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran*

E-mail: marzebrahimi@tabrizu.ac.ir

Take home message Improving body weight and simultaneously improving body parameters can improve reproductive performance of Ghezel ewe lambs.

Introduction Although in most countries ewe lambs successfully breed at 7 to 9 months of age (Kenyon P. *et al.*, 2014), Iranian Ghezel sheep always breed at 16-18 months old and accordingly have a lower reproductive performance. This study was designed to evaluate whether low fertility and reproductive rate of ewe lambs are due to the lack of puberty or low body weight.

Material & methods During the breeding season (July, 2016), 30 ewe lambs were selected, weighed, and their blood samples (n= 11) were taken to evaluate progesterone concentration. Then, their body parameters were determined including: hock height, BCS, body depth, body length, chest diameter, elbow height, head length, head width, croup height, ischium width, pelvic width, shank width, shoulder width, body barrel, and withers height. One year later during the breeding season (July, 2017), 14 ewe lambs (two years old) were used for blood sampling and determining the same body parameters. Blood samples were centrifuged at 3000 rpm for 15 min and serum were separated. Progesterone in samples were determined using enzyme-linked immunoabsorbent assay (ELISA) kit (MonobindInc, USA) by ELISA reader (STAT-FAX 3200, USA). Data were then analysed using Proc GLM of SAS software (Ver. 9.2) and results presented as mean±SE. Duncan test was used for comparisons (P<0.05).

Results & discussion Results of the present study showed that body weight along with all body parameters were significantly higher (P<0.01) in year two in comparison with year one in Ghezel ewe lambs (Table 1). Also, serum progesterone concentration was significantly higher in year two in comparison with year one (P<0.01), (Table 1). It was indicated that when progesterone concentration of ewe lambs exceeded 1 ng/ml, they reached puberty (Valasi *et al.*, 2006). Accordingly, as almost all ewe lambs in year one had higher progesterone concentration than 1 ng/ml, it indicates that all lambs reached puberty during first year of the breeding season. Hence, low reproductive performance during the first year is directly related to low body weight and body parameters. It was reported that the onset of puberty related to the attainment of critical body mass (Rosales Nieto *et al.*, 2013), although this weight is not still sufficient for attaining successful reproduction rates (based on the present results). Rosales Nieto *et al.*, (2013) reported a positive correlation between fertility and reproductive rate with leptin concentration, depth of eye muscle, fat, and live weight. Also, they reported higher fertility and reproductive rate for ewe lambs with higher weight at mating (Rosales Nieto *et al.*, 2013).

Table 1 Body weight parameters and progesterone levels of Ghezel ewe lambs collected over two years.

Body parameters[1]	Ewe lambs (year 1)	Ewe lambs (year 2)	P-value
Hock height[2]	26.84±0.24^b	29.55±0.37^a	<0.01
BCS	2.19±0.05^b	3.08±0.07^a	<0.01
Body depth[2]	30.37±0.42^b	38.27±0.62^a	<0.01
Body length[2]	69.51±2.64^b	104.52±3.93^a	<0.01
Chest diameter[2]	75.39±1.13^b	103.87±1.68^a	<0.01
Elbow height[2]	39.68±0.46^b	44.03±0.68^a	<0.01
Head length[2]	20.45±0.44^b	27.18±0.66^a	<0.01
Head width[2]	11.29±0.25^b	15.61±0.36^a	<0.01
Croup height[2]	62.28±0.62^b	68.64±0.92^a	<0.01
Ischium width[2]	6.52±0.14^b	9.78±0.21^a	<0.01
Pelvic width[2]	17.24±0.42^b	24.34±0.62^a	<0.01
Shank width[2]	9.93±0.14^b	10.97±0.21^a	<0.01
Shoulder width[2]	15.92±0.48^b	22.06±0.71^a	<0.01
Body barrel [2]	86.42±1.40^b	114.25±2.08^a	<0.01
Withers height[2]	61.90±0.45^b	73.45±0.67^a	<0.01
Body weight[3]	29.25±0.88^b	59.25±1.37^a	<0.01
Progesterone[4]	1.18±0.61^b	5.54±0.54^a	<0.01

[1] Data are means ± SE. [2] cm. [3] kg. [4] ng/ml.

[a,b] Means within the same line with different superscripts differ significantly (P<0.05).

Conclusion It was concluded that improving growth of ewe lambs by either nutrition, improving environmental factors or genetic selection for higher body weight during the first year of their life can increase fertility and reproductive rate of Ghezel ewe lambs.

References

Kenyon PR, Thompson AN and Morris ST 2014. Small Ruminant Research 118, 2-15.

Rosales Nieto CR, Ferguson MB, Macleay CA, Briegel JR, Martin GB and Thompson AN 2013. Animal 7, 990-997.

Valasi I, Menegatos I, Papanikolaou T, Goulas P and Amiridis GS 2006. Theriogenology 66, 2144-2151.

The effect of pregnancy toxemia on gluconeogenesis in both maternal and fetal livers

Yanfeng Xue, Changzheng Guo, Fan Hu, Fei Xie, Junhua Liu, Shengyong Mao

Nanjing Agricultural University, Nanjing, Jiangsu province, China

E-mail: xueyanfeng1990@163.com

Take home message Gluconeogenesis is repressed in maternal livers but activated in the fetal livers of ewes with pregnancy toxemia.

Introduction Pregnancy toxemia (PT) is a metabolic disease caused by negative energy balance due to the rapid growth and development of a fetus during late gestation. PT is usually characterized by low glucose concentration. More than half of the glucose in ruminants comes from gluconeogenesis. However, remarkably little information is available about gluconeogenesis in ewes with PT. Thus, the objective of the present study is to research the expression of genes related to gluconeogenesis in maternal and fetal livers of ewes with PT.

Material & methods Twenty multiple pregnancy ewes (60.6 ± 4.9 kg) who gestated for 115 days were enrolled in this study and assigned to two groups randomly. Ewes in the control (CON) group (n=10) were fed normally while ewes in the treated (TR) group (n=10) were subjected to 30% feed restriction. Blood samples were collected from the jugular vein every five days to investigate beta-hydroxybutyrate (BHBA) and glucose levels. After 15 days' treatment, all the ewes were slaughtered to collect maternal and fetal liver samples. The expression of genes associated with gluconeogenesis in maternal and fetal livers were normalized to beta-actin and evaluated by the method of $2^{-\Delta\Delta Ct}$. Blood glucose was analyzed by two-way analysis of variance, and the expression of genes in livers was analyzed using independent-sample t-test in SPSS 19.0.

Results Blood BHBA in the TR group (4.1 mmol/L) was higher than the threshold (1.6 mmol/L) (Firat *et al.,* 2001; Ismail *et al.,* 2008), while that of the CON group remained at normal level (0.6 mmol/L). In addition, all the ewes in the TR group showed clinical signs of toxemia including grinding teeth, depression, blindness, and difficult walking and some even lost the ability to stand up. Blood GLC in the TR group dropped rapidly and reached 1.81 mmol/L at Day 15, which was lower ($P < 0.05$) than that of the CON group (Figure 1). In maternal livers of PT ewes, the expression of phosphoenolpyruvate carboxykinase (PCK) 1, PCK2, fructose 1,6-diphophatase (FBP), and glucose-6-phosphatase (G6PC) was decreased ($P < 0.05$), compared to that in the CON group, while pyruvate carboxylase (PC) was increased ($P < 0.05$) (Figure 2). In fetal livers of PT ewes, the expression of PC, PCK1, PCK2, FBP, and G6PC was increased ($P < 0.05$), compared to that in the CON group (Figure 3).

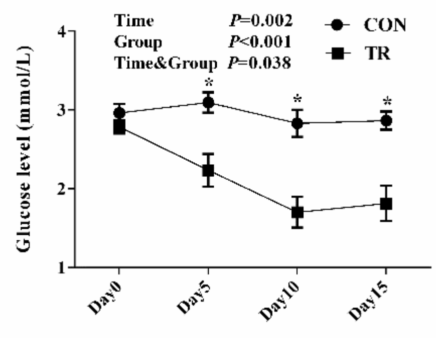


Figure 1 Glucose level of ewes in the two groups (mean ± s.e., n=20).

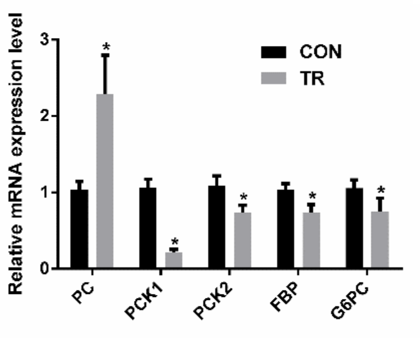


Figure 2 The expression of genes related to gluconeogenesis in maternal livers.

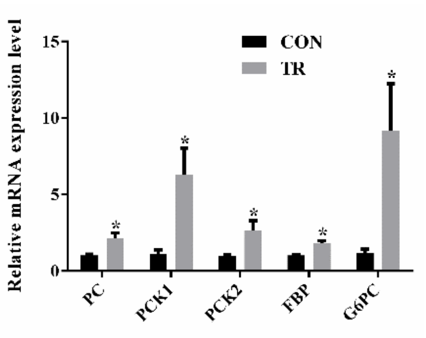


Figure 3 The expression of genes related to gluconeogenesis in fetal livers.

Conclusion High concentrate blood HBA and clinical signs in ewes in the TR group confirmed that the pathological model of PT had been induced successfully. Due to the insufficient energy supply, gluconeogenesis was activated in fetal livers of PT ewes. While the inhibited gluconeogenesis in maternal livers of PT ewes might result from the severe damage of hepatic structure and function.

Acknowledgements This research was supported by the National key research and development program (China; No. 2016YFD0501200).

References

Firat A, Ozpinar A, Ates A, and Durak H. 2001. International Symposium of Veterinary Laboratory Diagnosticians and Oie Seminar on Biotechnology.
Ismail ZA, Almajali AM, Amireh F and Alrawashdeh OF 2008. Veterinary Clinical Pathology 37, 434–437.

Enteric methane emission of lactating Holstein and Jersey cows fed two levels and two sources of forage neutral detergent fibre

Md Elias Uddin, Omar Ivan Santana, Taylor Wickert, Dominique D’huyvetter, Michel A. Wattiaux

Department of Dairy Science, University of Wisconsin-Madison, Madison, WI, USA

E-mail: muddin2@wisc.edu

Take home message Holstein cows fed high FNDF and high CS diet emitted less CH_4 per unit of MY or DMI than Jerseys’.

Introduction To reduce enteric CH_4 from ruminants, many feeding strategies have been developed. Among them, forage quality (*i.e.,* composition) and precision feeding have been identified as having the best prospects (Gerber *et al.,* 2013). A previous study from our group showed that enteric CH_4 per unit of digested NDF decreased with increasing alfalfa silage (AS) in the diet at the expense of corn silage (CS, Arndt *et al.,* 2015). Also, a Canadian group showed that total replacement of AS with CS reduces the enteric emissions expressed either per unit of DM intake (DMI) or milk yield (MY) (Hassanat *et al.,* 2013). However, results of these studies may have been influenced by a possible confounding effect of dietary starch. Therefore, our objective was to determine the effects of replacing AS NDF with CS NDF at two levels of forage NDF (FNDF) on enteric CH_4 emissions of lactating Holstein (H) and Jersey (J) cows fed iso-nitrogenous and iso-starch diets.

Material & methods Twelve H (mean ± SD, 606 ± 40 kg BW and 106 ± 17 d in milk) and twelve J (407 ± 43 kg BW and 112 ± 15 d in milk) lactating cows (all primiparous) housed in tie-stalls, fed once and milked twice daily, were fed four total mixed ration (TMR) in a 2×2 factorial arrangement with two levels of FNDF (200 and 260 g/kg of DM) and two sources of FNDF (70:30 and 30:70 ratio of AS:CS NDF). Crude protein (165 g/kg DM) and starch (240 g/kg DM) content were kept constant across diets. The design was a split plot triplicated 4×4 Latin square in which breed and diet formed the main and subplots, respectively. Experimental periods were four weeks (wk) in length with wk 3 and 4 used for sampling. All data reported here were collected in wk 3. In each period, BW was measured twice, DMI, and MY were recorded daily, and milk samples were obtained from 6 consecutive milking. Daily enteric CH_4 (g/d) was calculated from 8-time points measurements (5-min each), which covered every 3-hr of a 24-hr clock spread over three days, using GreenFeed System (C-Lock Inc.). Data were analysed using proc Mixed of SAS (version 9.4) and the explanatory variables included in the model were breed, level of FNDF, source of FNDF, and all possible interactions as fixed effects whereas square within breed, and cow within breed and square were considered as random effects. Statistical significance was declared at $P \leq 0.05$. Only significant results are presented.

Results Compared to J cows, H cows had greater DMI (mean ± pooled SE, 23.0 *vs.* 17.0; ± 0.71 kg/d), FNDFI (5.20 *vs.* 3.92; ± 0.16 kg/d), MY (33.0 *vs.* 21.0; ± 1.0 kg/d) and fat protein corrected milk (FPCM, 32.0 *vs.* 25.0; ± 1.0 kg/d). Cows fed low FNDF diet had greater DMI (21.0 *vs.* 19.0; ± 0.54 kg/d), MY (28.0 *vs.* 27.0, ± 0.74 kg/d), and FPCM (29.0 *vs.* 28.0; ± 0.78 kg/d) but lower FNDFI (4.0 *vs.* 5.0; ± 0.12 kg/d) compared to cows fed high FNDF diet. Cows fed AS based diet had lower DMI (19.0 *vs.* 21.0; ± 0.54 kg/d), MY (27.0 *vs.* 28.0; ± 0.74 kg/d), FPCM (28.0 *vs.* 29.0; ± 0.78 kg/d) and FNDFI (4.3 *vs.* 4.7; ± 0.12 kg/d) compared to cows fed CS based diet. Interaction term between level and source of FNDF was significant for DMI and FNDFI. Compared to J cows, H cows had greater daily CH_4 (471 *vs.* 385; ± 21 g/d) but lower CH_4/BW (0.77 *vs.* 0.93; ± 0.03 g/kg), and CH4/MY (14.0 *vs.* 18.0; ± 0.73 g/kg). Cows fed low FNDF diet had greater daily CH_4 (440 *vs.* 416; ± 15 g/d), CH_4/BW (0.87 ± 0.03 *vs.* 0.83 ± 0.03 g/kg), CH_4/metabolic BW (4.11 ± 0.13 *vs.* 3.89 ± 0.13 g/kg), and CH_4/FNDFI (108.0 *vs.* 86.0; ± 2.85 g/kg) compared to cows fed high FNDF diet. Source of FNDF influenced CH_4/DMI and CH_4/FNDFI (23.0 and 100.0, *vs.* 21.0; ± 0.68 and 94.0; ± 2.85 g/kg for high AS *vs.* high CS diets, respectively). Cow breed, level of FNDF and source of FNDF did not affect CH_4 per unit of fat yield or protein yield.

Conclusion Compared to J cows, H cows had greater milk performance and daily emission of CH_4 but lower emission of CH_4 per unit of BW or MY. Cows fed low FNDF produced more milk and FPCM and emitted more daily CH_4, CH_4 per unit of FNDFI or BW or MBW compared to high FNDF fed cows. Also, cows fed high CS diet had greater MY and FPCM and emitted less CH_4 per unit of DMI or FNDFI than cows fed high AS NDF-based diet. In this study, there was an effect of breed, but no effect of FNDF level and forage source on CH_4 per unit of milk or FPCM.

Acknowledgements This work is funded by USDA Hatch Multi-state research formula fund (# WIS01941, and USDA-NIFA # 2013-68002-20525).

References

Arndt C, Powell JM, Aguerre MJ and Wattiaux MA. 2015. Journal of Dairy Science 98, 418-430.

Gerber P, Hristov AN, Henderson B, Makkar HPS, Oh J, Lee C, Meinen R, Montes F, Ott T, Firkins J, Rotz A, Dell C, Adesogan AT, Yang WZ, Tricarico JM, Kebreab E, Waghorn W, Dijkstra J and Oosting SS 2013. Animal 7, 220-234.

Hassanat F, Gervais R, Julien C, Massé DI, Lettat A, Chouinard PY, Petit HV, and Benchaar C 2013. Journal of Dairy Science 96, 4553–456.

665

Comparison of blood metabolites and progesterone of Ghezel ewe lambs at one and two years old

Marziyeh Ebrahimi, Banafshe Fardoost, Gholamali Moghaddam, Seyed Abbas Rafat

[1]*Department of Animal Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran*

E-mail: marzebrahimi@tabrizu.ac.ir

Take home message Reproductive failure observed in one-year old ewe lambs can be explained by their metabolic profile.

Introduction Iranian Ghezel ewe lambs always bred at 16 to 18 months old (year two), while other breeds successfully bred at the age of 7 to 9 months (Kenyon P. *et al.,* 2014). This study was designed to compare blood nutritional parameters between one and two years old ewe lambs in order to explore reasons for low reproductive performances observed in one year old ewe lambs.

Material & methods On July 2016 (breeding season), blood samples (one sample per ewe lamb) from 21 ewe lambs (8.02±0.75 months old) were collected individually. Blood samples were centrifuged (3,000×g for 15 min at 4°C) and serum was separated and kept at -20°c until serum metabolites (n=21) and progesterone concentration (only in 11 one year old ewe lambs) were measured. One year later, during the next breeding season (July, 2017), blood samples from 17 two-year old ewe lambs (20.10±0.79 months old, some ewe lambs were the same lambs monitored in the first year and some new ewe lambs of the same age) were collected and serum was separated and stored at -20°C until they were analysed for metabolite (n=17) and progesterone concentration (n=14). It should be mentioned that ewe lambs' feeding in both years was based on pasture grazing. The concentrations of serum metabolites or progesterone were determined for both years Cholesterol, glucose, HDL, LDL, triglyceride, total protein, and urea reagents were supplied by Sigma Diagnostics (Zist Shimi Co, Iran). Blood metabolites were detected using the Enzymatic, Colorimetric method by spectrophotometric assays. Serum progesterone concentration was determined by ELISA reader (STAT-FAX 3200, USA) using enzyme-linked immunoabsorbent assay (ELISA) kit (MonobindInc, USA). Body weight of ewe lambs in year one (n=30) and year two (n=14) was also recorded. Normality test was performed for all data using UNIVARIATE procedure (data were normal, then there was no need for data transformation) and data were analyzed by the GLM procedure of SAS 9.2 software. Results are presented as mean±SE and Duncan's multiple range test used for comparisons (P<0.05).

Results & discussion Results of the present experiment indicated no significant effect of time (year) on serum cholesterol, HDL, and LDL concentrations (P>0.05, Table 1). Total protein was significantly higher in year two in comparison with year one (P<0.01, Table 1). On the other hand, serum urea concentration was higher in year one in comparison with year two (P<0.01, Table 1). Higher urea, but lower total protein concentrations in year one indicates higher protein degradation (Macias-Cruz *et al.,* 2017). Moreover, lower blood urea concentration with higher protein concentration in the second year suggests higher protein synthesis (Macias-Cruz *et al.,* 2017). Then, most probably nutritional status in the first year (feeding was based on grazing on weak pastures) did not comply with lambs' requirement and resulted in low protein synthesis and high protein degradation in which energy imbalance may be an important cause of low reproductive performance during the first year (Schneider, 2004). Based on the results, glucose concentration was significantly higher in year one in comparison with year two (P<0.05, Table 1). As glucose availability is considered to be one of the metabolic regulators in the onset of puberty (Schneider, 2004; Shahat *et al.* 2014); then, increasing glucose concentration of ewe lambs in the first year may be the reason for the onset of puberty (based on progesterone results, three lambs did not reach puberty). Also, progesterone concentration was higher in year two compared with year one (P<0.01, Table 1). Based on the study, ewe lambs with higher progesterone concentration than 1 ng/ml reached puberty (Valasi *et al.,* 2006); so, almost all Ghezel ewe lambs (except for three) reached puberty during their first year of the breeding season. As the body weight of ewe lambs was significantly higher (P<0.01) in year two in comparison with year one (P<0.01, Table 1), energy imbalance may be the reason for blood metabolite changes and so low reproductive performance in the first year (Schneider, 2004).

Table 1 Serum metabolites and progesterone concentrations of Ghezel ewe lambs at 1 and 2 years old, respectively.

Parameters[1]	Ewe lambs (year 1)	Ewe lambs (year 2)	P-value
Cholesterol [2]	70.38±3.99	72.71±4.44	0.70
Glucose[2]	59.38±3.61^{a}	46.23±4.01^{b}	0.02
HDL[2]	31.79±1.88	30.73±2.09	0.71
LDL[2]	36.20±2.88	38.31±3.13	0.62
Triglyceride[2]	21.33±1.84	18.29±2.05	0.28
Total protein[3]	8.11±0.28^{b}	9.06±0.31^{a}	0.03
Urea [2]	42.62±1.55^{a}	36.71±1.72^{b}	0.01
Progesterone[4]	1.18±0.61^{b}	5.54±0.54^{a}	<0.01
Body weight[5]	29.25±0.88^{b}	59.25±1.37^{a}	<0.01

[1] Data are means ± SE. [2] mg/dl. [3] g/dl. [4] ng/ml, [5]kg.
[a,b] Means within the same line with different superscripts differ significantly (P < 0.05).

Conclusion The overall results showed higher body weights, serum glucose and urea, and lower progesterone and total protein concentrations in one year old in comparison with two years old Ghezel ewe lambs.

References

El-Shahat KH, Khaled NF and El-Far FI 2014. Asian Pacific Journal of Reproduction 3, 224-230.

Kenyon PR, Thompson AN and Morris, ST 2014. Small Ruminant Research 118, 2-15.

Macías-Cruz U, Vicente-Pérez R, Correa-Calderón A, Mellado M, Meza-Herrera CA and Avendaño-Reyes L 2017. Livestock Science 205, 64-69.

Schneider JE 2004. Physiology & Behavior 812, 289-317.

Valasi I, Menegatos I, Papanikolaou T, Goulas P and Amiridis GS 2006. Theriogenology 669, 2144-2151.

Variability in growth of weaned beef cattle during the dry season in northern Australia

Simon Quigley[1], Sarah Streeter[2], Tim Schatz[2], Stephen Anderson[1], Dennis Poppi[1]

[1]*The University of Queensland, Gatton, Queensland, Australia,* [2]*Department of Primary Industry and Resources, Northern Territory, Australia*

E-mail: s.quigley@uq.edu.au

Take home message Variation in the growth of steers immediately after weaning had little impact on subsequent growth when fed low and moderate quality diets in pens or grazing wet season tropical forages.

Introduction In northern Australia weaner beef cattle graze pastures of declining quality [30 to 40 g crude protein (CP) and 5 to 6 MJ ME/kg dry matter (DM)] during a protracted dry season (May to December) each year. Calves are typically weaned at the start of the dry season and experience either weight loss or stasis unless supplements are provided. However, even when supplements are provided large within herd variation in growth rates occurs during the dry season after weaning. This experiment examined factors that may be associated with such variability in liveweight gain of weaners during the dry season, and whether a divergence in post-weaning growth persisted under diets that differed in quality.

Material & methods Male *Bos indicus* calves (n=203) from a single commercial cattle herd were weighed, castrated, branded and weaned at the commencement of the dry season (May) in the Northern Territory, Australia. The weaned calves were fed *Centrosema pascurum* hay and a mixture of copra meal and cracked sorghum for 14 days before grazing native pastures (63 g CP and 6.8 MJ ME/kg DM) as a single mob with access to a dry season supplement (120 g/head.day) for 91 days at which time liveweight was measured (Phase 1). Steers of similar weaning liveweight but divergent post-weaning liveweight gain (LWG) [moderate (Mod-LWG, 0.21 kg/day) or low (Low-LWG, 0.03 kg/day)] were pair-matched and allocated to low (*Brachiaria brizantha* hay; 30 g CP and 931 g organic matter (OM)/kg DM) or medium (*Centrosema pascuroum* hay; 108 g CP and 939 g OM/kg DM) quality diets. Thirty-six pairs of steers were generated and allocated to one of 24 pens with 3 steers/pen with diets allocated at random to pens. Steers were weighed and intake determined each week over 70 days (Phase 2). Upon completion of Phase 2 steers then grazed a *Digitaria eriantha* dominant pasture over the subsequent wet season for 180 days with liveweight measured every 30 days (Phase 3). Blood samples were collected at weaning, 91 days after weaning and at the end of the pen feeding period and were analysed for insulin-like growth factor-1 (IGF-1), urea, glucose, creatinine and albumin. Data was analysed using the GLM procedure in SAS (SAS v9.2). The model included post-weaning growth (Mod-LWG or Low-LWG) in Phases 1 and 3 and post-weaning growth, diet and their interaction in Phase 2. The individual steer was considered the experimental unit in Phases 1 and 3 and the pen was considered the experimental unit in Phase 2. Differences between treatments were considered significant when $P < 0.05$.

Results & discussion Brahman steers that were of similar liveweight (145 kg) at weaning but differed in liveweight 91 days after weaning (148 *vs* 163 kg for Low-LWG and Mod-LWG, respectively) had similar LWG when fed a low (*Brachiaria brizantha*, 0.28 kg/day) or medium (*Centrosema pascuroum*, 0.47 kg/day) quality hay in pens. Overall steers fed the medium quality hay had a higher rate of LWG than steers fed the low quality hay. During the subsequent wet season Mod-LWG steers had significantly higher LWG than Low-LWG steers (0.58 *vs* 0.55 kg/day), although the differences were practically small, such that at the end of the wet season Mod-LWG steers were only 12 kg heavier than Low-LWG steers. There were no carry-over effects of diet quality during Phase 2 on LWG during wet season grazing (Phase 3). The concentration of IGF-1 in the plasma of steers was significantly higher at weaning (42 ng/mL) compared to 91 days after weaning (14 ng/mL) and did not differ between Mod-LWG and Low-LWG steers at either time point. The low concentration of IGF-1 in the plasma of steers post-weaning is likely due to a dietary protein and/or energy restriction resulting in decreased protein accretion and poor muscle growth, and possibly explains the low growth rates observed across all steers after weaning. In Phase 2, steers in pens fed the higher quality *Centrosema pascuroum* hay had significantly higher plasma IGF-1 concentration (52 ng/mL) compared to steers fed the lower quality *Brachiaria brizantha* hay (33 ng/mL), again with no difference between Mod-LWG and Low-LWG steers. The concentration of urea (4.6 and 3.9 mM) and albumin (37 and 34 mM) in the plasma of steers decreased between weaning and 91 days after weaning, whilst the concentration of creatinine (148 and 161 mM) increased over the same period, with no differences between Mod-LWG and Low-LWG steers at either time point. As Mod-LWG and Low-LWG steers responded similarly to the medium and low-quality diets (Phase 2) and to wet season grazing (Phase 3) we speculate that genetic variation is unlikely to be the cause of the within herd variation in post-weaning growth rates observed in this experiment.

Conclusion The variability in LWG of steers in the dry season after weaning is most likely related to the stress response to weaning, differences in grazing behaviour and/or supplement intake, rather than genetics.

Acknowledgements We gratefully acknowledge Meat and Livestock Australia for funding this work. We thank Mr Jack Wheeler and staff at the Katherine Research Station for assistance throughout the experiment and Brian Bynon at The University of Queensland, for conducting the urea, glucose, creatinine and albumin assays.

Forage intake and foraging strategy of gestation beef cows with changes in herbage allowance and cow genetic group grazing Campos grassland

Pablo Soca[1], Martin Claramunt[2], Santiago Scarlato[1], Martín Do Carmo[1]

[1]*Agronomy Faculty. Universtiy of Uruguay, Montevideo, Uruguay,* [2]*CURE Universty Uruguay, Treinta y Tres, Uruguay*

E-mail: psocape@gmail.com

Take home message Improving forage height and changes in foraging strategy contributed to explaining the increment in forage intake and body condition score of cross gestating beef cows grazing grassland with 4.5 kgMS/kg LW during winter.

Introduction Increase herbage allowance (HA, Sollenberger *et al*., 2005) between 2.5-4.5 kgDM/kg LW and F1 cows allowed to improve the efficiency of energy use in grassland beef cows systems (Do Carmo *et al*., 2016). During winter (mid to late gestation) a negative energy balance is established due to low energy intake and high energy requirements (Do Carmo *et al.*, 2016). The forage intake of cow grazing depends on pasture height and was modulated by changes in grazing behaviour. We postulated that changes in foraging strategy at different spatial and temporal scales was involved in forage intake and energy balance in response to increase in HA and cow genetic group. The objective was to study the effect of HA and cow genetic group (GG) on forage intake, body condition score (BCS) and foraging strategy characterized by daily grazing (GT), rumination time (RT), bite rate (BR), selection of patches (PH), feeding station (FS) of gestating beef cows grazing Campos grassland.

Material & methods Multiparous beef cows (n=24) were used in a complete randomized design with a factorial arrangement of herbage allowance (2.5 *vs.* 4.5 kg DM/kg BW LO *vs.* HI) of Campos grassland and cow genotype (PU Angus-Hereford *vs.* F1 reciprocal crosses; CR [BW=462±29, BCS=4±0.7] *vs.* PU [BW=437 ±53 BCS=4±0.5]). The HA was adjust based in estimation of forage quantity and cow live weight (Do Carmo *et al.*, 2016). From 88 to 77 ± 11 DPP in six cows of each HA*GG were collocated IGER ® to record during 24 hours grazing (GT) and rumination (RT) time. During the grazing session we estimated the time to take 100 prehension bites (bite rate [BR]) number of patches (PH) and feeding stations (FS) per hour. Forage intake was estimated based on an internal indicator (*n*-alkanes). The effect of HA, GG, y HA*GG on forage intake, BCS, BR PH y EA and relationships between HA and GG with GT and RT were analyzed by repeated measure and regression, respectively.

Results & discussion Increased HA improved pasture quantity (Hi = 1500 vr Lo = 800 kgDM/ha) and height (Hi = 3 vr Lo = 1.7 cm). Forage intake was affected by HA but not by CG (Table 1). The BCS were higher in HI cows whereas CR cows had higher BCS than PU (Table 1). The HA did not modify GT, FS, PH but increased RT in 40 minutes / day (Table 1). The HI increased PH and FS. Patches per hour was lower for CR cows than for PU cows but FS per hour was higher for CR cows than for PU cows The BR increased in LO–PU with respect to HI–CR, HI–PU, and LO–CR.

Table 1 Effect of HA and GG on GT, RT, BR, FS, PH and grazing site of beef cows in gestation (least square means ±standard error).

	HiPu	HiCr	LoPu	LoCR	HA	GG	HA*GG
Forage intake (%LW)	2.8±0.05	2.7±0.1	2.1±0.06	2.2±0.07	0.05	0.15	0.22
Body condition Score	4.00±0.45	4.25±0.5	3.5±0.4	3.75±0.5	0.06	0.05	0.18
GT (min/day)	695±17	754±17	710±17	730±17	0.86	0.06	0.55
RT (min/day)	467±13	469±13	428±13	428±13	0.05	0.98	0.98
BR (bite/minutes)	50±2	53±2	58±2	49±2	0.31	0.22	0.06
FS (Feeding station/hour)	246±20	320±20	279±20	318±20	0.62	0.09	0.59
PH (patches/hour)	17±2	13±	18±	7±	0.48	0.02	0.14

Conclusion Foraging strategy was oriented to increased energy intake in HI HA cows or improved energy efficiency in CR cows, which could explain improved BCS during gestation.

References

Do Carmo, M., Claramunt M, Carriquiry M and Soca P 2016 Journal of Animal Science 94 (Suppl. 6), 85–92.
Sollenberger LE, Moore JE, Allen VG and Pedreira CGS 2005. Crop Science 45, 896–900

Retention marker excretion suggests incomplete digesta mixing across primates

Ikki Matsuda[1], Fabiola Espinosa-Gómez[2], John Sha[3], Diana Marlena[4], Joeke Nijboer[5], Sylvia Ortmann[6], Angela Schwarm[7], Marcus Clauss[8]

[1]Chubu University, Kasugai-chi, Japan, [2]Universidad Veracruzana, Veracruz, Mexico, [3]Sun Yat-sen University, Guang Zhou, China, [4]Wildlife Reserves, Singapore, Singapore, [5]Nijboer Consultancy, Bergschenhoek, The Netherlands, [6]Leibniz-Institute of Zoo and Wildlife Research, Berlin, Germany, [7]Institute of Agricultural Sciences, ETH Zurich, Switzerland, [8]Clinic for Zoo Animals, Exotic Pets and Wildlife, University of Zurich, Switzerland

E-mail: mclauss@vetclinics.uzh.ch

Take home message In contrast to other herbivores, the digesta of primates is less thoroughly mixed in the gastrointestinal tract.

Introduction Understanding digestive tracts as reactors has increased the functional understanding of digesta kinetics (Jumars, 2000). Marker excretion patterns of many herbivores correspond to expectations from reactor models that imply complete mixing of contents (digesta), exemplified by relatively smooth excretion curves in ruminants (Figure 1a). Spiky excretion curves (with 'secondary peaks') are often interpreted as indication for coprophagy (Clauss *et al.*, 2007). However, in several experiments with primates, we observed spiky curves (*e.g.,* Figure 1b) without concomitant observations of coprophagy. To test how frequent this finding is, we performed new experiments with colobines, and surveyed existing literature and datasets.

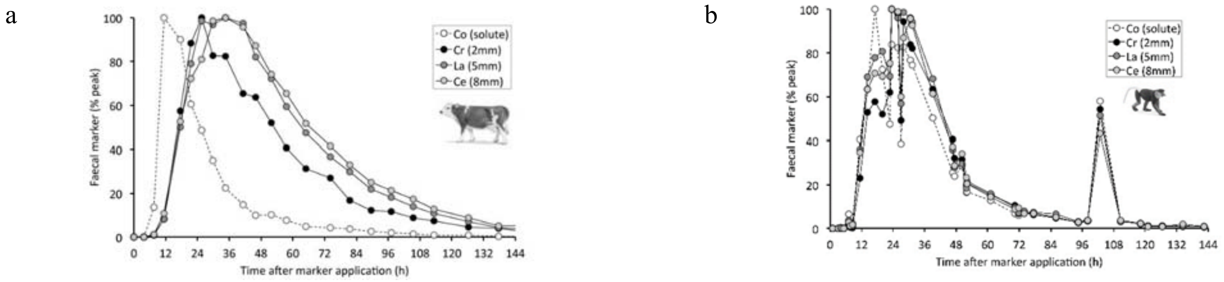


Figure 1 Excretion curve for a solute (Co) and three particle markers in (a) cattle *vs.* (b) proboscis monkey (from Matsuda *et al.*, 2015). Note not only the isolated secondary peak in (b) but also the irregular initial marker excretion pattern.

Material & methods We performed digesta kinetics experiments in 8 individual langurs from 3 species, using identical techniques (one solute and 3 different-sized particle markers, high-frequency faecal sampling, feeding a diet with a high proportion of browse, near-constant monitoring) as applied previously to proboscis monkeys (Matsuda *et al.*, 2015). Results were plotted as recommended in that study as marker concentrations, expressed as % of the peak concentration. Graphs or data from previous studies on colobine monkeys (*e.g.,* Kirkpatrick *et al.*, 2001) and a hindgut-fermenting monkey (*e.g.,* Espinosa-Gómez *et al.*, 2013) were collected and inspected for smooth *vs.* spiky/'irregular' excretion patterns.

Results We inspected 23 individual marker excretion graphs from members of 9 foregut-fermenting and 17 individual graphs from of 3 hindgut-fermenting primate species (8 publications and our own experiment). Spiky/'irregular' marker excretion patterns were observed in 22 individual graphs of foregut- and 15 of hindgut-fermenters, representing 92.5 % of all graphs.

Conclusion Secondary marker excretion peaks are often interpreted as coprophagy (Clauss *et al.*, 2007; Espinosa-Gómez *et al.*, 2013); although occasionally reported as a behavioural abnormality in captive primates, this is not typically considered a hallmark of primate digestive strategies. Coprophagy was not observed in our own study (where 7 of 8 individuals showed an irregular excretion) nor in a study on howler monkeys (Espinosa-Gómez *et al.*, 2013, 11 out of 12 graphs) nor a study on proboscis monkeys (Matsuda *et al.*, 2015, 4 out of 4 individuals). Another reason could be confusion of samples during the study, but the large number of findings across studies (and the fact that the observations did not relate to single stray peaks only) makes this unlikely. The most parsimonious explanation for the irregular excretion patterns is a systematic lack of complete digesta mixing in the gastrointestinal tract of many primates. This interpretation matches the finding that primates, as a group, appear to lack the clear distinction between fluid and particle passage observed in many other herbivores (Müller *et al.*, 2011). This might mean that a comparative shortage of fluid in the digesta makes homogenisation of the digesta difficult. Reasons and consequences of this primate peculiarity remain to be elucidated.

Acknowledgements This study was conducted in compliance with animal care regulations and applicable Singaporean laws.

References

Clauss M, Besselmann D, Schwarm A, Ortmann S and Hatt JM 2007. Comparative Biochemistry and Physiology A 147, 453-459.

Espinosa-Gómez FC, Gómez-Rosales S, Wallis IR, Canales-Espinosa D and Hernández-Salazar L 2013. Journal of Comparative Physiology B 183, 1089-1100.

Jumars PA 2000. American Naturalist 155, 544-555.

Kirkpatrick RC, Zou RJ, Dierenfeld ES and Zhou HW 2001. American Journal of Physical Anthropology 114, 156-162.

Matsuda I, Sha JC, Ortmann S, Schwarm A, Grandl F, Caton J, Jens W, Kreuzer M, Marlena D, Hagen KB, Clauss M 2015. Physiology & Behavior 149, 45-52.

Müller DW, Caton J, Codron D, Schwarm A, Lentle R, Streich WJ, Hummel J and Clauss M 2011. Comparative Biochemistry and Physiology A 160, 207-220.

The role of salivary proteins in the consumption of tannin-rich foods in a neotropical monkey (*Alouatta pigra*)

Fabiola Carolina Espinosa-Gómez[1,2], Juan Carlos Serio-Silva[3], Eliel Ruiz-May[4], Carlos Sandoval-Castro[5], Juan Diego Santiago-García[6], John Aristizábal[7], Colin Austin Chapman[1,8]

[1]McGill School of Environment and Dept. of Anthropology, McGill University, Montreal, Quebec, Canada, [2]Facultad de Medicina Veterinaria y Zootecnia, Universidad Popular Autónoma del Estado de Puebla, Puebla, Puebla, Mexico, [3]Red de Biología y Conservación de Vertebrados, Instituto de Ecología AC, Xalapa, Veracruz, Mexico, [4]Red de Estudios Moleculares Avanzados, Instituto de Ecología AC, Xalapa, Veracruz, Mexico, [5]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán, Merida, Yucatán, Mexico, [6]de Investigaciones Biológicas, Universidad Veracruzana, Xalapa, Veracruz, Mexico, [7]Posgrado, Instituto de Ecología AC, Xalapa, Veracruz, Mexico, [8]Wildlife Conservation Society, Bronx, New York, USA

E-mail: fabiolacarolina.espinoza@upaep.mx

Take home message Black howler monkeys produce salivary cystatins related to their intake of condensed tannins.

Introduction Some mammals can counter dietary tannins by secreting tannin-binding salivary proteins (TBSPs) which reduce the astringency and bitter taste of the food and minimize the effects of the tannins (Shimada 2006). In the wild, howler monkeys (5.5-9.5 kg BM, hindgut fermenting herbivore) regularly eat tannin-rich leaves and unripe fruits raising the possibility that they use TBSP to cope with dietary tannins. We have shown that black howler monkeys do secrete TBSPs (Espinoza-Gomez *et al* 2018) but the types of TBSP and their relationship with tannin intake remain unclear. The objectives of this study were to: (i) analyze in four groups of wild howler monkeys, the protein profile and the relative density of protein bands having tannin-binding capacity; (ii) relate patterns of production of TBSPs with dietary tannin intake; and (iii) identify proteins that were precipitated by tannic acid by Nano LC-MS/MS.

Material & methods We calculated the condensed tannin (CT) intake (g/d) of four groups (n = 14) of wild monkeys in south of Mexico for eight consecutive days by recording all food eaten and measuring the CT concentration in these foods by the vanillin assay (Price, *et al.* 1978) using catechin as a standard. We collected whole saliva and calculated flow rate (ml/min) in 14 adult monkeys after stimulating salivary flow with pilocarpine-hydrochloride. We analyzed the salivary proteins by 1D SDS electrophoresis coupled with densitometry. In each gel, we measured the absolute quantity (µg) of each protein band, using BSA as a standard, and calculated the relative contribution of each protein band to the total salivary protein. Image Lab software version 5.2.1 (Bio-Rad, CA, USA) was used for image acquisition and densitometry analysis on the gels. Finally using in-gel digestion and shotgun proteomics we identified the salivary proteins that were precipitated by tannic acid using Nano LC-MS/MS.

Results The four groups of monkeys ate similar quantities of CT (X^2= 3.361, gl=3, p=0.338) (Fig. 1) and had similar salivary flow rates, total protein concentrations (mg/ml) and protein profiles. In all individuals, four protein bands showed the highest density and these were identified as TBSPs (Fig. 2). Their relative quantity was significantly correlated with tannin concentration in the diet (R^2=0.497; p= 0.005). Data from peptide mass fingerprinting allowed us to identify 22 different proteins, some involved in tannin-defense and bitter taste reception, among them: cystatin A, B; IgM, serum albumin, lactate peroxidase and carbonic anhydrase.

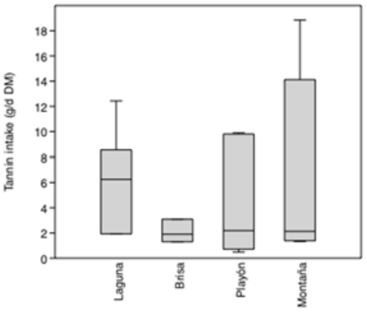
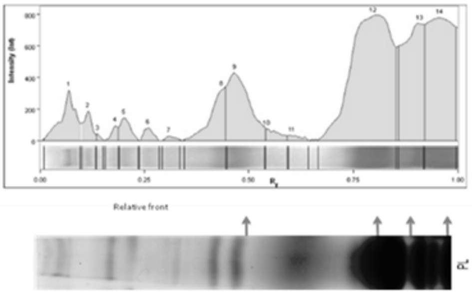


Figure 1 Condensed tannin intake (g/d DM) in four groups of monkeys.

Conclusion We showed that howler monkeys are able to eat plants with high concentrations of tannins and that some salivary proteins with tannin-binding affinity increase in their relative importance when the animals eat more tannins. Salivary protein composition correlates with the feeding behavior of herbivorous generalist as showed by the presence of salivary carbonic anhydrase VI and two types of cystatin (A-B), also it was important the identification of proteins related with astringency and bitterness acceptance as cystatin, lactoperoxidase and histidine-rich proteins. In spite of other work claiming the presence of proline-rich proteins in saliva of howler monkeys, these salivary proteins were not identified in whole saliva by MS/MS in this study. Our results show that salivary proteins play an important role in explaining the dietary flexibility of howler monkeys, and allow them to feed on the tannin-rich foods that are available in disturbed habitat.



Figue 2 Densitometry analysis of bands of salivary proteins on ID SDS-PAGE and Coomasie R250.

Acknowledgements FCEG was supported by a postdoctoral fellowship from National Council of Science and Technology, México CONACYT 232395 and 263706. This study was conducted in compliance with animal care regulations and applicable Mexican laws.

References

Espinosa Gómez FC, Serio-Silva JC, Santiago-García JD, Sandoval-Castro CA, Hernández-Salazar LT, Mejía-Varas F Ojeda-Chávez J and Chapman AC 2018 American Journal of Primatology 80, e22737.
Price ML Van Scoyoc S and Butler LG 1978. Journal of Agricultural and Food Chemistry 26, 1214–1218.
Shimada T 2006. Journal of Chemical Ecology 32, 1149-63.

Diet overlap between water buffaloes and Lesser White-fronted Goose

Illias Kaemiris, Thomas Papachristou, Panagiotis Platis, Savas Kazantzidis

Hellenic Agricultural Organisation "DEMETER", Forest Research Institute, GR 570 06 Vassilika, Thessaloniki, Central Macedonia, Greece

E-mail: ilias@fri.gr

Take home message Feeding niche partitioning between water buffaloes and Lesser White-fronted Goose minimizes the potential of emerging competitive interactions for food resources.

Introduction The aim of this study was to estimate the diet overlap of water buffaloes (*Bubalus bubalis*) and Lesser White-fronted Goose (*Anser erythropus* – LWfG), grazing in common wetlands surrounding Kerkini Lake (Northern Greece). Evaluating the feeding niches of coexisting herbivores is important for understanding not only their impact on forage resources but also their role in ecosystem processes (Krebs, 1999).

Material & methods Fresh faecal samples of water bufflaoes and LWfG were collected at Kerkini Lake from October 2012 to February 2013. Due to the temporarily flooding character of the study area, plant species usually start growing in August-September and complete their annual life cycle in January-February, thus this study took place throughout their whole life cycle. Diet composition was determined by microscopic analysis at 100-200X magnification, using the frequency addition procedure (Holechek *et al.,* 1984). Each plant species was assigned to one of the following forage categories: (1) grasses, (2) other graminoid species (*Cyperaceae* and *Juncaceae* families), (3) aquatic plants, *i.e.,* submerged, emerged and amphibious species and (4) forbs, *i.e.,* all the other broadleaved herbs present in the study area. Diet overlap was estimated at the species level using the Horn's overlap index R_o (Krebs, 1999). Overlap coefficients higher than 0.6 are considered to be indicative of a biologically significant overlap (Magurran, 1988).

Results & discussion Both herbivores heavily grazed on grasses as this forage category comprised more than the half of their total diet composition, whereas other graminoids and aquatic species were consumed by LWfG in higher percentages compared to water buffaloes (Figure 1). Concerning the forage categories level a similarity of the composition of diet of the studied herbivores is observed. However, at the species level it is a clear differentiation in diet composition as Horn's index $R_o = 0.3585$ is quite lower than the threshold (0.6) which indicates a significant overlap.

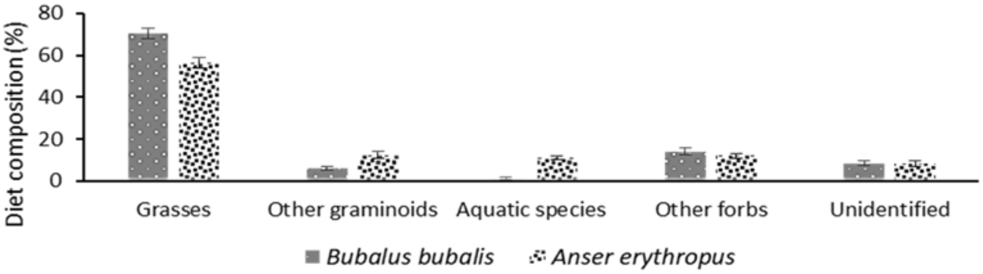


Figure 1 Average food consumption of major forage categories by water buffaloes and LWfG (mean ±SD) at Kerkini Lake (northern Greece) during the wintering period 2012-2013.

Conclusion The dissimilarity in the diets of water buffaloes and LWfG, lead to the conclusion that their coexistence in the same time-place is feasible with a limited potential of emerging competitive interactions between them for food. Grazing by water buffaloes maintains relatively low vegetation height which facilitates goose species as it has been observed in the northwestern European coast where cattle grazing benefits species of *Anseriformes* (van der Graaf *et al*. 2002). In addition, common grazing by these herbivores may contribute to a more efficient utilization of the available forage resources than a single-species grazing. Taking into consideration the fact that the study area is a typical Mediterranean temporary wetland, these conservational approaches can be applied to decision-making strategies for range and wildlife management over other areas with similar ecological conditions.

Acknowledgements This research was supported by the EU LIFE + project "LIFE 10 NAT/GR/000638 and funded by the European Commission and the Norwegian Directorate for Nature Management.

References

Krebs CJ 1999. Ecological methodology, 2nd ed. Addison-Welsey Educational Publishers, California.
Holechek JL, Vavra M and Pieper RD 1984. In: Van Dyne G.M., Brotnov J., Burch B., Fairfax S., Huey B. (Eds.), Developing Strategies for Rangeland Management, Westview Press, Boulder, Colorado.
Magurran AE 1988. Ecological diversity and its measurement, Princeton University, Princeton, USA
van der Graaf AJ, Bos D, Loonen MJ, Engelmoer M and Drent RH 2002. Journal of Coastal Conservation 8, 179-188.

671

Feeding behaviour of alpaca and llamas co-grazing on Andean highlands in Peru and the interactions with spatial distribution of available vegetation

Joaquin Castro-Montoya[1], Daniel Hoehn[1], Carlos Gómez[2], Uta Dickhoefer[1]

[1]*University of Hohenheim, Animal Nutrition and Rangelands Management in the Tropics and Subtropics, Stuttgart, Germany,* [2]*Universidad Agraria La Molina, Departamento de Zootecnia, Lima, Peru*

E-mail: jcm@uni-hohenheim.de

Take home message Llamas and alpacas have different grazing behaviour and strategies that result in different growth rates by each species depending on feed availability. No differences are observed between sexes.

Introduction Despite the economic relevance of South American camelids (SACs) across the vast Andean region relatively few efforts have been made to study their feed intake (FI) and animal behaviour under grazing conditions. Data available on FI and nutrient requirements derive mainly from animals kept in barns or studies involving sheep, goats and cattle (National Research Council, 2007), highlighting the knowledge gap on grazing behaviour of SACs in conditions typical of Andean farming systems (Gauly, 2011). Hence, a study with alpacas and llamas co-grazing on Andean pasture in Peru was conducted aiming at (i) Exploring group specific differences (*i.e.,* alpaca/llama, male/female) on activity patterns, FI, and growth; and at (ii) Relating grazing behaviour, FI and digestibility parameters with growth and vegetation characteristics.

Material & methods The study was conducted in 2016 between October and December (transition from dry to rainy season) in the Pasco region (4,350 m.a.s.l.) of Peru with vegetation predominantly as bofedales. From a herd of 634 alpacas and 74 llamas co-grazing on an area of 341 ha, twelve eight-month-old alpacas (huacaya) and llamas (q'ara) (six females and six males, respectively) were randomly selected for the study. Global positioning devices attached to the animals were used setting speed thresholds to monitor animal activity for twelve consecutive days. Internal (Titanium dioxide, 2.5 g/d) and external markers (acid detergent insoluble ash) were used to estimate FI and organic matter digestibility. Faecal grab samples were collected during five days from each animal. Diet simulation sampling was performed for four alpacas and four llamas by direct observation. Vegetation samples for estimation of biomass production and nutrients concentration were taken at the end of October and the end of November from 14 transects across the grazing area. Live weight of animals was recorded every four weeks. The data was analysed by an anova using the Mixed procedure of SAS with species, sex and their interaction as main effects and animal as repeated measurement.

Results Llamas spent more time grazing than alpacas, while alpacas spent more time resting, and both species having similar travelling time. Llamas walked longer distances than alpacas. No differences between sexes were found (Table 1). Alpacas travelled longer early, indicating more time dedicated to find a place to graze, opposite to llamas. Feed intake (g/kg $BW^{0.75}$) did no differ between species and sexes. Alpacas showed a higher growth rate during the first month of the transition from the dry to the rainy season than llamas, which could be a biological response to worse body condition after the scarcity period, and/or because of an advantageous grazing strategy when better quality feeding sites became available; interestingly, during the second month llamas had a higher growth rate than alpacas. Over the two months, alpacas had greater relative growth rate than llamas (Table 1). Based on diet simulation, nitrogen intake (NI, g/d) was higher for alpacas than for llamas, but available nitrogen (g/100 g NI) appeared to be higher for llamas (data not shown).

Table 1 Grazing time, distance travelled, dry matter intake and growth performance of female and male alpacas and llamas co-grazing during the transition of the dry to the rainy season in the Pasco region of Peru (Means±standard deviation; n = 6).

Parameter	Alpacas		Llamas		P-value	
	Female	Male	Female	Male	Species	Sex
Grazing time (h/d)	8.3 ± 0.9	8.2 ± 0.9	9.9 ± 0.1	9.9 ± 0.3	<0.01	0.94
Distance walked (km/d)	7.12 ± 0.36	6.86 ± 0.36	7.93 ± 0.68	7.66 + 0.97	<0.01	0.26
Dry matter intake (kg/d)	0.95 ± 0.14	1.02 ± 0.29	1.43 ± 0.35	1.45 ± 0.20	<0.01	0.36
Dry matter intake (g/kg $BW^{0.75}$)	65.6 ± 12.2	71.0 ± 16.0	64.1 ± 3.3	65.5 ± 7.3	0.40	0.45
Relative growth rate (g/d/kg BW)	1.93 ± 0.91	2.49 ± 0.24	1.74 ± 0.71	1.22 ± 0.96	0.03	0.95

Conclusion Alpacas seem to be more efficient on the selection of good quality pasture than llamas and on energy conservation, strategies that could favour them to recover faster than llamas once fresh biomass was available after the onset of the rainy season. However, llamas seem to have a physiological advantage to better utilize nutrients from roughages.

Acknowledgements To the community San Pedro de Racco, Pasco, Perú, to Carlos Quispe and Edith Gómez.

References

Gauly M, Vaughan J and Cebra C 2011. Neuweltkameliden - Haltung, Zucht, Erkrankungen. 3rd edition, page 62.
National Research Council. 2007. Washington, D.C. National Academies Press, page 238.

Nutrients composition of feedstuffs fed to animals in the National Gardens of South Africa

KyUngu Ulrich Mwimbi, Khanyisile Mbatha

Unisa, Pretoria, South Africa

E-mail: ulrichkm@gmail.com

Take home message Variations in nutritional contents of some feed items and their systematic nomenclature can contribute to formulating the diet sheets and the management of feeding programmes in the zoo.

Introduction A feeding programme without chemical evaluation of dietary constituents or assessment of utilization of a particular feedstuff has limited value in the management of captive animals (Dierenfeld, 1997). While the appearance and preference of feeds are important, nutrient analyses will more accurately define the nutritional value of captive animal diets (Bernard, 1997). There are different kinds of animal species in the National Zoological Garden (NZG) of South Africa (herbivores, carnivores, insectivores, and piscivores). The feeds include dry forages, fish, pellets, meats, fruits, and invertebrates. The composition of these feeds fluctuates either due to season, development stages, age, or types of species (fish), etc. Not enough or nutrient composition of the feedstuffs fed in the NZG is known and this needs to be documented. Lastly and not least, the nutritional composition may affect the wild animal species responses based on their requirements.

Material & methods A feed item list, fed to captive animals, was provided by the store and nutrition service of the NZG of South Africa. The diet evaluation by survey and review technique (Fidgett & Plowman, 2009) was adopted and modified to answer to the objectives of the study, which was coupled to laboratory analyses for proximate analysis according to Bernard and Dempsey (1999) guidelines. Zootrition ™ (Version 2.6) is a software package and database designed as a tool for zoological, aquarium and training institutions to assist in the analyses and preparation of diets for captive wild animals (Wildlife Conservation Society, 1999). Animal species, to be evaluated, were chosen based on their morpho-physiological type of feeder and their conservation status. Data on the nutrient composition of feedstuffs was first captured into the program; thereafter a number of selected NZG diet sheets were uploaded to the programme database and saved as so-called in-house mix diets.

Results & discussion The International Network of Feed Information Centres (INFIC) nomenclature were adopted to describe the feed items. Lucerne in wet season presented high levels of crude protein, consequently it is categorised as protein source (class 4: protein supplements) than hay and roughages. Comparing batches within fish species, hake, and maasbanker showed significant difference on dry matter, crude protein level, and on fat content. Day-old chicks eviscerated and non-eviscerated, presented nutritional variation are subjected to the process of removing all the viscera from their body. The evaluation of different animal species diet sheet with Zootrition™ programme, in-house mix diet showed both excess and deficiency of macro-elements and microelements in the case Greater Flamingo Phoenicopterus ruber roseusBlack rhinoceros Diceros bicornis, African wild dog Lycaon pictus, African buffalo Syncerus caffra and Chimpanzee Pan troglodytes. The INFIC feedstuff identification indicated that the items fed to zoo animals were too anthromorphic since they can only be characterised by three of the six descriptors.

Conclusion Human nutritional practices adopted at some zoological gardens do not consider the effects of food processing and preparation on nutrition and seasonal variations of forages which affect the quality and bioavailability of nutrients. This may have negative consequences on animal health and cost of feed formulation. Items fed to animal in the NZG presented specific nutritional variation, which need to be considered when formulating the diet. NZG used to formulate diets for captive animals based on empirical combination of extrapolations and questions that included, among others the morpho-physiological criteria for the diet formulation. However, variations in nutritional contents of some feed items and their systematic nomenclature can contribute to formulating the diet sheets and the management of feeding programmes in the zoo.

Acknowledgements Dr Ellen Dierenfeld for assistance, as well as Lorna Fuller (Johannesburg Zoo) for her encouragement and her assistance with training on Zootrition™ software, the librarian Mrs Brenda Swanepoel and Adriana Pienaar, Frank Rowland and all staff from the animal kitchen at the National Zoological Gardens.

References

Dierenfeld ES 1997. Captive Wild Animal Nutrition: Historical Perspective. Proceeding Of The Nutrition Society 56: Pp 989-999

Bernard JB and Dempsey JL 1999. Quality Control of Feedstuffs: Nutrient Analyses. In: Ullrey, D. E., Ward, A. M. & Allen, M. E. (Eds.). Nutrition Advisory Group Handbook. Fact Sheet 010. Chicago Zoological Society, Brookfield Zoo, Chicago, Illinois. pp1- 7.

Fidgett AL and Plowman A 2009. Zoo Nutrition Guidelines: Nutrition Evaluation. Bristish And Irish Association Of Zoos And Aquariums, London, UK.

Wildlife Conservation Society. 1999. Zootrition ™ Version 2.6 User's Guide. Bronx New York, USA.

Mixed grazing systems with horses and cattle: an alternative to control nematode infection

Louise Forteau[1,2], Bertrand Dumont[1], Guillaume Sallé[3], Geneviève Bigot[4], Géraldine Fleurance[2,1]

[1]*Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, Saint Genès Champanelle, France,* [2]*Ifce, pôle développement innovation et recherche, Exmes, France,* [3]*INRA et Université François Rabelais Tours, UMR Infectiologie et Santé Publique, Nouzilly, France,* [4]*Université Clermont Auvergne, AgroParisTech, INRA, Irstea, VetAgro Sup, UMR Territoires, Clermont-Ferrand, France*

E-mail: louise.forteau@inra.fr

Take home message Young saddle horses conducted in mixed grazing systems with beef cattle had lower strongyle faecal egg count than their counterparts from specialized systems.

Introduction Controlling strongyle nematode infection is a key challenge in horse grassland-based systems. They are responsible for major health issues in horses and systematic drenching programs have selected for anthelmintic-resistant strongyle populations. This questions the use of anthelmintics for horses, and calls for alternative methods to control strongyles. Many studies demonstrated that associating cattle and sheep in mixed grazing systems successfully controlled nematode infection in sheep (Brito *et al*., 2013; Marley *et al*., 2006). On mixed farms, horses and cattle are frequently grazed together. Here, we aim to test the dilution effect on horse strongyle infections that could result from the presence of cattle on the same pastures (simultaneously or alternatively), since cattle and horses only have one strongyle parasite in common.

Material & methods Strongyle faecal egg count (FEC; results given in number of egg per g) were measured in 46 horses from mixed grazing systems (n=23, six farms) or specialized systems (n=23, five farms). Horses aged between 18 months and 3.5 years old, the age class when horses are the most sensitive to parasites (Love and Duncan 1992). Faecal material were recovered from fresh horse dungs in pasture. Horses had not been treated with anthelmintic within the last 3 months preceding the study. No differences were observed on anthelmintic treatment frequency between mixed and specialized systems. Faaeces were kept at 4°C less than 48 hours, and egg counts were performed by the official local veterinary services (DDCSPP du Puy de Dôme). Data were analysed with a mean comparison test (on lognormal distribution, t-test on 2 independents samples with a 95% confidence interval) under R studio software.

Results Strongyle faecal egg counts was highly variable between horses in each system (Figure 1). However, young horses grazing the same pastures than as cattle in mixed systems displayed twice as low mean FEC two in comparison to their counterparts from specialized systems: 313 eggs per g *vs.* 636 eggs per g respectively (P<0.01).

Conclusion Mixed grazing with cattle was associated with reduced FEC in young horses. This brings the first evidence that mixed grazing allows for a decreasing parasitic burden in grazing horses. Mixed grazing together with the short-term use of tannin-rich pellets in horse diet (Collas *et al*., 2018) could thus be be part of an integrated management of saddle horse health in grassland-based systems, in line with agroecological principles. This work is part of a larger project in which we analyze the health, environmental and economic benefits offered by mixed grazing systems, as well as obstacles to their development (working load, farmers' skills, etc.).

Acknowledgements This research is supported by grants from IFCE and INRA-Phase Division, in partnership with Irstea. We are grateful to the farmers who agreed to leave us collecting dung samples in their fields and answered our questions.

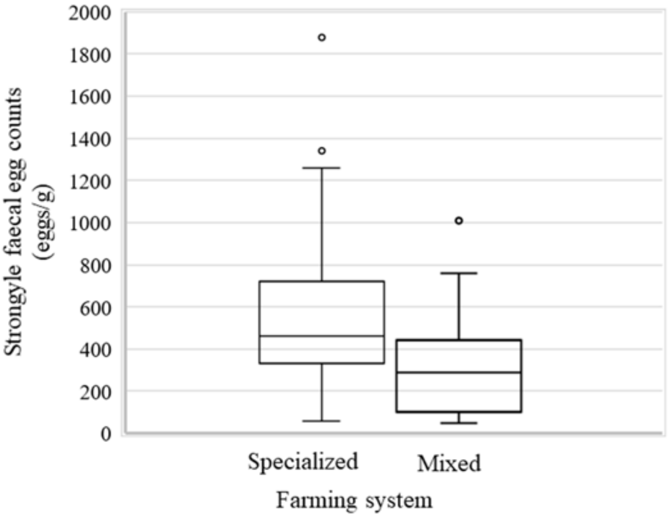


Figure 1 Box and whisker plot of Faecal Egg Count in horses (in egg per g) from specialized (specialized, median = 460 eggs per g) or mixed (mixed, median = 290 eggs per gram) systems.

References

Brito DL, Lima Dallago BS, Louvandini H, Verdolin dos Santos VR, Figueirêdo de Araújo Torres SE, Gomes EF, Talamini do Amarante AF, Barros de Melo C and McManus CM 2013. Revista Brasileira de Parasitologia Veterinária 22, 485–494.
Collas C, Sallé G, Dumont B, Cabaret J, Cortet J, Martin-Rosset W, Wimel L and Fleurance G 2018. Animal 12, 359-365.
Love S and Duncan JL 1992. Veterinary Parasitology 44, 127–142.
Marley CL, Fraser MD, Davies DA, Rees ME, Vale JE and Forbes AB 2006. Veterinary Parasitology 142, 134-41.

Conjugation of *leucaena*-toxins plays an important role in protection against hydroxypyridone toxicity in ruminants on high leucaena diets

Michael Halliday[1,2], H. Max Shelton[2], Chris McSweeney[3], Jagadish Padmanabha[3], Graham Kerven[2]

[1]The University of New England, Armidale, Australia, [2]The University of Queensland, Brisbane, Australia, [3]The Commonwealth Scientific and Industrial Research Organisation, Brisbane, Australia

E-mail: michael.halliday@une.edu.au

Take home message Conjugation of hydroxypyridone (DHP), and not microbial detoxification, plays a vital role in protecting animals from leucaena toxicity when adapting to and consuming high leucaena diets.

Introduction The forage tree legume leucaena is a high-quality ruminant feed vitally important as a source of protein for livestock production in tropical regions. However, it contains the acutely toxic non-protein amino acid mimosine in leaves and seeds. While ruminants are able to degrade mimosine if they are introduced slowly to high leucaena diets, its breakdown to chronically toxic hydroxypyridones (DHP) was believed to limit its productivity and adversely affect animal health. The Australian development of a fermenter-cultured oral inoculum containing the DHP-degrading ruminal bacterium *Synergistes jonesii* in the 1980s was thought to overcome this issue; however, evidence of leucaena toxicity occurring in inoculated animals remained.

In response, a program of research was initiated to investigate the efficacy of *S. jonesii* as well as other methods of protection against toxicity in ruminants. Broadly, this involved study of: (a) the effectiveness of microbiological degradation via *S. jonesii*; and (b) non-microbial metabolic detoxification. Key objectives were to: (a) profile the toxicity status of ruminants in eastern Indonesia; (b) determine the efficacy of inoculation with *S. jonesii* in both Indonesia and Australia; and, (c) determine the extent of *in vivo* hepatic conjugation of DHP and its role in protecting ruminants against toxicity.

Material & methods In Indonesia, the leucaena toxicity status of ruminants was assessed in a survey across the four islands of Lombok, Sumbawa, Sumba and West Timor. Secondly, an inoculation study involving the transfer of rumen fluid from “protected” Indonesian ruminants to naïve Bali bulls was conducted. In Australia, a controlled animal house experiment was conducted to measure the impact of inoculation with the commercial *S. jonesii* inoculum on naïve steers consuming high leucaena diets. Finally, the role and mechanisms of hepatic conjugation of toxins were investigated. This involved a 4-week longitudinal assessment of the toxicity status and productivity of Indonesian Bali bulls on 100% leucaena diets.

Results & discussion The survey of toxicity status in Indonesia revealed the ubiquitous presence and a number of different strains of *S. jonesii*, including the ATCC type strain (78.1). However, *S. jonesii* was always at low population levels and always accompanied by high levels of undegraded 2,3-DHP in urine. Despite this apparent failure of *S. jonesii* to degrade DHP, there was no observed impact on animal productivity or health.

In the Indonesian inoculation study, sequential monitoring of DHP levels in urine indicated no effect of inoculation from “protected” donors to recipient animals. Key findings from the Australian inoculation study were: (a) inoculation had no effect on total DHP excretion; (b) indigenous *S. jonesii* strains were present in naïve animals prior to inoculation; (c) DHP did not suppress thyroid hormone production; and (d) high levels of the isomer 2,3-DHP were present without accompanying signs of toxicity.

In the longitudinal assessment of toxicity status, bulls recorded high liveweight gains even though they persistently excreted high levels of the toxin 2,3-DHP. Using HPLC and HDMS it was found that up to 97% of DHP was excreted as 2,3-DHP in conjugated form. The conjugate was identified as glucuronic acid, forming a 2,3-DHP-*O*-glucuronide, with ongoing work investigating other potential conjugates. It was concluded that *S. jonesii* was not capable of degrading all DHP and that conjugation played the key role in preventing DHP toxicity, due to the active detoxification of the compound, inhibiting chelation of essential metals, and the increase in the speed of clearance of conjugated toxins from the body. Further, previously unidentified mono-hydroxypyridines (HP) were also found to be conjugated and could indicate possible intermediates in the isomerisation of 3,4-DHP (from mimosine) to 2,3-DHP.

Conclusion It was concluded that hepatic conjugation, and not microbial detoxification, was the main pathway for control of toxicity in ruminants consuming high leucaena diets, and that inoculation with *S. jonesii* may no longer be necessary. However, appropriate management of ruminants being introduced to leucaena diets is necessary to allow adaptation to the mimosine in diet, and then to build metabolic tolerance to DHP. The findings of this research greatly advances our knowledge of how ruminants adapt to the toxins when consuming diets containing leucaena.

Acknowledgements This research was supported by funding from the Australian Centre for International Agricultural Research (ACIAR).

Feeding sugar rich hay can support milk production and stabilize the ruminal pH in early lactating Simmental cows

Fenja Klevenhusen, Maria-Theresia Kleefisch, Qendrim Zebeli

Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Vienna, Austria

E-mail: fenja.klevenhusen@vetmeduni.ac.at

Take home message Reducing the amount of concentrate by increasing hay quality can maintain milk production and stabilize ruminal pH in early lactation.

Introduction Feeding high grain diets to dairy cows in early lactation has become a common practice. However, the risk of metabolic disorders like subacute ruminal acidosis grows with increasing starch intake. Accordingly, alternative feeding strategies have to be evaluated in order to decrease the use of concentrates in ruminant feeding and to improve ruminal and animal health while maintaining production efficiency. A first experiment with non-lactating cows showed that feeding sugar rich hay significantly increased nutrient digestibly and concentration of ruminal short chain fatty acids (Kleefisch *et al*., 2017; Klevenhusen *et al*., 2017). In combination with concentrate feeding sugar rich hay decreased rumination, while effects on ruminal pH were small (Kleefisch *et al*., 2017). However, dry matter intake (DMI) of the non-lactating cows was low and effects on rumination and ruminal pH might be different at higher intake levels. In this respect a feeding trial with 24 Simmental cows in early lactation was carried out to test the effects of feeding incremental proportions of high-quality, sugar rich hay while reducing the amount of concentrate on DMI, ruminating activity, ruminal pH and milk production.

Material & methods Twenty-four Simmental cows were blocked by lactation number and previous milk yield and distributed to four dietary groups (n=6) beginning 10 days before the expected calving date until 28 days thereafter. Diets were 100HQ (100% sugar rich hay), 75HQ (75% sugar rich hay + 25% concentrate) and 60HQ (60% sugar rich hay + 40% concentrate). As control diet 60LQ containing 60% fibre-rich hay + 40% concentrate was used. Diets differed in content of sugars (100HQ = 19.8%, 75HQ = 16.1%, 60HQ = 13.9%, 60LQ = 9.6%), neutral detergent fibre (100HQ = 42.3%, 75HQ = 37.3%, 60HQ = 34.3%, 60LQ = 45.1%) and crude protein (100HQ = 22.6%, 75HQ = 20.4%, 60HQ = 19.3%, 60LQ = 14.2%). Daily DMI and milk yield were electronically recorded. Ruminating and chewing behaviour of each cow was measured on four consecutive days using noseband sensors (Rumiwatch, ITIN & HOCH GmbH, Fütterungstechnik, Liestal, Switzerland). Four cows per group additionally received indwelling wireless pH sensors (smaXtec premium, smaXtec animal care GmbH, Graz, Austria) before parturition to allow continuous measurement of ruminal pH. Data were analysed with the Mixed procedure of SAS (Version 9.4) using the Repeated statement to account for a potential day effect.

Results & discussion Intake of DM and milk yield increased continuously postpartum. No differences between groups were found in daily time spent eating (on average 456 min/d; $p = 0.539$) and ruminating (on average 517 min/d; $p = 0.472$). However, DMI was approx. 3.5 kg lower in cows fed 100HQ compared to the others, resulting in increased time spent ruminating per kg DM intake (significant against 75HQ, $p = 0.018$). Milk yield four weeks post partum was higher ($p < 0.001$) when cows were fed 60HQ (39.3 kg/d) compared to the others (on average 33.3 kg/d). Mean ruminal pH measured with indwelling sensors was not affected by diet (average pH = 6.3, $p = 0.621$). However, daily time spent below pH 6 was longest in cows fed the control diet 60LQ (381 min/d), whereas in the other cows time averaged 70 min/d ($p = 0.0998$). In line with that contrast analysis revealed a significant effect of hay quality ($p = 0.017$) on time below pH 6, regardless of the concentrate level.

Conclusion Beneficial effects of feeding sugar rich hay on milk production and ruminal pH were found. Feeding sugar rich hay maintained milk production with low or no concentrate addition in comparison to the control diet. Daily time spent ruminating was not affected by feeding sugar rich hay, but feeding sugar rich hay alone resulted in lower DMI, likely due to the physical rumen fill. Regardless of concentrate level, feeding sugar rich hay stabilized the ruminal pH by decreasing the daily time of ruminal pH below 6.

Acknowledgements This project was funded by the Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management under Grant BMLFUW100928. The high-quality hay was provided by ARGE Heumilch Austria, which is gratefully acknowledged.

References

Kleefisch MT, Zebeli, Q, Humer E, Kröger I, Ertl P and Klevenhusen F 2017. Archives of Animal Nutrition 71, 21-36.
Klevenhusen F, Petri RM, Kleefisch M-T, Khiaosa-ard R, Metzler-Zebeli BU and Zebeli Q 2017. FEMS Microbiology Ecology, 93.

Effect of parity and age at first calving of dairy cows on dynamics of milk calcium contents and blood biomarkers of bone accretion and resorption throughout lactation.

Pierre Gaignon[1], Philippe Faverdin[1], David Sidaner[1], Anca Laza-Knoerr[2], Karine Legrand[2], Catherine Hurtaud[1], Anne Boudon[1]

[1]*UMR PEGASE, INRA, 35590, Saint-Gilles, France,* [2]*CMI, 18 avenue F. Roosevelt, BP80139, 35400, Saint-Malo, France*

E-mail: pierre.gaignon@inra.fr

Take home message Analyses of plasma biomarkers of bone accretion and resorption throughout lactation illustrates that older cows have less bone remodelling and lower capacity to mobilise their bones.

Introduction At the beginning of lactation, dairy cows face a huge increase in their calcium (Ca) requirements due to their important milk production. These requirements cannot be satisfied by Ca ingestion. It has been shown that cows mobilize Ca from their bone and that Ca reserves in bone can be restored later during lactation. The amplitudes of these cycles of bone reserve are difficult to quantify on important number of cows and for that reason, its consequences on cows' health remains to be determined. It has been shown that the mammary gland can be involved in the regulation of calcemia by decreasing milk Ca content and increasing bone resorption when calcemia decreased (Van Houten, 2004). The objective of this study was to determine if a link could exist between individual dynamics of milk Ca content and amplitude of bone mobilisation cycles.

Material & methods Thirty-three dairy cows were involved (n=17 in first lactation, n=10 for second lactation and n=6 for third or higher rank of lactation). Cows were fed *ad libitum* with a constant total mixed diet throughout lactation. Milk and blood samples were collected 15 days before expected date of calving and from 15 days after calving and every 4 weeks after. Ca and phosphorus (P) contents were analysed in milk. Ca, inorganic P, osteocalcin (biomarker of bone accretion) and CTX (biomarker of bone resorption) were also measured in plasma. Data were analysed with proc MIXED of SAS, including effects of parity and stage of lactation and their interaction as fixed effects, and using the repeated statement on cows. Individual milk Ca content dynamics were also characterized using factorial approach (PCA), and effect of dynamics was analysed with the interaction of stage of lactation using proc MIXED of SAS.

Results & discussion Both plasma contents of biomarkers of bone accretion and resorption were lower as the parity increased ($P < 0.05$, Figure 1). These results suggested that older cows had less bone remodelling. The amplitude of the increase of plasma CTX after calving was also higher in primiparous than in multiparous cows (interaction parity × lactation stage, $P < 0.05$). Three years old primiparous cows had also lower plasma osteocalcin contents than 2 years old primiparous cows, with values near to 2nd lactation cows. All primiparous cows had similar CTX contents.

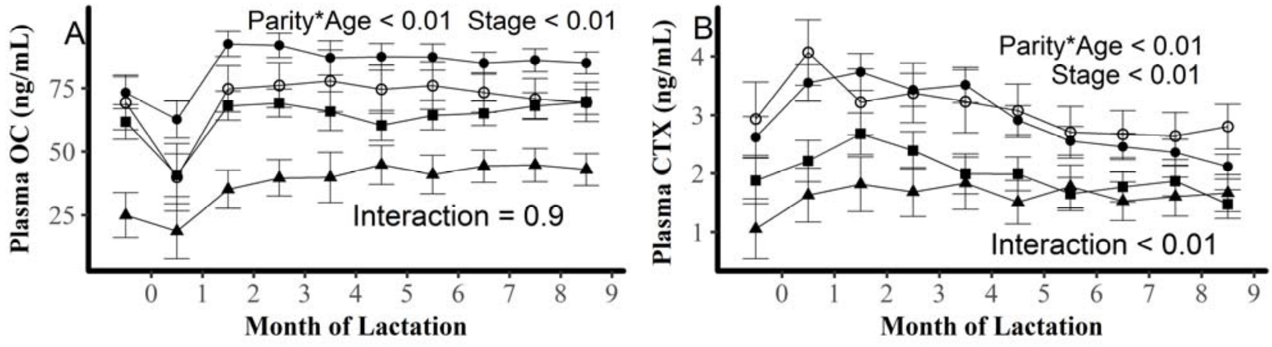


Figure 1 Effect of parity and stage of lactation on A) plasma osteocalcin concentration; B) plasma CTX concentration ●Primiparous with calving at 2 years old; ○Primiparous with calving at 3 years old; ■ 2nd lactation; ▲ 3rd lactation.

Amplitude of variation of milk Ca content at the evening milking, throughout lactation, also tended to be higher ($P < 0.05$) in primiparous than in multiparous cows suggesting a weak link between milk Ca content and plasma CTX content. Three groups of milk Ca dynamics have been characterized by PCA. Neither these groups, nor the interaction between these groups and the stage of lactation affected plasma OC or CTX contents. This suggested that the evolution of milk Ca content throughout lactation is not linked to the dynamics of biomarkers when individual data are considered.

Conclusion The dynamics of plasma biomarkers of bone accretion and resorption clearly show that bone modelling is higher in younger cows and that first parity cows had higher amplitude of plasma content of CTX during lactation. However, the link between plasma biomarkers of bone accretion and resorption and milk Ca content seemed weak when considering individual variation.

Acknowledgements This research was supported by INRA, Région Bretagne and CMI-AII.

References

Van Houten J, Dann P, McGeoch G., Brown E, Krapcho K, Neville M and Wysolmerski J 2004. The Journal of Clinical Investigation 4, 598-608.

Feed restriction modifies mammary miRNome of mid-lactation cows

Pierre-Alexis Billa[1], José Pires[1], Yannick Faulconnier[1], Tao Ye[2,3], Christine Leroux[1,4]

[1]Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès-Champanelle, France, [2]Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), 67404 Illkirch, France, [3]Centre National de la Recherche Scientifique, UMR7104, 67404 Illkirch, France, [4]Department of Food Science and Technology, University of California Davis, Davis, CA, 95616, USA

E-mail: pierre-alexis.billa@inra.fr

Take home message Feed restriction modifies mammary gland miRNome of midlactation Holstein cows.

Introduction Feed restriction is frequently used as an experimental model to induce negative energy balance (NEB), altering milk production and composition, and plasma metabolite profiles in dairy cows (Bjerre-Harpøth *et al.*, 2012). MicroRNAs (miRNA; small non-coding RNA) regulate the expression of genes involved in many cellular processes (Ambros, 2004), including mammary gland (MG development and lactation (Le Guillou *et al.*, 2014). Recent research show that feed-deprivation alters the miRNome of goat MG (Mobuchon *et al.*, 2015). The objective of this study was to assess the effects of 6 days of feed restriction on MG miRNome of mid-lactation Holstein and Montbéliarde cows.

Material & methods Nineteen mid-lactation (165 ± 21 DIM) cows (9 Holstein and 10 Montbéliarde) underwent 6 days of feed restriction during which feed allowance was reduced to meet 50% of NE_L requirements (calculated before initiation of the challenge). Phenotypic measurements were performed from day -2 to +20 relative of initiation of restriction, corresponding to the following periods: Control (CONT; day-2 and -1), restriction (REST; day 1 to 6), week 1 (WK1; day 7 to 13) and week 2 (WK2; day 14 to 20). Morning jugular plasma was analysed for non-esterified fatty acid (NEFA) and β-hydroxybutyrate (β-OH) concentrations were measured using an automatic analyser (ARENA 20XT). MiRNA expression profiles were analysed from MG biopsies performed one day before and on day 6 of feed restriction. Total RNA was extracted using miRVana kit, and miRNAs were sequenced by RNAseq using Illumina HiSeq 4000. Statistical analyses for repeated measures and known miRNAs were performed using Mixed models of SAS and the DESeq2 package of R, respectively. Significance was considered at $p \leq 0.05$ and tendency at $0.05 < p \leq 0.10$.

Results & discussion Feed restriction induced NEB, altered milk production and plasma metabolic profile, as expected for mid-lactation cows. Breed effects were not observed for plasma NEFA and β-OH concentrations (Table 1). Preliminary analyses of miRNome showed that feed restriction modified the expression of 93 miRNAs (54 with $p \leq 0.05$ and 39 with $0.05 < p \leq 0.10$) when both breeds were analysed together. Fifty-two miRNAs (33 with $p \leq 0.05$ and 19 with $0.05 < p \leq 0.10$) were differentially expressed in response to restriction in MG of Holstein cows. Only 2 miRNAs presented a tendency to change in MG of Montbéliarde cows, which is probably due to the greater variance observed in Montbèliarde. Common miRNA were found among these comparisons (Figure 1).

Table 1 Effects of restriction on energy balance, milk and plasma variables in mid-lactation Holstein and Montbéliarde cows.

		Period				*p*-value		
		Cont	Rest	wk 1	wk 2	Breed	Period	Breed x Period
Energy balance (MJ/d)	Holstein	47	-44	36	44	0.03	0.001	0.004
	Montbéliarde	28	-41	24	38			
Milk (kg/d)	Holstein	29	20	25	28	0.002	0.001	0.21
	Montbéliarde	24	16	21	24			
NEFA (mmol/L)	Holstein	0.11	0.64	0.08	0.09	0.27	0.001	0.87
	Montbéliarde	0.11	0.67	0.09	0.10			
B-OH (mmol/L)	Holstein	0.59	0.64	0.63	0.50	0.28	0.001	0.31
	Montbéliarde	0.58	0.61	0.59	0.52			

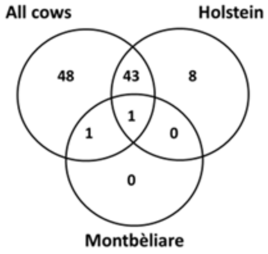


Figure 1 Comparison of three lists of differentially expressed miRNA ($p \leq 0.10$) in response to restriction.

Conclusion This model of feed restriction induced expected changes in milk production and plasma metabolic profiles. Mammary miRNA profiles showed that bovine mammary miRNomes are modified by feed restriction. If specific miRNA are secreted in the milk, they may constitute easily available biomarkers of undernutrition and NEB.

Acknowledgements Authors thank the staff at Herbipôle (INRA, UE1414, Marcenat, France) for animal care and sample collection, S. Bes, D. Chadeyron and A. Delavaud for technical assistance, and IGBMC (Illkirch, France) for miRNA sequencing. This research was funded in part by the GISA program of INRA (LongHealth project).

References

Bjerre-Harpøth V, Friggens NC, Thorup VM, Larsen T, Damgaard BM, Ingvartsen KL and Moyes KM 2012. Journal of Dairy Science 95, 2362–2380.
Ambros V 2004. Nature 431, 350–355.
Le Guillou S, Marthey S, Laloë D, Laubier J, Mobuchon L, Leroux C and Le Provost F 2014. PLoS One 21, e91938.
Mobuchon L, Marthey S, Le Guillou S, Laloë D, Le Provost F, Leroux C 2015. PLoS One 10, e0140111.

LC-MS based urine metabolic profiling of sheep with pregnancy toxemia

Changzheng Guo, Shengyong Mao

Nanjing Agricultural University, Nanjing, China

E-mail: guocz312@163.com

Take home message The pathogenesis of Pregnancy toxemia is not completely clear. Metabolomics can fully reflect metabolic differences, thus using blood metabolomic technology could be beneficial in understanding the pathogenesis.

Introduction Pregnancy toxemia is a metabolic disease of polytocous sheep and goats, which occurs in late gestation following inappropriate nutrition and stress. It is characterized by hypoglycaemia and hyperketonemia (Marteniuk and Herdt, 1988). Previous studies suggest that the treatments described for the pregnancy toxemia outcomes are variable because of the unclear mechanisms behind the condition. Thus, we aimed to assess the metabolic profile of urine samples from sheep with pregnancy toxemia.

Material & methods Sixteen healthy pregnant ewes (Hu sheep, 60.6 ± 4.9 kg) were randomly allocated to either a normal feeding pattern in the control group (CON, n = 8) or feed restriction to 30% of the baseline feed intake (FR, n = 8) for 15 days. Blood samples were collected from the jugular vein before the morning feed on the slaughter day, and were stored at -20 °C until analysis. The concentration of glucose and β-hydroxybutyrate (BHBA) of the blood samples was determined. Urine samples were collected from bladder after slaughter and stored at -80 °C until analysis by LC-MS. The urine metabolites were analyzed using SIMCA-P software (version 13.0, Umetrics AB, Umea, Sweden). Multivariate statistical analyses included principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA). The blood parameters were analyzed with SPSS (version 20.0 for windows, IBM) using the independent sample t-test.

Results Blood BHBA of the FR group exceeded the threshold of normal level (0.8 mM), which was higher ($p < 0.0001$) than that of the CON group (0.61 mM). Plasma glucose of the FR group was lower ($p = 0.001$) compared with the CON group (Table 1). PCA and PLS-DA were used to identify the key compounds responsible for the differentiation. Score plots based on the first two components showed that the two groups were well separated (Figure 1). The most relevant metabolic differences between the CON and FR groups are illustrated in Figure 2.

Table 1 The effect of FR on the concentration of glucose and β-hydroxybutyrate in blood in pregnant ewes (mean ± SEM).

Items	CON	FR	P value
Glucose, mM	2.87 ± 0.11	1.81 ± 0.22	0.001
β-hydroxybutyrate, mM	0.61 ± 0.06	4.11 ± 0.34	< 0.0001

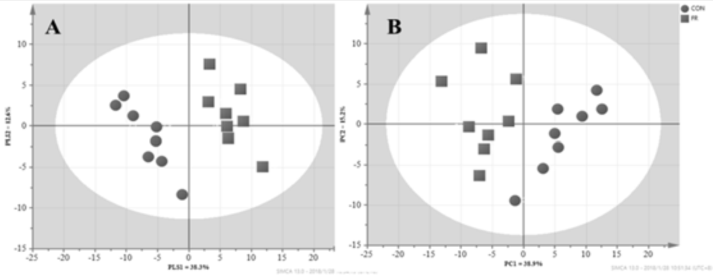


Figure 1 PCA (A) and PLS-DA (B) scores plot of urine metabolites based on LC-MS from the CON and FR groups.

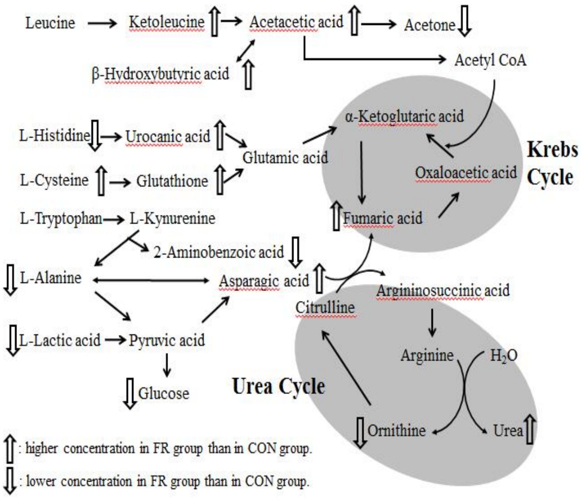


Figure 2 This schematic representation depicts the most relevant metabolic differences between the CON and FR group.

Conclusion Pregnancy toxemia was successfully induced by feed restriction in the present study. As compared with the CON group, LC-MS based urine metabolomics revealed that the levels of lactic acid and glucose were decreased in FR group, in addition, the content of amino acids, including alanine and histidine decreased while cysteine and the intermediate metabolites of ketogenic amino acids (ketoleucine) increased, which were accompanied by the increase in the levels of fumaric acid and urea. Generally, these results suggest that pregnancy toxemia may result in mobilization of body protein.

Acknowledgements This research was supported by National key research and development program (China No. 2016YFD0501206).

References

Marteniuk JV and Herdt TH. 1988. Veterinary Clinics of North America: Food Animal Practice 4, 307-315.

679

Comparison of hepatic mineral concentrations between grass-fed and conventional-fed beef cattle

Robert Van Saun

Pennsylvania State University, University Park, Pennsylvania, USA

E-mail: rjv10@psu.edu

Take home message Appropriate mineral supplementation is necessary to optimize grass-fed feeding systems.

Introduction Exclusive grass-fed feeding practices for finishing beef cattle are becoming a popular management system in meeting niche consumer markets. Forage, depending upon quality, can provide nearly all required nutrients in meeting beef cattle needs, though trace mineral status is often inadequate. The study objective was to determine if hepatic liver mineral concentrations are different between beef cattle raised under conventional or exclusive grass-fed feeding systems.

Material & methods All liver samples for mineral analyses were collected from either a regional abattoir, to represent the grain supplemented Conventional-Fed (CF; n=39) group, or local abattoirs catering to the Grass-Fed (GF; n=55) producer market. Inductively coupled plasma atomic emission spectroscopy (ICP/MS) was used to assay 9 minerals (Ca, Cu, Co, Fe, Mg, Mn, Mo, Se, Zn) in all samples. Mineral concentrations were determined on a wet weight (WW) and converted to a dry weight (DW) basis. Forage, soil, and mineral supplements were collected from the 5 participating GF herds and analysed for nutrient content. Analysis of variance methods (ANOVA) were used to determine significant effects of source (CF vs GF), herd effects, or their interaction on liver, forage, or soil mineral concentrations.

Results & discussion Conventional-fed samples had higher liver Fe (P=.003), Cu (P<.0001), Zn (P<.0001), and Se (P<.0001) compared to GF samples suggesting inadequate trace mineral supplementation or dietary interactions reducing trace mineral availability (Suttle, 2010; Table 1). A similar pattern was seen when accounting for herd effects. All GF herds had lower (P<.05) Se status compared to CF samples. All but 1 GF herd had lower (P<.05) Cu and Zn compared to CF samples. This GF herd had moderately low forage Mo content. Differences in forage nutrient content were minimal and did not account for all observed liver mineral differences between herds. Soil nutrient content was not different between herds. Mineral supplement content was variable across GF herds; however, all supplements had lower Cu (<420 ppm) and Se (<50 ppm) content than is typically recommended in Pennsylvania.

Table 1 Comparison of mean hepatic mineral concentrations (µg/g DW) for beef cattle raised using either conventional supplementation (n=39) or all grass-fed feeding (n=55) practices.

Mineral[1]	Conventional Fed			Grass Fed			P>F[2]
	Mean	Range	Std Dev	Mean	Range	Std Dev	
DM	0.32	0.28 - 0.37	0.02	0.32	0.27 - 0.48	0.03	NS
Ca	158.0	78.9 - 235.3	37.3	140.3	40.4 - 974.0	122.1	NS
Co	0.341	0.043 - 5.91	0.92	0.202	0.026 - 0.546	0.11	NS
Cu	245.7	2.92 - 1204.4	293.6	66.7	10.0 - 203.5	51.3	<.0001
Fe	249.6	106.2 – 700.6	139.6	199.9	62.4 - 447.3	63.7	0.0034
Mg	518.1	434.4 – 623.3	46.3	552.9	202.7 – 1109.6	113.7	0.13
Mn	8.23	5.7 - 14.4	1.91	9.21	2.72 - 14.4	2.08	NS
Mo	3.09	1.86 - 4.62	0.70	3.38	1.4 – 4.40	0.67	0.15
Se	1.35	0.12 - 3.55	0.90	0.55	0.121 - 1.48	0.306	<.0001
Zn	174.6	89.3 - 783.2	116.2	107.5	48.6 - 169.6	23.9	<.0001

[1] Abbreviations: DM = dry matter ratio; Ca = calcium; Co = cobalt; Cu = copper; Fe = iron; Mg = magnesium; Mn = manganese; Mo = molybdenum; Se = selenium; Zn = zinc.
[2] NS = not significant (P> 0.25).

Conclusion Although not determined, deficiencies in Cu, Zn and Se potentially increase disease risk and reduce productive efficiency through their effects on immune response and metabolism. These data show grazing beef cattle to be trace mineral challenged and highlight opportunities to improve mineral status with appropriately formulated mineral supplements.

Acknowledgements The study was partially funded by a Pennsylvania Department of Agriculture grant. The author wishes to thank the beef cattle producers for their participation and Carolyn Burns for her technical support in managing all logistics of sample and data collection for the study. Louise Byler is recognized for statistical support.

References

Suttle NF 2010. The mineral nutrition of livestock, 4th ed. Wallingford, Oxfordshire, UK.

Various condensed tannins from tropical plants as potential multi-purpose nutraceutic in ruminant feed

Carine Marie-Magdeleine[1], Didier Macheboeuf[2], Lucien Philibert[1], Javier Arece Garcia[3], Lyn Udino[4]

[1]INRA, URZ Recherches Zootechniques, Petit-Bourg, Guadeloupe, France, [2]INRA, UMR1213 Herbivores, Saint-Genès-Champanelles, France, [3]Estación Experimental de Pastos y Forrajes Indio Hatuey, Universidad de Matanzas, Central Espana Republicana, Matanzas, Cuba, [4]Université des Antilles, COVACHIM M2E, pointe-à-Pitre, Guadeloupe, France

E-mail: carine.marie-magdeleine-chevry@inra.fr

Take home message Natural multi-active nutraceutics from tropical plant resources: a sustainable alternative to chemicals.

Introduction Condensed tannins (CT) are bio-reactive complex plant secondary metabolites. Protein-CT complexes could affect rumen fermentation (Min *et al*., 2001), allow availability of amino-acids and induce a gastrointestinal nematocidal action in ruminants (Marie-Magdeleine *et al*., 2010). In this study the CT composition of 5 tropical plants were quantified and elucidated to provide some natural multi-active nutraceutics for animal production.

Material & methods CT were extracted using acetone/water (3:7; v/v) and derivatised with benzyl mercaptan before HPLC (water:acetonitrile gradient with 1% acetic acid) and LC-MS analysis for flavan-3-ols and benzyl mercaptan adducts quantification at 280 nm, and terminal and extension units identification. CT content, polymerisation degree (mDP) and procyanidin / prodelphinidin ratio (PC/PD) were determined. CT extracts were assayed for bioactivity against the exsheathment of the nematode *Haemonchus contortus*, using up to 6 concentrations and repetitions with Phosphate Buffer Sample (PBS) as a control. The fermentation profiles (CH_4 and NH_3-N produced, organic matter digestibility (OMD)) were determined by incubating pure plant substrates in rumen mixed bacterial cultures (buffer solution:rumen fluid at 2:1, v/v) for 24h at 39°C; with 3 repetitions and 2 samples of perennial ryegrass (PRG*)* as a control. Chemical composition and CT content (vanillin-H_2SO_4 assay) were determined. Data was analysed using the linear mixed model with plant species and concentrations as fixed effects, repetition as a random effect and then expressed as a ratio of mean PRG (fermentation profile) or PBS (nematocidal effect) values.

Results Thiolytic analysis showed that CT extracts contained from 1.7 to 59 g of CT per 100g. mDP ranged from 3.3 to 15.3, PC/PD ratios from 0 to 10.7, cis/trans flavan-3-ol ratios from 1.7 to 24.7, and galloyl groups from 0 to 37.4 %. CT had a high effect ($p<0.001$) on nematocidal activity (Figure 1) and fermentation profile (Figure 2). At high levels of CT, differences were observed between plants: less nematocidal efficacy and more NH_3-N production for *L. Leucocephala*; higher N protection and OMD for *T. catappa*.

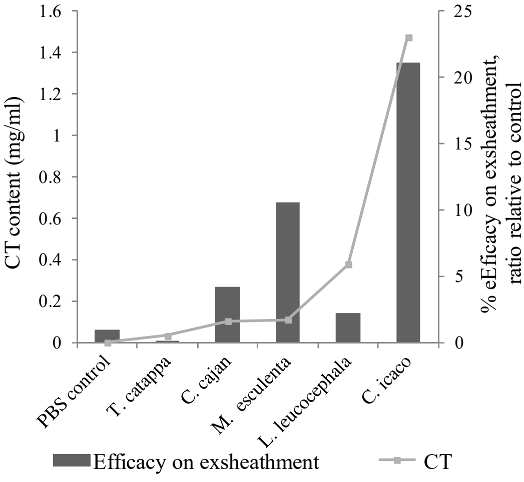


Figure 1 Effect of CT extracts from 5 tropical plants on *H. Contortus* L3 exsheathment.

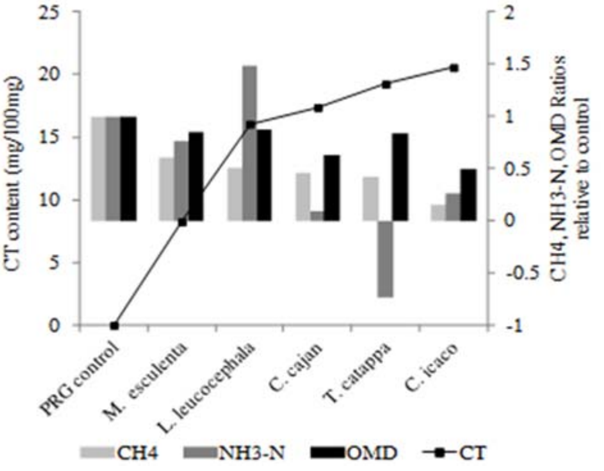


Figure 2 Effect of 5 CT-containing tropical plants on OMD, CH_4 and NH_3-N production.

Conclusion This is the first step of a study for CT multipurpose use in animal production. To our knowledge, this is the first comprehensive CT analysis for these plants. The complexity of composition of CT in tropical plants and their multipurpose potential for animal production were highlighted. It seems that CT composition may influence bio-reactivity. These results encourage further investigations to determine optimal strategies for the use of CT-rich tropical plants as multi-active nutraceutics.

Acknowledgements This research was partly supported by the Region Guadeloupe FEDER project Agroecodiv.

References

Marie-Magdeleine C, Boval M, Philibert L, Borde A and Archimède H 2010. Livestock Science 131, 234-239.
Min BR, Attwood GT, McNabb WC and Barry TN 2001. Journal of Animal Science 79 (Suppl. 1), 163.

681

Evaluation of antioxidant status and trace elements in dairy cows at different physiological stages

Jiaqiao Zhu[1,2], Heng Wang[1,2], Jianji Li[1,2], Zongping Liu[3,4]

[1]Yangzhou University, Yangzhou, Jiangsu, China, [2]Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou, Jiangsu, China, [3]International cooperation laboratory of agriculture and agricultural products safety, Yangzhou, Jiangsu, China, [4]Institute of agricultural science and technology development yangzhou universtiy, Yangzhou, Jiangsu, China

E-mail: jqzhu1998@163.com

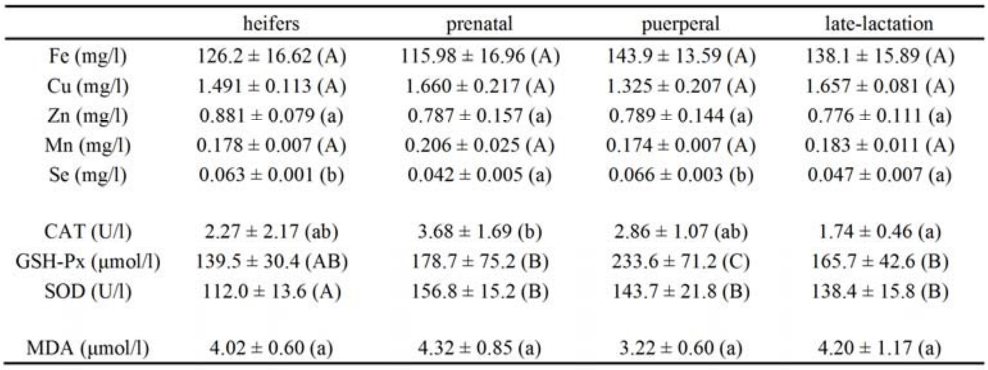
Take home message Balance of oxidant/antioxidant status is associated with the physiological stages and the concentration of trace elements in dairy cows.

Introduction The sufficient trace elements are necessary for optimal performance of production. Several trace elements also act in the antioxidant system as an essential component of several enzymes, various enzyme activities and the oxidation-reduction process. The aim of the present study is to test the antioxidant status and the concentration of trace elements in dairy cows at different physiological stages, and to investigate the association between antioxidant status and the physiological stages.

Material & methods Eighty animals at different physiological stages (20 for each stage) were randomly selected in a commercial dairy herd in China (Yangzhou, Jiangsu, China). The physiological stages are replacement heifers, prenatal cows (30 days before parturition), puerperal cows (30 days after parturition), and late-lactation cows. The diet for all animals consisted of a base ration fed as a daily total mixed ration comprising 10 kg corn silage, 13 kg grass silage, 3 kg dehydrated alfalfa and 3 kg of a commercial concentrate composed of cereal grains and oilseeds. All animals were kept under identical conditions. Blood samples were collected from the coccygeal vein. Iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), and selenium (Se) were determined by inductively coupled plasma mass spectrometry analysis of serum. Glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) were measured on UV/VIS spectrophotomerer by the commercial ELISA kits. All samples were measured at least three times each. Data were presented as means ± SD and statistically analysed by ANOVA using the SPSS software.

Results & discussion The results are summarized in Table 1 Concentration of Fe, Cu, Zn and Mn were comparable in all samples. While there was significant difference for Se levels between animals of different stages. The serum content of CAT, GSH-Px and SOD was maintained at a high level in the transition period (prenatal and puerperal). CAT levels were lower at late-lactation than that at other the stages, while GSH-Px and SOD were lower in heifers than in other animals. MDA content was maintained at a stable level in all samples. We will further test markers of oxidant status in all samples and all parameters in the cow feed to investigate the correlation between each other.

Table.1 Concentration of trace elements and parameters levels of oxidant/antioxidant. Data were presented as means ± SD (n=20). Lower case and capital letters in parentheses indicate significant difference at $p < 0.05$ and 0.01, respectively.



	heifers	prenatal	puerperal	late-lactation
Fe (mg/l)	126.2 ± 16.62 (A)	115.98 ± 16.96 (A)	143.9 ± 13.59 (A)	138.1 ± 15.89 (A)
Cu (mg/l)	1.491 ± 0.113 (A)	1.660 ± 0.217 (A)	1.325 ± 0.207 (A)	1.657 ± 0.081 (A)
Zn (mg/l)	0.881 ± 0.079 (a)	0.787 ± 0.157 (a)	0.789 ± 0.144 (a)	0.776 ± 0.111 (a)
Mn (mg/l)	0.178 ± 0.007 (A)	0.206 ± 0.025 (A)	0.174 ± 0.007 (A)	0.183 ± 0.011 (A)
Se (mg/l)	0.063 ± 0.001 (b)	0.042 ± 0.005 (a)	0.066 ± 0.003 (b)	0.047 ± 0.007 (a)
CAT (U/l)	2.27 ± 2.17 (ab)	3.68 ± 1.69 (b)	2.86 ± 1.07 (ab)	1.74 ± 0.46 (a)
GSH-Px (μmol/l)	139.5 ± 30.4 (AB)	178.7 ± 75.2 (B)	233.6 ± 71.2 (C)	165.7 ± 42.6 (B)
SOD (U/l)	112.0 ± 13.6 (A)	156.8 ± 15.2 (B)	143.7 ± 21.8 (B)	138.4 ± 15.8 (B)
MDA (μmol/l)	4.02 ± 0.60 (a)	4.32 ± 0.85 (a)	3.22 ± 0.60 (a)	4.20 ± 1.17 (a)

Conclusion Antioxidant status in dairy cows at the transition period were high, which may be partially attributable to the trace elements.

Acknowledgements This work was supported by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD) in China.

References

Abuelo A, Hernandez J, Alves-Nores V, Benedito JL and Castillo C 2016. Biological Trace Element Research 174, 319-324.
Omur A, Kirbas A, Aksu E, Kandemir F, Dorman E, Kaynar O and Ucar O 2016. Polish Journal of Veterinary Sciences 19, 697-706.

Effect of calcium and magnesium supplementation on energy balance regulation in merino ewes around parturition

Forough Ataollahi[1,2], Shawn McGrath[1,2], Michael Friend[1,2], Marie Bhanugopan[1,2]

[1]*School of Animal and Veterinary Sciences, Charles Sturt University, PO Box 588, Wagga Wagga, Australia,* [2]*Graham Centre for agricultural innovation, Locked Bag 588, Wagga Wagga, Australia*

E-mail: fataollahi@csu.edu.au

Take home message Prevention of metabolic diseases and improving energy balance regulation of ewes at pre-lambing and post lambing is critical for animal health and welfare.

Introduction The critical period for maintaining energy balance in ewes is from two weeks before parturition to two weeks after parturition because of high energy demands of ewes during this period. Depending on fetal requirements for energy and demands for milk production, ewes may experience negative energy balance, since they may not be able to consume sufficient energy to counter the energy output (Hunter *et al*., 2015). Metabolic conditions of animals in negative energy balance shift to catabolic metabolism of triglycerides which in turn, gives rise to Non Esterified Fatty Acids (NEFA), which are an indicator of the degree of adipose tissue mobilization (Ingvartsen and Moyes, 2013). This study assessed the effect of calcium (Ca) and magnesium (Mg) supplementation on energy regulation of ewes from late gestation to early lactation through measurement of NEFA concentration.

Material & methods Forty-eight twin bearing ewes were randomly allocated to one of four treatments: Control group (Basal pellets), Ca group (Basal pellets+ 50g limestone/head/day), Mg group (Basal pellets+ 50 g magnesium oxide/head/day) and Ca+Mg group (Basal pellets+25g limestone/head/day+25g magnesium oxide/head/day). Ewes in treatment groups were supplemented with Ca and Mg from 2 weeks before lambing until one-month post lambing. Blood samples were collected from ewes at two weeks before lambing (-2 W), 12 hours after lambing (+12 hours), two weeks post lambing (+2 weeks) and four weeks post lambing (+4 weeks). Plasma NEFA concentration were measured to assess energy balance in ewes using a commercial kit (Wako Diagnostic, Japan) and data analysed using linear mixed models in IBM SPSS Statistics (Version 20.0) for Windows.

Results & discussion Mean plasma NEFA concentration was greater for all treatment groups at lambing compared to the other time points ($p<0.001$). The interaction between time×treatment was significant, with the control group having greater NEFA concentration at +12 hours compared to the other treatments and time points (Table 1). Ewes supplemented with Ca, Mg and Ca+Mg showed higher ability in energy regulation at +12 hours in comparison to the control group which was not supplemented with these minerals. Calcium acts as a second messenger for energy metabolism in hepatocytes. As cytosolic Ca level increases, it gives a parallel rise to Ca concentration in mitochondria which is critical for the stimulation of mitochondrial oxidative metabolism and energy production (Martinez *et al*., 2012). Moreover, Mg acts as a second messenger in synthesis, storage and conformational integrity of insulin (Cinar *et al*., 2008).

Table 1 Effect of calcium and magnesium supplementation over time on non esterified fatty acids concentration (µEq/ml) in ewes (time × treatment effect, $P < 0.05$).

Time	Treatment				P-value
	Control	Calcium	Magnesium	Calcium + Magnesium	Time×Treatment
-2 Weeks	416.5±88.24	400.23±111.87	366.9±113.92	365.23±109.07	n.s.
+12 Hours	1079.9±84.48^{a}	572.4±116.73^{b}	533.1±101.89^{b}	348.0±94.46^{b}	0.002
+2 Weeks	395.1±84.48	369.3±98.66	283.1±98.66	240.7±96.28	n.s.
+4 Weeks	303.7±96.28	381.5±96.28	190.3±101.89	268.3±93.01	n.s.

The superscripts with different alphabets are significantly different to each other.

Conclusion Supplementation with Ca and Mg improved energy regulation in ewes at 12 hours post lambing as shown by the lower NEFA concentration in blood, suggesting better energy balance immediately after parturition which could have significant effect on prevention of metabolic disorders that occurs around parturition. Thereby improving animal health and welfare.

Acknowledgements We gratefully acknowledge School of Animal and Veterinary Science for funding this work.

References

Cinar V, Polat Y, Mogulkoc R, Nizamlioglu M and Baltaci AK 2008. Pakistan Journal of Pharmaceutical Sciences 21, 237-240

Hunter TE, Suster D, DiGiacomo K, Dunshea FR, Cummins LJ, Egan AR and Leury, BJ 2015. Small Ruminant Research 131, 123-129

Ingvartsen KL and Moyes K 2013. Animal 7, 112-122

Martinez N, Risco CA, Lima FS, Bisinotto RS, Greco LF, Ribeiro ES, Maunsell F, Galvão K and Santos JEP 2012. Journal of Dairy Science 95, 7158-7172

Milk restricted lambs have greater colonic butyrate and a transitory modified anti-oxidant status

Javier Frutos[1], F. Javier Giráldez[1], Julio Benavides[1], Erminio Trevisi[2], Nuria Santos[1], Alba Santos[1], Sonia Andrés[1]

[1]Instituto de Ganadería de Montaña, CSIC-Universidad de León, Finca Marzanas s/n, 24346, Grulleros, León, Spain, [2]Faculty of Agriculture, Food and Environmental Science, Institute of Zootechnics, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122, Piacenza, Italy

E-mail: sonia.andres@eae.csic.es

Take home message Milk restriction of suckling lambs promotes an anti-inflammatory response in the colon and a transitory modified anti-oxidant status.

Introduction Nutritional interventions during early life may affect the development of the gastrointestinal tract and the maturation of the immune system, compromising metabolism and health status in the later stages of life (Bach, 2012). Thus, the aim of this study was to investigate the impact of moderate milk restriction during the suckling period of Assaf lambs on colonic parameters and long-term effects on biochemical parameters related to inflammatory response and oxidative stress.

Material & methods Forty Assaf lambs (average BW 4.73 kg) were penned individually, twenty of them were fed milk replacer (200 g dry matter/L) *ad libitum* (ADL; 192 mL/kg LBW) whereas the other group (restricted, RES) only received 120 mL/kg LBW. Eight lambs from each group were slaughtered at 35-days-old and colonic samples were collected for volatile fatty-acid analysis, analysis of microbial composition (qPCR), study of gene expression of toll-like receptors and cytokines (qRT-PCR), quantification of secretory IgA (ELISA), morphometric analysis (thickness of colonic layers) and immunohistochemistry examination (*e.g.,* lymphocytes infiltrated in the lamina propria). The rest of the lambs (12 ADL *vs.* 12 RES) were weaned progressively, offered alfalfa hay and a starter concentrate *ad libitum* during 6 weeks and then a total mixed ration (TMR) *ad libitum* until the end of the experiment. Animals were blood sampled at the end of the feed restriction period (1st month) and once when the ewe lambs were being fed exclusively the TMR (6th and 8th months). Colonic parameters were analysed using GLM procedure of SAS, with the milk replacer intake level as the only source of variation. Biochemical parameters were analysed as a repeated measures design using the MIXED procedure of SAS.

Results & discussion During the suckling period, a greater proportion of butyrate (4.47 % *vs.* 7.07 %, P=0.014) and a trend towards an increased expression of the anti-inflammatory cytokines TGF-β (P=0.055) and IL-4 (P=0.081) were found in the colon of RES lambs. These results could be associated with an increase of *Butyrivibrio spp.* and the lower relative abundance of *Prevotella spp.* observed in the colonic mucosa of restricted lambs. The rest of colonic parameters and two positive acute-phase proteins (*e.g.,* haptoglobin and ceruloplasmin) related to inflammatory response were not affected by milk replacer restriction, but levels of albumin, paraoxanase and total antioxidant activity (FRAP) were significantly reduced in RES lambs at the end of the 1st month of life, probably due to a decreased nutrient intake (Table 1).

Table 1 Biochemical parameters of lambs fed *ad libitum* (n=12, ADL) or restricted (n=12, RES) during the suckling period.

	1st month		6th month		8th month				*P*-value		
	ADL	RES	ADL	RES	ADL	RES	SED_1	SED_2	T	D	T*D
Haptoglobin, g/L	0.51	0.56	0.27	0.40	0.17	0.39	0.170	0.177	0.450	0.341	0.893
Ceruloplasmin, µmol/L	3.86	4.24	2.70	2.97	2.53	2.76	0.445	0.308	0.514	<0.001	0.967
Paraoxanase, U/L	173^b	134^a	159^b	149^{ab}	166^b	152^b	12.5	4.86	0.106	0.455	0.012
ROM, mg/100 mL	20.5	20.8	15.3	16.3	14.3	15.3	1.74	1.24	0.672	<0.001	0.951
FRAP, µmol/L	155^b	125^a	134^a	133^a	134^a	131^a	5.77	5.32	0.065	0.289	0.018
SOD, U/L	1.03	0.962	1.25	1.22	1.34	1.46	0.125	0.086	0.952	<0.001	0.500
Retinol, ug/100 mL	22.0	22.5	53.3	45.2	58.0	49.1	3.92	3.21	0.174	<0.001	0.286
Tocopherol, ug/mL	1.16	1.42	0.782	0.700	0.681	0.620	0.115	0.071	0.732	<0.001	0.036
Albumin, g/L	33.6^b	30.4^a	37.1^d	35.8^c	36.7^{cd}	36.0^c	0.39	0.36	<0.001	<0.001	0.005

SED_1, standard error of the difference to compare dietary treatments; SED_2, standard error of the difference to compare days; T, dietary treatment; D, day; T*D, interaction between treatment and day.

Conclusion Moderated milk replacer restriction of artificially reared suckling lambs promoted both, an anti-inflammatory response of the colon and a transitory modified antioxidant status that was corrected after weaning.

Acknowledgements This research was supported by the Spanish Ministry of Economy, Industry and Competiveness (MINECO, AGL2014-54124R), CSIC (Project 201540E084), Ayudas Puente Formativas (Universidad de León) and the Spanish Ministry of Education, Culture and Sport (MECD, FPU15/01630).

References

Bach A 2012. Journal of Animal Science 90, 1835-1845.

Effect of an acute metabolic challenge on ingestive behaviour and brushing activity of grazing dairy cows

Esther Müller[1], Lukas Eggerschwiler[1], Roi Mandel[3,4], Ann-Catherine Schwinn[2], Josef J. Gross[2], Rupert M. Bruckmaier[2], Hans Dieter Hess[1], Frigga Dohme-Meier[1], Andreas Münger[1]

[1]Agroscope, Posieux, Switzerland, [2]Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [3]Koret School of Veterinary Medicine, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University, Rehovot, Israel, [4]ETH Zürich, Institute of Agricultural Sciences, Zurich, Switzerland

E-mail: frigga.dohme-meier@agroscope.admin.ch

Take home message Due to the strong impact of weather conditions, the observation of outdoor brushing activity cannot be recommended for identifying short-term alterations in the metabolic state of grazing cows.

Introduction Herbage from pasture is the most cost efficient nutrient source for dairy cows, and feeding maximum herbage implies less competition with human food resources. However, the quality and availability of pasture herbage can show considerable variation, and temporary nutrient shortages are likely. Therefore, herbage-fed cows not receiving a supplementary concentrate may experience a high metabolic load (Zbinden *et al*., 2017) and are particularly susceptible to metabolic disorders and, hence, impaired well-being. In order to decrease the risk of reduced animal welfare in grazing dairy cows, non-invasive markers that immediately reflect changes in the animal's metabolic state are desirable. Core activities such as eating and lying are essential for short-term survival and presumably not appropriate for assessing an animal's welfare. For this purpose, observing low-resilience behaviours such as the use of automated cow brushes was suggested (Mandel *et al*., 2013). The aim of the present study was to analyse grazing cows' core activities and their use of an automated brush during an acute metabolic challenge.

Material & methods The experiment started mid May 2016 with 15 multiparous cows (parity: 3.2 ± 1.6) being on average 24 (± 7.4) days in milk and was for a duration of three weeks. In the first experimental week, cows grazed and were supplemented with concentrate according to their estimated individual energy and nutrient requirements. On the first day of the second experimental week concentrate was withdrawn for one week in order to simulate a period of nutrient shortage, which can occur frequently in pasture-based milk production systems with no or restricted concentrate supplementation. In the third experimental week, cows received a quantity of concentrate similar to that of the first experimental week. Milk yield, body weight, and concentrate consumption were recorded daily. Blood metabolites and hormones were analysed on d 5 of each experimental week. Eating and ruminating as well as locomotion were also recorded using the RumiWatch® system (Itin&Hoch GmbH, Liestal, Switzerland). Furthermore, the use of an automated brush (VPB2, Buri AG, Hasle-Rüegsau, Switzerland) was monitored on pasture using 2 cameras (DH61E, ANNKE, City of Industry, USA) which were installed outside the paddocks. The climatic conditions were recorded daily at a meteorological station, located close to the experimental site. Statistical analysis was done with parametric linear mixed models using Systat 13.0.

Results Total yield of milk and protein decreased ($P < 0.05$) and fat yield, fat to protein ratio, and acetone content increased ($P<0.05$) when concentrate was withdrawn. Plasma glucose and insulin concentrations were lower ($P < 0.05$) and concentrations of non-esterified fatty acids and beta-hydroxybutyrate were higher ($P < 0.05$) during concentrate withdrawal. While time spent eating and ruminating increased ($P < 0.05$) when concentrate was withdrawn, time spent idling decreased ($P < 0.05$). Concomitantly, time standing and moving increased ($P < 0.05$) during concentrate withdrawal whereas walking time decreased ($P < 0.05$). The daily proportion of cows using the automated brush and the duration of brushing per day decreased ($P < 0.05$) when concentrate was withdrawn. Temperature-humidity index ($P < 0.01$), wind velocity ($P < 0.05$) and occurrence of precipitation ($P < 0.05$) had a strong impact on brushing activity

Conclusion The time spent eating appears to reflect the short-term variation in grazing cows' nutrient supply better than other behavioural characteristics. Brushing activity may be linked to a cow's metabolic state but due to strong day-to-day variation it is not suitable as a marker for detecting the onset of a metabolic challenge.

Acknowledgements The authors acknowledge the financial support of the State Secretariat for Education, Research and Innovation (SERI), the support by the COST network action FA1308 DairyCare, and the H. Wilhelm Schaumann-Stiftung (Hamburg, Germany).

References

Mandel R, Whay H, Nicol C and Klement E, 2013. Journal of Dairy Science 96, 6506–6513.

Zbinden R, Falk M, Münger A, Dohme-Meier F, Dorland H, Bruckmaier R and Gross JJ 2017. Journal of Animal Physiology and Animal Nutrition101, 767–778.

685

Use of *Bacillus subtilis* C-3102 as probiotic feed additive in dairy cows

Noriko Nakamura[1], Marta Terré[2], Belen Martin[3], Teresa Aymerich[3], Alex Bach[2,4]

[1]*Asahi Calpis Wellness Co., Ltd., Tokyo, Japan,* [2]*IRTA - Department of Ruminant Production, Barcelona, Spain,* [3]*IRTA - Food Safety, Monells, Spain,* [4]*ICREA, Barcelona, Spain*

E-mail: noriko.nakamura@calpis.fr

Take home message Supplementation of *B. subtilis* C-3102 to dairy cows is beneficial for health and performance and does not cause contamination in milk.

Introduction *Bacillus subtilis* C-3102 is known as a probiotic which improves milk yield and somatic cell counts in dairy cows. Meanwhile, presence of spore-forming bacteria in milk has been regarded as a problem in the dairy manufacturing process due to their high heat stability. Questions remain if the use of *Bacillus* probiotics in dairy cow feed increases the risk of milk contamination. We performed two feeding trials in 2014 and 2017 in Spain. The first trial focused on the long-term effects of *B. subtilis* C-3102 on the dairy cow health and performance. The second trial was performed as a short-term study to evaluate the risk of milk contamination by probiotic *Bacillus*.

Material & methods The first trial involved 375 lactating cows divided into 3 groups (n=125) receiving either control feed supplement (T0), supplement containing 3×10^9 CFU/day (T1) or 6×10^9 CFU/day (T2) of *B. subtilis* C-3102. Milk production and composition was recorded daily and somatic cell count on a monthly basis. Data were analysed using a mixed-effects model with treatment, week of study, parity and their 3-way interactions. Week of study entered the model as a repeated measure. The second trial was performed with 20 lactating cows divided into two groups receiving control supplement (n=10) or supplement containing 6×10^9 CFU/day (n=10) of *B. subtilis* C-3102. *Bacillus spp.* was enumerated from milk and faeces taken on days 0, 14 and 28 by culture method. Colonies of *B. subtilis* C-3102 were identified by its morphology. Data were analysed using a mixed-effects model with treatment, week of study and their 2-way interactions. Week of study entered the model as a repeated measure.

Results In the first trial, there was an interaction ($P < 0.05$) among treatment, parity and week of study for milk yield. Primiparous cows on T2 tended to have a greater persistency than primiparous cows on T1 and T0; whereas multiparous cows on both T1 and T2 had greater ($P < 0.05$) persistency than multiparous cows on T0 (Figure 1). An interaction between treatment and week of study was observed in milk somatic cell counts ($P < 0.05$; Figure 2). They remained fairly stable in T1 and T2 throughout the study, but increased in T0. Primiparous cows had a lower ($P < 0.05$) milk somatic cell count ($118\pm12.9 \times 10^3$ cells/ml) than multiparous cows ($162\pm9.98 \times 10^3$ cells/ml), but there was no interaction with treatment or week of study. In the second trial, *Bacillus* spp. was detected in faeces and milk of both control and test cows throughout the study. There were effects ($P < 0.05$) of week on the total *Bacillus* counts in faeces (6.6 log CFU/g on day 0, 6.2 log CFU/g on day 14 and 6.9 CFU/g on day 28; s.e.=0.06) as well as in milk (1.5 log CFU/ml on day 0, 1.1 log CFU/g on day 14 and 1.0 log CFU/ml on day 28; s.e.=0.20), but no interaction with treatment was observed. *Bacillus subtilis* C-3102 was only present in faeces of test cows after probiotic supplementation (5.3 log CFU/g on day 14 and 5.2 log CFU/g on day 28) and was not found in any milk samples throughout the study.

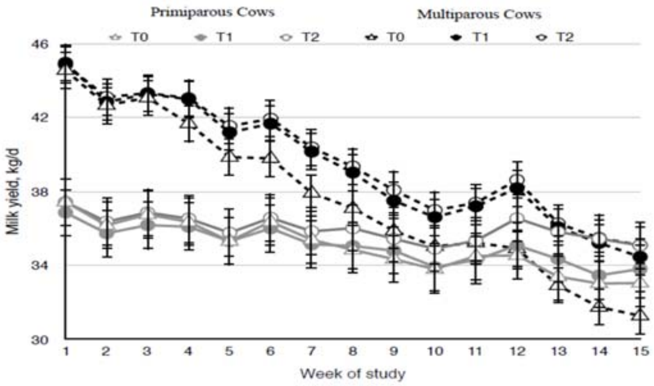


Figure 1 Evolution of milk yield (mean ± s.e.) (First trial).

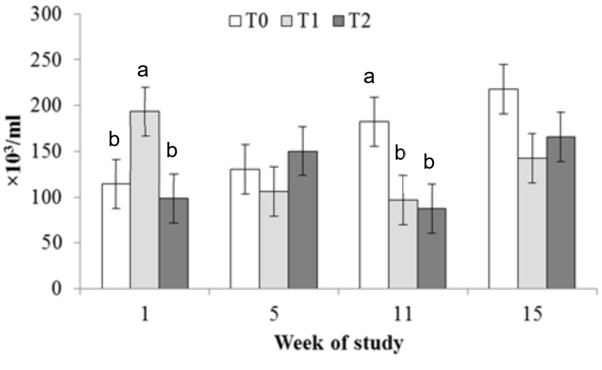


Figure 2 Milk somatic cell count (mean ± s.e.)
[a,b] Different at P<0.05 within same week (First trial).

Conclusion Supplementation of *B. subtilis* C-3102 had positive effects on milk persistency in multiparous cows. Although *Bacillus* spp. was prevalent in faeces and existed in low quantity in milk as well, supplementation of *B. subtilis* C-3102 did not cause contamination in milk when fed at the dosage of 6×10^9 CFU/day. Thus, *B. subtilis* C-3102 is a suitable probiotic to be used as a feed additive for dairy cows.

Combination of rumen peripheral parameters to detect sub-acute ruminal acidosis in dairy cows

Clothilde Villot[1], Cécile Martin[1], Jonathan Bodin[2], Denys Durand[1], Benoît Graulet[1], Anne Ferlay[1], Marie-Madeleine Mialon[1], Erminio Trevisi[3], Mathieu Silberberg[1]

[1]Université Clermont Auvergne, INRA, Vetagro Sup, UMR Herbivores, Saint-Genès-Champanelle, France, [2]BR3 Consultants, Lyon, France, [3]Instituto di Zootecnica, Facoltà di Agraria, Università Cattolica del Sacro Cuore, Piacenza, Italy

E-mail: mathieu.silberberg@inra.fr

Take home message Combinations of rumen peripheral parameters would be relevant to detect Sub-Acute Ruminal Acidosis (SARA) on-farm.

Introduction In ruminants, SARA is a common digestive disorder usually induced by an overload of rapidly fermentable carbohydrates ingested by animals. This disorder is characterized by abnormal and intermittent drops in rumen pH that alter animal production and welfare, and consequently it increases economic losses for the farmer. It was recently demonstrated that relative rumen pH indicators obtained from high-resolution kinetics measured with sensors are highly accurate in characterizing experimental SARA (Villot *et al*., 2017). However, this expensive technique cannot be widely deployed on farms. It is also well documented that numerous but nonspecific rumen peripheral parameters can be impacted during SARA. Nevertheless, no study has examined the potential interest to combine statistically several parameters to detect SARA more accurately than taken one by one. In this pilot study, our aim was to propose an original approach to better detect SARA by developing multi-parametric models based on peripheral parameters in dairy cows.

Material & methods A longitudinal study was conducted with 11 primiparous dairy cows including 6 rumen fistulated. Animals were fed with 2 different diets during 3 successive periods: a 4-wk control period (P1) with a low-starch diet (LSD: 13% starch), a 4-wk SARA period (P2) with a high-starch diet (HSD: 35% starch), and a 3-wk recovery period (P3) again with the LSD. Animal behaviour was monitored throughout the experiment and faeces, urine, saliva, milk and blood were sampled simultaneously for each animal at least once a week for analysis. A total of 136 rumen peripheral parameters (milk fatty acids, biomarkers of metabolism, inflammation…) was screened by different statistical approaches: (i) linear mixed model with Dunnett post-hoc test to compare control period to the other weeks of the experiment, and (ii) multivariate Principal Component Analysis (PCA and PLS-DA) to select the most relevant parameters screened. Finally, (iii) a generalized linear mixed model (GLMM) was applied to highlight parsimonious combinations of parameters able to identify animals under SARA.

Results & discussion Among 136 parameters measured, 85 were significantly modified between SARA *vs* control periods. Among them, most of parameters were measured in milk (41% of total modified parameters), blood (30%) and saliva (15%), and in a lesser extent in faeces (7%), behaviour (5%), and urine (<5%). Parameters reported on the PCA (Figure 1) were selected as the most representative to discriminate SARA from control periods (P1 and P3). Combinations (n= 20) of parameters were selected from the GLMM with specificity and sensitivity above 90% and 75%, respectively, to detect SARA. In particular, 1 combination involving DMI, blood bicarbonate and milk urea concentrations was able to detect cows undergoing SARA and but also when cows totally recovered from the disease. Parameters like the number of drinking acts, fat:protein ratio in milk, saliva and faecal pH were the most frequently used in other combinations.

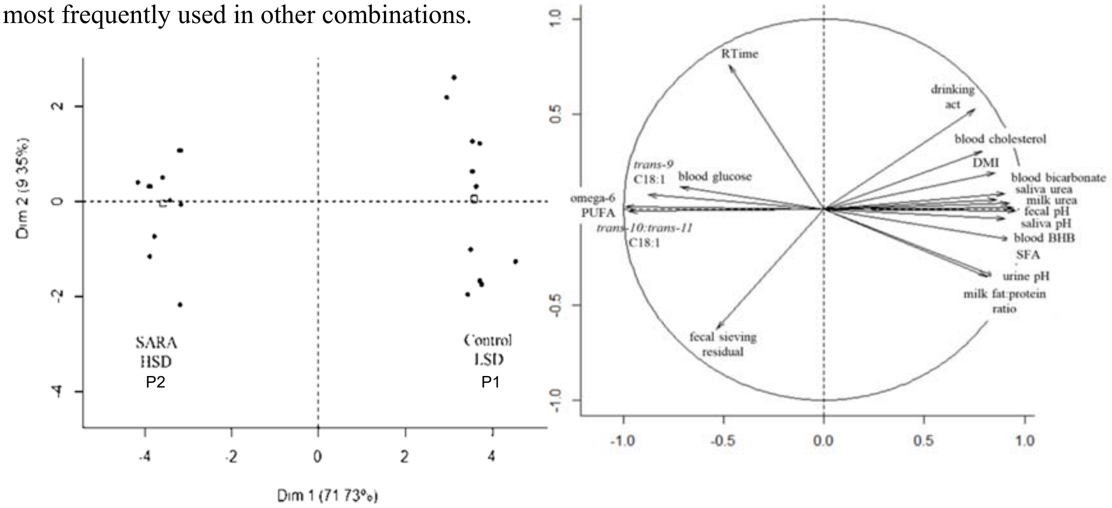


Figure 1 Principal component analysis (PCA) of selected rumen peripheral parameters discriminating cows with SARA from cows in the control period.

Conclusion The present pilot study demonstrates the possibility of multi-parametric models to detect on-farm SARA. Next step will be to test the robustness of the proposed models using a greater number of animals as well as different SARA inducing diets before being applied in on-farm conditions.

Acknowledgements This research was financially supported by Lallemand, Terrena and Valorex and the French Association Nationale Recherche Technologie through a “Convention Industrielle de Formation par la REcherche” fellowship grant.

References

Villot C, Meunier B, Bodin J, Martin C and Silberberg M. 2017. Animal 12, 481-490

Naturally produced lovastatin at a high level impairs muscle development in goats

Sani Garba[1], Teik Kee Leo[2], Awis Qurni Sazili[1,2], Su Chui Len Candyrine[3], Yong Meng Goh[1], Anjas Asmara Samsudin[2], Ron Ronimus[4], Stefan Muetzel[4], Juan Boo Liang[1]

[1]Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, Serdang, Selangor, Malaysia, [2]Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia, [3]Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Sandakan, Sabah, Malaysia, [4]Rumen Microbiology, AgResearch, Palmerston North, New Zealand

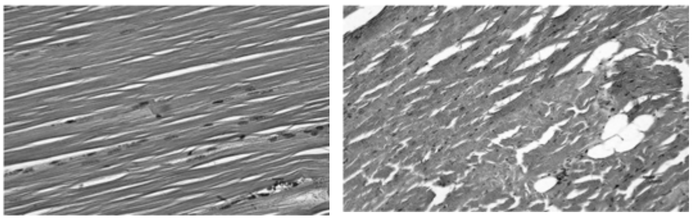
E-mail: jbliang@upm.edu.my

Take home message Dietary supplementation of lovastatin mitigates enteric methane production but at a high level (6 mg/kg BW) impairs muscle development in goats.

Introduction We have reported that naturally produced lovastatin, at a dosage of 4 mg/kg body weight (**BW**) reduces enteric methane production by 20% in goats (Liang *et al.*, 2017), however, feed intake of goats fed a higher dosage (6 mg/kg BW) was significantly reduced. Long-term consumption of statins to treat hyperlipidemia can result in myotoxicity of skeletal muscle in humans (Di Stasi *et al*., 2010). We know of no data on muscular myotoxicity due to consumption of statins in farm animals except that supplementation of red yeast rice containing lovastatin at up to 2.6 mg/kg BW resulted in adverse digestive and physiological disorders in cattle (Ramirez-Restrepo *et al*., 2014). This paper reports on the examination of muscles from goats fed zero and 6 mg lovastatin/kg BW using histological and proteomic analyses.

Material & methods *Longissimus dorsi* muscles were collected from growing goats fed with control diet (no lovastatin, n=5) and a similar diet supplemented with 6 mg lovastatin/kg BW (n=5) following 12 weeks of a feeding trial (Liang *et al.*, 2017). For histological analysis, the muscle samples were fixed in 10% buffered formalin solution, processed for paraffin embedding, sectioned at 5 μm, mounted on slides and stained with haematoxylin-eosin. Images were viewed using light microscopy. For proteomic analysis, crude protein was extracted from each muscle sample and digested with trypsin. The digested peptides were separated using liquid chromatography and analysed using tandem mass spectrometry. Quantitative analysis of the data was conducted using Perseus (v.1.6.0.2) and bioinformatics analysis was performed on the differentially expressed proteins using Ingenuity® Pathway Analysis (IPA®, Qiagen, Germany).

Results & discussion Histological analysis showed that 6 mg/kg BW supplementation resulted in severe muscular degeneration compared to the control group (Figure 1). Proteomic analysis indicated that 24 proteins were down-regulated in the 6 mg/kg BW group. These findings support the results of the histological analysis suggesting possible myotoxicity in goats fed with high dose (6 mg/kg BW) of lovastatin. Further analysis showed that the proteins involved were associated with skeletal muscle development and other vital cellular functions (Table 1).



A: zero lovastatin

B: 6 mg lovastatin/kg BW

Figure 1 Longitudinal section of *longissimus dorsi* muscles. 6 mg/kg sample showed severe degeneration of muscle structure.

Table 1 Molecular functions of proteins down regulated in 6 mg/kg muscle sample.

Molecular functions	P-value
Carbohydrate metabolism	4.22E-02 – 1.24E-09
Small molecule biochemistry	4.66E-02 – 8.19E-06
Energy production	2.00E-02 – 5.93E-05
Cellular movement	3.73E-02 – 1.16E-04
Molecular transport	4.66E-02 – 1.28E-04

Conclusion Results of our studies have shown that dietary supplementation of 6 mg lovastatin/kg BW results in severe muscular degeneration. It is thus not a viable option to use this level for mitigation of enteric methane production in goats.

Acknowledgements This study was funded by the New Zealand Government to support the objectives of Livestock Research Group of the Global Research Alliance on Agricultural Greenhouse Gases and the Universiti Putra Malaysia.

References

Di Stasi SL, MacLeod TD, Winters JD and Binder-Macleod SA. 2010. Physical Therapy 90, 1530-1542.

Liang JB, Mahadzir MF, Garba S, Jahromi,MF, Candyrine SCL, Ronimus R, Muetzel S and Samsudin AA 2017. 2017 Congress on Gastrointestinal Function. Chicago, Illinois, USA.

Ramirez-Restrepo CA, O’Neill CJ, López-Villalobos N, Padmanabha J and McSweeney C 2014. Animal Production Science 54, 1294-1299.

Precision livestock farming tools reveal behavioural modifications in ruminants under Sub-Acute Ruminal Acidosis (SARA)

Marie-Madeleine Mialon, Mathieu Silberberg, Bruno Meunier, Isabelle Veissier

Université Clermont-Auvergne, INRA, VetAgro Sup, UMR Herbivores, Saint-Genès-Champanelle, France

E-mail: mathieu.silberberg@inra.fr

Take home message New tools of precision livestock farming based on general activity monitoring may be promising to improve SARA detection in ruminants.

Introduction High producing ruminants, *e.g.,* fattening beef cattle or high yielding dairy cows, are fed diets rich in concentrates. This can modify the rumen fermentative patterns and in turn alter ruminal pH, leading to SARA and subsequent diseases. Several behavioural modifications have been observed during SARA. For instance, sheep under SARA are more agitated (more time standing awake during the day) and more aggressive towards each other, they eat more salt and drink more water, and limit their concentrate intake (Commun *et al.*, 2012). Under SARA bulls in the finishing period, spend less time eating and more time drinking (Mialon *et al.*, 2008). Today, precision livestock tools allow a close monitoring of the activity of animals so that disorders can be detected at an early stage (Veissier *et al.*, 2017). Our aim was to assess if behavioural modifications associated to SARA could be detected with such tools.

Material & methods We conducted two experiments:
1). During a 138-d finishing period, 24 Blond d'Aquitaine bulls were divided in 3 groups. Each group had *ad libitum* access to either a 92% concentrate + 8% straw diet (n=8), or 56 % concentrate + 44% hay diet (n=8) or a 43% concentrate + 57% maize silage diet (n=8). The feed was distributed twice a day and feeding behaviour was recorded *via* electronic feeding gates.

2). During 14 days of lactation, 14 Prim' Holstein cows had *ad libitum* access to a SARA challenge diet containing 46% concentrate whereas 14 control cows were kept on a diet containing 25% concentrate; Cows were monitored using a real time locating system (CowView®, GEA) from which the cow activity was inferred (eating, standing, resting); then the level of activity was calculated as in Veissier *et al. (*2017).

Results & discussion In Experiment 1, the bulls fed 92% concentrate progressively modified their feeding behaviour by spreading their ingestion all along the diurnal period of the day whereas the other groups maintained two major peaks of eating activity. As a result, the standard deviation of the time spent eating/hour was significantly lower (0.058, $p<0.001$) than for those fed the other diets (0.123 and 0.157 for the concentrate + maize silage diet and the concentrate + hay diet). We hypothesise that it was a mean to cope with the digestive discomfort induced by the diet.

In Experiment 2, the cows under SARA were less active after the morning feed distribution (Figure 1), which was partly explained by less time spent eating. In addition, SARA cows spent more time licking salt blocks, which makes them ingest buffering carbonates and stimulates insalivation, both of which probably help them to cope with a low ruminal pH.

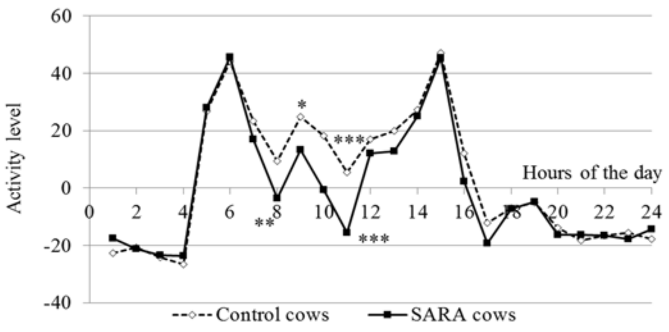


Figure 1 Circadian variations of activity level in SARA (n=14) and control cows (n=14) during the SARA challenge. Activity level is a weighted sum of the percentage time spent in the different activities, *, $P<0.05$. **, $P<0.01$, ***, $P<0.001$.

Conclusion Continuous behaviour monitoring is a promising tool to improve the early detection of SARA, however sensitivity and specificity of such PLF tools need to be evaluated.

Acknowledgements Experiment 2 was supported by the FP7 European project funding (EU-PLF, KBBE.2012.1.1-02-311825).

References

Commun L, Silberberg M, Mialon MM, Martin C and Veissier I 2012. Animal 6, 2011-2022.
Mialon MM, Martin C, Garcia F, Menassol JB, Dubroeucq H, Veissier I, Micol D 2008. Animal 2, 1682-1691.
Veissier I, Mialon MM and Sloth KH 2017, Journal of Dairy Science 100, 3969-3974.

Changes in maternal blood beta-hydroxybutyrate and plasma non-esterified fatty acids concentrations in periparturient commercial dairy goats bearing single and twin foetus

Fernanda Zamuner[1], Kristy DiGiacomo[1], Alexander W.N. Cameron[2], Brian J. Leury[1]

[1]*Faculty of Veterinary and Agricultural Sciences - The University of Melbourne, Melbourne, Victoria, Australia,* [2]*Meredith Dairy Pty Ltd, Meredith, Victoria, Australia*

E-mail: fzamuner@student.unimelb.edu.au

Take home message Pregnant does bearing multiple foetuses experience a more intense negative energy balance during the peripartum period compared to those bearing singletons.

Introduction Periparturient dairy goats often enter a state of negative energy balance (NEB) when the energy demand for maintenance and lactation exceeds that of dietary energy intake. The energy imbalance causes the animal to mobilise body lipid stores to meet energy requirements. Multiple pregnancy may further exacerbate NEB due to rumen compression and increased energy requirements (NRC, 2007). The main blood indicators of lipomobilization are beta-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA). Examination of differences in maternal energy status of goats bearing single or twin fetuses is especially important due to goats' high incidence of multiple births (Haldar *et al*., 2014).

Material & methods This study was conducted at the Meredith Dairy commercial farm (Meredith, Australia, 37°50′S; 144°04'E), during four consecutive kidding seasons, from June 2016 to March 2017. In total, 1000 (n = 250 per kidding season) pregnant Saanen and Saanen-cross goats (1-7 years, LW; 66 ± 17.0 kg, and BCS; 2.5 ± 0.3) were enrolled in the study. Litter size was determined 60-80 days post-breeding using transabdominal ultrasound. Animals were *ad libitum* fed a total mixed ration (9.7 MJ/kg ME and 160 g/kg CP DM). Blood samples were collected *via* jugular venipuncture into 10 mL vacuum tubes (BD Vacutainer), before the morning feeding on weeks -3, -2, -1, 0, 1, 2 and 3 (relative to delivery) and whole blood BHB concentration was measured via a hand-held BHB analyser (FreeStyle Optium, Abbott) prior to centrifugation (1250 g, 12 min, 4° C) to isolate plasma. Isolated plasma samples were stored at -20°C until analysis. Plasma NEFA concentrations were determined spectrophotometrically using a commercial kit (WAKO). Data obtained was analysed using the Linear Mixed Models procedure of GenStat (version 18.1). Litter size, weeks from delivery, kidding season, parity and age were included as fixed effects and goat was included as a random effect.

Results The concentration of BHB was greater (*P*<0.01) throughout the sampling period in twin compared to singleton-bearing goats. In twin-bearing goats, plasma NEFA was greater (*P*<0.01) in the pre-partum period and at parturition compared to singleton-bearing goats. The effects of litter size on maternal lipomobilization persisted for several days after parturition. However, differences between the two groups for concentrations of both metabolites gradually declined as females advanced into lactation.

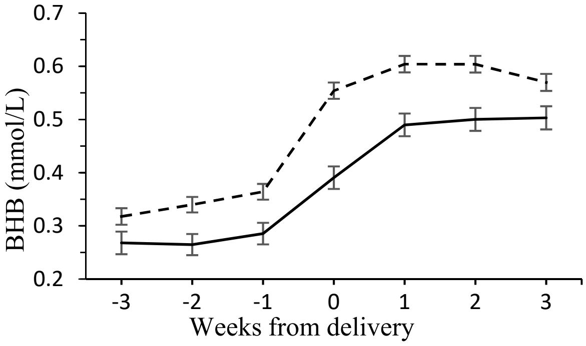


Figure 1 Temporal changes in whole blood BHB (mmol/L) in singleton (dashed line) and twin-bearing (soild line) goats (mean ± S.E.).

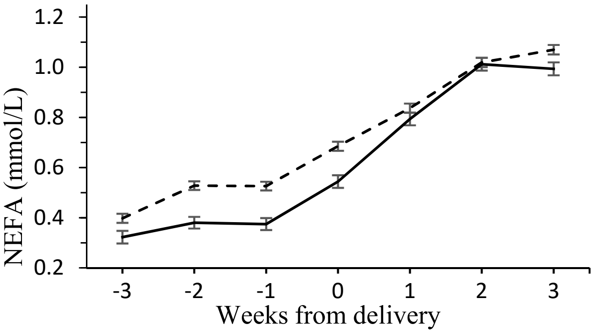


Figure 2 Temporal changes in plasma NEFA (mmol/L) in singleton (dashed line) and twin-bearing (soild line) goats (mean ± S.E.).

Conclusion The findings of this experiment suggest that twin-bearing goats underwent a more intense NEB, especially in the pre-partum period, compared to singleton-bearing goats. This is likely driven by the combined effects of physical restriction of the rumen, and the increased metabolic demand imposed by two fetuses (Khan & Ludri, 2002). The determination of the impact of litter size on maternal energy status can contribute to establishing specific care and nutritional management plans to prevent production diseases in the peripartum period in commercial dairy goats.

Acknowledgements This research is supported by an Australian Government Research Training Program Scholarship and Meredith Dairy Ltd.

References

Haldar A, Pal P, Datta, M, Paul, R and Pal SK 2014. Asian-Australasian Journal of Animal Sciences 27, 628.
Khan JR and Ludri RS 2002. Asian-Australasian Journal of Animal Sciences 15, 504-508.
NRC 2007. Nutrient Requirements of Small Ruminants. National Academy of Science, Washington, DC 347p.

Rumen epithelial adaptation to severe feed restriction during late gestation involves the coordinated regulation of papillae growth and nutrient metabolism in pregnant ewes

Fan Hu, Shengyong Mao

Nanjing Agricultural University, Nanjing, China

E-mail: 2016105046@njau.edu.cn

Take home message DE genes by transcriptome analysis suggest that feed restriction can affect the expression of genes involved in growth and epithelium metabolism, which may lead to the alteration of rumen epithelial morphology.

Introduction During late pregnancy in sheep, the voluntary feed intake declines because rumen volume decreases due to the fetuses growth and fat deposits in the abdominal cavity, which lead to negative energy balance. Previous studies found that the morphology of the rumen epithelium could be changed by different energy densities (Steele *et al*., 2015). However, the effect of severe feed restriction (SFR) on rumen epithelial morphology is unclear. In the present study, transcriptomic profiling was used to investigate the effects of feed restriction on morphology of rumen epithelium and the underlying mechanism during late gestation.

Material & methods Sixteen pregnant ewes (108 days of gestation) were randomly assigned to a control group (CON, $n = 8$) or severe feed restriction (SFR, $n = 8$) group. Ewes were fed a common diet with a 60:40 forage to concentrate ratio for 7 days for the baseline period followed by a SFR challenge period (30% of the basal diet for 15 days). Sheep were slaughtered after morning feeding. The rumen fluid was sampled and used to determine ruminal pH and volatile fatty acids (VFAs). Rumen epithelium was collected and used to assess the alterations in mRNA expression profiles.

Results Ruminal pH was greater in SFR group ($P = 0.040$) compared with CON group, while SFR decreased ($P < 0.05$) the concentrations of ruminal acetate, propionate, butyrate and total VFA (TVFA). The papillae length, width and surface area decreased ($P < 0.05$) in SFR group. Transcriptome analysis revealed that 106 genes were differed significantly (≥ 1.5 fold change; FDR < 0.05) between two groups. Furthermore, the expressions of 14 genes were significantly correlated with the fermentation parameters (VFAs) or pH (Table 1). A proposed relationship between transcriptional mechanism and phenotype of the morphology of RE affected by feed restriction in sheep was showed in figure 1.

Table 1 Correlationship between the concentration of VFAs and expression of DEGs in ewes with severe feed restriction ($P< 0.05$).

	r value			
Gene	pH	Acetate	Propionate	TVFA
ADSSL1	—	-0.75	-0.76	-0.69
ATP12A	0.80	-0.64	—	-0.65
CLDN17	0.64	—	—	—
COL18A1	0.71	0.71	0.70	0.66
COL1A2	—	0.81	0.76	0.75
CPT1B	0.73	—	-0.67	—
CSF1	0.67	0.65	0.68	—
CYP8B1	—	-0.78	-0.90	-0.79
DGKA	0.69	0.75	0.76	0.72
DPYD	0.85	-0.81	-0.86	-0.77
FZD7	0.83	—	-0.71	—
HK2	0.67	—	-0.73	—
JAK3	—	0.70	0.80	0.67
THBS1	0.69	—	—	—

— means no significance

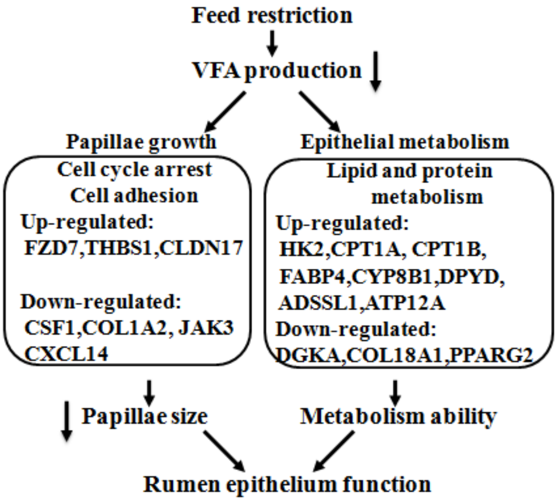


Figure 1 The network of feed restriction and rumen epithelium function, ↓ means decreased when the feed intake restricted to 30%.

Conclusion KEGG pathway analysis indicated that the differentially expressed genes (DEG) were associated with focal adhesion, PI3K-Akt signaling pathway, and cell cycle, which were mostly associated with cell growth, death, and cell adhesion. The integrated interpretation of the results suggested an overall change in nutrient metabolism after feed restriction, particularly PPAR signaling pathway, protein digestion and absorption, and fatty acid metabolism. Our results indicated that severe feed restriction elicited a strong transcriptomic response in the ruminal epithelial tissue in pregnant ewes.

Acknowledgements This research was supported by National key research and development program (China No.2016YFD0501206).

Reference

Steele MA, Schiestel C, AlZahal O, Dionissopoulos L, Laarman AH, Matthews JC and McBride BW 2015 Journal of Dairy Science 98, 2583–95.

691

Impact of type of milk on behaviour, health and performance in artificially-fed young meat lambs

Marie-Madeleine Mialon[1], Raymond Nowak[2], Xavier Boivin[1], Denys Durand[1], Alain Boissy[1], Thierry Fassier[3], Eric Delval[1], Anne-Sophie Bage[1], Frédéric Bouvier[3], Fabien Cornilleau[2], Céline Parias[2], Laurence Guilloteau[4]

[1]Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, 63122 Saint-Genès-Champanelle, France, [2]INRA-CNRS-Université de Tours-Haras Nationaux, UMR85, UMR7247, PRC, 37380 Nouzilly, France, [3]INRA, UE 0332 Bourges-La Sapinière, 18390 Osmoy, France, [4]INRA, UR 0083 Unité de Recherches Avicoles, 37380 Nouzilly, France

E-mail: marie-madeleine.richard@inra.fr

Take home message Revisiting the quality of milk replacer in young meat lambs is critical to limit problems in growth, health and behaviour.

Introduction In sheep meat production, artificial rearing with commercial milk replacer is frequently used to save lambs born from large litters. It is often connected with poor animal health and behavioural deficits, issues needing further scientific investigation (Napolitano *et al*, 2008). The objective of this exploratory study was to evaluate the effects of feeding lambs with ewe's milk instead of milk replacer on performance, health and behaviour compared to maternally reared lambs.

Material & methods Forty-three lambs (Romane breed) weighing at least 2.5 kg at birth were involved. They were either left with the mother (Control group, C), or separated at 3 days of age and fed either milk replacer (MR), following manufacturer specifications, or fresh milk (FM) from dairy Lacaune ewes at the same lactation stage than the dams. In both MR and FM lambs, milk was distributed twice a day in buckets in equal amounts with volumes increasing from 1L per lamb on the first week to 3L between weeks 4 and 7. All the lambs also received concentrates from day 7 onwards. At weaning (45 days), all lambs were mixed in single sex groups. Chemical milk composition was measured once at day 21. On day 90, lambs were submitted to a subcutaneous vaccination against chlamydiosis. Various physiological measures or samples were regularly collected: dag score as a diarrhoea indicator, blood to measure the total antioxidant status (TAS), the immune response to vaccination, and faeces to analyse intestinal pathogens present. In addition, lambs were weighed at different ages. Likewise, they were exposed to behavioural tests during the nursing period (phase 1 = lamb left alone in the test pen; phase 2 = lamb in presence of an unknown human), and a qualitative behaviour assessment (QBA; AWIN, 2015) was performed from video recordings made in the rearing pen after simulating straw mulching.

Table 1 Effect of type of milk on performances, health and behaviour of lambs reared artificially (deviation (%) to dam nursed group C). * P<0.05, † P<0.1.

Item	Type of milk (1)		
	MR	FM	P
Weight gain D1 to D21	-21*	-13	0.01
Dag score D21 (2)	+77†	-22	0.09
Intensity of immune response (3)	-20	+34	0.04
Total antioxidant status	-1	-2	0.13
Activity level (QBA) (4)	-231*	-77	<0.001
Attraction for human (5)	+365	+984*	<0.01

(1) MR: Milk Replacer; FM: Fresh Milk

(2) rear end clean (0), dirty (1) or very dirty (2)

(3) response to vaccine (serum positive percentage)

(4) axe 1 of PCA of 21 descriptors (AWIN, 2015)

(5) time spent in human zone (phase 2 - phase 1)

Results & discussion Overall, lambs fed MR had impaired early growth rate, worse dag scores and were more active/agitated in their nursing pen compared to C lambs (Table 1). When replacing MR by fresh milk, performances and health were partially restored. In the behavioural test, FM lambs were the most attracted by a human despite an amount of human contact similar to MR lambs and they did not behave differently from control lambs in their nursing pens. A closer examination of the results shows that the critical period was the first month of age. Fresh milk was richer in fat (80% *vs* 43%) and in protein (56% *vs* 34%) but less rich in lactose (43% vs 65%) than MR. This could explain its positive impact in early life. Interestingly this seems to influence also behaviour. This milk could have been more rewarding as it improved lambs' responses to humans. The best results in the FM group are consistent with Sevi *et al* (1999) working on dairy sheep who promote a gradual transition from ewe's milk to milk replacer to minimize the detrimental effects of artificial rearing.

Conclusion Our findings highlight the need to revisit the importance of the quality of the milk replacer, at least in early age, not only for growth but also for lamb's health and behaviour.

Acknowledgements This research was supported by a grant from the INRA metaprogram on "Integrated Management of Animal Health" for the 'WHELP' project (www.gisa.inra.fr/en). The authors would like to express their gratitude to the personal of the INRA farm UE 332 (Bourges) and the technicians of the UMR 1213 (Theix) and UMR 7247 (Nouzilly).

References

AWIN, 2015. AWIN welfare assessment protocol for Sheep. DOI:10.13130. 72 p.

Napolitano F, De Rosa G and Sevi A 2008. Applied Animal Behaviour Science 110, 58-72.

Sevi A, Napolitano F, Casamassima D, Annicchiarico G, QuarantellieT and De Paolad R 1999. Applied Animal Behaviour Science 64, 249–259.

A descriptive study of natural nursing procedures in French dairy farms

Yannick Le Cozler[1,2], Catherine Disenhaus[1,2], Elisa Arnaud[1], Lucie Beugnet[1], Marie Charleuf[1], Pauline Denis[1], Marie Roig-Pons[1], Dominique Pomies[3,4], Bruno Martin[3,4], Chantale Chassaing[4,3], Audrey Michaud[4,3]

[1]*Agrocampus-Ouest, 65 rue de St-Brieuc, 35 042 Rennes cedex, France,* [2]*INRA, UMR 1348 Physiology, Environment and Genetics for Animal and Livestock Systems, 35590 St-Gilles, France,* [3]*INRA, UMR 1213 Herbivores, 63163122 Saint-Genès-Champelle, France,* [4]*Vet AgroSup, 89, avenue de l'Europe - BP 35, 63370 Lempdes, France*

E-mail: audrey.michaud@vetagro-sup.fr

Take home message Various methods and practices are available to efficiently reared calves through suckling in dairy cows, using specific dairy nurse cows or not.

Introduction On most dairy farms, calves and cows are separated shortly after birth, but animal welfare associations are questioning such practices more and more. Alternative methods (Johnsen *et al.*, 2016), based on calves suckling their mother or a specific nurse, have been adopted for a long time by some dairy farmers, for purposes such as a reduction of labor or improvement of calves growth and health. However, practical (how these methods are applied within farms) and human (motivations, perception…) aspects are scarce and not very well-known in France. The present study, based on a descriptive survey, aimed to fulfill such lack of information.

Material & methods A semi-quantitative survey was designed and conducted from January to March 2018 and comprised results from 102 farms. Farms where calves were not artificially fed were identified by different technicians and operators. Farmers were selected if calves stayed with their mother ("Fed by their dam, FBD; n = 59), a nurse cow ("Fed by a nurse cow", FBN; n = 13) or both (*i.e.,* fed first by their dam and then by a nurse cow; "mixed"; n = 30) at least 24 hours. To get the highest variability in breeds (Holstein, Montbeliarde…), type of farming (organic or not), facilities (tie-stall or not, milking parlor…), survey was conducted in west (n = 50), middle (n = 11) and east (n=41) of France (Figure 1).

The survey comprised general description, nursing procedures, perception of performances of calves and cows, behavioral aspects and farmers' perception. It was completed either though phone call (n = 56) or face to face interview (n = 46). Only descriptive results are presented in this paper.

2.2 Labor Unit (min 0.5; max 8.5)
114 ha (min 11; max 500)
66 dairy cows (min 8; max 210)
56% Organic farming

Figure 1 Localization of the 102 farms included in present study and variability of surveyed farms.

Results & discussion Herd size varied from 8 to 210 dairy cows and out of the 102 farms studied, 56 % of them were organic farms. A wide diversity of practices among farms was noted, observed in both small (≤ 70 dairy cows) and large herds. FBD was more frequent in small herds compared to FBN or "mixed", but equally distributed in large sized herds. In detail, 5 different types of nursing were observed, from 58 farms where such details were available (Figure 2). According to farmers, these practices were efficient to save time and improved working conditions (quoted by 47 and 29 farmers, respectively). Eighty per cent of farmers considered that, regardless of the nursing procedure, these methods resulted in a decrease of diarrhea frequency in comparison of previous artificial nursing. If they agree for a better welfare for calves, they also considered that weaning is a stressful situation especially for the cows.

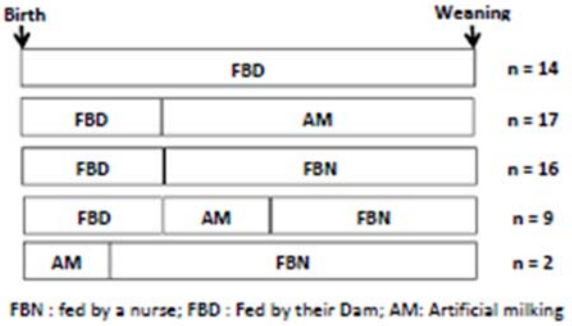


Figure 2 Schematic representation of nursing procedures observed in 58 farms where detailed procedure was available.

Conclusion A diversity of systems and methods exists to rear calves through suckling, either by their mother or another nurse cow. According to most of the farmers in the present study, these practices are easy to use and manage, in a large variety of farms. They also considered that calves' health and welfare are improved, even though weaning is a hard and stressful situation for the cows. Moreover, it is an opportunity for the farmer to save time, money and improve working conditions.

Acknowledgements This investigation was supported by performed by master students from both Agrocampus Ouest and VetAgro Sup high School. Authors thanks Gala Association for its financial support.

References

Johnsen JF, Zipp KA, Kälber T, Passillé AM de, Knierim U, Barth K and Mejdell CM 2016. Applied Animal Behaviour Science 181, 1–11.

Effects of feeding regime pre-puberty on growth and first lactation performance of Holstein Friesian dairy heifers

Joaquin Castro-Montoya, Elizabeth Velasco, Uta Dickhoefer
University of Hohenheim, Animal Nutrition and Rangelands Management in the Tropics and Subtropics, Stuttgart, Germany

E-mail: jcm@uni-hohenheim.de

Take home message Heifers transitioning from a total mixed ration to grazing during their pre-pubertal phase achieve higher daily gains, whereas heifers spending more time grazing during pre-puberty have higher first lactation milk yield.

Introduction In typical dairy heifers rearing systems in Europe the feeding is determined by the season and, thus, availability of pasture for grazing. Depending on the date of birth, heifers will be fed the majority of their pre-pubertal phase grazing on pasture, feeding on a total mixed ration (TMR) indoors, or would transition from one of those feeding regimes to the other. The life-time potential for milk production of dairy cattle is strongly influenced by their body weight gain and mammary gland development before reaching puberty (Serjsen and Purup, 1997). Therefore, we studied the influence of pre-pubertal feeding regimes of heifers on growth, reproduction and production parameters.

Material & methods Data from 105 Holstein Friesian heifers born between 2009 and 2014 who completed their first lactation between 2012 and 2017 was collected from the experimental farms of the University of Hohenheim (UHO). At UHO farm heifers are reared under two feeding regimes: grazing (plus supplementation with 0.5 kg of concentrate daily) and TMR (indoors, barley, wheat, oats, oilseeds, grass silage). The grazing season lasts between May and October, while the indoor feeding lasts between November and April. For this study, the pre-pubertal phase was set between the 5th and the 10th months of age (Akins, 2016), thus, a heifer would spend a maximum of 150 days under any feeding regime in its pre-pubertal phase. Depending on the start of their pre-puberty, heifers were classified into four feeding regimes: Grazing (> 120 days grazing), TMR (> 120 days feeding on TMR), Grass-TMR (> 30 initial days grazing, transitioned to TMR feeding), and TMR-Grass (> 30 initial days feeding on TMR, transitioned to grazing). Monthly data on body weight, reproductive parameters, and daily milk yield records for the first lactation of all heifers were gathered. Energy corrected milk yield adjusted for 305 days (ECM 305) was estimated. All parameters were evaluated by an analysis of variance with feeding regime as fixed effect and year of rearing as random effect using the Mixed procedure of SAS.

Results Heifers transitioning from a TMR feeding to grazing during pre-puberty achieved greater average daily gain (ADG) until pregnancy, while heifers starting their pre-puberty under grazing condition had the lowest ADG. Heifers transitioning from grazing to TMR during pre-puberty needed longer to reach their first artificial insemination (AI), while heifers under a TMR feeding regime needed the fewest AI's to achieve pregnancy (Table 1). Milk yield during first lactation was highest for animals spending the majority of the pre-puberty on grazing, and lowest for those who transitioned from grazing to TMR. Moreover, heifers transitioning from grazing to TMR needed also a longer time to achieve a second pregnancy.

Table 1 Average daily gain (ADG), and productive and reproductive parameters of Holstein Friesian heifers in South Germany spending the pre-pubertal phase on one of four feeding regimes.

Feeding regime	Grazing	Grass-TMR	TMR-Grass	TMR	SEM
ADG until pregnancy (g)	705^{b}	707^{b}	752^{a}	740^{ab}	16.8
Age at first artificial insemination (Months)	16.8^{b}	18.0^{a}	17.2^{b}	17.2^{b}	0.13
Number of artificial inseminations	1.93^{b}	1.57^{ab}	1.91^{b}	1.38^{a}	0.095
Age at pregnancy (Months)	18.1^{b}	18.6^{b}	18.5^{b}	17.8^{a}	0.21
ECM 305 (kg)	9621^{a}	8966^{b}	9594^{ab}	9236^{ab}	140.6
Open days	127^{ab}	150^{a}	92^{c}	108^{bc}	5.45

Conclusion The feeding regime seems to have an effect on growth, production and reproduction of dairy heifers until the first lactation. Animals grazing during the majority of their pre-puberty might promote an optimal balance between the body growth and mammary gland development that reflects later on a higher first lactation milk yield. The transition of grazing to TMR feeding during pre-puberty may have a negative impact on the heifers' development that is carried until the first lactation. Conversely, the transition of TMR to grazing does not seem to affect the heifers' performance and reproduction. Other interactions with diet during further physiological stages may play an additional role in the findings of this study.

Acknowledgements To the experimental farms Unterer Lindenhof and Meiereihof of the University of Hohenheim.

References

Akins MS 2016. Veterinary Clinics Of North America-Food Animal Practice 32, 303-317.
Sejrsen K and Purup S 1997. Journal of Animal Science 75, 828-835.

Morphological adaptation of sheep's rumen epithelium to high-grain diet entails alteration in the expression of genes involved in cell cycle regulation, cell proliferation and apoptosis.

Lei Xu, Yue Wang, Junhua Liu, Weiyun Zhu, Shengyong Mao

Jiangsu Key Laboratory of Gastrointestinal Nutrition and Animal Health, Laboratory of Gastrointestinal Microbiology, Department of Animal Science and Technology, Nanjing Agricultural University, Nanjing, China

E-mail: njauxulei@163.com

Take home message These findings provide new insights into the morphology adaptation and cell-cycle-regulating adaptation of the rumen epithelium (RE) to HG feeding are procedural adaptations. These findings enhanced our understanding of the molecular adaptive mechanisms of the RE in response to HG diet feeding, which may be helpful in developing technologies or feeding strategies to increase productivity in feedlot sheep and cattle.

Introduction Previous studies revealed that RE adaptation to highly fermentable diets entails morphological adaptations associated with tissue proliferation (Penner G, 2011; Steele M, 2015). However, to date, little is known about the molecular basis of epithelial proliferation and apoptosis in the RE cells of sheep during grain adaptation. The changes in their cell cycle have not been clarified yet. Therefore, the objectives of this study were to characterize changes in the relative mRNA expression of candidate genes and proteins involved in cell cycle regulation, cell proliferation and apoptosis in the ruminal epithelium of sheep during high-grain diet adaptation.

Material & methods Twenty sheep were assigned to four groups with five animals each. These animals were assigned to different periods of HG diet (containing 40% forage and 60% concentrate mix) feeding. The HG groups received an HG diet for 7 (G7, n=5), 14 (G14, n=5) and 28 d (G28, n=5), respectively. In contrast, the control group (CON, n=5) was fed the forage-based diet for 28 days. All experimental designs and procedures for this study were approved by the Animal Care and Use Committee of Nanjing Agricultural University (SYXK(Su)2015-0656). Comparisons between the groups were carried out with one-way ANOVA, followed by Tukey's multiple comparison test in SPSS software packages (SPSS version 16.0.1 for Windows; SPSS Inc., Chicago, IL, USA). Linear, quadratic or cubic polynomial contrasts were performed to test for a trend in the treatment means. Differences were considered significant at $P < 0.05$, and trends were discussed at $0.05 < P < 0.10$.

Results & discussion The results showed that high grain feeding linearly decreased ($P < 0.001$) the ruminal pH, and increased the concentrations of ruminal total volatile fatty acid (linear, $P = 0.001$), butyrate (linear, $P < 0.001$), valerate (quadratic $P = 0.029$) and the level of IGF-1 (quadratic, $P = 0.043$) in plasma. In response to increased VFA production and decreased ruminal pH, our data revealed that the RE responded to these challenges in a coordinated manner. The length (quadratic, $P = 0.004$), width (cubic, $P = 0.015$) and surface of the ruminal papillae (linear, $P = 0.003$) were all enlarged after 14 d of HG diet feeding. An enlargement of the RE surface area could stimulate VFA absorption and regulate intracellular pH. High grain feeding cubically increased the number of cell layers forming the stratum corneum ($P < 0.001$) and the thickness of the stratum corneum ($P < 0.001$) and stratum basale ($P < 0.001$). The proportion of basal layer cells in the rumen epithelium decreased (linear, $P < 0.001$) in the G0/G1-phase, but it increased linearly ($P = 0.006$) in the S-phase and cubically ($P = 0.004$) in the G2/M-phases. This indicated that the time duration of the G0/G1-phase was shortened, and then, cell cycle progression was accelerated. These changes may lead to an increase in rumen papillae size, which further enhances the absorption ability of the RE. The proportion of apoptosis cells in G7, G14 and G28 was reduced compared to the CON (quadratic, $P < 0.001$). High grain diet feeding linearly decreased the mRNA expression of Cyclin E1 ($P = 0.021$) and CDK-2 ($P = 0.001$) and ($P = 0.027$) the protein expression of Cyclin E1. Feeding an HG diet linearly increased the mRNA expression of genes IGFBP-2 ($P = 0.034$) and IGFBP 5 ($P < 0.009$), while linearly decreasing ($P < 0.001$) the IGFBP 3 expression. The expression of cell apoptosis gene Caspase 8 decreased (quadratic, $P = 0.012$), while Bad mRNA expression tended to decrease (cubic, $P = 0.053$) after HG feeding. Thus, it is reasonable to suppose that IGF-1 may trigger the proliferation of the RE and block apoptosis to increase growth after two weeks of HG feeding. In addition, butyrate may trigger the downregulation of IGFBP 3 in the RE and block apoptosis and increase growth.

Conclusion These results demonstrated sequential changes in rumen papillae size, cell cycle regulation and the genes involved in proliferation and apoptosis as time elapsed in feeding a high-grain diet to sheep.

Acknowledgements This work was supported by the National Natural Science Foundation of China (No.31572436) and Natural Science Foundation of China (No. 31372339).

References

Penner G, Steele M, Aschenbach J and McBride B 2011. Journal of Animal Science 89, 1108-1119.

Steele M, Schiestel C, AlZahal O, Dionissopoulos L, Laarman A, Matthews J, and McBride BW 2015. Journal of Dairy Science 98, 2583-2595.

695

Potential of four different sources of non-starch soluble polysaccharides to modulate the immune response and metabolic state in the intestine

Alexandre Budan[1], Marcel Hulst[2], Denis Métais[1], Marinus van Krimpen[2]

[1]Cargill Premix & Nutrition, Trégueux, France, [2]Wageningen Livestock Research, Wageningen, The Netherlands

E-mail: alexandre_budan@cargill.com

Take home message Macro and micro algae may be evaluated as additives to prevent infectious diseases.

Introduction Non-starch soluble polysaccharides (NSPs) produced by yeasts are used in ruminant nutrition to improve health and performance (Broadway *et al.,* 2015). However, the magnitude of the effect may be dependent upon the quantity and the composition of the polysaccharides. As seaweeds are potent sources of NSPs, this study was set up to evaluate their potential to improve intestinal health.

Material & methods Intestinal epithelial cells J2 (IPEC-J2) were incubated *in vitro* with 6 different treatments in the presence and absence of inflammation induced by the enterotoxigenic bacterium *Escherichia coli* K99 strain (ETEC). The treatments were negative control (no addition to incubation medium), 0.0001% zinc oxide (ZnO, positive control) and fractions containing 0.05% NSPs prepared from, *Saccharomyces cerevisiae* containing β-glucan and mannan (SC), micro algae containing β-glucans (MA), brown macro algae containing fucoidan and laminarin (BA) and green macro algae containing ulvans (GA). RNA was extracted from IPEC-J2 after 6 hours of incubation and gene expression was measured using "whole genome" microarrays as described by Hulst *et al.,* 2017. Bioinformatics programs were used in order to identify induced and repressed cytokines (23) and pathways (244) by ETEC and treatments.

Results The analysis of the transcriptomics datasets indicated that 18 acute phase proteins and 49 pathways involved in immune response were modulated in the IPEC-J2 cells by the NSP's in the presence of ETEC, and 2.5 fold less in the absence of ETEC. Contrary to algae, SC did not modify gene expression of acute phase proteins without challenge. The level of expression of Interleukin 6 , Colony Stimulating Factor 2A and C-C Motif Chemokine Ligand 5 was increased with all tested algae and was not modified by ETEC and SC. Only GA upregulated Interleukin 1β, with multiplication factor of 45 without challenge and 9 with challenge. Without challenge, algae modulated 3 (BA) to 13 (MA) pathways involved in the immune response, *versus* 1 with SC. With challenge, algae modulated 18 (GA) to 36 (BA) pathways involved in the immune response, *versus* 13 with SC. Other affected pathways included energy metabolism, intestinal carriers and the oxidative response. Feeding MA and BA led to higher expression of genes favoring gluconeogenesis such as pyruvate kinase (-2.74 fold) and phosphoenolpyruvate carboxykinase 1 (+16.5 fold). MA activated reactive oxygen species (ROS) reduction pathway genes (NAD(P)H dehydrogenase, quinone 2-NQO2; chemokine (C-X-C motif) ligand 2-CXCL8; cytochrome P450 1A1-CYP1A1) compared to BA and SC, while GA also activated the vitamin C antioxidation pathway through higher expression of NQO1 and nitric oxide synthase 2 (+8.63 fold) with or without challenge. This metabolic state slows down utilization of glucose for ATP production, which also reduces production of ROS and rescues cells from apoptosis induced by ROS.

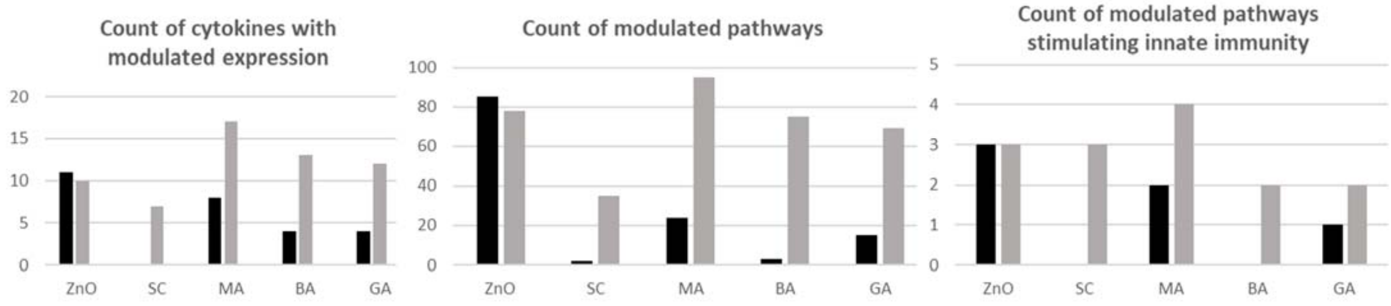


Figure 1 Modulated cytokines and pathways with (grey) and without (black) ETEC.

Conclusion In this study, the sources of NSPs can be classified in the following order MA>GA>BA>SC according to their capacity to modulate the gene expression of acute phase proteins and pathways involved in the innate immune response with and without challenge. These new data suggest that some algae may be efficient to alert the immune system before infection and in case of infection by *Escherichia coli* K99. *In vivo* trials are necessary to evaluate this hypothesis.

Acknowledgements This research was supported by grants from the Dutch government.

References

Broadway PR, Carroll JA and Burdick Sanchez NC 2015. Microorganisms 3, 417-427

Hulst M, Jansman A, Wijers I, Hoekman A, Vastenhouw S, van Krimpen M, Smits M and Schokker D 2017. Genes Nutrition 13, 12-11

Supplemental bovine lactoferrin and a probiotic in Ghezel lambs during the pre-weaning phase

Mokhtar Mallaki, Ali HosseinKhani, Akbar Taghizadeh, Hamid Paya

University of Tabriz, Tabriz, Iran

E-mail: mallaki.mokhtar@yahoo.com

Take home message Supplemental Bovine Lactoferrin and probiotic could improve the health and growth performance of Ghezel lambs during the pre-weaning phase.

Introduction Poor lamb survival rate is detrimental to a sheep farms income (Yeoman *et al*., 2014). So, ruminant neonates which are naturally hypogammaglobinaemic must consume colostrum as a source of bioactive energy in order to reduce lamb mortality rates (10-30%, Vetter *et al*., 1960, Turkson, 2003). We hypothesise that bovine lactoferrin (bLF) and a probiotic GIT modulator could promote performance and health in the initial stage of a lambs life.

Material & methods Thirty-six Ghezel suckling male lambs (3.9±0.65 kg body weight (BW)) were selected for the experiment from age 3 days and housed in group pens. Lambs were assigned randomly to one of the 6 Groups: 1) control (without bLF and probiotic), 2) 1 g/d probiotic, 3) 0.25 g/d bLF, 4) 0.25 g/d bLF and 1 g/d probiotic, 5) 0.5 g/d bLF, 6) 0.5 g/d bLF and 1 g/d probiotic. Bovine lactoferrin (Shangqiu Kangmeida Bio-Technology Co. Ltd) and a probiotic (Primalac TM) were given orally every day (0900) for 56 days. Suckling lambs were fed fresh milk from ewes by nipple bottles three times per day (0600, 1400 and 2200) up to 56 days. Starter feed and water were available *ad libitum* from two weeks of age. Feed intakes (FI) and BW were recorded daily and weekly respectively. Starter feed, milk and orts were sampled weekly, composited, and frozen at -20 °C for analysis. The health status was evaluated based on clinical traits including fecal score, rectal temperature, suckling, days medicated, nasal and eye discharge, respiratory sounds, cough and appetite. These traits were measured and monitored throughout the experimental period. Feces were scored 3 days per week on a scale of 1 through 7, with 1 = separate hard lumps and 7 = liquid consistency without solid space diarrhea. Rectal temperatures were determined in lambs 3 times per week. Days medicated were recorded as each day a lamb received treatment. Statistical analysis was performed as repeated measures data using the MIXED PROC model of SAS software 9.2 (SAS, 1999). If the P value was significant, differences among means were evaluated by Tukey.

Results Despite the same initial weight among lambs, the final body weight was more significantly in bLF and bLF plus probiotic groups (Table 1, P<0.05). Albeit bLf and probiotic supplementation increased weight gain, however, probiotic alone did not significantly increase weight gain. The bLF and probiotic could increase and promote FI and FCR respectively, but the results shown that different levels of bLF and bLF plus probiotic are more effective compared with probiotic alone. Although rectal temperatures decreased during the experimental period, there was no significant change in rectal temperature with the application of treatments. Fecal score was not affected by treatment, in spite of the fluctuation on the medicated days.

Table 1 Performance and health status of Ghezel lambs supplemented with bovine lactoferrin and/or probiotics (Primalac TM).

Measurement	Treatments^{1}						SEM	P-value		
	1	2	3	4	5	6		bLF	probiotic	bLF*probiotic
Initial Body Weight (kg)	3.96	3.98	3.98	3.95	3.95	4.03	0.16	ns	ns	ns
Final Body Weight (kg)	11.89^{c}	12.24^{c}	13.73^{b}	14.65^{a}	13.86^{b}	14.74^{a}	0.37	*	ns	*
Weight gain (kg/day)	0.141^{c}	0.148^{c}	0.174^{b}	0.191^{a}	0.176^{b}	0.191^{a}	0.01	**	ns	**
Feed intake (kg/day)	0.239^{c}	0.255^{b}	0.272^{a}	0.285^{a}	0.285^{a}	0.293^{a}	0.01	**	*	**
FCR	3.07^{a}	2.67^{b}	2.14^{c}	1.98^{c}	2.02^{c}	2.01^{c}	0.16	**	*	**
Feces score	2.62	2.53	2.48	2.27	2.34	2.36	0.31	ns	ns	ns
Rectal temperature	39.30	39.10	39.12	38.81	38.67	38.69	0.24	ns	ns	ns
Medicated days	10.17	8.00	7.17	6.83	5.27	5.20	2.78	ns	ns	ns

[1] 1) Control (without bLF and probiotic), 2) 1g probiotic, 3) 0.25g bLF, 4) 0.25g bLF + 1g probiotic, 5) 0.5g bLF, 6) 0.5g bLF + 1g probiotic, ns=nonsignificant (P>0.10), *P<0.05, and **P<0.01 for a difference of treatment groups.^{a,b,c,d} LS means with different letters in superscripts are different at P<0.05.

Conclusion To our knowledge, there isn't any study with use bLF plus probiotic in ruminant neonates, however the results of present study agreement with Joslin *et al.* (2002) found that fed bLF for calves in preweaning phase effective on final body weight, ADG and FCR. It could be hypothesized that healthier lambs had higher DMI which caused to improved ADG and other performance properties and health indexes. On the other hand, growth factor activity as one of the most important functions of LF and GIT morphology developed by probiotic (Yeoman *et al*., 2014), lead to increase intestinal growth and nutritional absorption, could support ADG and FCR changes in our study.

References

Joslin, R, Erickson PS, Santoro HM, Whitehouse NL, Schwab CG and Rejman JJ 2002. Journal of Dairy Science 85, 1237-1242.
Turkson P 2003. Tropical Animal Health and Production 35, 477-490.
Vetter R, Norton H and Garrigus U 1960. Journal of Animal Science 19, 616-619.
Yeoman CJ and White BA 2014. Annual Review of Animal Biosciences 2, 469-486.

Catharanthus Roseus can ameliorate *Escherichia Coli* toxin induced diarrhoea: an *in vitro* model on isolated buffalo jejunum

Qurrat ul Ain, Khalid Abdul Majeed, Habib ur Rehman, Imtiaz Rabbani

University of Veterinary and Animal Sciences, Lahore, Pakistan

E-mail: imtiaz.rabbani@uvas.edu.pk

Take home message *Catharanthus roseus* plant extract has the potential to ameliorate *E. coli* toxin induced diarrhoea in buffalos.

Introduction The livestock sector contributes to the livelihood and food security of billions of people (Robinson *et al*., 2014) and aids the sustenance of 600 million farmers in the developing world (Thornton, 2010). Diarrheal disease in livestock is a leading global health problem with an estimated 2 to 4 billion episodes (Chen *et al*., 2007). Pathogenic *E. coli* is responsible for severe diseases in both humans and animals (Escherich, 1988) accounting 20% of all diarrheal diseases (Islam *et al*., 2008). In recent years, there has been an increased focus on lowering the use of drugs in livestock (Liu *et al*., 2016) leading to use of plants in disease conditions (Hassan, Brenda, Patrick, & Patrick, 2011). *Catharanthus roseus* is known for its therapeutic effects as antidiabetic, analgesic, antioxidant, antimicrobial and antidiarrheal (Hassan *et al*., 2011). *In vitro* studies with Ussing Chamber provides an inexpensive way to determine electrophysiology and can assist in estimation of nutrients transport across the epithelial tissue (Clarke, 2009). It is therefore, the aim of the study to evaluate the effect of *C. roseus* on electrophysiology of isolated jejunum of buffalo treated with *E. coli* toxin.

Material & methods This study involved isolated jejunum epithelium from buffalo (n = 5) mounted in the Ussing Chamber and divided in to four groups; Group A (Control), Group B (1.5% *C. roseus* extract), Group C (10% *E.coli* toxin) and Group D (10% *E.coli* toxin followed by 1.5% *C. roseus* extract) added on mucosal sides in all cases. The tissues were incubated under open circuit conditions for 20 minutes followed by short circuit condition (Voltage clamp). Transepithelial potential difference (PDt in mV) and Short Circuit Current (Isc in $\mu A/cm^2/hr$) was determined. Data were analysed using one way ANOVA with Tukeys post hoc test.

Results & discussion Following exposure to *E. coli* toxin on the mucosal side, the Isc ($\mu A/cm^2/hr$) rose from 6.12±1.22 to 12.04±3.21 ($p<0.01$ n=5). Addition of *C. roseus* extract followed by the toxin reduced the current from 10.31±1.63 to 4.24±0.89 ($p<0.01$ n=5) suggesting a possible role of plant extract on chloride channels that were opened due to exposure of the toxin.

Conclusion *Catharanthus roseus* treatment affects the electrophysiological indices of isolated jejunum epithelium of buffalo presumably by blocking the chloride channels and ameliorate the effect of secretary diarrhoea. This may provide a better alternative to use of conventional antidiarrheal drugs.

Acknowledgements The lab for this research was established by the grant provided by DAAD.

References

Chen JC, Chang YS, Wu SL, Chao DC, Chang CS, Li CC and Hsiang CY 2007. Journal of Ethnopharmacology 113, 233-239.
Clarke LL 2009. American Journal of Physiology-Gastrointestinal and Liver Physiology 296, G1151-G1166.
Escherich T 1988. Review of Infectious diseases 10, 1220-1225.
Hassan KA, Brenda AT, Patrick V and Patrick OE 2011. African Journal of Pharmacy and Pharmacology 5, 1797-1800.
Islam MA, Mondol AS, De Boer E, Beumer RR, Zwietering MH, Talukder KA and Heuvelink AE 2008. Applied and environmental microbiology, 74, 5414-5421.
Liu G, Guan G, Fang J, Martínez Y, Chen S, Bin P, Al-Dhabi NA 2016. BioMed Research International 2016, 1-7.
Robinson TP, Wint GR, Conchedda G, Van Boeckel TP, Ercoli V, Palamara E, Cinardi G, D'Aietti L, Hay SI and Gilbert M 2014. PloS one, 9, e96084.
Thornton PK 2010. Philosophical Transactions of the Royal Society B: Biological Sciences, 365, 2853-2867.

The effect of calcareous marine algae, with or without marine magnesium oxide, and sodium bicarbonate on milk production in mid-lactation dairy cows

Enda Neville[2,1], Alan Fahey[1], Brendan Molloy[1], Stephen Taylor[2], Finbar Mulligan[1]

[1]*University College Dublin, Dublin, Ireland,* [2]*Celtic Sea Minerals, Cork, Ireland*

E-mail: e.neville@celticseaminerals.com

Take home message The inclusion of rumen buffers, in a TMR fed to Holstein dairy cows, improved milk components. Calcareous marine algae increased milk fat and protein yield, offering dairy farmers a solution to improve milk production.

Introduction High producing dairy cows consuming highly fermentable diets often experience low rumen pH (Plaizier *et al*., 2008), initiated by the accumulation of volatile fatty acids in rumen fluid (Whelan *et al*., 2013). Rumen buffers are commonly added to lactating cow diets to avoid prolonged episodes of low rumen pH and the associated production losses. The addition of sodium bicarbonate (SB) to the diets of high producing dairy cows, as a rumen buffer, has become a regular practice in many parts of the world (Rauch *et al*., 2012). In recent years' calcareous marine algae (*Lithothamnion calcareum*) has been used to stabilise rumen pH and improve fermentation (Cruywagen *et al*., 2015). The objective of this experiment was to evaluate different dietary buffers on the milk production, milk composition, dry matter intake and feed efficiency of mid-lactation dairy cows.

Material & methods The supplements included were: calcareous marine algae (*Lithothamnion calcareum*), with or without marine magnesium oxide (precipitated magnesia derived from seawater), and SB. Fifty-two multiparous and four primiparous cows (62.7 ± 3.4 DIM) were assigned to four experimental treatments based on calving BCS (3.1 ± 0.03; scale 1 to 5), pre-experimental milk yield (34.7 ± 0.79 kg/d) and previous 305-day yield (7073 ± 198 kg). Cows were housed in a free stall barn and had *ad-libitum* access to total mixed ration (TMR) and water. The diets were based on a forage: concentrate ratio of 46:54. Dietary treatments consisted of the control (283 g starch and sugar, and 230 g neutral detergent fibre (NDF) from forage per kg dry matter (DM)) including no dietary buffer (CON); the CON plus 3.5 g/kg DM calcareous marine algae (CMA); the CON plus 3.5 g/kg DM calcareous marine algae and 0.9 g/kg DM marine magnesium oxide (CMA+MM); the CON plus 7 g/kg DM SB. The experiment lasted for 80 days (d), which included 7 d acclimatisation to the diet and 73 d of data collection. Milk production data were analysed using the MIXED procedure (SAS, version 9.4). The model included fixed effects of treatment, week and parity as well as treatment by week interaction with cow considered as the random effect. Week was considered as a repeated measure. Pre-experimental milk yield and calving BCS were considered as covariates.

Results & discussion The DMI of cows consuming SB tended to be higher than cows on the CON diet (+ 1.9 kg, $P<0.10$). CMA increased the production of milk solids (fat and protein kg/d) compared to CON (+ 0.16 kg, $P<0.01$), CMA+MM (+ 0.09 kg, $P<0.05$) and SB (+ 0.10 kg, $P<0.05$). Both CMA (+ 0.09 kg, $P<0.01$) and CMA+MM (+ 0.06 kg, $P<0.01$) increased milk fat yield compared to CON but were not different to each other and SB. Cows supplemented with CMA (+ 0.19 %, $P<0.01$), CMA+MM (+ 0.25 %, $P<0.01$) and SB (+ 0.19 %, $P<0.01$) increased milk fat concentration compared to CON but were not different from each other. The CMA treatment increased milk protein yield compared to CON (+ 0.04 kg, $P<0.01$), CMA+MM (+ 0.06 kg, $P<0.01$) and SB (+ 0.04 kg, $P<0.01$). The SB treatment reduced the efficiency of milk production, energy-corrected milk (ECM) per kg of DMI, compared to CON (- 0.11 kg, $P<0.01$), CMA (- 0.12 kg, $P<0.01$) and CMA+MM (- 0.13 kg, $P<0.01$).

Conclusion Results indicate that the addition of rumen buffering products can increase milk fat concentration when included in lactating dairy cow diets. The use of CMA when compared to sodium bicarbonate, in such diets, can increase milk production efficiency and combined fat and protein yield (kg/d). This offers dairy producers, with milk pricing based on milk solids, the opportunity to increase the value of their milk.

Acknowledgements The authors gratefully acknowledge funding from Celtic Sea Minerals, Cork, Ireland.

References

Cruywagen CW, Taylor S, Beya MM and Calitz T 2015. Journal of Dairy Science 98, 5506-5514.
Plaizier JC, Krause DO, Gozho GN and McBride BW 2008. Veterinary Journal 176, 21-31.
Rauch RE, Robinson PH and Erasmus LJ 2012. Animal Feed Science and Technology 177, 180-193.
Whelan SJ, Mulligan FJ, Callan JJ, Flyn B and Pierce KM. 2013. Animal Feed Science and Technology 183, 62-66.

First study on volatile organic compounds exhaled by dairy cows under experimental sub-acute ruminal acidosis

Mathieu Silberberg[1], Jérémy Ratel[2], Lucas De Ofeu Aguia Prado[1], Erwan Engel[2], Marie-Madeleine Mialon[1], Cécile Martin[1]

[1]*Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, 63122, Saint-Genès-Champanelle,, France,* [2]*INRA, UR QuaPA, 63122, Saint-Genès-Champanelle, France*

E-mail: mathieu.silberberg@inra.fr

Take home message Volatolome in the exhaled air differs consistently between dairy cows experiencing Sub-Acute Ruminal Acidosis and those that are not.

Introduction High producing ruminants are fed rich-concentrate diets, which can lead to digestive disorders such as Sub-Acute Ruminal Acidosis (SARA). This disorder characterized by abnormal and intermittent drops in rumen pH alters animal production and welfare and increases economic losses. The reference method to assess SARA is to use ruminal boluses measuring pH, however this technology remains expensive and little used on farms. When an animal is under SARA, metabolic disturbances may occur in some organs, with ultimately changes in composition including its volatolome that can be recovered in the exhaled air. Many studies show in humans that the volatolome is particularly promising to reveal physio-pathologic situations, such as the development of diseases (Shirasu and Touhara, 2011), or the exposure to some contaminants (Defois *et al.*, 2017). In this pilot study, we propose to make the proof-of-concept that volatile organic compounds (VOCs) in the exhaled air of dairy cows undergoing SARA may be different from control cows.

Material & methods Four lactating rumen cannulated Prim' Holstein cows were conducted in a longitudinal study. In a first 5-week period (P1), all cows received an acidogenic diet containing 30% starch, followed by a 4-week control period (P2) with a 6% starch diet. The 2 diets contained the same ingredients (hay, maize silage, grass silage and concentrates) but in different proportions. Diets were distributed twice a day (2/3 at 09:00 and 1/3 at 17:00). Before the experimental period, all cows were fed the control diet. All cows were fitted with an intra-ruminal bolus measuring pH continuously during the whole experiment (eCow, Exeter, UK). Relative ruminal pH indicators (NpH) were calculated as described by Villot *et al.* (2017). During the last week of P1 & P2, cows were equipped with 2 prototype systems to collect (i) exhaled air, and (ii) ruminal gas through the rumen cannula. Collection was performed during 4 hours, from 2 hours after morning meal, on 2 consecutive days. VOCs in samples were extracted by SPME and analysed by GC-MS as described in Ratel *et al.* (2017). Identification of VOCs was performed using mass spectra and retention indices. Analysis of variance was performed on the VOCs abundances to reveal candidate markers of SARA in both gases. Principal component analyses were performed on the most discriminant VOCs.

Results & discussion All relative NpH indicators were discriminant between P1 and P2 (amplitude of NpH was 0.75 *vs* 0.58 NpH, time spent under NpH<-0.5 was 208 *vs* 6 min, respectively) and clearly demonstrate that cows were under SARA challenge during P1. Regarding the VOCs analysis, the PCA showed a good discrimination between the 2 periods (91% of the variability expressed on 1st component). After processing of the volatolomic information, 57 VOCs were determined as discriminative of the 2 experimental periods. These tentatively identified compounds belong to the following chemical families: branched alkanes and alkenes, alcohols, aldehydes, ketones, acids, esters, terpenoids, aromatic compounds. Among the 57 VOCs modified in the exhaled gas, 35 were also modified in the rumen gas indicating that those compounds may be linked to the diet or to the ruminal fermentative processes. The 22 VOCs specific from the exhaled gas could be more related to the host biology. However, it remains necessary to validate these results facing other acidogenic diets and to elucidate the reasons of such modifications during SARA in order to focus on the most interesting VOCs for SARA detection.

Conclusion This proof-of-concept study shows that the exhaled volatolome contains VOCs that can be specifically altered by SARA. VOCs candidate markers can be of major interest for SARA diagnosis since they are non-invasive and easy to collect. Recent developments in nanotechnologies offer great promise in developing functionalized sensors specific to VOC markers.

Acknowledgements This study was supported by the PHASE div. of INRA trough incentive funding.

References

Defois C, Ratel J, Denis S, Batut B, Beugnot R, Peyretaillade E, Engel E and Peyret P 2017. Frontiers in Microbiology 8, 1562.
Ratel J, Planche C, Mercier F, Blinet P, Kondjoyan N, Marchand P, Fournier A, Travel A, Jondreville C and Engel E 2017. Chemosphere 189, 634-642.
Shirasu M and Touhara K 2011. Journal of Biochemistry 150, 257-266.
Villot C, Meunier B, Bodin J, Martin C and Silberberg M 2017. Animal 12, 481-490

Use of *Calliandra calothyrsus* and *Artocarpus heterophyllus* leaves against parasites and the effects on performance of Bligon goats in Indonesia

Kustantinah Kustantinah, Wahyu Setyono, Zuprizal Zuprizal, Edwin Indarto, Nanung Danar Dono, Insani Hubi Zulfa

Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada, Sleman, Yogyakarta, Indonesia

E-mail: kustantinah@ugm.ac.id

Take home message *Calliandra calothyrsus* and *Artocarpus heterophyllus* leaves are anti-parasitic functional feeds and sources of nutrients for livestock.

Introduction The diverse forage plant species in Indonesia provides natural ingredients, such as: tannin, which have anti-parasitic properties for ruminant animals (Daryatmo *et al*., 2008). Current study determined the support of potential nutrients and the effectiveness of forages containing tannin as natural anticoccidial to improve the health and performance of female Bligon goats.

Material & methods This study used 15 female 1-year old Bligon goats that were randomly divided into 3 groups, in a oneway completely randomized design. The control group (C) were fed king grass soley; Group I (G I) were fed 50% king grass and 50% *Calliandra calothyrsus* leaves; and Group II (G II) were fed 50% king grass and 50% *Artocarpus heterophyllus* (Jackfruit) leaves, all on DM basis. The feed was given 5% (DM basis) of body weight. The treatment diets were given for 8 weeks, with 10 initial days for adaptation. Total faeces, as well as, given and feed refusal, were collected for the last 14 consecutive days. The data were used to calculate consumption and digestibility of dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), extract ether (EE), and total digestible nutrients (TDN). Observation of parasite (oocyte) in feces on days 0, 7, 21, 28, 35, 42, and 49, according to the McMaster method. The nutrient contents of feed were analyzed using proximate method (AOAC, 2005). All data were subject to variance analysis using CRD Design, followed by DMRT to separate different means between groups.

Results & discussion Goats that were fed a combination of king grass and *Calliandra calothyrsus* leaves (G I) or and Jackfruit leaves (G II) had higher DM, OM, CP, and TDN intakes ($P<0.05$) and digestibility ($P<0.05$) than those of fed te control diet. These results showed beneficial findings that substitution of king grass with *Calliandra* or Jack fruit leaves improved nutrient intake. The intake of DM for both control and treatments was higher than that of the previous study, which were 35.35 g/kg BW/day (Daryatmo *et al*., 2008). The average intake of DM according to the body weight was 4% of BW. Results also showed that daily body weight of goats in G I and G II was higher than in the control group ($P<0.05$).

Table 1 Nutrient Digestibility (%) of goats fed king grass (control) king grass and (G I) or king grass and jack fruit leaves (G II).

Nutrient	Control	G I	G II
DM	57.17^{a}	67.89^{b}	74.00^{b}
OM	58.06^{a}	67.06^{b}	74.80^{b}
CP	4.38^{a}	8.57^{b}	8.80^{b}
CF	12.18	13.44	15.20
EE	0.73^{a}	1.04^{b}	1.30^{b}
TDN	52.32^{a}	62.87^{b}	69.79^{c}

^{a,b,c} Different superscript on the same line showed significant differences ($P<0.05$).

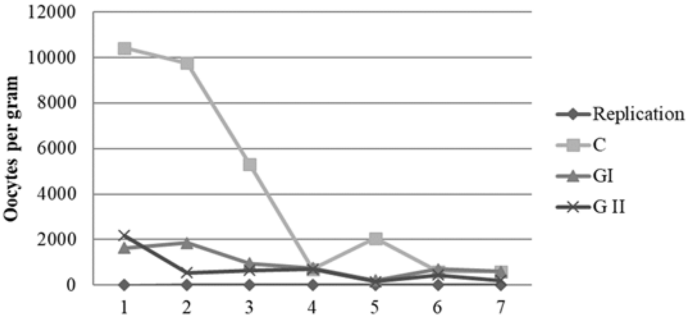


Figure 1 Number of oocytes per gram feces of goats fed king grass (control) king grass and (G I) or king grass and jack fruit leaves (G II).

Goats in G II had the highest value of tannin intake (0.93 g/kg BW), therefore supplied highest natural coccidiostat for the goats. Moreover, at that level, tannins bind to the feed proteins during mastication and protect proteins from microbial rumen attack, the goats therefore were able to use proteins more efficient, improved nutrient digestibility (Table 1), and reduced the number of internal parasites (Figure 1).

Conclusion Substitution of King Grass with *Calliandra calothyrsus* or *Artocarpus heterophyllus* leaves which containing tannins increased the intake and digestibility of DM, OM, CP, TDN, and reduced more intestinal parasite, when compared to those of fed King grass as a sole feed. Further study is needed to investigate the effect of bioactive substances in King grass as natural anti-parasite.

References

AOAC. 2005. Official Methods of Analysis Published by the AOAC, Maryland, USA.

Daryatmo J, Kustantinah, Ørskov ER, Hartadi H and Nurcahyo W 2008. Nutrition potential of various forages that contain active compound and their effectiveness as antiparasite. Proc. The 13th Animal Science Congress of the AAAP. Sept 22-26. Hanoi, Vietnam.

701

Effects of subacute ruminal acidosis on ruminal epithelial morphology and permeability in dairy goats

Hu Honglian, Gao min, Cheng Meng, Sun Yanyong

Inner Mongolia Academy of Agricultural&Animal Husbandry Sciences, Hohhot, China

E-mail: honglianhu2010@163.com

Take home message Subacute ruminal acidosis significantly altered the eletrophysiological properties and increased the permeability of the ruminal epithelium.

Introduction The ruminal epithelial barrier is responsible for maintaining concentration gradients required for ion absorption, and preventing the translocation of LPS and other toxins (Penner *et al*,2010). Thus, maintaining the integrity of the structure and function of rumen epithelium is very important. The objective of this study was to investigate whether sub acute ruminal acidosis (SARA) had persistent effects on the barrier function of the rumen epithelium.

Material & methods Twelve healthy lactating *Saanen* dairy goats were randomly divided into three groups (control group, SARA group and recovery group). Dairy goats in control group were fed a basal diet (non-fiber carbohydrates/neutral detergent fiber (NFC/NDF)=1.40), those in SARA group and recovery group were fed experimental diets with different NFC/NDF (1.40, 1.79, 2.31 and 3.23 in order, each for 15 days), which gradually induced SARA , and those in recovery group were fed *ad libitum* green hay for 30 days after SARA. Ruminal tissue samples were collected post-saughter from the ventral sac to observe under a transmission electron microscope, and instantly be subjected to Ussing chamber experiments, where electrophysiological properties were determined in parallel with the permeability of marker molecules of different sizes [fluorescein isothiocyanate (FITC) and horseradish peroxidase (HRP)] from luminal to apical side. All experiments were performed according to Chinese laws for the protection of animals.

Results & discussion The total thickness and thickness of stratum spinosum and basale were significantly decreased ($P<0.05$) in the SARA group, while the thickness of stratum corneum significantly increased ($P<0.05$, Table 1). Electrophysiological data indicated that the short-circuit current, tissue conductance and both marker molecules permeability of rumen epithelium in SARA and recovery groups were significantly increased ($P<0.05$) compared with control group, and potential difference is significantly decreased ($P<0.05$, Table 2).

Table 1 Effects of sub acute ruminal acidosis on thickness of different cellular stratum of rumen epithelium (mean±s.e).

Items	Control group	SARA group	Recovery group
Total epithelia(μm)	122.17±4.65^{a}	99.16±5.16^{b}	129.28±4.24^{a}
Stratum corneum(μm)	16.68±0.90^{b}	25.54±2.69^{a}	20.04±1.35^{b}
Stratum granulosum(μm)	15.01±0.68^{a}	16.32±0.98^{a}	17.46±0.60^{a}
Stratum spinosum and basale(μm)	90.48±4.36^{a}	57.29±5.00^{b}	91.78±3.83^{a}

^{a,b} values with different superscripts in the same row differ significantly ($P<0.05$). The same as below.

Table 2 Effects of sub acute ruminal acidosis on the electrophysiological properties and tissue permeability as measured as flux rate of horseradish peroxidise (HRP) and fluorescein-isothiocyanate (FITC, mean ±s.e).

Items	Control group	SARA group	Recovery group
Short-circuit current (Ma/cm^2per hour)	0.25±0.06^{b}	1.02±0.74^{a}	0.95±0.04^{a}
Tissue conductance (mS/cm^2 per hour)	3.89±0.12^{b}	4.95±0.21^{a}	4.49±0.87^{a}
Potential difference (mV/cm^2per hour)	1.26±0.02^{a}	0.58±0.15^{b}	0.75±0.04^{c}
HRP flux rate (μmol/ cm^2per hour)	3.64±0.06^{b}	10.22±0.83^{a}	8.25±0.72^{a}
FITC flux rate (mmol/cm^2per hour)	16.19±0.24^{c}	20.71±0.10^{a}	17.86±0.41^{b}

Conclusion SARA impaired the integrity of ruminal epithelial morphological structure and caused the permeability of the rumen epithelium increase and the rumen epithelium still maintained higher permeability in recovery group, which resulted in long-term impairment of the rumen epithelial barrier function, thereby possibly increasing the opportunity for the translocation toxins and bacteria from the rumen into the systemic circulation.

Acknowledgements This research was supported by Fund of the National Natural Science Foundation of China (grant nos.31101739, 31472124) and China Agriculture Research System (grant no.CARS-36).

References

Klevenhusen F, Hollmann M, Podstatzky-Lichtenstein L, Krametter-Frotscher R, Aschenbach JR and Zebeli Q 2013. Journal of Dairy Science 96, 2293-2302.

Penner GB, Oba M, Gabel G and Aschenbach JR 2010. Journal of Dairy Science. 93, 4838-4845.

Inoculation of young goat kids with rumen fluid from adult animals as a strategy for early weaning

Alejandro Belanche, Juan Manuel Palma-Hidalgo, Ibtissam Nejjam, Rosa Serrano, Elisabeth Jiménez, A. Ignacio Martín-García, David R. Yáñez-Ruiz

Estación Experimental del Zaidín (CSIC), Granada, Spain

E-mail: a.belanche@csic.es

Take home message Inoculation of newborn goats with fresh rumen fluid has positive effects on the rumen development.

Introduction There is increasing evidence showing that the nutritional management in the pre-weaning period determines to a great extent the potential of milk production during subsequent lactations (Soberon *et al*., 2012). Artificial milk feeding is the most common strategy used in intensive dairy goats systems, which implies no contact with adult animals and therefore limited rumen microbial colonization. Moreover, feeding cost represents another constrains in artificial rearing, thus new alternatives need to be further explored to minimize these drawbacks. This study investigates the inoculation of different types of rumen fluid to newborn goats as a strategy to accelerate the rumen development.

Material & methods A total of 72 newborn goats kids were randomly distributed into 4 groups. Kids were daily inoculated from birth up to 11 weeks of age with autoclaved rumen fluid as prebiotics (PRE), fresh rumen liquid adapted to concentrate (RLC), fresh rumen liquid adapted to forage (RLF) or absence of inoculation (CTL). Rumen inocula were obtained at 3h after feeding from adult rumen-cannulated goats fed either concentrate (33:67 F:C ratio) or 100% forage diets. Inoculum was filtrated, kept in thermal flasks and orally inoculated to the young animals (1 mL/kg BW). Experimental groups were kept physically separated and animals had *ad libitum* access to milk replacer (for 7 weeks), commercial concentrate and oat hay. Body weight and feed intakes (in groups of 4 animals) were recorded. Rumen samples were obtained by oesophageal tubbing at 5 weeks (equivalent to early-weaning) and 7 weeks of age (conventional weaning) to determine pH, ammonia and volatile fatty acids (VFA). Plasma samples were collected to determine β-hydroxybutyrate and glucose levels. Data were analysed by ANOVA using the SPSS software.

Results & discussion Although no differences were noted in terms of body weight, inoculation of fresh rumen fluid (RLC and RLF) promoted higher feed intake (P=0.011) at 5 weeks of age leading to greater rumen concentrations of total VFA (P=0.003), ammonia (P=0.013) and molar proportion of butyrate (P=0.013) than in CTL and PRE-animals. As a result, RCL and RLF kids tended to have a higher plasmatic β-hydroxybutyrate concentration (P=0.052) denoting an acceleration of the development of the rumen function, which could favour the success of early weaning strategies. Most of these differences tended to disappear at 7 weeks of age (conventional weaning), possibly as a result of more advanced rumen maturity across treatments. Non treated kids (CTL) had higher rumen proportion of propionate (P=0.048) and plasmatic levels of glucose (P=0.027) than the rest of the treatments, possibly as a result of a delay on the establishment of a acetic fermentation and a dominance of ketonic bodies instead of glucose as the main shift from pre-ruminant to ruminant energy metabolism (Baldwin *et al*., 2004).

Table 1 Effect of early life inoculation with rumen fluid on the colon fermentation of adult goats.

Age	5 weeks old						7 weeks old					
Treatment	CTL	PRE	RLC	RLF	SED	*P*	CTL	PRE	RLC	RLF	SED	*P*
BW (kg)	7.49	7.69	7.14	7.32	0.306	ns	10.03	10.26	9.58	9.91	0.371	ns
Feed intake (g DM/d)	15.0^{b}	13.1^{b}	29.4^{a}	25.4^{a}	4.620	*	66.8	67.0	76.8	68.7	12.87	ns
Rumen fermentation												
pH	6.61	6.68	6.68	6.70	0.081	ns	6.40	6.51	6.50	6.50	0.114	ns
NH_3-N (mg/dL)	25.4^{b}	24.9^{b}	29.5^{b}	35.4^{a}	3.420	*	29.8	33.0	27.8	30.2	3.940	ns
Total VFA (mM)	27.7^{ab}	18.6^{b}	30.3^{a}	35.5^{a}	4.650	**	102	99	101	101	9.230	ns
Molar proportion (%)												
Acetate	74.0	74.4	74.1	73.6	1.693	ns	70.3^{ab}	66.3^{b}	70.2^{ab}	72.3^{a}	2.132	*
Propionate	13.7	14.9	15.0	13.7	1.108	ns	15.2	16.5	15.0	14.7	1.136	ns
Butyrate	4.92^{a}	2.66^{b}	4.82^{ab}	6.35^{a}	1.115	*	8.99	10.3	9.67	8.08	1.606	ns
Plasmatic levels												
β-hydroxybutyrate (µM)	0.96	0.65	1.23	1.15	0.222	†	2.12	2.77	2.60	3.04	0.372	ns
Glucose (mg/dL)	85.6	85.5	84.5	78.6	4.430	ns	88.1^{a}	85.3^{ab}	82.1^{b}	84.2^{b}	1.976	*

*** P<0.001; ** P<0.01, * P<0.05, † P<0.1, ns not significant (*n*=18)

Conclusion Our findings suggested that inoculation of young animals with fresh rumen fluid from adult animals as probiotics had some positive effects on the rumen development which could favour the successful implementation of early weaning strategies. The persistency of these effects later in life is addressed in a companion paper (Belanche *et al*., 2018).

Acknowledgements This study was funded by the Economy and Competitiveness Spanish Ministry (AGL2017-86938).

References

Baldwin R, McLeod K, Klotz J, and Heitmann R 2004. Journal of Dairy Science 87, E55-E65.
Belanche A, Palma-Hidalgo JM, Jimenez E, Martín-García AI and Yáñez-Ruiz DR 2018. Proceedings ISNH
SoberonF, Raffrenato E, Everett RW and Van Amburgh ME 2012. Journal of Dairy Science 95, 783-793

Effects of either nutritional restriction or intake of concentrate feedstuffs prior to adaptation period on rumen pH and nutrients digestibility of Nellore cattle consuming high-energy diets

Gustavo Bertoldi, Ana Carolina Pinto, Breno Demartini, Evandro Dias, Luana Felizari, Mariana Squizatti, Antonio Silvestre, Danilo Millen

São Paulo State University (UNESP_), Dracena, São Paulo, Brazil

E-mail: danilomillen@dracena.unesp.br

Take home message Regardless of their nutritional background at feedlot arrival, the introduction of the finishing diet does not affect rumen pH and digestibility of nutrients of Nellore cattle.

Introduction Nutritionally deprived cattle may eat faster when they first arrive at a feedlot and this overconsumption may lead to rumen acidosis and decrease digestibility of nutrients. Likewise, cattle that were previously consuming concentrate feedstuffs before arriving at the feedlot may also experience rumen acidosis due to previous microbial colonization. Thus, the objective of this study was to evaluate if ruminal pH, total tract digestibility and ruminal degradability of the nutrients were similar for cannulated Nellore cattle previously exposed to nutritional restriction or intake of concentrate feedstuffs when the finishing diet was introduced.

Material & methods Six 20-month-old Nellore bulls cannulated in the rumen (236 ± 23 kg), were randomly assigned to one of two 3 x 3 Latin squares and consequently to the following treatments: Control (forage *ad libitum* + mineral supplement), Restriction (forage restricted to 1.4% of body weight + mineral supplement) and Concentrate (forage *ad libitum* + 0.5% of the body weight of concentrate feedstuffs + mineral supplement). Each period lasted 33 days and it was divided as follows: 14 days of exposure to treatments, 12 days of adaptation (72% and 79% concentrate diets containing 29,0% and 24.5% NDF, respectively, for 6 days each), and 7 days of a finishing diet containing 86% concentrate with 19.4% NDF (7% sugarcane bagasse, 7% Tifton hay, 73.5% cracked corn grain, 9.0% cottonseed meal, 1.2% urea, 0.8% limestone and 1.5% of a mineral/vitamin supplement containing sodium monensin). The study lasted 115 days, including a washout interval of 8 days between periods 1 and 2 and 2 and 3. A data logger was used to record rumen pH on days 7 (when treatments were applied) and 29 (when finishing diet was fed) of each experimental period. Mean, minimum and maximum ruminal pH, as well as pH 4 hours after feeding were reported. Nylon bags were incubated for 24 hours in the rumen on days 5, 6, 7 (when treatments were applied), 29, 30 and 31 (when finishing diet was fed) of each experimental period to determine degradability of dry matter, starch, crude protein, and neutral detergent fibre. Likewise, samples of diets, orts and feces were obtained on days 10, 11, 12, 13, 14 (when treatments were applied), 29, 30, 31, 32 and 33 (when finishing diet was fed) of each experimental period for further determination of total tract digestibility of same nutrients just described for rumen degradability. Titanium dioxide was used as external marker (added at 0.1% of diet dry matter). Data was analysed using PROC MIXED of SAS. The effect of treatment was considered fixed in the model, whereas effects of square, period, square*period, square*treatments, animal (square) and period*animal*treatment (square) were considered random. Tukey test was used to compare means and significance was declared when $P < 0.05$.

Results Prior to adaptation, when treatments were applied, cattle that were quantitatively restricted had greater mean (Control: 6.45^b, Restriction: 6.59^a, Concentrate: 6.33^b; SEM = 0.05; $p = 0.01$), minimum (Control: 5.98^b, Restriction: 6.26^a, Concentrate: 5.80^b; SEM = 0.08; $p < 0.01$) and maximum ruminal pH (Control: 6.77^b, Restriction: 6.86^a, Concentrate: 6.73^b; SEM = 0.05; $p < 0.01$) than cattle fed concentrate or in the control group. In addition, four hours after feeding, cattle consuming concentrate had lower rumen pH values (Control: 6.63^a, Restriction: 6.69^a, Concentrate: 6.50^b; SEM = 0.07; $p < 0.01$). Regarding degradability of nutrients, cattle receiving concentrate prior to adaptation had higher degradability of dry matter (Control: 0.62^b, Restriction: 0.65^b, Concentrate: 0.71^a; SEM = 0.03; $p < 0.01$) and starch (Control: 0.80^c, Restriction: 0.85^b, Concentrate: 0.94^a; SEM = 0.04; $p = 0.02$) than cattle from other groups, and higher crude protein degradability than cattle nutritionally restricted (Control: 0.58^{ab}, Restriction: 0.57^b, Concentrate: 0.61^a; SEM = 0.03; $p = 0.05$). No effects of treatments ($p = 0.56$) were observed for neutral detergent fibre degradability. With respect to total tract digestibility prior to adaptation period, cattle quantitatively restricted had lower digestibility of dry matter (Control: 0.57^a, Restriction: 0.50^b, Concentrate: 0.58^a; SEM = 0.02; $p < 0.01$), starch (Control: 0.76^b, Restriction: 0.62^c, Concentrate: 0.87^a; SEM = 0.07; $p < 0.01$), and crude protein (Control: 0.57^a, Restriction: 0.48^b, Concentrate: 0.57^a; SEM = 0.02; $p < 0.01$) than cattle from other treatments, and also lower digestibility of neutral detergent fibre (Control: 0.62^a, Restriction: 0.57^b, Concentrate: 0.56^b; SEM = 0.03; $p = 0.04$) than cattle from control group. However, when finishing diet was introduced, no effects of treatments were observed ($p > 0.10$) for any of the rumen pH, degradability and total tract digestibility variables evaluated.

Conclusion After the introduction of the finishing diet, ruminal pH, as well as total tract digestibility and ruminal degradability of nutrients were similar for Nellore cattle that were either nutritionally restricted or consuming concentrate feedstuffs prior to adaptation period. Thus, adaptation protocols do not need any adjustments when Nellore cattle arrive at the feedlot from different nutritional status.

Acknowledgements This research was supported by São Paulo Research Foundation (FAPESP), grant 2014/26210-4.

Effects of subacute ruminal acidosis on tight junctions protein expression in the ruminal epithelium of dairy goats

Gao Min, Hu Honglian, Sun Yanyong, Cheng Meng, Song Liwen

Inner Mongolia Academy of Agricultural&Animal Husbandry Sciences, hohhot, China

E-mail: gmyh1588@126.com

Take home message Subacute ruminal acidosis caused the permeability of the rumen epithelium increase which was associated with down-regulation of intercellular junction proteins mRNA express and protein expression.

Introduction Tight junctions (TJs) are an important way of intercellular connection, and they play an important role in regulating the permeability of the epithelial barrier, maintaining the rumen mucosal barrier integrity and preventing the translocation of LPS and other toxins. The TJs consists of transmembrane proteins (claudins and occludin) and plaque proteins such as ZO-1, ZO-2 and ZO-3. Recent studies have shown that the impairment of the epithelial barrier was mainly caused by the changes in TJ protein expression. The objective of this study was to investigate whether subacute ruminal acidosis (SARA) had persistent effects on the ruminal epithelial tight junctions' protein mRNA and proteins expression.

Material & methods Twelve healthy lactating *Saanen* dairy goats with similar body weight were randomly divided into three groups (control group, SARA group and recovery group). Dairy goats in control group were fed a basal diet (non-fiber carbohydrates/neutral detergent fiber (NFC/NDF)=1.40), those in SARA group and recovery group were fed experimental diets with increasing NFC/NDF ratios (1.40,1.79,2.31and 3.23) each for 15 days, which gradually induced SARA , and those in recovery group were *ad libitum* fed green hay for 30 days after SARA. Samples of the ruminal epithelium from ventral sac were collected to detect gene mRNA and proteins expression levels of intercellular junction protein using RT-PCR and Western blot technology.

Results & discussion Compared with the ruminal epithelial tissue of control goats, the ruminal epithelial tissue of SARA goats had an increase in the levels of Claudin-4 mRNA and protein expressions, while, a decline in the levels of claudin-1 ($P < 0.05$), claudin-7 ($P<0.05$), occludin ($P<0.05$), ZO-1 ($P<0.05$),connxin43 ($P<0.05$) and desmoglein1($P < 0.05$) mRNA and protein expressions (Tables 1 and 2).

Table 1 Effect of SARA on mRNA express levels of intercellular junction of ruminal epithelium in dairy goats (relative expression).

Items	Control group	SARA group	Recovery group
Claudin-1	1.45±0.09^a	0.63±0.07^b	0.82±0.07^b
Claudin-4	1.01±0.09^a	1.40±0.02^a	1.21±0.05^a
Claudin-7	1.39±0.07^a	0.70±0.04^b	0.75±0.02^b
Occludin	1.19±0.09^a	0.43±0.05^b	0.68±0.05^b
ZO-1	1.28±0.04^a	0.53±0.08^b	0.69±0.08^b
Connxin43	1.33±0.09^a	0.81±0.05^b	1.00±0.05^b
desmoglein1	1.05±0.07^a	0.49±0.07^b	0.90±0.11^a

^{a,b} values with different superscripts in the same row differ significantly ($P < 0.05$).

Table 2 Effect of SARA on intercellular junction protein expression in ruminal epithelium of dairy goats (relative expression).

Items	Control group	SARA group	Recovery group
Claudin-1	0.85±0.05^a	0.24±0.09^b	0.42±0.03^b
Claudin-4	0.54±0.03^a	0.94±0.06^a	0.85±0.14^a
Claudin-7	0.99±0.04^a	0.45±0.25^b	0.55±0.17^b
Occludin	0.82±0.07^a	0.36±0.02^b	0.49±0.04^b
ZO-1	0.83±0.11^a	0.49±0.08^b	0.58±0.01^b
Connxin43	0.74±0.10^a	0.60±0.03^b	0.66±0.07^b
desmoglein1	0.49±0.07^a	0.15±0.04^b	0.41±0.04^a

Conclusion These data demonstrated that SARA caused profound alterations in ruminal epithelial intercellula junction proteins claudins, occludin, ZO-1, connxin43 and desmoglein1 mRNA and proteins expression.

Acknowledgements This research was supported by Fund of the National Natural Science Foundation of China (grant nos.31101739, 31472124) and China Agriculture Research System (grant no.CARS-36).

References

Liu JH, Xu TT, Liu YJ,, Zhu WY and Mao SY 2013. American Journal of Physiology-Regulatory 305 232-241.
PennerGB, Oba M, Gabel G and Aschenbach JR 2010. Journal of Dairy Science 93, 4838-4845.

Expression of lipid metabolism related genes in adipose tissue of transition Holstein cows with different body condition

Priit Karis[1], Hanno Jaakson[1], Katri Ling[1], Jaak Samarütel[1], Andres Waldmann[1], Pille Pärn[1], Rupert Bruckmaier[2], Josef Gross[2], Ene Reimann[3], Maksim Runin[1], Merike Henno[1], Meelis Ots[1]

[1]Estonian University of Life Sciences, Tartu, Estonia, [2]University of Bern, Bern, Switzerland, [3]University of Tartu, Tartu, Estonia

E-mail: priit.karis@emu.ee

Take home message Higher lipid turnover potential of subcutaneous adipose tissue in prepartum obese and all postpartum cows throws light on the mechanisms of lipid-induced health problems**.**

Introduction There is little information on the effect of the amount of subcutaneous adipose tissue (SAT), assessed as body condition score (BCS), on adipose gene expression in cattle. Sub-optimal BCS as well as over-conditioning before parturition is associated with intensive lipolysis and more pronounced insulin resistance (Jaakson *et al.,* 2018). The aim was to examine the effect of BCS during the dry period on the SAT expression of genes controlling lipolysis/lipogenesis in Holstein cows.

Material & methods Multiparous Holstein cows were grouped according to the BCS 28 days (d-28) before calving: ≤3.0 (Thin – T, n=14); 3.25-3.5 (Optimal – O, n=14); ≥3.75 (Over-conditioned – OC, n=14). Cows were fed TMR according to Estonian feeding recommendations. SAT biopsies were taken on d -21 and d 21 from the pin bone region and analysed with RT qPCR method for lipoprotein lipase (*LPL*), fatty acid transport protein 1 (*SLC27A1*), diacylglycerol-O-acyltransferase 2 (*DGAT2*) and hormone sensitive lipase (*LIPE*) mRNAs. *GADPH* was used as a reference gene. Relative gene expression level was calculated using the ΔCt method. General linear mixed model and ANOVA were used for the statistical analysis.

Results & discussion The day of biopsy was a significant factor for all the genes' mRNA abundance ($P < 0.01$), which was higher on d 21 for *SLC27A1*, *DGAT2* and *LIPE*, and lower for *LPL* (Table 1). Lower *LPL* and higher *LIPE* abundance postpartum indicate shift to lipolysis and agree with raised plasma NEFA levels in the same cows (Jaakson *et al.,* 2018). Higher abundance of *SLC27A1* and *DGAT2* on d 21 suggests increased uptake and intracellular (re-)esterification of FAs, presumably due to high intracellular NEFA levels (Zechner *et al*., 2012). There was a group effect on mRNA abundance for *LPL* ($P = 0.002$) and *DGAT2* ($P = 0.037$); in OC cows the abundance on d -21 was the lowest for *LPL* and highest for *DGAT2* (Table 1). According to our earlier finding OC cows had raised plasma NEFA levels on d -21 (Jaakson *et al*., 2018), together these results indicate that obese cows have intensifyed lipolysis concurrently with greater esterification potential prepartum compared to thinner cows.

Table 1 The expression of mRNA in SAT 21 days before (d -21) and after calving (d 21) for thin (T), optimal (O) and over-conditioned (OC) cows. Values expressed as LSM. Letters represent difference between groups (Gr; $P \leq 0.05$), asterisks tendency to differ ($P \leq 0.1$).

	d-21			d21						
Gene	T	O	OC	T	O	OC	SEM	Day	Gr	Day x Gr
LPL	3.62^{a*}	2.72^{ab*}	2.26^{b}	2.12	1.31	1.40	0.283	<0.001	0.001	0.444
SLA27A1	1.40	1.30	1.74	2.22	2.01	2.16	0.170	<0.001	0.231	0.440
DGAT2	1.85^{a}	2.74^{ab}	3.33^{b}	4.41	5.24	4.73	0.359	<0.001	0.037	0.137
LIPE	3.58	3.11	3.24	4.31	3.59	3.97	0.308	0.008	0.169	0.896

Conclusion Expression of lipid metabolism related genes is different pre- and postpartum. Regardless of the amount of SAT in dry period, there is lower availability of lipoproteins-derived FAs (decreased *LPL*) and a metabolic shift towards lipolysis (increased *LIPE*, raised plasma NEFA) postpartum. Potentially enhanced uptake and intracellular (re-)esterification of FAs (increased *SLC27A1* and *DGAT2*) together with the prevalence of lipolysis suggest increased lipid turnover rate in SAT postpartum. The same – decreased cellular uptake of lipoproteins-derived FAs, more pronounced lipolysis, and increased esterification potential of intracellular FAs – applies to over-conditioned cows compared to thinner cows prepartum.

Acknowledgements The study was supported by the Estonian Ministry of Education, Research grant IUT 8-1.

References

Jaakson H, Karis P, Ling K, Ilves-Luht A, Samarütel J, Henno M, Jõudu I, Waldmann A, Reimann E, Pärn P, Bruckmaier R M, Gross JJ, Kaart T, Kass M and Ots M 2018. Journal of Dairy Science 101, 752-766.

Zechner R, Zimmermann R, Eichmann T O, Kohlwein S D, Haemmerle G, Lass A and Madeo F 2012. Cell Metabolism 15, 279-291.

Exploration of the potential of a novel *Pediococcus acidilactici* strain as a direct-fed microbial in ruminants

Zhigang Zhu, Giuseppe Copani, Erik Juncker Boll, Nina Milora, Gunnar Oeregaard, Bea K. K. Nielsen

Chr.Hansen, Hørsholm, Denmark

E-mail: dkzhzh@chr-hansen.com

Take home message A novel *Pediococcus acidilactici* strain is a promising candidate for use as a direct-fed microbial (DFM) for improving health and production performance of dairy cows.

Introduction Application of DFMs is an alternative approach for enhancing health and production performance of ruminants without the negative effects associated with antibiotic usage. *Pediococcus acidilactici* related lactic acid bacteria have been applied in the area of food and clinical practice due to their excellent capacity for bacteriocin production *(*Porto *et al.*, 2017). It has been reported that two *Pediococcus* strains have positive effects on growth performance and nutrient digestibility of lambs around the weaning period (Saleem *et al.*, 2017). This preliminary study attempts to explore the potential of a novel *Pediococcus acidilactici* strain for being used as a DFM in ruminants.

Material & methods Following a screening project where the features of 600 bacterial strains were evaluated, a novel *Pediococcus acidilactici* strain isolated from the caecum wall of Holstein dairy cows was selected. The growth properties of this strain was assessed using media containing different types of sugars (1.8%) or under conditions with different pH (2 to 6) or in the presence of 0.3% oxy-bile acid. In addition, the ability of the strain to form biofilm was evaluated by adopting a well-established method for auto-aggregation (Janković *et al.*, 2012). Furthermore, to investigate the impact of the strain on gut permeability, we exposed monolayers of human intestinal Caco-2 cells to the strain and measured trans-epithelial electrical resistance (TEER) in real-time (Majima *et al.*, 2017).

Results & discussion Results from growth assessment showed that glucose, fructose, maltose and xylose were favourite carbon sources for the *Pediococcus acidilactici* strain. The growth of this strain at low pH (pH = 4) or in the presence of 0.3% oxy-bile acid suggests that it might be able to survive and grow under conditions inside the gastrointestinal (GI) tract. The results from the auto-aggregation assay (Table 1) showed a time-dependent increase in auto-aggregation ability, implying that this strain is likely to successfully colonize in the GI tract. Preliminary results from the gut permeability assay indicate that the strain causes a TEER increase (20%) after 4h incubation compared to the control group, which in turn suggests that the strain has a beneficial effect on gut barrier function. According to results from a previous *in vivo* study, this strain showed a significantly positive effect on milk yield in lactating dairy cows (Thomas *et al.*, 2017). Based on these data, we speculate that the strain improves gut function and thus nutrient absorption, which might be a driving factor for increased milk yield.

Table 1 Auto-aggregation assay (%). Over-night culture of *Pediococcus acidilactici* was dispensed in phosphate buffer (100mM, pH=6.7) and OD values at 540 nm were determined after 2, 4, 22 and 24h of incubation.

Auto-aggregation for *Pediococcus acidilactici*	Time (h)			
	2	4	22	24
Mean (%)	7.75	13.17	23.58	23.75
standard error	0.57	0.38	0.68	1.01

Conclusion This novel *Pediococcus acidilactici* strain is a promising candidate for being used as a DFM for improving health and production performance in ruminants. However, more investigations in ruminants are required to document the efficacy as well as mode of action of this strain.

References

Janković T, Frece J, Abram M and Gobin I 2012. International Journal of Sanitary Engineering Research 6, 19–24.
Majima A, Handa O, Naito Y, Suyama Y, Onozawa Y, Higashimura Y, Mizushima K, Morita M, Uehara Y, Horie H, Iida T, Fukui A, Dohi O, Okayama T, Yoshida N, Kamada K, Katada K, Uchiyama K, Ishikawa T, Takagi T, Konishi H, Yasukawa Z, Tokunaga M, Okubo T and Itoh Y 2017. Journal of Digestive Diseases 18, 151–159.
Porto MCW, Kuniyoshi TM, Azevedo POS, Vitolo M and Oliveira RPS 2017. Biotechnology Advances 35, 361–374.
Saleem AM, Zanouny AI and Singer AM 2017. Asian-Australasian Journal of Animal Sciences 30, 523–530.
Thomas D A, Shouse S C, Dooley B C, Copani G, Nielsen K K B, Milora N, Cernat C R, Ramirez-Ramirez A H. 2017 ADSA Annual meeting, Pittsburgh, Pennsylvania. Abstract #T241.

707

Associating hoof disorders with sub-optimal mobility in dairy cows in pasture-based systems

Aisling O'Connor[1,2], Eddie Bokkers[2], Imke de Boer[2], Henk Hogeveen[3], Riona Sayers[1], Noel Byrne[1], Elodie Ruelle[1], Laurence Shalloo[1]

[1]Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Cork, Ireland, [2]Animal Production Systems, Department of Animal Sciences, Wageningen University & Research, Wageningen, The Netherlands, [3]Business Economics, Department of Social Sciences, Wageningen University & Research, Wageningen, The Netherlands

E-mail: aisling.oconnor@teagasc.ie

Take home message Cows with hoof disorders, are more likely to have a sub-optimal mobility score in pasture-based systems.

Introduction Sub-optimal mobility in dairy cows is considered by the dairy industry worldwide to be among the most significant challenges (Huxley, 2012), and the third most important health-related economic loss, after fertility and mastitis (Bruijnis *et al*., 2010, Huxley, 2013). Sub-optimal mobility can be broadly defined as abnormal gait which causes a deviation from the optimal walking pattern of a cow. The main cause of sub-optimal mobility is hoof disorders (HD), which are associated with pain (Bruijnis *et al*., 2010) and responsible for 92% of the cases (Murray *et al*., 1996). This study aims to characterise sub-optimal mobility, by using HD prevalence to predict mobility score (MS).

Material & methods A dataset with 7,602 cows from 52 spring-calving pasture-based dairy farms in Ireland was used. For the HD data, cows were scored as either 0 or 1 (absence or presence of a HD respectively). HD included overgrown claw, bruised claw, whiteline disease, ulcer and digital dermatitis. For this study the DairyCo mobility scoring method (DairyCo, 2007) was used. The DairyCo mobility scoring tool is a 4 point scale ranging from 0 to 3, where 0 describes a cow with optimal mobility and 3 describes a cow with very poor mobility (classified as severely lame). For the statistical analysis, the effects of the different HD on MS were assessed using a forward stepwise regression approach. Output variables were analysed with logistic regressions as multinomial (MS 0, 1, 2, or 3). Included in the model used to predict cow MS were HD presence/absence (denoted by 0 if absent and 1 if present). Analyses were performed using R statistical software (R Development Core Team, 2009; functions multinomial logistic regressions).

Results & discussion Overgrown claw affected 16.13% of the cows and had an impact on MS wherein a cow with an overgrown claw was more likely to have a MS>0 versus MS=0 compared to a cow with no overgrown claw at all levels of MS ($P<0.001$). Whiteline disease, ulcer and digital dermatitis had a similar impact to MS with a $P<0.001$). Bruised claw had an impact on both MS 1 *vs.* 0 and MS 2 *vs.* 0 ($P<0.001$), however did not impact MS 3 *vs.* 0 ($P=0.08$). The odds ratio for bruised claw was smaller compared to the other HD which may suggest bruised claw causes less pain to the cow while walking, thus causing less deviation from how the cow should optimally walk. Interestingly cows with some form of ulcer or digital dermatitis only made up 1.32% and 1.49% respectively of all the cows; however ulcer and digital dermatitis had the largest impact on MS in terms of odds ratio.

Conclusion This analysis confirms an association between MS and HD. The results indicate that any form of HD presence is a relevant predictor of MS. From the results, it is clear that more severe type HD, for example; ulcers and digital dermatitis have a significantly greater impact on MS. Based on HD presence, the results also indicate the thresholds wherein MS is likely to be sub-optimal (MS>0) thus causing a deviation from the optimal walking pattern of a cow.

Acknowledgements An Irish Department of Agriculture Food and the Marine funded study across spring calving dairy herds with the objective of collecting animal health data was used as the data source.

References

Bruijnis MRN, Hogeveen H and Stassen EN 2010. Journal of Dairy Science 93, 2419-2432.

DairyCo, A. 2007. DairyCo Mobility Scoring. DairyCo, Kenilworth, UK. (https://dairy.ahdb.org.uk/technical-information/animal-health-welfare/lameness/husbandry-prevention/mobility-scoring/#.WXnhULuFOr8 ; accessed 27 July 2017)

Huxley J 2012. Lameness in cattle: An ongoing concern. WB Saunders.

Huxley J 2013. Impact of lameness and claw lesions in cows on health and production. Livestock Science 156, 64-70.

Murray R, Downham D, Clarkson M, Faull W, Hughes J, Manson F, MerrittJ, Russell W, Sutherst J, and Ward W. 1996. The Veterinary Record 138, 586-591.

The response to an oral drench of propylene glycol is modified in dairy goats previously fed straw for two days compared to control fed goats

Andrew A. Ponter[2], Alexandra Eymard [3], Ophélie Dhumez [3], Christine Duvaux-Ponter [3], Nicolas C. Friggens [3]

[1]UMR BDR, INRA, Ecole Nationale Vétérinaire d'Alfort, Université Paris Saclay, 78350, Jouy en Josas, France, [2]Ecole Nationale Vétérinaire d'Alfort, 94700, Maisons-Alfort, France, [3]UMR Modélisation Systémique Appliquée aux Ruminants, INRA, AgroParisTech, Université Paris-Saclay, 75005, Paris, France

E-mail: andrew.ponter@vet-alfort.fr

Take home message The composition of the diet given to goats influences their response to a propylene glycol drench.

Introduction Plasma concentrations of glucose and insulin are known to increase in response to dietary propylene glycol (PG) (Miyoshi *et al.,* 2001). PG is a gluconeogenic precursor widely used in the prevention and treatment of ketosis. It increases the molar percentage of ruminal propionate in postpartum dairy cattle (Christensen *et al.,* 1997). After oral administration, a portion of PG is fermented in the rumen to propionate, but the majority escapes the rumen untransformed and is converted to glucose by the liver (Rizos *et al.,* 2008). High levels of glucose stimulate pancreatic insulin secretion which in turn reduces plasma non-esterified fatty acids (NEFA) if animals are mobilising adipose tissue reserves. We tested whether causing lipomobilisation by giving a straw diet had an influence on the metabolic response to PG.

Material & methods Sixteen dairy goats were selected at parturition and fed *ad libitum* a TMR to cover dietary requirements for lactation. On day 21 after parturition the goats received an initial PG drench (1 mL/kg LW). Starting on day 26 the goats received straw *ad libitum* instead of the regular diet and on day 28 the goats received a second PG drench (1 mL/kg LW). Blood samples were taken at -10, 0, 15, 30, 45, 60 and 120 min in relation to each drench and plasma was analysed for insulin, glucose and NEFA. Statistical analysis was performed with PROC Mixed in SAS using the Repeated statement to account for a time effect.

Results The glucose and insulin response to the PG drench was rapid in control animals but delayed when they received straw for 2 days prior to the drench (Fig. 1). Plasma NEFA were significantly higher in straw + PG compared to control + PG goats (respectively 1.65 ± 0.06 mmol/L *vs.* 0.56 ± 0.06 mmol/L, $p < 0.0001$) but did not decrease after the PG drenches in either group (results not shown).

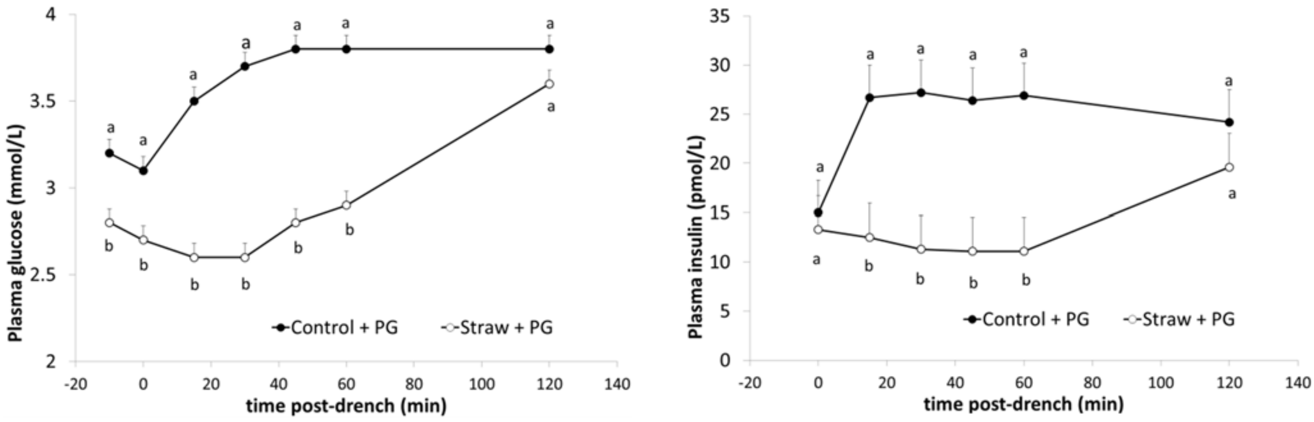


Figure 1 Plasma glucose and insulin after a propylene glycol drench in dairy goats fed either a control diet (control + PG) or straw for 2 days (straw + PG) (n=16, lsmean ± s.e., a,b at a time point indicate a difference between treatments, $p < 0.05$).

Conclusion Feeding straw for 2 days prior to a PG drench delayed the increase in plasma glucose and insulin compared to control fed goats. This may be due to a slower rumen emptying rate caused by the straw compared to the lactation diet and this may mean that more time was required for the PG to reach the liver and be converted into glucose. It is unclear why NEFA concentrations were unaffected by PG.

Acknowledgements The authors would like to thank the goat unit of UMR INRA-AgroParisTech MoSAR for care of the animals, B. Lagofun for technical assistance and DIFAGRI, France for the PG.

References

Christensen JO, Grummer RR, Rasmussen FE and Bertics SJ 1997. Journal of Dairy Science 80, 563-568.
Miyoshi S, Pate JL and Palmquist DL 2001. Animal Reproduction Science 68, 29-43.
Rizos D, Kenny DA, Griffin W, Quinn K, Duffy P, Mulligan FJ, Roche JF, Boland MP and Lonergan P 2008. Theriogenology 69, 688-699.

Monitoring of milk fatty acid during the build-up of compound feed in early lactation allows to identify subacute ruminal acidosis sensitive and tolerant dairy cows

Longhui Jing[1], Lore Dewanckele[1], Bruno Vlaeminck[1], Wilfried van Straalen[2], Veerle Fievez[1]

[1]*Laboratory for Animal Nutrition and Animal Product Quality (Lanupro), Department of Animal Sciences and Aquatic Ecology, Ghent University, Ghent, Belgium,* [2]*Schothorst Feed Research B.V, Lelystad, The Netherlands*

E-mail: longhui.jing@ugent.be

Take home message Cows sensitive to develop subacute ruminal acidosis are characterised by increased levels in milk fat of C15:0 and C18:1 *trans*-10 and a significant drop in C18:1 *trans*-11 concentration during the build-up of compound feed.

Introduction Subacute ruminal acidosis (SARA) represents one of the most important metabolic disorders in intensive dairy farming with a suggested incidence between 19% and 26% in early and mid-lactation (Plaizier *et al*., 2008), but susceptibility for SARA development seems animal-dependent (Krause and Oetzel, 2006). Our previous work documented that odd and branched chain fatty acids, *e.g.,* C15:0 and some trans isomers such as C18:1 *trans*-10, increased during SARA development (Colman *et al*., 2010). Thus, we selected two groups of cows showing distinctive concentrations of these milk fatty acids during the build-up of compound feed in the first four weeks in milk (WIM) and validated whether these two groups differed in susceptibility for SARA development.

Material & methods This experiment was carried in two parts. During the first trial, milk fatty acid composition of 125 cows was monitored once every week during the first four weeks in lactation. Five cows (HT10s) were selected showing C18:1 *trans*-10 beyond 0.31 g/100 g FA in the third WIM, an average level of C15:0 of 1.18 g/100 g FA or higher over the first four WIM and a sharp decrease of C18:1 *trans*-11 ($\Delta \geq 0.25$g/100 g FA during the first four WIM). Their counterparts (LT10s) had a milk fat C18:1 *trans*-10 concentration below 0.23 g/100 g FA in the third WIM, an average C15:0 concentration of 0.99 g/100 g FA or lower and a rather stable C18:1 *trans*-11 concentration. Both groups were stratified in parity as well as lactation stage at the start of the SARA induction trial. This trial lasted for 28 days and was divided in three periods based on the relative amount of rapidly fermentable carbohydrates (RFC) in the diet: i/ low RFC (d0-18), ii/ increasing RFC (incr; d19-24) and iii/ high RFC (d25-28). In order to increase the amount of RFC in the diet, a standard concentrate was gradually and partly replaced by a concentrate high in RFC (*i.e.,* wheat) during the increasing period to remain at a constant level during the high period. The reticular pH was measured every 10 minutes using a smaxTec Premium bolus (smaXtec animal care GmbH, Austria). Data were analysed using the General linear model.

Results & discussion Reticular pH values monitored in the second trial revealed SARA only could be induced in the HT10s group (reticular pH below 6.0 for more than 6 hours per day). Strikingly, these animals were defined as suffering of SARA already during the low RFC period, while the LT10s group showed a higher mean reticular pH and never experienced SARA according to the formerly mentioned definition. Meanwhile, LT10s cows also showed lower acidosis index compared with sensitive cows throughout the trial.

Table 1 Mean reticular pH, time of reticular pH < 6.0 (h/d) and acidosis index (pH × min/kg, DM) of the HT10s and LT10s group during three experimental periods of the SARA induction trial.

	HT10s			LT10s				*P*-value		
Item	Low	Incr[1]	High	Low	Incr	High	SEM[2]	G[3]	Pe[4]	G× Pe
Mean reticular pH	6.21	6.07	6.19	6.40	6.47	6.54	0.365	<0.001	0.128	0.005
Time pH < 6.0	6.05	10.33	6.56	1.88	2.73	1.75	0.016	<0.001	0.008	0.140
Acidosis index[5]	3.19	6.09	3.11	1.15	1.96	0.95	3.900	<0.001	<0.001	<0.001

[1]One HT10s cow died due to an accident at the beginning of the increasing period; [2]SEM= standard error of the mean; [3]G=group; [4]Pe=period; [5]Acidosis index was calculated as the area under pH 6.0 divided by dry matter intake.

Conclusion Milk fatty acid composition could be used to identify the relative SARA sensitive and tolerant cows among a herd. The SARA sensitive could be distinguished by the higher levels of milk fat C15:0, C18:1 *trans*-10 and a drop of C18:1 *trans*-11 within the build-up of compound diet.

Acknowledgements

PhD research of Longhui Jing is supported by Chinese Scholarship Council (CSC, China) and by the Special Research Fund of Ghent University (Bijzonder Onderzoeksfonds, Belgium).

References

Plaizier JC, Krause DO, Gozho GN, and McBride BW. 2008. The Veterinary Journal 176, 21-31.
Krause KM and Oetzel GR 2006. Animal Feed Science and Technology 126, 215-236.
Colman E, Fokkink WB, Craninx M, Newbold JRB and Fievez V 2010. Journal of Dairy Science 93, 4759-4773.

Effects of ruminal phytol infusion on phytanic acid production and lipid metabolism in sheep

Masaru Matsumura, Taketo Obitsu, Toshihisa Sugino

Hiroshima University, Higashihiroshima-shi, Japan

E-mail: b146765@hiroshima-u.ac.jp

Take home message One-third of dietary phytol would be converted to phytanic acid in the rumen. Excess provision of phytol or phytanic acid is associated with the risk of hyperlipidemia in sheep.

Introduction Green forages abundantly contain plant pigments such as chlorophyll. When forage is ingested by ruminants, the phytol part of chlorophyll is converted to phytanic acid in the rumen by ruminal microorganisms (Patton and Benson, 1966); however, quantitative information for ruminal phytanic acid production is lacking. Phytol and phytanic acid are known as ligands of peroxisome proliferator-activated receptors, which stimulate hepatic fatty acid oxidation (Hellgren, 2010). In contrast, previous studies (Steinberg *et al*., 1966) showed that excess dietary phytol (>20 g/kg diet) induced hepatic lipid accumulation and body weight loss in rodents. However, the effect of phytol and phytanic acid on lipid metabolism in ruminants is not yet known. Thus, we investigated the extent of phytol that can be converted to phytanic acid in the rumen and whether phytol and phytanic acid affect lipid metabolism in sheep.

Material & methods Four wether sheep (average body weight, 54 kg) fitted with ruminal and duodenal cannula were used in a 4 × 4 Latin squire design. The experimental treatment included infusion with phytol (mixture of trans/cis isomers; Sigma-Aldrich) into the rumen at 0, 4, 8, and 12 g/kg dry matter (DM) intake in two equal portions at feeding (0900 and 2100 h). Sheep were fed a mixed diet composed of oat hay and barley grain in a ratio of 2:3 on a DM basis at maintenance energy level. Chromium oxide (1 g/d) as a digestion marker was also added through the rumen cannula. Each experiment lasted 14 days. Following a 7-day adaptation period, faeces and duodenal digesta were collected from days 8 to 11 to measure the duodenal flow and faecal output of DM, phytol, and phytanic acid. On day 13, samples of jugular venous blood and rumen content were collected at 0800, 1100, 1300, 1500, 1700, and 1900 h. Plasma samples were analysed for the concentration of metabolites related to lipid metabolism and insulin. Digestion data were analysed as a mixed model by using JMP software (SAS International Inc), with treatment and period as fixed effects and sheep as random effect. The data for plasma and ruminal parameters were analysed as repeated measures.

Results The DM intake and digestibility in the rumen and intestine as well as ruminal volatile fatty acid concentrations were not affected by phytol infusion. Duodenal flows of phytol and phytanic acid linearly increased with increasing phytol infusion (Figure 1-a,b). The slope of a linear regression equation suggested that the ruminal production rate of phytanic acid from phytol was 0.36±0.040, while 0.37±0.073 of infused phytol entered the duodenum. Plasma concentrations of glucose, non-esterified fatty acid (NEFA), ketone body, and insulin were not affected by phytol infusion. However, plasma triglyceride level for the 8 and 12 g/kg infusion was higher ($P < 0.01$) than that for the 0 g/kg infusion (control). Conversely, plasma HDL-cholesterol for the 12 g/kg infusion was lower ($P < 0.01$) than that for the other treatments.

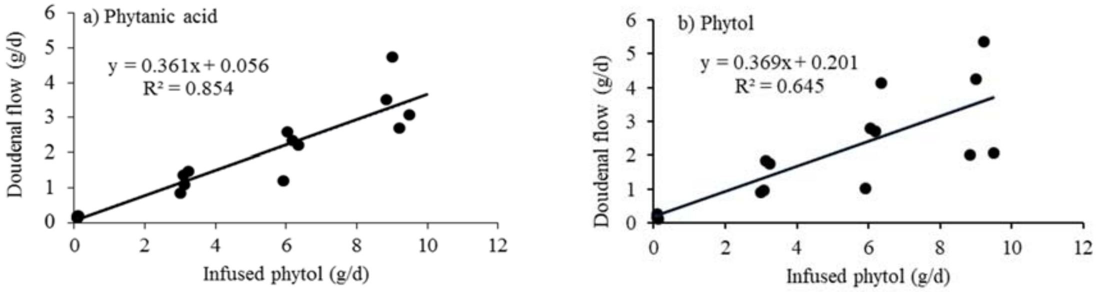


Figure 1 Relationship between the amounts of phytol infusion and amount of duodenal flow of a) phytanic acid and b) phytol. Duodenal phytanic acid represents ruminal phytanic acid production.

Conclusion One-third of dietary phytol was converted to phytanic acid in the rumen. Even though ruminal phytol infusion did not affect rumen fermentation, the higher level of phytol or phytanic acid supply induced hyperlipidemia in sheep.

References

Patton S and Benson AA 1966. Biochimica et Biophysica Acta 125, 22-32.
Hellgren LI 2010. Annals of the New York Academy of Sciences 1190, 42-49.
Steinberg D, Avigan J, Mize CE, Baxter JH, Cammermeyer J, Fales HM and Highet PF 1966. Journal of Lipid Research 9, 684-691.

711

Milk replacer program and postweaning feeding level on ruminal environment in female Holstein calves

Germán Antúnez[1], Cecilia Cajarville[1], Cinthya Fernández[1], Juan Dayuto[1], Laura Artús[1], Martina Fernández[1], Leticia Hornos[1], Florencia Correa[1], Iván Filip[2], Rodrigo Bres[2], José Luis Repetto[1]

[1]Instituto de Producción Animal de Veterinaria, Facultad de Veterinaria, Universidad de la República, Libertad, San José, Uruguay, [2]Facultad de Recursos Naturales, Universidad Nacional de Formosa, Formosa, Argentina, Argentina

E-mail: antuneztort@gmail.com

Take home message The milk replacer program of female Holstein calves affected the ruminal environment in the preweaning period but this effect was not evidenced after weaning, independently of the postweaning feeding level.

Introduction Several previous work has shown that accelerated milk replacer (MR) programs reduce starter intake and therefore, affect the ruminal pH and NH_3-N concentration in preweaning period, but there is limited work that evaluates the effect of varying both the pre- and postweaning feeding program. The aim of this study was to evaluate whether the amount of milk replacer and postweaning feeding level affect ruminal environment around the weaning period.

Material & methods Thirty-two newborn female Holstein calves (41 ± 4.3 kg of BW) were blocked according to birth date and randomly assigned (n = 8 per treatment) to high (H) or low (L) milk replacer (MR) programs (MR powder equivalent to 1.25 % or 2.50 % of initial BW since 2nd to 8th week of life) and high (H) or low (L) postweaning feeding level (From the 9th to 11th week of life). In both preweaning MR (25% CP, 20% fat, at 12.5% solid and offered at 0800 and 1630 h) programs calves had free access to a pelleted starter (PS; 21% CP, 15% NDF) and water. Calves were weaned along 8th weeks and calves received decreasing amounts of MR, free access to PS and 0.2 kg of mixture chopped hay (CH; 18% CP, 40% NDF) in a separated bucket. From the 9th to 11th week calves were fed amounts of PS and CH feed to achieve high (800-900 g/d) or low (500-600 g/d) daily weight gain. In 7th and 11th weeks of life ruminal fluid samples were taken through a stomach tube and ruminal pH (Antúnez *et al*., 2017) was measured immediately with a digital pH meter. The ruminal NH_3–N concentration was analysed by spectrophotometry according to Weatherburn (1967) and VFA by HPLC (Dionex Ultimate 3000, Sunnyvale, CA) as described by Adams *et al*., (1984). Ruminal pH, NH_3-N and VFA were analysed using a mixed model including the fixed effect of treatment in period 1 (P1; High or Low milk replacer program since 2nd to 8th week of life), period 2 (High or Low feeding level since 9th to 11th week of life) and interactions P1 × P2. The block was included as a random effect. Means were compared with Tukey test. Differences were declared at $P < 0.05$.

Results & discussion Ruminal pH and NH_3-N concentration were higher in H milk replacer programs (5.98 *vs.* 5.78, $P < 0.05$ and 16.5 *vs.* 11.5 mg/dl, $P < 0.01$ respectively). An interaction in ruminal pH between treatments in P1 and P2 were observed (see Table 1). It seems that that the high MR program and the consequent reduction of starter intake (Antúnez *et al*., 2017) led to slight changes on ruminal fermentation activity in the preweaning period, which reverted during the weeks after weaning.

Table 1 Ruminal parameters of female calves in high (H) or low (L) milk replacer programs (P1; 2 to 8 wk of life) and high (H) or low (L) postweaning (P2; 9 to 11 wk of life) feeding level (500-600 or 800-900 grams average daily weight gain).

Item	Treatment				SE	P-value[1]		
	HH	HL	LH	LL		P1	P2	P1 × P2
Ruminal pH	6.00^{x}	5.97^{x}	5.67^{y}	5.90^{xy}	0.074	0.015	0.185	0.097
NH_3-N, mg/dl	15.2	17.8	10.4	12.5	1.57	< 0.01	0.154	0.903
Total VFA[2], mmol/l	75.3	86.8	79.3	88.9	6.83	0.836	0.592	0.451
Lactic, mmol/l	14.8	16.8	14.0	13.1	2.57	0.300	0.821	0.522
Acetic, mmol/mol	0.46	0.46	0.45	0.48	0.021	0.835	0.487	0.677
Propionic, mmol/mol	0.44	0.43	0.46	0.43	0.033	0.816	0.474	0.626
Butyric, mmol/mol	0.11	0.11	0.09	0.10	0.022	0.504	0.719	0.713

$^{x, y}$ Within a row, means with different superscripts are tendency to interaction P1 × P2 ($0.05 > P < 0.10$). [1] Effect of treatment in Period 1 (P1), effect of treatment in Period 2 (P2) and interaction between P1 × P2. [2] Total VFA as the sum of acetate, propionate and butyrate concentration (mmol/l).

Conclusion Milk replacer programs applied in female Holstein calves affected ruminal pH and NH_3-N concentration during the preweaning period, but this effect was no evident after weaning in both high or low postweaning feeding level.

References

Adams RF, Jones RL and Conway PL 1984. Journal of Chromatography B: Biomedical Sciences and Applications. 336, 125-137.

Antúnez G, Cajarville C, Fernández CM, Dayuto JE, Artús LM, Fernández M, Hornos L, Correa F, Biasiolo R, Vicente A, Repetto JL 2017. ADSA Annual Meeting, Pittsburgh, Pennsylvania.

Weatherburn MW 1967. Analytical Chemistry 39, 971–974.

The immunomodulation effects of glycine in primary bovine mammary epithelial cells induced by *E. coli* lipopolysaccharide

Ruiping Du[1], Xingfu Zhang[1], Minghui Tang[2], Ni Dan[2], Changjin Ao[2], Min Gao[1]

[1]Animal Nutrition Institute, Agriculture and Animal Husbandry Academy of Inner Mongolia, Huhhot City, Inner Mongolia Region, China, [2]College of Animal Science, Inner Mongolia Agricultural University, Huhhot City, Inner Mongolia Region, China

E-mail: duruiping1989@163.com

Take home message Glycine may be a new strategy to control mastitis and provide a viable alternative to less effective mastitis control procedures based on traditional chemotherapy.

Introduction Given today's public health and food safety concerns, approaches to improve the animal's immune system to increase its effectiveness against invading mastitis pathogens, and thus minimize use of antibiotics have gained considerable attention. Glycine is a well-documented cytoprotective agent whose anti-inflammatory function is supported by *in vitro* studies in a variety of cells types and in vivo studies in various species. However, whether glycine has an immunomodulatory effect against mastitis remains unknown. In this study, we detected the modulation of proliferation, apoptosis and pro-inflammatory cytokine release by glycine in primary bovine mammary epithelial cell (pBMEC) with or without LPS.

Material & methods We first established primary bovine mammary epithelial cell model, cell isolation and modified primary cell culture methods were performed as described in previous reports (Jedrzejczak and Szatkowska, 2013), the fourth passage cells were used in all experiments. The cells were incubated in glycine-free DMEM/F12 medium until 80% confluence and then washed once in PBS before the simultaneous addition of different doses of glycine (0, 1, 2, 5 and 10 mM) with (+, 1 µg/ml) or without (-, 0) *Escherichia coli* lipopolysaccharide for 24 h. Briefly, the treatments were divided into G(0)LPS(-), G(0)LPS(+), G(1)LPS(+), G(2)LPS(+), G(5)LPS(+) and G(10)LPS(+). Each experiment included at least three replicates per treatment and was repeated at least twice. The effect of glycine on cell proliferation was assessed by MTT assay, cell cycle analysis was performed by flow cytometry, a flow cytometry annexin-V FITC/PI double stain assay was used to examine the effects of glycine on cell apoptosis, and the effects of glycine on the relative pro-inflammatory cytokine gene abundance of TNF-α, IL-6, IL-8 and IL-1β were determined by real-time PCR. Data were analysed by one-way ANOVA using the MIXED procedure of SAS.

Results & discussion Firstly a significant decrease in cell proliferation ($P < 0.05$), the maximum apoptosis rate and a significant increase in the percentage of G2/M -phase cells and cytokines mRNA expression were observed in the G(0)LPS(+) treatment compared with the G(0)LPS(-)treatment. Interestingly, as expected, the supplementation of culture medium with glycine produced a significant blunting effect ($P < 0.05$) in a dose-dependent manner (2, 5 and 10 mM) by affecting the G2/M phase compared with the G(0)LPS(+) treatment. In addition, the apoptosis rate was significantly decreased in the cell populations simultaneously incubated with glycine (2, 5, and 10 mM) and LPS compared with G(0)LPS(+) treatment ($P < 0.05$). Furthermore, 2 and 5 mM glycine significantly reduced the mRNA expression of the pro-inflammatory cytokine of IL-1β, TNF-α, IL-6 and IL-8 in pBMEC induced by LPS ($P < 0.05$).

Conclusion Our findings suggest that glycine performs an immunomodulatory function in inflammatory pBMEC by promoting cell growth and inhibiting apoptosis and cytokine release, thus providing promising insights into the development of natural immunomodulatory strategies involving glycine for the control of bovine mastitis.

Acknowledgements This research was supported by the National Basic Research Program of the China Ministry of Science and Technology (Beijing, China; grant No. 2011CB100803) and partly supported by the "Western Light" program of the Chinese Academy of Sciences.

References

Alarcon-Aguilar FJ, Almanza-Perez J, Blancas G, Angeles S, Garcia-Macedo R, Roman R and Cruz M 2008. European Journal of Pharmacology 599, 152-158.
Froh M, Thurman RG and Wheeler MD. 2002. American Journal of Physiology-Gastrointestinal and Liver Physiology 283, G856–G863.
Garcia-Macedo R, Almanza JC, Blancas G, Sanchez F, Duran-Reyes G, Alarcon-Aguilar F and Cruz M 2008. European Journal of Pharmacology 587, 317–321.
Jedrzejczak M and Szatkowska I 2014. *In Vitro* Cellular & Developmental Biology-Animal. 50,389-398.
Tsune I, Ikejima K, Hirose M, Yoshikawa M, Enomoto N, Takei Y and Sato N 2003. Gastroenterology 125, 775–785.

Evaluation of the impact of water quality on the nutritional value of various willow ecotypes

Hussein Muklada[1], Tzach Glasser[2], Joshua Klein[1], Najah Halabi[1], Tova Deutch[1], Serge (Yan) Landau[1]

[1]Agricultural Research Organization, Rishon LeZion, Israel, [2]The Ramat Hanadiv Nature Park, Zikhron Yaakov, Israel

E-mail: hussein@agri.gov.il

Take home message There are significant differences in nutritional value between the different willow ecotypes in response to water quality. Watering willow with treated wastewater doubles the biomass production and increases the percentage of protein and *in vitro* DM digestibility.

Introduction The willow tree (*Salix* spp.) grows naturally in riparian landscapes and combines rapid growth and high rates of evapotranspiration. In a previous study, we found that willow (*Salix acmophylla* and/or *S. alba*) irrigated with treated wastewater could be safely used as fodder for goats. Willow foliage is rich in nutraceuticals, including anti-inflammatory and analgesic molecules. Providing willow to dairy goats inhibited the increase in somatic cell count in milk at late lactation (Muklada *et al.,* 2017). The aim of this experiment was to compare the productivity and nutritional value of willow ecotypes, depending on water quality. We tested the leaf and stem weighted content (on DM basis) of CP, Ash, NDF, ADF, IVDMD, Ca, and P for ten ecotypes irrigated with treated wastewater (TWW) and fresh water (FW) The treated wastewater with secondary level of purification featured 70.1±12.1 mg/l COD, 14.5±3.3 mg/l BOD and 11.9±1.2 mg/l TSS.

Material & methods Branch cuttings from ten ecotypes of willow collected from different regions in Israel were rooted in 100 pots of 50 liters each, encompassing five replicates of ten ecotypes for each type of water (FW/TWW). We harvested them after a three-month growth period. We tested total biomass production, DM content and, CP, NDF, ADF, Ca, P, and *in vitro* DM digestibility (on a DM basis) based on customized NIRS calibrations (Muklada *et al*., 2017). A bi-factorial ANOVA, with water type "FW/TWW" and "ecotype" and their interaction, were used to compare the nutritional attributes of willow silage. Paired t-tests were used to compare the nutritional attributes of willow fodder and silage.

Results & discussion The biomass production greatly differed between ecotypes (Figure 1). TWW irrigation resulted in two-fold biomass production, compared with FW. Willow foliage contained 45%-50% leaves, with no difference between treatments in the percentage of leaves. The contents of CP and IVDMD was 2 and 3%, higher in TWW, respectively, compared with FW. Similar changes were noted for ADF and NDF, probably due to the increase in the main stem diameter (Table 1).

Conclusion There is a wide variation between the different willow ecotypes in the production of biomass independent of water quality. Irrigation with TWW improves the growth performance and nutritional value of all the different ecotypes to a similar extent.

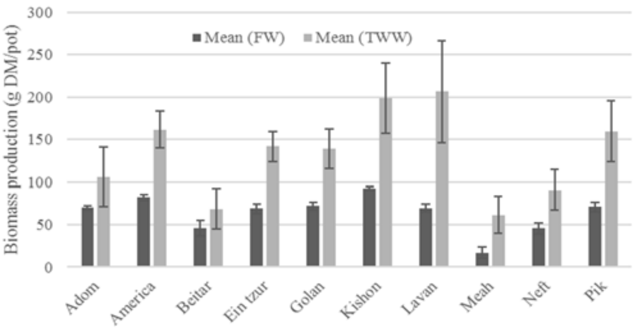


Figure 1 Biomass production [gr DM/pot] of the ecotypes under fresh water (FW) and treated wastewater (TWW) irrigation.

Table 1 Biomass production, dry matter content (%), leaf proportion (% of DM), the percentages of CP, Ash, NDF, ADF, IVDMD, P, Ca and main stem diameter [mm] of willow ecotypes irrigated with FW and TWW.

	Dry weight [gr]	Dry matter [%]	Leaves [% DM]	CP [% DM]	Ash [% DM]	NDF [% DM]	ADF [% DM]	IVDMD [% DM]	P [% DM]	Ca [%DM]	Main stem diameter [mm]
FW	63.4	30.5	47.7	9.3	6.4	50.9	41.3	35.3	0.24	1.13	7.9
TWW	133.7	28.2	46.8	11.7	6.5	55.6	44.4	38.4	0.29	0.89	10.8
SEM	7.2	0.01	0.7	0.24	0.1	0.56	0.48	0.75	0.004	0.01	0.2
P value	<.0001	0.029	0.46	<.0001	0.56	<.0001	<.0001	0.0037	<.0001	<.0001	<.0001

Acknowledgements This research was supported by MERC (Middle East Regional Cooperation - U.S. Agency for International Development - project 33-018). We are indebted to Ramat Hanadiv Nature Park and in particular to Mr. Hugo Yan Trago for allowing us to plant the willow pots.

References

Muklada H, Klein JD, Glasser TA, Dvash L, Azaizeh H, Halabi N, Davidovich-Rikanati R, Lewinsohn E and Landau SY 2018. Small Ruminant Research 163, 76–83.

The effect of porous ZnO as compared with regular ZnO and ZnSO4 on rumen fermentation and nutrient degradability in lactating cows

Leen Vandaele[1], Johan De Boever[1], Agathe Roméo[2], Sam De Campeneere[1]

[1]*Flanders Research Institute for Agriculture, Fisheries and Food, 9090, Melle, Belgium,* [2]*ANIMINE, 74330, Sillingy, France*

E-mail: leen.vandaele@ilvo.vlaanderen.be

Take home message The source of zinc fed to cows slightly above their requirements does not affect rumen fermentation.

Introduction Zinc is an essential trace element for cows as it plays a role as a cofactor for many proteins and enzymes in the immune system, stimulating wound healing, reduction of somatic cell numbers in milk and recovery from foot-rot (Jung *et al.*, 2013). Furthermore, Zn at high levels has been reported to slow down the degradation of urea in the rumen of steers fed low quality hay (Arelovich *et al.*, 2000) and to alter rumen fermentation patterns in high quality diets fed to non-lactating cows (Bateman *et al.*, 2002). In this study a porous ZnO was compared with other zinc sources to test the effect on rumen fermentation and nutrient degradability of lactating cows at a lower supplementation dose.

Material & methods The experiment was setup as a 3x3 Latin square with 3 Zn sources and 3 cows during 3 successive periods of 2 weeks. We used 3 rumen-cannulated HF-cows, which were housed in a tie-stall with individual mangers and were milked twice a day. The animals were fed *ad libitum* grass silage and maize silage in a ratio of 50/50 on dry matter (DM) base in 2 meals, around 8.30 am and 7.00 pm. The basal ration was supplemented with concentrates according to the individual requirements of energy and protein, fed in 2 equal meals at milking about 7.30 am and 6.30 pm. The Zn sources concerned porous ZnO (Hizox, Animine), a standard feed grade ZnO and $ZnSO_4$ and were provided pro rato of 50 mg Zn per kg concentrates.

During 2 consecutive days at the end of each experimental period rumen fluid was sampled just before the concentrate feeding in the morning and at 1, 2, 3.5, 5.5, 8 and 11 h thereafter. For each sample pH and NH_3 concentration was measured. In a pooled rumen sample per day volatile fatty acids (acetic, propionic and butyric acid) and lactic acid were determined. On the penultimate day of each experimental period nylon bags filled with 5 g DM-equivalent of a mixed feed (890 g DM, 227 g CP, 272 g starch and 177 g NDF per kg) were incubated in the rumen of each cow for 3, 8, 24 and 48 h to study the effect on degradability of the organic matter (OM), crude protein (CP), starch and NDF. The rumen fermentation parameters were analysed by ANOVA with 2 factors, Zn source and cow, and their interaction. The nutrient degradability was analysed by one-way ANOVA with Zn source as main factor an with the 3 cows as repetitions.

Results & discussion The mean daily milk production of the 3 cows over the whole experiment amounted to 36.3, 29.2 and 22.2 kg. The ration consisted of about two-thirds roughage and one third concentrates. The Zn content in the total ration varied between 53 and 60 mg per kg DM and was some 20% above the animal requirements according to NRC. Zn source had no significant ($p>0.05$) effect on pH nor on NH_3 content in rumen fluid at all sampling times. The pH before the concentrate meal and at 1, 2, 3.5, 5.5, 8 and 11 h thereafter, averaged over Zn sources and cows, amounted to 6.89, 6.47, 6.19, 6.16, 6.04, 6.41 and 6.77 respectively. There was a tendency ($p<0.10$) for an effect of Zn source on pH at 5.5 h after the meal, corresponding with the lowest pH level during the day. Hizox showed a 0.2 units higher pH than the other Zn sources, but the difference among treatment means was not significant after post-hoc test. The NH_3 concentration at the distinct times averaged 13, 24, 29, 27, 15, 11 and 13 mg/dl rumen fluid. There was a tendency ($p<0.10$) for an effect of Zn source at 11 h after the meal, with Hizox showing a significantly higher level of about 4 mg/dl than the other two Zn sources. Zn source had no effect on molar percentage of acetic, propionic and butyric acid in total fatty acids with on average 60, 19 and 17%, nor on lactic acid, on average 81 mg/dl rumen fluid. Zn source did not affect ($p>0.05$) rumen degradability of OM, CP, starch and NDF. The overall mean degradability of OM after 3, 8, 24 and 48 h of incubation amounted to 25, 35, 63 and 88%.

Conclusion Compared with feed grade ZnO and $ZnSO_4$, Hizox supplemented to lactating cows appeared to have no significant effect on rumen fermentation parameters nor on nutrient degradability, but tended to result in a higher minimum pH and NH_3 concentration. The supplementation level in this study (at 20% above animal requirements) might be the reason for the contrasting results with earlier studies in which much higher concentrations were used.

Acknowledgements This research was carried out by ILVO on request of Animine SAS (Silligny, France).

References

Arelovich HM, Owens FN, Horn GW and Vizcarra JA 2000. Journal of Animal Science 78, 2972-2979.
Bateman HG, Williams CC and Chung YH 2002. The Professional Animal Scientist 18, 363-367.
Jung KJ, Ko YH, Bae GS, Kim EJ, Lee SS, Paik IK, Kil DY, Chang JS, Kim CH, Song JY and Chang MB 2013. Journal of Animal and Veterinary Advances 12, 1048-1054.

Evaluation of nutraceutical properties of *Leucaena leucocephala* leaves pellets fed to kids

Steve Ceriac, carine Marie Magdeleine, Fred Periacarpin, Harry Archimede

INRA, Petit bourg, Guadeloupe

E-mail: harry.archimede@inra.fr

Take home message *Leucaena leucocephala* leaves have nutraceutical properties which are maintained when used as pellets.

Introduction Today, the main method to control gastrointestinal nematode (GIN) is still the use of chemical anthelmintics but the resistance of parasites to chemical molecules weakens this control strategy. Today, there is an urgent need to develop integrated control combining genetic selection of resistant and resilient animals, pasture management, adapted nutrition and anthelmintic plants. The present study was conducted to study the nutraceutical and anthelmintic properties of *leucaena leucocephala* leaves pellet fed to Creole kids.

Material & methods Fifty-five kids aged about 4 months were used during the 2-month trial. The kids were placed partly in individual boxes on the ground (4/lot) except the non-infested control lot. The others kids (5 /lot) were in metabolic cages for the collection of urine (5 consecutive days every 15 days). All animals were equipped with fecal bags. Artificial infestations with 2 strains of *Haemonchus contortus* (sensitive *versus* insensitive to chemical anthelmintic) were used to compare infected versus uninfected kids. Four iso-nitrogenous diets were formulated: Diet 1, hay *ad libitum* + 300 g (62% alfalfa + 38% soybean); Diet 2, hay *ad libitum* + 300 g (47 % alfalfa + 25 % *leucaena* + 28 % soybean); Diet 3, hay *ad libitum* + 300 g (30% alfalfa + 50 % *leucaena* + 20 % soybean); Diet 4, hay *ad libitum* + 300 g *leucaena*. The kids were assigned to one of the following 7 lots: Lot 1, none infested kids feeding diet 1; Lot 2, none infested kids feeding diet 4; Lot 3, infested kids with sensible strain diet 1; Lot 4, infested kids with sensible strain feeding diet 2; Lot 5, infested kids with sensible strain feeding diet 3; Lot 6, infested kids with sensible strain feeding diet 4; Lot 7, infested kids with resistant strain feeding diet 4.

Daily total intake and total tract digestibility of DM, OM, NDF, ADF and CP were determined. Average daily gains were estimated by weighting kids every 2 weeks. The feed efficiency was estimated for each treatment. Every week, the number of circulating eosinophils, packed cell volume, eggs per gram of faeces were performed. Intake, total tract digestibility, ADG, eosinophil, PCV and FEC were analyzed as a randomized design using the mixed procedure of SAS (2000). The statistical model included the fixed effect of diet (4), infestation status (2), Haemonchus strains (2), interaction diet* infesation status, time and the random effect of animal (model 1). Differences between means were tested using the pdiff option.

Results & discussion Compared with alfalfa diets, the anthelmintic activity of *leucaena* was highlighted by its potential to reduce egg excretions (1524 *vs.* 3651 eggs/g) and to maintain and lower larval development of eggs excreted (3.5 *vs* 24%). Anthelmintic properties of *leucaena* were expressed when the quantities were highest in the diets. In addition to its anthelmintic properties, *leucaena* had a high feed value highlighted by voluntary DM intake (79.3 *vs.* 77.0), total tract DM digestion (0.585 *vs.* 0.620) and growth (57.1 *vs.* 71.3 g/d) of kids fed *leucaena* compared to alfalfa. No sign of poisoning was recorded even with the highest amounts of *leucaena*. In conclusion the *leucaena* fulfilled the conditions to be a good nutraceutical and pelleting is a good way for its use in goats.

Conclusion *Leucaena leucocephala* pellets have the two main properties of a nutraceutical: feed and anthelmintic. As a feed *Leucaena leucocephala* is a high protein resource allowing Creole goats to reach about 80% of their growth potential. As anthelmintic, *Leucaena leucocephala* reduced hatching rate of eggs in larvae therefore limiting environmental contamination and reduction in the fertility of female worms.

Acknowledgements This study was completed with the financial support of, INTERREG IV Caraïbes (IEPAC project), FEDER (AGROECODIV project), INRA metaprogramme (GISA StreP project), other Region Guadeloupe and European funding: FSE, FEADER.

A supplement including 25-(OH) D3 and additional calcium improves calcium status of late-pregnant twin-bearing ewes

Shawn McGrath[1,2], Thomas Keogh[1,2], Kate McCarthy[1], Michael Friend[1,2], Bruce Allworth[1,2], Marie Bhanugopan[1,2]

[1]Charles Sturt University, Wagga Wagga, NSW, Australia, [2]Graham Centre for Agricultural Innovation, Wagga Wagga, NSW, Australia

E-mail: shmcgrath@csu.edu.au

Take home message Supplementation with 25-(OH) D3 plus calcium in late pregnancy improves ewe calcium status.

Introduction Transfer of calcium (Ca) to the growing fetus and milk results in a negative maternal Ca balance during late pregnancy and early lactation, and has implications for ewe health. Susceptibility to hypocalcaemia increases from about 6 weeks prior to lambing to about 3 to 4 weeks after lambing. Clinical hypocalcaemia mostly occurs in the last few weeks of gestation when the fetal skeletons are mineralising. In the sheep industry, supplementation with vitamin D3 can be used to improve the vitamin D status and regulation of Ca and P. The absorptive characteristics of 25-(OH) D3 are greater relative to vitamin D3, with absorption occurring independent of fat adsorption and bile acids. This study sought to determine the effect of supplementation with a commercial metabolite 25-(OH) D3 (branded HyD®; DSM Nutrition) in late pregnancy on calcium and bone markers in ewe plasma.

Material & methods Naturally joined pregnant single and twin bearing Merino ewes (n=120 sourced from two flocks) were grazed on a dry legume and grass pasture at the Charles Sturt University farm near Wagga Wagga, Australia from 3 June 2017. During the "pre-loading" phase (3 -21 June) pregnant ewes were fed either a mineral supplement that included HyD® and additional calcium (limestone) or a control supplement (including magnesium and trace minerals but no HyD® or limestone). Ewes were moved to the lambing paddock on 21 June (i.e. end of pre-loading; start of grazing lambing paddock) and were allocated to one of 12 plots sown to dual-purpose wheat in three replicates. Treatments were: 1) Control-Control (control supplement during both pre-loading and lambing paddock); 2) Control-HyD (control supplement during pre-loading; HyD® supplement in lambing paddock); 3) HyD-Control (HyD® supplement during pre-loading; control supplement in the lambing paddock); and 4) HyD-HyD (HyD® supplement in both pre-loading and lambing paddock). Blood samples collected on 10-12 July from pregnant ewes that subsequently gave birth to twin lambs were analysed for plasma Ca and bone markers (n=42; 47±10 days prior to mean lambing date at the commencement of pre-loading phase). Data was analysed using linear mixed models, with interaction between pre-loading and lambing paddock supplementation as fixed model and random model of replicate/plot/source flock.

Results & discussion Plasma Ca at the start of the experiment was a significant ($P<0.001$) co-variate for plasma calcium at late pregnancy. Treatments that included HyD in the supplement in either the pre-loading or lambing paddock had higher plasma Ca at late pregnancy than ewes that did not receive HyD in the supplement (Table 1), and a high proportion of ewes in the control treatment were identified as sub-clinically hypocalcaemic, with plasma Ca below 90 mg/l (Suttle, 2010). Supplement fed had no significant effect on osteocalcin in plasma at late pregnancy, and osteocalcin in plasma at the start of the trial was a significant co-variate ($P<0.001$). Ewes that received the control supplement in the lambing paddock had significantly higher crosslaps in plasma at late pregnancy compared to the HyD supplemented ewes (0.86 v. 0.49 ng/ml); $P<0.001$), indicating greater resorption from bone at this time; however pre-loading treatment only approached significance (0.60 v. 0.75; $P=0.075$), and the interaction of pre-loading and lambing paddock treatment was not significant for crosslaps.

Table 1 Calcium (Ca; mg/l) and bone markers (ng/ml) in plasma of twin-bearing Merino ewes fed supplements during pre-loading and on entry to the lambing paddock.

	Treatment ('pre-loading'-'lambing paddock')					
	Control-Control	Control-HyD	HyD-Control	HyD-HyD	s.e.d.	P-value (interaction)
Plasma Ca	90.3	107.1	111.6	105.6	6.6	0.027
Osteocalcin	22.0	18.7	17.2	16.5	3.0	n.s.
Crosslaps	0.81	0.39	0.90	0.61	0.12	n.s.

Conclusion Feeding HyD plus additional Ca to late-pregnant twin-bearing ewes may improve calcium status in late pregnancy. Feeding this supplement prior to ewes entering the lambing paddock may assist to maintain higher plasma Ca in late pregnancy, even if ewes stop consuming the supplement. Feeding HyD with additional calcium during late pregnancy compared to the control may reduce the risk of hypocalcaemia.

Acknowledgements This research was supported by MLA Donor Company and DSM Nutrition International.

References

Suttle NF 2010. 'Mineral Nutrition of Livestock.' CAB International: Wallingford, UK.

Comparative assessment of supplemental zinc on solubility and rumen fermentation

Vivek Fellner[1], Valérie Kromm[2], Jerry Spears[1]

[1]North Carolina State University, Raleigh, North Carolina, USA, [2]ANIMINE, Sillingy, France

E-mail: vkromm@animine.eu

Take home message Supplemental zinc alters rumen microbial metabolism and improves nutrient utilization.

Introduction Zinc (Zn) is essential for ruminants and is also required by rumen microbes for cellulose degradation (Martinez and Church, 1970). Rumen inert Zn meets animal requirements whereas the rumen soluble fraction likely meets the microbial requirement. Solubility varies among sources and poses a challenge for ruminants. Microbes reportedly have a low Zn requirement of 1 ppm for cellulose digestion (Woods, 1965). However, greater amounts of Zn have improved cellulose digestion (Eryavuz and Dehority, 2009). Effect of dietary Zn on microbial fermentation is not clear and data are inconsistent. Meeting animal and microbial requirements may depend on the degree of interaction of mineral complexes within the rumen environment. The objective of this study was to compare the effects on rumen fermentation of a standard zinc oxide source (ZnO) and a potentiated zinc oxide source (HiZox®; Animine, Sillingy, France), at two supplemental levels.

Material & methods Five dual-flow continuous cultures of rumen microbes were used to determine the effect of supplemental Zn. Diets were 1) control (no supplemental Zn), 2) HiZox®, 30 mg of Zn/kg of DM, 3) HiZox®, 120 mg of Zn/kg of DM, 4) ZnO, 30 mg Zn/kg of DM, and 5) ZnO, 120 mg Zn/kg of DM. Diets were formulated to meet requirements for lactating dairy cows (NRC 2001). Each experiment consisted of 8 days with 4 days for stabilization followed by 4 days for data collection. Each experiment was repeated three times. Zinc concentration was determined with an inductively-coupled-plasma (ICP) spectrophotometer. Headspace gas was collected in gas-tight syringes and immediately analysed for methane using GLC. Culture pH was recorded before and 2 hours after feeding. Rumen soluble Zn, short chain fatty acids (SCFA) and NH_3-N were analysed after centrifugation. Dietary samples (0.5g) were weighed in Dacron bags and placed in respective fermentors for 24 h to estimate DMD. Data were analysed according to a randomized complete block design with repeated measures using the PROC MIXED procedures of PC SAS (SAS Inst., Inc., Cary, NC). Preplanned orthogonal contrasts were used to compare means.

Results Rumen soluble Zn increased with supplemental Zn and with level of Zn inclusion ($p < 0.001$; Figure 1). Compared to ZnO, soluble Zn tended ($p < 0.10$) to be greater with HiZox®. HiZox® increased ($p<0.01$) ruminal pH, methane, acetate and isoacids compared to ZnO (Table 1).

Table 1 The effect of source (Zinc oxide ZnO; potentiated zinc HiZox) and level (30 or 120 ppm) of supplementary Zn on rumen fermentation.

	Treatments						
		ZnO (ppm)		HiZox (ppm)			
	Control	30	120	30	120	SE	Constrasts[1]
Total SCFA, mM	44.4	44.0	47.1	43.1	2.28	2.28	NS
Individual SCFA, mol%							
Acetate	51.6	51.1	49.5	51.2	0.52	0.52	B**
Propionate	23.6	23.6	26.5	23.1	21.7	0.83	B**
Isobutyrate	0.9	0.	0.6	0.8	0.9	0.04	A**B***
Isovalerate	4.0	3.5	2.4	4.2	4.7	0.31	B***
A:P ratio	2.2	2.2	1.9	2.2	2.5	0.09	B**
Culture pH	5.9	6.1	6.1	6.3	6.2	0.05	A***B*
Methane, nmol/ml	560.3	493.3	407.7	565.3	597.2	45.78	B**

[1]A = control *vs.* Zn, B = ZnO *vs.* HiZox, C = 30 *vs.* 120 ppm. *p<0.05; **p<0.01; ***p<0.001.

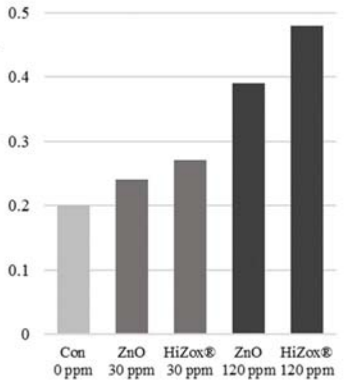


Figure 1 Soluble Zn (µg/ml).
Con *vs.* Zn ($P < 0.001$)
Zn Source ($P < 0.10$)
Zn Level ($P < 0.001$)

Conclusion Supplemental zinc improved fermentation and microbial energetics. Mode of action between Zn sources differed. HiZox® favoured cellulolytic microbes whereas ZnO seemed to inhibit gram positive fiber digesters.

References

Eryavuz A and Dehority BA 2009. Aninaml Feed Science and Technology 151, 175-183.
Martinez A and Church DC 1970. Journal of Animal Science 31, 982-990.
Woods W 1965. Study of the efficiency of various sources of trace elements for ruminant animals. Dept. Animal Sci. Rep. No. 3 on Project No. ZC87. Univ. of Nebraska, Lincoln.

In silico construction of a bovine milk proteome atlas to identify signatures of early lactation

Mylène Delosière, Laurence Bernard, Isabelle Cassar-Malek, José Pires, Muriel Bonnet

INRA, Clermont-Ferrand, France

E-mail: mylene.delosiere@inra.fr

Take home message Among an atlas of 4510 proteins established by a computational aggregation, 65 proteins are specific of early lactation and potential biomarkers of negative energy balance.

Introduction Noninvasive methods based on easily available animal fluids (plasma, milk) and rapid analytical tools are needed to phenotype animal traits related to performance, welfare, health and product quality. Milk is an accessible biological fluid and its molecular characterization is a prerequisite to design tools for the phenotyping or the diagnosis of animal diseases (Roncada *et al.*, 2012). The sensitivity of proteomics technology made possible the identification of thousands of proteins in milk during the last 10 years. We hypothesized that computation of available proteomic data may enable the identification of milk protein biomarkers of traits such as energy balance and body reserve mobilization during early lactation, which are major drivers of dairy performance, robustness and functional longevity of dairy cows (Zachut and Moallem, 2017).

Material & methods Based on a computational approach, we aggregated datasets from 18 publications on milk proteomics from healthy cows whatever the husbandry factors studied. The lists of protein identifiers were extracted from articles and/or the supplementary data files, converted into gene names and aggregated in an atlas. This atlas was divided in 5 periods of the lactation cycle: colostrum (0-5 days in milk (DIM)), early lactation (5-21 DIM), lactation peak (21-60 DIM), mid-lactation (60-80 DIM) and late lactation (> 80 DIM). The early lactation period was narrow in order to ensure a negative energy balance of cows. Protein lists were mined using ProteINSIDE (http://www.proteinside.org/; Kaspric *et al.*, 2015) allowing the identification of metabolic pathways in which proteins are implicated. ProteINSIDE webservice relies mainly on Gene Ontology (GO) enrichment tests (p value_Benjamini and Hochberg < 0.05) among the categories: Biological Process, Molecular Function and Cellular Component.

Results & discussion Among the 4510 different proteins identified in bovine milk, 65 gene names were identified exclusively in early lactation milk, 58 of which were annotated by 442 enriched GO terms. The most significantly ($p < 0.01$) enriched terms in the Biological Process category were “oxidation-reduction process”, 6 terms related to “transport” and 4 terms related to “immune system”. The most enriched GO terms in the Molecular Function category was “oxido-reductase activity”. The 58 proteins found in early lactation milk were mainly related to oxidative processes or oxidative metabolism such as mitochondrial cytochrome c oxidase subunit (COX5A, COX5B, COX7A2), aldehyde dehydrogenase family 3 member B1 (ALDH3B1), catalase (CAT), mitochondrial succinate dehydrogenase flavoprotein subunit (SDHA). Mitochondrial acetyl-CoA acetyltransferase (ACAT1) and mitochondrial phosphoenolpyruvate carboxykinase 2 (PCK2) were also found exclusively in early lactation milk. The biological significance of their presence in the milk remains to be defined. It may result from modifications of mammary epithelium permeability, may be linked to the secretion of metabolites related to oxidative pathways in milk during early lactation (communication Pires *et al.*, this symposium), and may constitute molecular signatures of physiological negative energy balance typical of this period. In agreement with these hypotheses, the abundance of PCK2 mRNA (a neoglugogenic gene expressed in liver) was increased in the mammary tissue of high genetic merit dairy cows (Weikard *et al.*, 2012), and may contribute to glyceroneogenesis during fasting or compromised feed intake, as already proposed for lipogenic tissues.

Conclusion The aggregation of milk proteomics from public data allowed the construction of a large milk protein atlas. This atlas can be mined to identify proteins that regulate the physiological adaptations and allow the phenotyping of dairy ruminants. Data suggest a proteomic signature specific of early lactation which is mainly related to “oxidation-reduction processes”. Two milk proteins associated to early lactation, ACAT1 and PCK2, may be biomarkers of negative energy balance typical of this period. Nonetheless, eventual associations remain to be confirmed by studies designed to relate their abundance, the energy balance and classic indicators of metabolic status in early lactating cows.

References

Kaspric N, Reichstadt M, Picard B, Tournayre J and Bonnet M 2015. Plos One 10, 1-24.
Roncada P, Piras C, Soggiu A, Turk R, Urbani A, Bonizzi L 2012. Journal of Proteomics 75, 4259-4274.
Weikard R, Goldammer T, Brunner RM and Kuehn C 2012. Physiological Genomics 44, 728-739.
Zachut M and Moallem U 2017. Journal of Dairy Science 100, 3143-3154.

719

Milk metabolites as noninvasive indicators of physiological state and energy balance of early lactation cows

José Pires[1], Christine Leroux[1,2], Torben Larsen[3]

[1]*INRA, Université Clermont Auvergne, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès-Champanelle, France,* [2]*Dept. of Food Science and Technology, University of California Davis, CA 95616, USA,* [3]*Dept. of Animal Science, Aarhus University, DK-8830 Tjele, Denmark*

E-mail: jose.pires@inra.fr

Take home message Selected milk metabolites are noninvasive indicators of physiological state of early lactation cows.

Introduction Previous research has shown that animal husbandry factors such as diet composition and intake can alter milk content of selected metabolites (Larsen *et al.*, 2016). The objectives were to determine the effects of days in milk (DIM) and experimentally induced undernutrition on milk metabolite concentrations, and to study their associations with classic indicators of energy balance and metabolic status of early lactation cows.

Material & methods To determine the effects of DIM, 17 multiparous Holstein cows were allowed *ad libitum* intake of a lactation diet (7.1 MJ/kg DM NEL, 17.4% CP) and were studied until 7 weeks of lactation. Samples were collected at 8, 15, 21, 23, 35, 42 and 49 ± 3 DIM. To assess the effects of experimentally-induced undernutrition, a subset of 8 cows received a diet containing 48% (DM basis) of straw (REST; 5.2 MJ/kg DM NEL, 12.2% CP) for 4 days, starting at 24 ± 3 DIM (Pires *et al.*, 2016), and 8 cows maintained on the standard lactation diet served as controls (CONT). Samples were collected from day -1 to 3 of feed restriction (or control), corresponding to 23, 24, 25 and 26 ± 3 DIM. Morning milk samples were analysed for β-hydroxybutyrate (BHBA), isocitrate, glucose, galactose, glutamate, creatinine, and uric acid (Larsen *et al.*, 2016, 2017). Plasma samples were analysed for insulin, glucose, nonesterified fatty acids (NEFA) and BHBA. Statistical analyses were performed separately to assess the effects of DIM and feed restriction using Mixed models of SAS with repeated measures. Relationships among variables were explored by Spearman correlations. Significance was considered at $p \leq 0.05$ and trends towards significance at $0.05 < p \leq 0.10$.

Results & discussion Dry matter intake, milk yield, NE_L balance (EBAL), plasma insulin, glucose, NEFA and BHBA concentrations did not differ between CONT and REST before diet change (21.8, 39.0 kg/d, -5.6 MJ/d, 22 µIU/mL, 3.78, 0.415 and 0.66 mM, respectively, at day -1), but were significantly altered in REST at day 3 (9.8, 28.3 kg/d, and -74 MJ/d, 11 µIU/mL, 2.77, 1.672 and 2.98 mM, respectively). Milk isocitrate and creatinine decreased, and BHBA tended to decrease ($p = 0.08$), whereas glucose and galactose concentrations increased ($p < 0.001$) with DIM. Conversely, feed restriction increased BHBA and isocitrate ($p < 0.05$), tended to increase creatinine ($p = 0.06$), and decreased ($p < 0.05$) glutamate, glucose and uric acid concentrations in milk. Therefore, milk profiles of BHBA, isocitrate, glucose, and creatinine were consistent during spontaneous physiological imbalance typical of early lactation, and in response to experimentally induced undernutrition. Milk glutamate was correlated with EBAL only during feed restriction (Table 1).

Table 1 Correlations among energy balance, milk and plasma metabolite concentrations in early lactation cows (DIM effect), and in response to partial feed restriction. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, † $p < 0.10$.

		Milk BHBA	Milk Isocitrate	Milk Glucose	Milk Galactose	Milk Glutamate	Milk Creatinine	Milk Uric Acid
DIM	Energy balance	-0.23 *	-0.37 ***	0.37 ***	0.27 **	ns	-0.51 ***	ns
	Plasma NEFA	0.28 **	0.52 ***	-0.25 **	ns	ns	0.61 ***	-0.23 *
	Plasma BHBA	0.51 ***	0.32 ***	-0.34 ***	ns	0.20 *	ns	ns
	Plasma Glucose	-0.38 ***	-0.36 ***	0.56 ***	ns	ns	ns	ns
Feed Restriction	Energy balance	-0.66 ***	-0.66 ***	0.48 ***	ns	0.42 ***	-0.48 ***	ns
	Plasma NEFA	0.67 ***	0.73 ***	-0.50 ***	0.33 **	-0.40 **	0.35 **	-0.40 ***
	Plasma BHBA	0.87 ***	0.74 ***	-0.54 ***	0.27 *	-0.25 *	0.34 **	-0.33 **
	Plasma Glucose	-0.74 ***	-0.59 ***	0.56 ***	ns	0.22 †	-0.22 †	0.47 ***

Conclusion Metabolites secreted in milk may serve as noninvasive indicators of physiological state and energy balance of early lactation cows. Milk glutamate was an indicator of negative EBAL in response to experimentally-induced undernutrition and resulting subclinical ketosis, but was not associated with spontaneous NEB and DIM.

Acknowledgements Authors thank the Herbipôle research station (INRA, UE1414, Theix, France) for animal care and sampling. This research was funded in part by the GISA program of INRA (Ruminflame and LongHealth projects).

References

Larse T, Alstrup L and Weisbjerg MR 2016. Journal of Dairy Research 83, 12-19.
Larsen T and Fernández C., 2017. Journal of Dairy Research 84, 32-35.
Pires J, Pawlowski K, Rouel J, Delavaud C, Foucras G, Rainard, P, Germon P and Leroux C 2016. Journal of Dairy Science 99, E-Suppl. 1, 64-65.

Subacute ruminal acidosis (SARA) affects fermentation and endotoxin concentration in the ruminal fluid and relative expression of the CD14/TLR4/MD2 genes involved in lipopolysaccharides systemic immune response in dairy cows

Barbara Stefanska[1], Ewa Pruszynska-Oszmalek[2], Dawid Szczepankiewicz[2], Jolanta Komisarek[3], Michal Gasiorek[1], Wlodzimierz Nowak[1]

[1]Poznan University of Life Science, Department of Animal Nutrition, Poznan, Poland, [2]Poznan University of Life Science, Department of Animal Physiology and Biochemistry, Poznan, Poland, [3]Poznan University of Life Science, Department of Animal Breeding and Product Quality Assessment, Poznan, Poland

E-mail: barbaras@up.poznan.pl

Take home message SARA increased concentration of free LPS in the ruminal fluid and stimulated the expression of CD14/TLR4/MD2 complexes and concentration of serum amyloid A (SAA).

Introduction It is common practise to feed high-yielding dairy cows high-grain diets. However, the disproportion between a high proportion of rapidly fermented carbohydrates and low physically effective NDF may cause impaired ruminal health through variation in the VFA and decreased ruminal fluid pH, which leads to SARA (Humer, 2017). Several studies have investigated the ethology and pathophysiology of SARA (Khafipour, 2009; Danscher, 2016), but still no clear definition of SARA exists. Additionally, current recommendations for the diagnosis of SARA in the dairy herds are mainly based on ruminal pH measured in fluid sampled by rumenocentesis (Nordlund and Garett, 1994). However, many authors have suggested that the use of ruminal pH as the only indicator of SARA should be avoided. The first objective of our study was to investigated the effects of SARA on fermentation, ruminal free LPS, and expression of the CD14/TLR4/MD2 complex involved in the systemic immune response in dairy cows. The second objective was a study of whether increased expression of the LPS receptor complex led to increases in the concentrations of plasma HDL and serum Ca.

Material & methods The study was conducted on 13 commercial dairy farms. In total, 305 Polish Holstein-Friesians, clinically healthy dairy cows were selected according to their DIM, 305-d milk yield, and the number of lactations. Rumen fluid samples were collected for each dairy cow at 3 to 6 h after the morning TMR delivery *via* rumenocentesis and the value of pH used to confirm occurrence of SARA was <5.6. The herds were selected according to the classifications proposed by Garrett *et al*., (1999) based on the percentages of cows with an assigned value of ruminal fluid pH and segregated into 3 groups as SARA-positive, -risk, and -negative. Moreover, 305 selected dairy cows were divided according to the classifications of Nordlund and Garrett (1994) based on ruminal fluid pH into 3 groups as healthy (HC), risk (RC), and acidotic (AC) cows. Ruminal fluid samples were analyzed for concentrations of lipopolysaccharide (LPS), volatile fatty acids (VFA) and total counts of bacteria and protozoa (*Entodiniomorpha* and *Holotricha*). Blood samples were collected from the jugular vein for the determination of concentrations of lipopolysaccharide-binding protein (LBP), haptoglobin (Hp), serum amyloid A (SAA), high-density lipoproteins (HDL) and Ca. Moreover, in the white blood cells analyzed expression of the cluster of differentiation 14 (*CD14*), toll-like receptor 4 (*TLR4*), and myeloid differentiation protein 2 (*MD2*) complex involved in the systemic immune response in dairy cows. The means were subjected to ANOVA and Duncan's multiple range test using the PROC GLM procedure of SAS 9.4 (SAS Institute, 2014).

Results & discussion In both classification, in the AC group cows and SARA-positive herds, lower total counts of bacteria, *Holotricha*, *Entodiniomorpha*, ratio of rumen acetate to propionate acid concentration (C_2:C_3; $P \leq 0.01$) and free ruminal LPS ($P \leq 0.05$) were noted. Ruminal fluid pH and its daily fluctuation characteristics are important factors in the regulation of the microbe fluctuation. We could suggest, similarly to another study, that intensive fermentation during SARA leads to an increase in the concentration of VFA, and a decrease in ruminal fluid pH and the total count of ciliates and bacteria, which affect the increase in LPS concentration. In the current study, a higher relative mRNA abundance of genes associated with the function of the LPS receptor complex, such as *CD14*, *TLR4*, and *MD2* ($P \leq 0.01$) in both classifications were noted. In the AC group, we recorded higher concentrations of SAA ($P \leq 0.05$) and HDL ($P \leq 0.01$), as well as a tendency to a higher level of serum LBP ($P = 0.06$) and lower levels of Hp ($P \leq 0.05$) and Ca ($P \leq 0.01$) compared with the HC group. Similar results were obtained in the SARA herd status analysis, but concentrations of LBP differed between SARA-positive and -negative herds ($P \leq 0.05$).

Conclusion SARA increased the release of free LPS into the rumen and stimulated the expression of CD14/TLR4/MD2 complexes and concentration of serum amyloid A (SAA). In addition, increase plasma HDL and reduction of serum Ca could be an effective protective mechanism against endotoxemia. However, the biological significance of these results needs to be investigated further in larger field trials.

Acknowledgements This study was supported by grant financed by Polish Federation of Cattle Breeding and Dairy Farmers.

References

Danscher AM, Li S and Plazier JC 2016. Acta Veterinaria Scandinavica 57-39.
Garrett EF, Pereira MN, Nordlund KV, Armentano LE, Goodger WJ and Oetzel GR 1999. Journal of Dairy Science 82, 1170-78.
Humer E, Aschenbach JR, Neubauer V, Kröger I, Khiaosa-ard R, Baumgartner W and Zebeli Q 2018 Journal of Animal Physiology and Animal Nutrition 102, 380-392.
Khafipour E, Krause DO and Plaizier JC 2009. Journal of Dairy Science 92, 1060– 1070.
Nordlund KV and Garrett EF 1994. Bovine Practitioner 28, 109–112.

Effect of oral drench of glycerol or propylene glycol on hepatic mRNA expression of ApoB100 and MTP in transition primiparous dairy cows

Zygmunt M. Kowalski, Anna Barteczko, Jadwiga Flaga, Pawel Gorka

University of Agriculture in Krakow, Krakow, Poland

E-mail: rzkowals@cyf-kr.edu.pl

Take home message Oral drench of glycerol or propylene glycol increases hepatic mRNA expression of ApoB(100) and MTP in transition primiparous dairy cows.

Introduction Apolipoprotein B100 (ApoB100) and microsomal triglyceride transfer protein (MTP) are proteins involved in hepatic secretion of very low density lipoproteins (VLDL). Liu *et al.* (2014) showed that non-esterified fatty acids (NEFA) significantly inhibit the expression of ApoB100 and MTP, thereby decreasing the synthesis and assembly of VLDL. Dietary glycerol (G) or propylene glycol (PG) increase blood glucose and decrease blood NEFA. If so, they may increase hepatic formation of ApoB100 and MTP. However, the effect of G or PG on the liver gene expression of ApoB100 and MTP in transition dairy cows is unknown. The objective of this study was to examine the effects of oral drench of G or PG during the transition period on mRNA expressions of ApoB100 and MTP in the liver of transition primiparous dairy cows.

Material & methods Thirty primiparous Holstein cows were divided into 5 groups of 6 animals each: control (not supplemented) and 4 groups fed diets supplemented with G or PG at a rate of 450 or 900 mL per cow/day (G450, G900, PG450, PG900), from −21 through +28 d relative to calving. The supplements were given as an oral drench before the morning feeding. Control cows were given 450 mL of water (W450). Liver tissue was sampled via puncture biopsy at −7, +3, and +21 d relative to calving at 1 or 2 hours after oral drench. The mRNA expression of ApoB100 and MTP was determined using real-time PCR with RPS9 used as a reference gene (Ostrowska *et al.,* 2014). Data were analyzed as repeated measurements, separately for each hour after drench. Statistical model included effect of treatment and day relative to calving and interaction between these two. Polynominal or orthogonal contrasts were used for data interpretation.

Results & discussion Irrespectively of treatment, expression of ApoB100 and MTP, measured at 2 h after oral drench, increased linearly from −7 through +21 d relative to calving (Figure 1). Oral drench of either G or PG increased plasma glucose but had no effect on serum NEFA (data not presented). Irrespective of a day around calving, neither oral supplements nor their dose affected the expression of ApoB100 and MTP at 1 h after oral drench (Table 1). However, at 2 h after drench, compared to W450, PG increased the expression of ApoB100 (P=0.06), especially PG450. The effect of G was less apparent. On the other hand, both G and PG increased expression of MTP at 2 h after oral drench.

Table 1 Effect of G or PG on the hepatic expression of mRNA of ApoB100 and MTP.

Item	Treatments					Contrasts [1]				
	W450	G450	G900	PG450	PG900	1	2	3	4	5
ApoB100										
1 h [2]	1.08	1.19	0.95	1.14	1.06	0.90	0.96	0.82	0.12	0.60
2 h	0.94	1.23	0.92	1.46	1.00	0.06	0.37	0.19	0.07	<0.01
MTP										
1 h	0.85	1.05	0.91	1.04	0.98	0.32	0.42	0.82	0.46	0.78
2 h	0.80	1.04	1.05	1.03	1.27	0.02	0.07	0.41	0.95	0.18

[1] 1 = W450 *vs.* PG; 2 = W450 *vs.* G; 3 = G *vs.* PG; 4 = G450 *vs.* G900; 5 = PG450 *vs.* PG900;
[2] 1 or 2 hour after oral drench

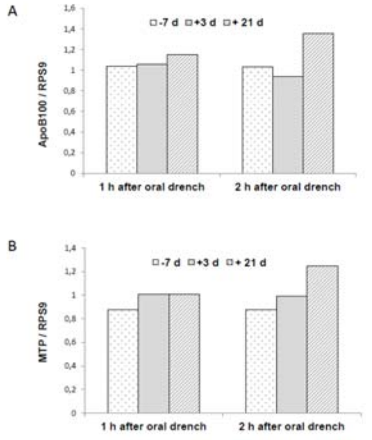


Figure 1 Effect of day around calving on hepatic mRNA expression of ApoB100 (A; linear effect at 2 h after G or PG drench; P=0.04) and MTP (B; linear effect at 2 h after drench; P<0.01)

Conclusion Oral drench of PG, precursor of glucose synthesis in the liver, especially at the lower dose (450 mL/d), increased the mRNA expression of ApoB100. The effect of G was less apparent. Both, G and PG increased the mRNA expression of MTP in the liver of transition primiparous dairy cows.

References

Ostrowska M, Żelazowska B, Słoniewski K, Kowalski ZM, Zwierzchowski L 2014. Journal of Dairy Science 97, 911-916.

Liu L, Li X, Li Y, Guan Y, Song Y, Yin L, Chen H, Lei L, Liu J, Li X, Wang Z, Yang X and Liu G 2014. Journal of Dairy Science 97, 1328-1335.

Hypertonic milk replacers induce an increase in gastrointestinal permeability to Cr-EDTA in healthy dairy calves

Juliette Wilms, Harma Berends, Javier Martín-Tereso

Trouw Nutrition Research and Development, Amersfoort, The Netherlands

E-mail: juliette.wilms@trouwnutrition.com

Take home message Hypertonic milk replacers increase gastrointestinal paracellular permeability and may compromise gut health in dairy calves.

Introduction In the gastrointestinal system of young calves, the effect of replacing lactose with glucose above 20% inclusion in milk replacer is not known, but it is hypothesized that increasing glucose concentration may affect intestinal osmotic pressure. In extremely premature infants, exposure of the gut to hypertonic solutions may lead to loss of mucosal integrity (Pearson *et al*., 2013; Travadi *et al*., 2003). In this study, the effect of increased osmolality of milk replacer through the replacement of lactose by monosaccharides (glucose and galactose) on gastrointestinal permeability was studied in healthy male dairy calves.

Material & methods In total, 30 male calves (60% Holstein-Friesian, 40% crossbreds with at least 40% Holstein-Friesian) were acquired from dairy farms which used a standardized protocol for colostrum management, including 3 feedings of colostrum in the first 24 h; 3 L as soon as possible followed by 2 feedings of 2 L. Calves were then transported to the research facility at 0 to 3 days of age and were fed L1 (0% glucose inclusion) twice daily for the first two weeks of age and then placed on their respective treatments from 3 until 7 weeks of age. Calves were randomly assigned to one of 5 treatments including 4 levels of glucose inclusion (replacing lactose): 0% (L1, n=5), 13.3% (L2, n=5), 26.7% (L3, n=5), and 40% (L4, n=5) and a treatment (G+D) including 20% galactose and 20% glucose (n=10). Carbohydrates were exchanged based on hexose equivalents. The G+D treatment helped distinguish whether calves are sensitive to high osmolality of calf milk replacer (CMR) or whether they are sensitive to a specific source of monosaccharides (glucose *vs.* galactose). Osmolality of the CMR ranged from 434 mOsm/kg (L1) to 610 mOsm/kg (L4 and G+D). Calves had free access to water throughout the study and no solid feed was provided. Gastrointestinal permeability was assessed using indigestible markers (lactulose, D-Mannitol and Cr-EDTA) delivered in the CMR meal as a single dose at 3 and 7 weeks of age. Marker recoveries were expressed as % of oral dose and assessed in a 24 h quantitative urinary collection period. The intestinal permeability (IP) index was determined as the ratio between urinary recovery (%) of lactulose and D-Mannitol.

Results Increasing CMR osmolality linearly increased urinary Cr-EDTA recovery ($P < 0.05$) and there was a trend for an increase of urinary lactulose recovery ($P = 0.10$) at 3 and 7 weeks of age (Table 1). Urinary recovery of D-mannitol increased with osmolality ($P = 0.05$) at 3 weeks of age. The IP index was not affected by osmolality neither at 3 nor at 7 weeks of age. The greater absorption of large size molecules (lactulose and Cr-EDTA) observed in this study may indicate a diminished gastrointestinal barrier function caused by an increased paracellular intestinal permeability.

Lactulose and Cr-EDTA recoveries as well as IP index did not differ between G+D and L4 (Table 1), suggesting that the monosaccharide source in CMR (40% glucose or 20% glucose and 20% galactose) has no effect on IP. This also indicates that the observed effect of osmolality on IP are only due to osmolality differences rather than a lack of galactose or a surplus of glucose.

Table 1 Effect of milk replacer osmolality (434 mOsm/kg (L1) to 610 mOsm/kg (L4 and G+D)) on indicators of gastrointestinal permeability as assessed by 24 h urinary recovery of lactulose, D-mannitol and Cr-EDTA in healthy calves (n=30) at 3 and 7 weeks of age.

Parameter	Age, weeks	Least square means					Pooled SEM	*P*-value	
		L1	L2	L3	L4	G+D		Linear response (L1 to L4)	Contrast L4 *vs.* G+D
Lactulose (L), % oral dose	3	2.25	2.89	2.79	3.02	2.60	0.33	0.10	0.30
	7	1.86	2.73	2.62	2.57	2.86	0.13	0.10	0.47
D-Mannitol (M), % oral dose	3	9.59	9.19	11.17	10.92	11.29	0.51	0.05	0.76
	7	7.95	7.80	8.84	7.45	9.53	0.44	0.76	0.11
IP index, (L/M)	3	0.23	0.32	0.25	0.23	0.28	0.01	0.96	0.19
	7	0.24	0.35	0.30	0.34	0.31	0.01	0.15	0.42
Cr-EDTA, % oral dose	3	3.91	4.30	4.84	5.04	4.33	0.47	0.04	0.18
	7	2.69	3.95	4.74	4.28	4.84	0.20	0.01	0.32

Conclusion The observed increase in permeability to large size molecules (Cr-EDTA and lactulose) with increased osmolality suggests that hypertonic CMRs affect gastrointestinal barrier function. Further research is needed to evaluate the clinical relevance of the observed differences for calf gut integrity and health.

References

Pearson F, Johnson MJ and Leaf AA 2013. Archives of Disease in Childhood. Fetal and Neonatal Edition 98, F166-169.
Travadi J, Patole S and Simmer K 2003. Journal of Perinatal Medicine 31, 523-5.

Effect of straw provision fed to pre-weaning dairy calves on performance, rumen fermentation and blood metabolites

Michal Gasiorek[1], Barbara Stefanska[1], Ewa Pruszynska-Oszmalek[2], Marcin Taciak[3], Wlodzimierz Nowak[1]

[1]Poznan University of Life Sciences, Department of Animal Nutrition, Poznan, Poland, [2]Poznan University of Life Science, Department of Animal Physiology and Biochemistry, Poznan, Poland, [3]The Kielanowski Institute of Animal Physiology and Nutrition Polish Academy of Sciences, Jabłonna, Poland

E-mail: barbaras@up.poznan.pl

Take home message Chopped wheat straw inclusion to starter concentrate (10%) in pre-weaning calves improved ruminal fermentation and blood BHBA concentration without affecting performance.

Introduction Physical and metabolic development of the rumen in young calves is the prerequisite to their transition from a pre-ruminant milk diet to a solid diet based on grain forage. During recent years, several studies have attempted to define the most appropriate solid diet for calves during the pre-weaning period (Castells *et al*., 2012). However, there is no agreement on optimum forage feed strategies for pre-weaned calves. It was assumed that the effect of the addition of roughage will be of crucial importance in the development of the rumen and fermentation process as well as performance. Recent studies have demonstrated positive affects feeding forage (straw, hay) to calves in preweaning period on feed consumption, growth performance (Imani *et al*., 2017), rumen development (Castells *et al*., 2013), rumen pH (Laarman and Oba, 2011) and preventing the occurrence of subacute ruminal acidosis (Terre *et al*., 2013). The aim of the study was to investigate the effect of different level of chopped wheat straw provision to calf starter feed on productive performance, rumen fermentation and blood metabolites during the pre-weaning period of dairy calves.

Material & methods Forty four Holstein male dairy calves were divided into 4 groups: CON (control, without straw, n=11), LS10 (low straw: 10 %, n=11), MS15 (medium straw: 15 %, n=11), HS20 (high straw: 20 %, n=11). During the experiment (from 4 to 56 d), the calves received 3 x 2L (150 g of powder/L) of milk replacer daily. All starter feeds were iso-nitrogenous (20 % CP) and were fed *ad libitum*. Productive performance such as body weight, average daily gain, starter intake and body measurements (heart girth; hip height) were recorded weekly. Rumen fluid was sampled 3 to 6 h after the morning feeding through a stomach tube on days 28 and 56. Ruminal fluid samples were analyzed pH value, concentrations of volatile fatty acids (VFA) and $N\text{-}NH_3$ and microbial analysis such as the total count of bacteria and protozoa (*Entodiniomorpha* and *Holotricha*). Blood samples were collected from the jugular vein on days 1, 28, 56 for determination concentrations of total protein (1d), blood urea nitrogen (BUN), β-hydroxybutyrate (BHBA), non-esterified fatty acids (NEFA) and insulin-like growth factor-I (IGF-I). The results obtained were analysed using the SAS computer software SAS v 9.4 (2014).

Results In the LS10 group, the highest ADG during 29-56d and whole experimental period ($P \leq 0.05$) compared to MS15 and HS20 were observed. In the same group, the highest growth measured in hip height during the 29-56d period was found. The inclusion of wheat straw (10%) to calf starter feed positively affected ruminal fluid pH, concentrations of acetate and butyrate acids ($P \leq 0.05$) and the total counts of bacteria and protozoa (*Entodiniomorpha* and *Holotricha*). The highest concentration of BHBA ($P \leq 0.05$) in blood in LS10 group could be associated with better rumen papillae development.

Conclusion Chopped wheat straw calf starter supplementation (10%) improved ruminal fermentation and blood BHBA concentration without affecting daily gain and starter dry matter intake compared to the control group. The highest level of straw in the starter (20%) feed decreased daily gain, growth and starter intake.

References

Castells L, Bach A, Araujo G, Montoro C and Terré M 2012. Journal of Dairy Science 95, 286–293.
Castells L, Bach A, Aris A and Terré M 2013. Journal of Dairy Science 96 ,5226–5236
Imani M, Mirzaei M, Baghbanzadeh-Nobari B and Ghaffari MH 2017. Journal of Dairy Science 100,1136–1150.
Laarman AH and Oba M 2011. Journal of Dairy Science 94, 5661–5664.
Terré M, Pedrals E, Dalmau A and Bach A 2013. Journal of Dairy Science 96, 5217-25.

Colostrum and calf serum IgG concentrations on commercial pasture-based Irish dairy farms

John Barry[1,2], Eddie Bokkers[2], Imke de Boer[2], Emer Kennedy[1]

[1]*Animal & Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co Cork, Ireland,* [2]*Wageningen UR Livestock Research, Wageningen University, PO Box 338, 6700 AH, Wageningen, The Netherlands*

E-mail: john.barry@teagasc.ie

Take home message Measuring colostrum quality on commercial farms is essential, as its quality has a large influence on calf welfare. Although data from commercial Irish dairy farms shows large within, and between, farm variability in colostrum quality, high blood serum IgG concentrations were found among calves on farms. This indicates colostrum management standards are good, which contributes to good welfare.

Introduction Poor colostrum management can negatively affect welfare, predisposing calves to an increased risk of morbidity and mortality (Weaver *et al*., 2000). Colostrum, the first mammary secretion following parturition, can provide immunologically naïve calves with passive immunity immediately after birth (Barrington and Parish, 2001). Welfare can be compromised when there is a failure to achieve adequate passive transfer of immunity (24 hr serum immunoglobulin G (IgG) < 10mg/ml), which occurs if colostrum quality is low (< 50 mg/ml IgG), or when a sufficient quantity is not received in a timely manner (Godden, 2009). As Irish herd sizes increase following milk quota removal, colostrum management practises could be modified to facilitate management of increasing calf numbers, which could affect calf welfare. The aim of this study was to evaluate the quality of colostrum produced by cows in pasture-based production systems, and investigate serum IgG concentrations among calves on commercial Irish dairy farms, of different herd sizes.

Material & methods Pasture-based commercial Irish dairy herds (n=47), operating spring calving systems and a herd size of > 70 cows, were enrolled in this study. Farms were located in the Munster region of Ireland, and each farm was visited on two separate occasions. Visit one was early in the calving season, while visit two occurred in the latter half of the calving season. During both visits, calves >24 hrs and < 6 days old were identified and blood samples were taken from the jugular vein using a plain serum tube (10 ml BD Vacutainer®, BD, Belliver Industrial Estate, Plymouth PL6 7BP. UK). During visit one, farm managers were given containers and requested to collect 100 ml of colostrum from six cows of their choice. Samples were then frozen and collected during the second visit. Colostrum and serum samples were analysed in duplicate, using radial immunodiffusion kits (Triple J Farms, Bellingham, Washington, USA). Variables associated with colostrum and serum IgG concentration were investigated using a mixed model (PROC MIXED, SAS Institute Inc., Cary, NC, Version 9.1). Visit number was included as a repeated measure. Herd sizes were categorised from 1 to 4, where 1 = 74-107 cows; 2 = 110-138 cows; 3 = 140-189 cows, and 4 = 210-385 cows.

Results & discussion Mean colostrum IgG concentration was 85 (SD 53.1) mg/ml, ranging from 4 to 325 mg/ml. Immunoglobulin G concentrations of < 50 mg/ml were identified in 21.5 % of colostrum samples, across 22 farms. Herd size category (P=0.84) and calving commencement week of year (P=0.25) had no effect on colostrum IgG concentration. Mean serum IgG concentration was 30 (SD 14.2) mg/ml and ranged from 0 to 86 mg/ml. The proportion of samples with < 10 mg/ml IgG was 8.5 %. Herd size category had an effect on serum IgG concentration (P<0.05), where category one had higher mean serum IgG concentrations (32 mg/ml) than category four herds (24 mg/ml; P<0.01). No differences were found between all other categories of herd size. Farm visit (P<0.05) had an effect on serum IgG concentration, IgG concentration was higher in visit one (31 (SD 13.9) mg/ml) than in visit two (30 (SD 14.2) mg/ml; P<0.05). Calving commencement week of year had no effect on serum IgG concentration (P=0.09).

Conclusion Large variation exists in quality of colostrum produced by cows in commercial Irish dairy herds, with approximately 20 % being unsuitable for feeding. Adequate passive transfer of immunity was achieved by > 90 % of calves tested on Irish dairy farms, and while above the threshold, the level achieved is influenced by herd size, and time point of birth in the calving season.

Acknowledgements The authors acknowledge funding from the Teagasc Walsh Fellowship Scheme and Dairy Research Ireland.

References

Barrington GM and Parish SM 2001. Veterinary Clinics of North America: Food Animal Practice 17, 463-476.
Godden SM, Haines DM, Konkol K and Peterson J 2009. Journal of Dairy Science 92, 1758-1764.
Weaver DM, Tyler JW, VanMetre DC, Hostetler DE and Barrington GM 2000. Journal of Veterinary Internal Medicine 14, 569-577.

Young Merino sheep from genetically fatter sires are more able to cope with sub-optimal nutrition

Andrew Thompson[1], Mark Ferguson[1,2], Gavin Kearney[3], Darren Gordon[4]

[1]*Murdoch University, Murdoch, WA, Australia,* [2]*neXtgen Agri Ltd, St Martins, Christchurch, New Zealand,* [3]*36 Payne Road, Hamilton, VIC, Australia,* [4]*Livestock Logic, Hamilton, VIC, Australia*

E-mail: andrew.thompson@murdoch.edu.au

Take home message Increasing genetic propensity for fat can improve the ability of young sheep to tolerate sub-optimal nutrition.

Introduction Improving the survival of young sheep between birth and 12 months of age is a priority for the sheep industry in Australia (Young *et al.* 2014). Managing ewe nutrition during pregnancy to increase the birth weight of twin born lambs, and maximising lamb growth rates to weaning and during the post-weaning period, are key strategies to improve lamb and weaner survival (Oldham *et al.* 2011; Thompson *et al.* 2011). However, achieving these nutritional targets is challenging in environments and years characterised by increasing variability in feed supply between seasons. Improving the genetic capacity of sheep to tolerate this variability will inevitably become more important with climate change, and this will include defining traits to identify Merino sheep that are more robust and that produce more progeny with higher survival rates both pre- and post-weaning. Increasing genetic fat is the prime candidate for increasing the robustness of Merino ewes and their progeny, and we tested the hypothesis that young weaner sheep from sires with higher genetic fat will be more likely to survive the post-weaning period.

Material & methods Merino weaners (n = 1,312) were sourced from the two experiments in south east Australia reported by Ferguson *et al.* (2011), Oldham *et al.* (2011) and Thompson *et al.* (2011). In brief, these weaners were generated by artificial insemination of Merino ewes using semen from 24 Merino rams with known Australian Sheep Breeding Values for fatness measured at yearling age (YFat; range -1.1 to +1.3 mm). The ewes were differentially fed on 30 plots with varying amounts of feed on offer during pregnancy and until the lambs were weaned at 12-13 weeks of age. The weaners were then managed together post-weaning, weighed every 1-2 months and any deaths were recorded up until 12 months of age. All statistical analyses were performed using GENSTAT. Weaner survival was assessed by fitting General Linear Mixed Models. The approach used a logit-transformation and binomial distribution. Using additive models, logits were predicted as a function of different variables, sire YFat, weaning weight (WWT), birth type and sex. Year, replicate (nested within year) and plot (nested within replicate) were fitted as random effects.

Results Weaning weight for the different nutritional treatments varied from 14.9 to 20.3 kg and 13.8 to 20.0 kg in the two experiments, and WWT was not significantly influenced by sire YFat. There as a significant effect of WWT and sire YFat on weaner survival (Fig. 1). Survival was greater for weaners from sires with higher fat, and the effects of YFat on survival were greater at lower weaning weights. In practical terms, 15 kg weaners from sires with a YFat of +1 mm achieved similar survival rates as 20 kg weaners from sires with YFat of -1 mm (92.8 *vs.* 92.1%). The average survival of ewe weaners was significantly greater than the survival of wether weaners (91.0 vs 87.9%; P<0.001), and the effects of sire YFat on survival was similar for both ewes and wether weaners.

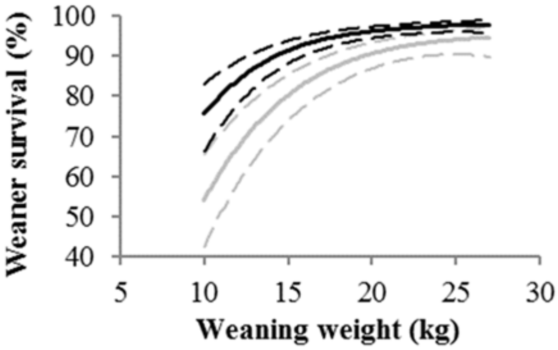


Figure 1 The relationship between weaning weight and post-weaning survival for wether weaners from sires with breeding values for yearling fat of + 1 mm (black) and - 1 mm (grey). Dash lines represent 95% confidence limits.

Conclusion These findings indicate that selection for increased fatness could improve weaner survival in Merino sheep. A more recent analysis has confirmed a significant genetic correlation between genetic fat and weaner survival (Walkom *et al.* 2018). In the current study the effects of sire YFat on survival were greater for lighter weaners, which are more typical of late lambing flocks and poorer seasons which are expected to become more frequent with climate change. Increasing sire Yfat has small effects on GR tissue depth and estimated carcass value (Ferguson *et al.* 2010), such that if new methods for valuing carcasses do result in penalties for high Yfat animals then the discounts would need to be large to counteract the positive effects of extra fat on survival of Merino weaners.

Acknowledgements This work was funded by Australian Wool Innovation Limited, Department of Primary Industries, Victoria and Department of Agriculture and Food, Western Australia.

References

Ferguson M, Young J, Kearney G, Gardiner G, Robertson I and Thompson A 2010. Animal Production Science 51, 866-872.
Ferguson M, Thompson A, Gordon D, Hyder M, Kearney G, Oldham C and Paganoni B 2011. Animal Production Science 51,763-775.
Oldham C, Thompson A, Ferguson M, Gordon D, Kearney G and Paganoni B 2011. Animal Production Science 51, 776-783.
Thompson A, Ferguson M, Campbell A, Gordon D, Kearney G, Oldham C and Paganoni B 2011. Animal Production Science 51, 784-793
Walkom S, Thompson A, Bowen E and Brown D 2018. Animal Production Science (in press).
Young J, Trompf J and Thompson A 2014. Animal Production Science 54, 645-655

Association between the number of consecutive days with heat stress and milk production of Holstein dairy cows in Eastern Canada

Veronique Ouellet[1], Victor Cabrera[2], Liliana Fadul-Pacheco[4], Patrick Grenier[3], Edith Charbonneau[1]

[1]*Departement des sciences animales, Universite Laval, Quebec, Quebec, Canada,* [2]*Department of dairy science, University of Wisconsin-Madison, Madison, Wisconsin, USA,* [3]*Ouranos Consortium, Montreal, Quebec, Canada,* [4]*Valacta, Saint-Anne-de-Bellevue, Canada*

E-mail: veronique.ouellet.6@ulaval.ca

Take home message The duration of heat stress has a corresponding negative impact on milk production of Holstein dairy cows in Eastern Canada.

Introduction Most heat stress (HS) research has been conducted in environments where animals are subjected to high temperature-humidity index (THI) over a long period. Thus far, little attention has been paid to HS effects on dairy cows in areas characterized by cooler yearly average temperatures, and large seasonal variations. With higher temperatures arising from climate change, HS will become more widespread with growing concern in regions actually characterized by cold continental climates (*e.g.*, Eastern Canada). Therefore, establishing actual effects of HS on dairy cows in cooler climates may help dairy producers to better prepare for the future. Our objective was to examine the relation between the number of consecutive days with HS and daily milk, fat, and protein yields (kg/d), and milk composition (fat, and protein %) in Holstein dairy cattle located in Eastern Canada.

Material & methods The initial data set used in this study comprised 606,031 test-day (TD) records from 16,446 Holstein dairy cows of different lactations (2.7 ± 1.7). Weather data (2010-2015) were retrieved from meteorological stations. Daily maximum temperature-humidity index was calculated using maximum ambient daily temperature (AT_{max}, °C) and average relative humidity (RH, %) following the equation reported by Kendall *et al.*, (2008) ($THI_{max} = (1.8 \times AT_{max} + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times AT_{max} - 26)]$) and merged with corresponding TD records. This was also done for the 16 d prior to TD. Consecutive day with HS were calculated from the TD to 7 days prior to TD and added to the data set. Regression analyses were conducted on these data with THI_{max} in the days before the TD as fixed effects, and DIM and genetic value as covariates. Consecutive days were defined as the number of successive days with a $THI_{max} > 65$. Number of consecutive days were then grouped in categories: 0 = no days with HS; 1 = 1 or 2 d with HS; 2 = 3 or 4 d with HS; 3 = 5 or 6 d with HS; 4 = 7 or 8 d with HS. Effects of the these categories on TD milk production and composition were assessed using the MIXED procedure of SAS. Fixed effects included in the model were DIM, exp DIM (calculated as $e^{-0.05DIM}$), genetic value, and categories of consecutive days with HS. Random effects were the herd nested within the year of calving, the interaction between calving month and the herd nested in the year of calving, the animal nested within the interaction between the herd, the year of calving, and the calving month.

Results & discussion Cows subjected to more consecutive days with heat stress ($THI_{max} > 65$) had lower ($P < 0.05$) ECM, fat, and protein yields (kg/d), and fat and protein milk % compared to cows subjected to conditions not susceptible to induce HS (Tables 1 and 2). Moreover, cows of third and higher lactations were more vulnerable to HS with higher milk performance decreases due to HS.

Table 1 Effects of number of days with heat stress categories on milk performance of cows of first lactation.

	First Lactation					
Variable[1]	0	1	2	3	4	*SE*
ECM (kg/d)	27.6^{a}	27.5^{b}	27.2^{c}	27.2^{c}	27.0^{d}	0.08
Fat (%)	4.09^{a}	4.03^{b}	3.96^{c}	3.95^{c}	3.90^{d}	0.007
Fat (kg/d)	1.11^{a}	1.10^{b}	1.09^{c}	1.08^{c}	1.07^{d}	0.003
Protein (%)	3.34^{a}	3.31^{b}	3.29^{c}	3.27^{d}	3.25^{e}	0.004
Protein (kg/d)	0.91^{a}	0.90^{b}	0.90^{b}	0.90^{b}	0.89^{c}	0.003

Table 2 Effects of number of days with heat stress on milk performance of cows of third plus lactation.

	Third plus lactation					
Variable[1]	0	1	2	3	4	*SE*
ECM (kg/d)	31.9^{a}	31.6^{b}	31.6^{b}	31.3^{c}	31.1^{d}	0.06
Fat (%)	4.10^{a}	3.98^{b}	3.91^{c}	3.89^{d}	3.85^{e}	0.01
Fat (kg/d)	1.27^{a}	1.25^{b}	1.25^{b}	1.23^{c}	1.22^{d}	0.006
Protein (%)	3.36^{a}	3.31^{b}	3.28^{c}	3.26^{d}	3.23^{e}	0.005
Protein (kg/d)	1.05^{a}	1.05^{a}	1.05^{a}	1.04^{b}	1.02^{c}	0.004

$^{a–e}$Means within the same parity group with different superscripts are significantly different ($P \leq 0.05$).

Conclusion The greater the number of consecutive days with a $THI_{max} > 65$ (heat stress) the more detrimental are the effects on dairy cows' productivity. Establishing actual effects of heat stress on dairy cows' milk performances in cooler climates may help dairy producers to better adapt to climate change and to develop appropriate management countermeasures.

Acknowledgements Ouranos is a scientific and financial partner to this project. The project was funded by the Fonds vert from *Plan d'action 2013-2020 sur les changements climatiques* of the Québec government.The author received a scholarship from the Natural Sciences and Engineering Research Council of Canada.

References

Kendall PE, Tucker CB, Dalley DE, Clark DA and Webster JR 2008. Livestock Science 117, 130–138.
Wheelock JB, Rhoads RP, Vanbaale MJ, Sanders SR and Baumgard LH 2010. Journal of Dairy Science 93, 644–655.

Lactational effects of shearing two breeds of dairy ewes under Mediterranean mild-winter conditions

Abdelaali Elhadi[1], Gerardo Caja[1], Ahmed A. K. Salama[1,2], Xavier Such[1], Elena Albanell[1]

[1]*Group of Research in Ruminants (G2R), Department of Animal and Food Sciences, Universitat Autonoma de Barcelona, Bellaterra, Barcelona, Spain,* [2]*Department of Dairy Science and Food Science, South Dakota State University, Brookings, South Dakota, USA*

E-mail: gerardo.caja@uab.es

Take home message Shearing lactating ewes during winter is a suitable management option for improving feed intake and milk production of high-yielding dairy ewes.

Introduction Shearing before summer is considered to be a necessary practice for sheep management in order to improve their welfare and performances. Shearing increases the critical low temperature and induces adaptive responses to cope with homeostasis. When conducted during winter or early spring, the shorn sheep are exposed to cold-stress because of losing their wool insulation. Few studies have evaluated the specific effects of shearing during lactation in dairy ewes (Aleksiev, 2008; Rassu *et al*., 2009), reporting inconsistent changes on feed intake and milk composition which varied according to experimental conditions. The aim of this study was to evaluate the lactational effects of shearing of 2 breeds of dairy ewes, differing in milk potential, during mid-lactation and under Mediterranean mild-winter conditions.

Material & Methods A total of 48 dairy ewes of 2 breeds (Manchega, MN; Lacaune, LC), with similar BW (MN *vs.* LC, 66.1 ± 1.6 *vs.* 65.2 ± 1.9 kg BW) and BCS (2.5 ± 0.1 *vs.* 2.2 ± 0.1) in mid lactation (83 ± 7 *vs.* 87 ± 8 DIM), respectively, were allocated in 8 groups, to which the treatments were applied in duplicate. Treatments were: CO (control, unshorn from previous May) and SH (shorn in February). Ewes were in pens of 6, fed alfalfa hay (ad libitum) and concentrate (15.2% CP, DM basis) at a flat rate (MN *vs.* LC, 0.45 *vs.* 0.65 kg/d as fed). Shelter temperature was monitored with a data logger (Opus 10, Lufft, DE). Milk yield was recorded at each milking (×2-daily) by electronic boluses (20 g; Datamars, ES) and milk meters (MM25, De Laval, SE). Milk was sampled for composition (NIR system; Foss, DE) and blood for plasma metabolic indicators, on d −7, −3, 3 and 7. Data were analyzed by the PROCMIXED of SAS v.9.4 (SAS Inst. Inc., Cary, USA).

Results & discussion Shelter temperatures were mild and did not vary before (12.6 ± 0.9°C) and after (13.0 ± 0.3°C) shearing. Shorn fleece was heavier in MN than LC (1.04 ± 0.10 *vs.* 0.75 ± 0.09 kg/ewe; $P = 0.009$). Rectal temperature decreased by shearing in MN (MN-CO *vs.* MN-SH, 38.88 ± 0.12 *vs.* 38.51 ± 0.11°C; $P < 0.001$), but did no vary in LC ewes (38.57 ± 0.08°C; $P = 0.89$). Lactational responses varied by breed, the results being more marked in LC than in MN. Shearing increased feed intake by 5% (2.67 ± 0.04 *vs.* 2.80 ± 0.07 kg DM/d; $P = 0.005$) and milk yield by 10% (1.32 ± 0.06 *vs.* 1.45 ± 0.05 kg/d; $P = 0.025$) in LC ewes, but did not vary in MN ewes (2.44 ± 0.02 kg DM/d, $P = 0.39$; 0.74 ± 0.03 kg/d, $P = 0.26$), as shown in Figure 1. No differences in milk composition, BW, BCS and metabolic and hormonal indicators were shown (Table 1), but LC-SH yielded more milk protein (9%; $P = 0.004$) and lactose (10%; $P = 0.012$) than LC-CO ewes.

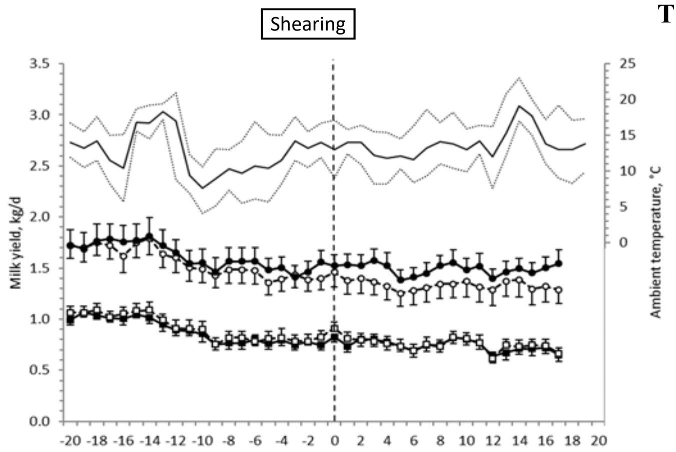


Figure 1 Ewe's milk yield before and after shearing. (○, LC-CO; ●, LC-SH; □, MN-CO; ■, MN-SH).

Table 1 Effect of shearing on blood metabolites and hormones.

Breed	Item	Shearing Before	 After	SEM[1]	Effect (*P* =)
LC[2]	Insulin, µg/L	0.43	0.44	0.08	0.893
	Cortisol, ng/ml	6.54	5.41	0.74	0.293
	NEFA, mmol/L	0.125	0.095	0.014	0.119
	Glucose, mg/dL	65.7	66.2	1.11	0.765
MN[3]	Insulin, µg/L	0.34	0.40	0.05	0.388
	Cortisol, ng/ml	5.64	3.81	1.13	0.268
	NEFA, mmol/L	0.115	0.100	0.008	0.172
	Glucose, mg/dL	63.2	65.2	0.66	0.050

[1] Standard error of the mean; [2] Lacaune; [3] Manchega.

Conclusion Shearing lactating ewes during a mild-winter was a suitable practice for improving management, feed intake and milk production of high-yielding dairy ewes, without deleterious effects on milk composition and on mid-yielding ewes.

Acknowledgements Spanish Ministry of Economy and Competitiveness (Project AGL2013-44061-R).

References

Aleksiev Y 2008. Bulgarian Journal Agricultural Science 14, 87−92.
Rassu SPG, Manca MG, Dias AH, Mereu A and Nudda A 2009. Italian Journal Animal Science 8 (Suppl. 2), 462.

Metabolic responses to heat stress of Lacaune dairy ewes in late lactation

Nabil Mehaba, Wellington Coloma, Ahmed Salama, Xavier Such, Gerardo Caja

Autonomous University of Barcelona, arcelona, Spain

E-mail: nabil-bra@hotmail.fr

Take home message Lactating dairy ewes submitted to heat stress conditions attempt to maintain their glycemia and body reserves by being less sensitive to lipogenic and lipolytic signals.

Introduction Countries bordering the Mediterranean Sea are characterized by exposure to considerable heat from 3 to 6 mo annually, reaching heat stress (HS) conditions for dairy sheep (Finocchiaro *et al*., 2005). Lacaune ewes are high yielding dairy animals that may be more sensitive than other Mediterranean sheep to HS. Hamzaoui *et al*. (2013) studied the effects of thermoneutral (TN) and HS conditions in dairy goats, but there is little research conducted in dairy sheep. This study aims to assess the physiological effects of HS on lactating Lacaune dairy ewes.

Material & Methods Four multiparous Lacaune ewes (75.5 ± 3.2 kg BW) in late-lactation (165 ± 4 DIM; 2.31 ± 0.04 L/d) were individually penned and sequentially submitted to TN and HS conditions (2×21 d periods, 6 d washout) with 12-12 h day-night schedule. Conditions and temperature-humidity index (THI; NRC, 1971) were: TN (indoors, 15 to 20ºC, 50 ± 5% HR; THI = 59 to 65) and HS (Climatic chamber 4 × 6 × 2.3 m, Eura, FI; day, 35ºC and night, 28°C, 45 ± 5% HR, THI = 75 to 83). Ewes were milked twice daily and fed a TMR *ad libitum* according to their requirements. Silicon catheters (Vygon, FR) were fitted into the jugular on d 15 and the ewes randomly submitted to glucose (GLU) tolerance test (GTT, 0.25 g/kg BW) and lipogenic (insulin, 4.6 µg INS/kg BW) and lipolytic (epinephrine, 2 µg/kg BW) hormonal challenges on d 17, 19 and 21 of each period. Blood samples were collected at 12-time points (min −30 to 120) in respect to jugular infusions. Plasma GLU was analysed by hexokinase (Olympus, Krefeld, IE), NEFA (non-esterified fatty acids) by enzymatic colorimetric (Wako, Neuss, DE) methods and INS by ELISA immunoassay for ovine-INS (Mercodia, Uppsala, SE). Data were analysed by the PROC MIXED of SAS v.9.4 (SAS Inst. Inc., Cary, USA).

Results & discussion INS responses of TN and HS ewes to GLU infusion during GTT are shown in Figure 1a. Basal and final INS values were similar in both ewe groups and peaked at min 10, although HS ewes tended to show higher INS levels than TN from min 5 to 30 min ($P = 0.09$). No differences between TN and HS ewes were detected in the GLU responses to INS challenge (Figure 1b), the level of GLU decreasing after INS infusion and reaching a similar level at min 30 (33.9 ± 2.4 mg/dL; $P < 0.001$). Thereafter, GLU concentration increased gradually until min 120. Furthermore, on average, the lipogenic response of NEFA to the INS challenge was lower in HS than in TN ewes (0.24 ± 0.03 *vs.* 0.36 ± 0.03 mmol/L, respectively; $P = 0.032$), whereas the lipolytic response of NEFA to the epinephrine challenge was greater in TN than HS ewes, the difference being observed from min 5 to 45 ($P < 0.001$).

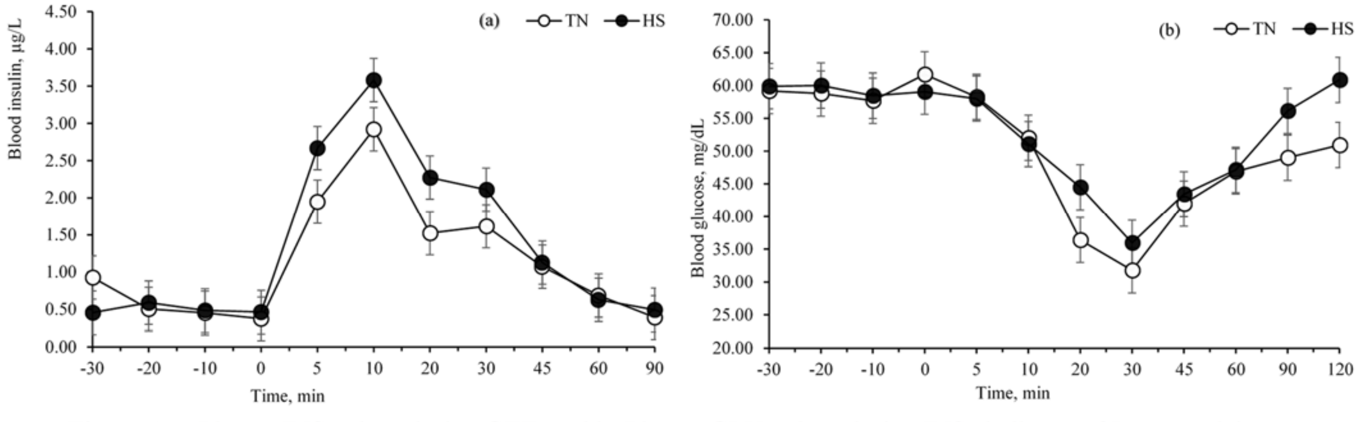


Figure 1 a) Plasma INS values during GTT, and b) Plasma GLU values during INS challenge of Lacaune dairy ewes at late lactation under TN and HS conditions. Values are means and standard errors for 4 ewes in each treatment.

Conclusion HS ewes tended to be less sensitive to INS for GLU uptake, and less responsive to catecholamines for fat mobilization, than TN ewes. This will result in more difficulties to cope with the energy restriction imposed by the decrease of feed intake induced by episodes of HS.

Acknowledgements Funded by the Ministry of Economy and Competitiveness of Spain (Project AGL2013-44061-R) and supported by a grant for early-stage research staff training to Nabil Mehaba from the Catalonian Government.

References

Finocchiaro R, van Kaam JB, Portolano B and Misztal I 2005. Journal Dairy Science 88, 1855-1864.
Hamzaoui S1, Salama AA, Albanell E, Such X and Caja G 2013. Journal Dairy Science 96, 6355-6365.

Metabolomic assessment of urinary biomarkers in heat stressed lactating dairy goats showed signs of gastrointestinal dysbiosis

Alexandra Contreras-Jodar[1], Nazri Nayan[2], Soufiane Hamzaoui[1], Gerardo Caja[1], Ahmed A. K. Salama[1,3]

[1]Group of Research in Ruminants, Departament of Animal and Food Sciences, Universitat Autonoma de Barcelona, Bellaterra, Barcelona, Spain, [2]Department of Animal Science, Faculty of Agriculture, University Putra Malaysia, Serdang, Malaysia, [3]Dairy and Food Science Department, South Dakota State University, Brookings, South Dakota, USA

E-mail: gerardo.caja@uab.es

Take home message Heat stress reduced the availability of Phenylalanine (Phe) and Tyrosine (Tyr) because of their catabolism by harmful gastrointestinal microbiota, as revealed by changes in the urinary metabolomic profile. This decrease compromised the synthesis of levodopa and thyroid hormones for thermoregulation and milk production of dairy goats.

Introduction Exposure of dairy animals to heat stress (HS) results in a decline of their productive and reproductive performances due to strong metabolic disruptions. Dairy animals respond to HS by increasing water consumption, thermoregulation traits (*i.e.*, respiratory rate and rectal temperature) and reducing feed intake, presumably in an attempt to reduce their metabolic heat production (Salama *et al*., 2016). Nevertheless, the reduction of dry matter intake only accounts for nearly half of the decline of milk yield in dairy cows (Rhoads *et al*., 2009). Therefore, there are specific pathways of the effects of HS in dairy ruminants that remain misunderstood. The objective of the current study is to compare the metabolomic changes of HS *vs.* thermoneutral (TN) lactating dairy goats through the application of ^{1}H Nuclear Magnetic Resonance (NMR) Spectroscopy in urine.

Material & Methods Sixteen adult Murciano-Granadina dairy does in mid-lactation (43.1 ± 1.5 kg BW; 90 ± 2 DIM; 2.00 ± 0.04 L/d) were randomly allocated to TN or HS conditions according to a cross-over design consisting of periods of 19 d. Does were in metabolic cages, submitted to 12-12 h day-night schedule, and fed and milked once-a-day. Ambient conditions and temperature-humidity index (THI; NRC, 1971) were: TN (indoors, 15 to 20ºC, 40 to 45% HR; THI = 59 to 65), and HS (Euroshield climatic chamber 4 × 6 × 2.3 m, Eura, FI; day, 37ºC and night, 30ºC; 40% humidity; THI = 86 and 77, respectively). Urine samples were collected at micturition on d 19 and analyzed by ^{1}H-NMR spectroscopy (600 MHz and 298°K; Bruker BioSpin, Rheinstetten, DE). Multivariate data analyses included PCA (principal component analysis) and PLS-DA (partial least square–discriminant analysis) assessment with cross validation to identify urinary biomarkers by using R v.3.2.3 software. Metabolites were identified using the Human Metabolome Data Base (www.hmdb.ca).

Results & discussion PLS-DA allowed separating TN- and HS-doe clusters. Most discriminating metabolites were Phe derivative compounds, such as OH-phenylacetate, OH-phenylacetylglycinate, phenylglyoxylate and hippurate, which increased in HS *vs.* TN does ($P < 0.01$). A greater urinary excretion of these toxic compounds indicated that HS induced a harmful gastrointestinal microbiota overgrowth, which sequestrates dietary Phe and Tyr for their metabolism. Consequently, the scarce Phe and Tyr in blood decreased the synthesis of levodopa and thyroid hormones, with negative effects on milk yield and composition of the goats.

Conclusion Heat stress caused significant changes in the urine metabolomic profile of dairy goats. These changes were related to an over-excretion of toxic compounds generated by gastrointestinal microbiota and decreases in the bioavailability of Phe and Tyr, which impaired the synthesis of levodopa and thyroid hormones and compromised milk production of dairy goats. On the other hand, further studies are needed to validate these candidate biomarkers with larger number of animals and under different conditions and thus, could potentially be applicable as a novel strategy for the non-invasive diagnosis of heat-stressed animals as well as the establishment of alleviation strategies for gastrointestinal dysbiosis.

Acknowledgements Work funded by the Ministry of Economy and Finance (Project AGL2009-09376) and supported by a research scholarship to Alexandra Contreras-Jodar from the Ministry of Economy and Competitiveness (BES-2012-052602) of Spain.

References

Hamzaoui S1, Salama AA, Albanell E, Such X and Caja G 2013. Journal Dairy Science 96, 6355-6365.

Rhoads ML1, Rhoads RP, VanBaale MJ, Collier RJ, Sanders SR, Weber WJ, Crooker BA and Baumgard LH 2009 Journal Dairy Science 92, 1986-1997.

Salama AAK, Caja G, Hamzaoui S, Badaoui B, Castro-Costa A, Façanha DE, Guilhermino MM and Bozzi R 2014. Small Ruminant Research 121, 73-79.

Salama AAK, Caja G, Hamzaoui S, Such X, Albanell E, Badaoui B and Loor JJ 2016. Chap. 2. Thermal stress in ruminants: Responses and strategies for alleviation. In: Animal Welfare in Extensive Systems. J. Villalbaand X. Manteca (eds.). The Animal Welfare Series, 5M Publishing, Sheffield, UK, pp. 11-34.

Contrasting the metabolic endocrine responses of beef steers under moderate thermal challenge with pair fed thermoneutral counterparts

Gene Wijffels[1], Megan Sullivan[2], Sally Stockwell[1], Susan Briscoe[1], Cinita de Mela Costa[1,3], Russell McCulloch[1], Judy Cawdell-Smith[2], Joesph Olm[2], Stephen Anderson[2], John Gaughan[2]

[1]CSIRO Food and Agriculture, Qld, Australia, [2]The University of Queensland, Qld, Australia, [3]UNESP, Sao Paulo, Brazil

E-mail: gene.wijffels@csiro.au

Take home message Even a moderate heat load can induce distinctive and persistent metabolic endocrine changes.

Introduction One of the most obvious impacts of increased heat load in cattle is a reduction of feed intake and as a consequence reduced productivity. This phenomenon has confounded attempts to elucidate the metabolic and endocrine responses unique to animals coping with high heat load. Progress has been made by using a model whereby control animals are maintained in thermoneutral conditions but are feed restricted or pair fed in correspondence with the reduced feed intake of their thermally challenged counterparts. Here we examined beef steers exposed to a moderate heat challenge.

Material & methods Two replicates of twelve Black Angus steers (~500 kg) backgrounded on a feedlot finisher ration were randomly allocated to a Control group (n=6) or a HOT group (n=6). The animals were housed in climate controlled rooms at the Queensland Animal Science Precinct (Gatton, Australia) for 19 d followed by 10 d in outdoor pens. The Control steers were maintained in thermoneutral conditions throughout the 19 d and were pair fed based on the refusals of the HOT group. The HOT steers were subjected to 3 climatic periods with daily cyclic temperature and humidity regimes as outlined in Figure 1. The bleed schedule is also presented. Plasma was assayed for leptin, adiponectin, prolactin, T3, T4 and TSH concentrations using in-house and commercial reagents in antibody based assays. Plasma insulin was determined by a commercial IRMA (DiaSource). One-way ANOVAs and multiple t-tests indicated significant differences between treatment periods, and groups.

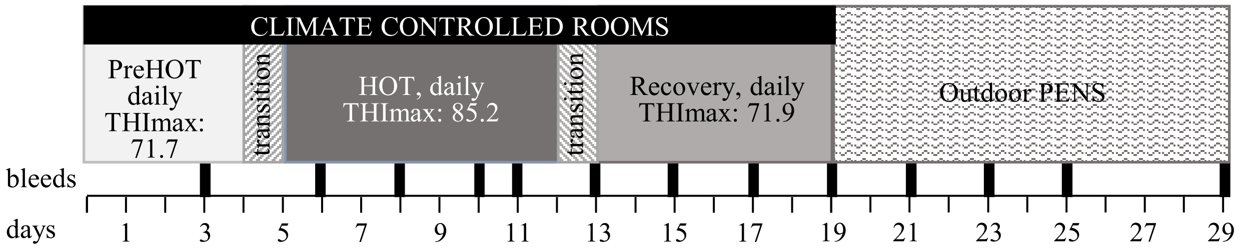


Figure 1 The climate regime, housing and bleed schedule.

Results & discussion The DMI of the HOT group was 33% lower under thermal challenge (HOT) and was still 18% lower during recovery relative to the PreHOT period. Significant changes in plasma concentrations of leptin, adiponectin, T3 and T4 were observed in both feed restriction (Control) and thermal challenge (HOT) relative to PreHOT concentrations (Table 1). Moreover, there were clear and significant differences in hormones responses between the two treatment groups. Plasma adiponectin levels were reduced in the HOT steers relative to the PreHOT baseline and compared to Controls for all periods. In contrast, plasma leptin was inhibited in response to feed restriction (Controls) and increased heat load but the suppression was dampened in the HOT steers. Plasma T3 and T4 responses were significantly different between treatments, but there were no significant changes in prolactin, insulin and TSH concentrations with feed restriction or heat load.

Table 1 Mean %change and direction of change relative to baseline (PreHOT).

Plasma analyte	Control – TN and feed restricted			HOT			One way ANOVA p-value*
	‘HOT’	Recovery	PENS	HOT	Recovery	PENS	
Adiponectin	3% ↑	17% ↓^{a}	21% ↓^{a}	19% ↓^{a}	3%↓	31% ↓^{c}	<0.0001
Leptin	27% ↓^{c}	28% ↓^{b}	47% ↓^{c}	17% ↓^{a}	15% ↓^{a}	35% ↓^{c}	<0.0001
Insulin	11% ↑	11% ↑	31% ↑^{a}	7% ↓	8% ↓	17% ↑	0.0039
T4	13% ↑^{a}	15% ↑^{a}	5% ↑	8% ↓	20% ↑^{b}	16% ↑^{b}	<0.0001
T3	34% ↑^{a}	42% ↑^{b}	18% ↓	4% ↓	16% ↓	9% ↓	<0.0001

*Superscripts indicate levels of significance: a, $p<0.05$; b, $p<0.005$; c, $p<0.0005$. *across all means*

Conclusion Plasma leptin, adiponectin and thyroid hormones concentrations exhibited altered responses to the impost of a moderate heat load and these response were distinct from those induced by feed restriction alone.

Acknowledgements This study was funded by Meat and Livestock Australia.

The dynamics of seasonal body conditions of Zebu Gobra cows, related to pastoral practices in Northern Senegal

Berenice Bois[1], Alexandre Ickowicz[1], Philippe Lecomte[1], Eliel Gonzalez-Garcia[2]

[1]*CIRAD, INRA, Montpellier SupAgro (Univ Montpellier), Montpeller, France,* [2]*INRA, CIRAD, Montpellier SupAgro (Univ Montpellier), Montpellier, France*

E-mail: berenice.bois@cirad.fr

Take home message In the Sahel, the body condition of the Zebu Gobra is mainly influenced by the seasons, but is also modulated by a set of other management, grazing or feeding practices adopted by the livestock keepers.

Introduction Rearing cattle in Senegal mainly relies on extensively managed natural pasturelands which impose seasonal feed shortages caused by fluctuating biomass availability and quality. *Pular* livestock keepers are specialised in livestock husbandry, whose practices are adapted to seasonal availability of natural forages. The *Pular* calendar comprises 5 seasons: *Ndugu* (July to September), *Kawlé* (October-November), *Dabundé* (December-February), *Ceedu* (March-May), *Ceetel* (May-June), which respectively correspond to: green abundant herbaceous forages, dry abundant herbaceous, dry herbaceous, dry scarce herbaceous, very scarce herbaceous and ligneous forages. In the local traditional transhumant (TRA) system, cows lose a lot of weight during the harshest periods. The first objective of this work was to get new insights into the progression of the body condition of cows over several consecutive seasons. The second objective was to evaluate the effects of incorporating alternative management and feeding practices on their liveweight (LW) and body condition score (BCS).

Material & methods Regular monitoring of Zebu Gobra cows (n = approx. 10 cows per system) was carried out in two contrasted systems (*i.e.,* TRA and intensified; INT) – each implying different management and feeding practices. The TRA is based on extensive grazing of natural grasses and ligneous forages, controlled access to drinking water and negligible feed supplementation (< 1 kg of cotton oilcake during the worst BCS periods of the cows). In contrast, the INT herders rent fields after harvests and buy some bran and cereals (rice and wheat) during *Ceedu* and *Ceetel*. A field visit every 2 months was carried out during 15 months (*i.e.,* from mid-July 2016 to late-October 2017). Measurements of individual LW and BCS were undertaken after hand milking early morning in cows equally distributed in 2 exploitations per system. The LW was estimated from replicated thoracic perimeter measures each visit (Dodo *et al.*, 2001). The BCS was estimated by visual assessment adapted from the scale proposed by Edmonson *et al.* (1989). R software was used for data processing.

Results & discussion. High intra-system variability between the cows monitored was observed for both parameters regardless of the herd or the system (Figure 1). Therefore, no statistical differences were detected throughout the whole experiment or within periods. The exception was a higher BCS of TRA cows (P<0.005) in October (*Kawlé*). However, as expected an overall tendency of decreasing cows' LW and BCS was observed during *Ceedu* and *Ceetel*, with an odd situation in August 2017 due to an interruption of early rains in June, leading to a break in the normal vegetation cycle and consequently a lack of forages (Figure 1). Globally, and contrarily to our initial hypothesis, a visual superiority of LW was observed in TRA cows. We speculate that rather than the feeding system *per se*, this was likely due to other factors such as particularities linked to the handling or management of the livestock. However, it must be taken into account that the dry seasons of 2016 and 2017 were not representative of harsh drought, thus we could speculate that in a more severe scenario, different results could be found.

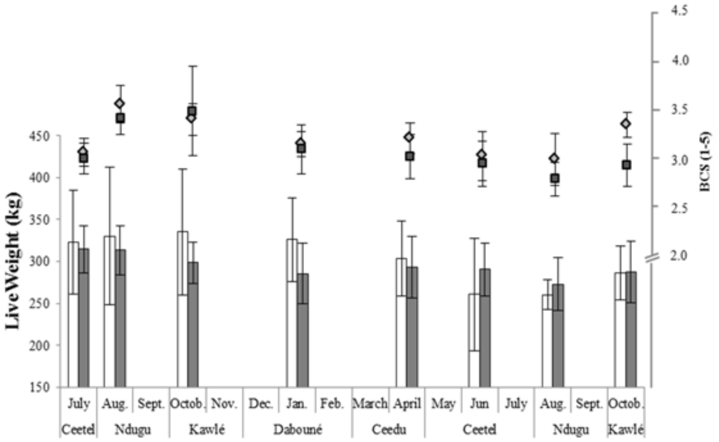


Figure 1 Seasonal dynamics of LW (bars) and BCS (dots) as affected by practices (TRA: white bars and diamond dots; INT: grey bars and square dots).

Conclusion The body condition of cows extensively reared under the conditions of the Northen Senegal is affected by typical factors such as seasons, and transitions through physiological status and their inherent energy balances. In addition, our results also suggest that livestock keeper skills and management of basal natural pastures and additional feed resources are key factors affecting the condition of the animals (verified by high variability between individuals).

Acknowledgments This Research was supported by CIRAD, INRA-SELMET Montpellier SupAgro (France) and the ISRA of Dakar (Senegal).

References

Edmonson AJ, Lean IJ, Weaver LD, Farver T and Webster G 1989. Journal of Dairy Science 72, 68–78.
Dodo K and Illiassou MS 2001. Rvue d'élevage et de Médicine Vétérinaire des Pays Tropicaux 54, 63-68.

Bone metabolism in growing cattle under severe heat stress

Stephen Anderson[1], Natasha Steiger[1], Megan Sullivan[2], Sally Stockwell[3], Russell McCulloch[3], Suzie Briscoe[3], Judy Cawdell-Smith[3], Joe Olm[2], John Gaughan[2], Gene Wijffels[3]

[1]*University of Queensland, St Lucia, Queensland, Australia,* [2]*University of Queensland, Gatton, Queensland, Australia,* [3]*CSIRO Agriculture and Food, St Lucia, Queensland, Australia*

E-mail: stephen.anderson@uq.edu.au

Take home message Excessive heat load adversely affects bone growth in steers.

Introduction Excessive heat load in feedlot beef cattle reduces feed intake, perturbs metabolism, and ultimately reduces growth. Bone growth appears to be affected by heat, with a reduction of the bone formation markers osteocalcin (OCN) and bone alkaline phosphatase (BALP) being observed in cattle under moderate heat stress (Kamiya *et al*. 2006; Anderson *et al*. 2017). Albeit the inhibition of OCN appears to be related to reduced feed intake rather than a direct effect of heat per se (Anderson *et al*. 2017). In the current study, we characterised bone metabolism in feedlot steers under severe heat stress.

Material & methods Angus yearling steers (599 ± 12 kg, n = 24) were placed in climate rooms with initial intake (as-fed) of 11.3 kg/hd/day of finisher ration. The cattle were fed twice daily at 0900h and 1300h. Climatic conditions commenced with 5 day adaptation period at thermoneutral (TN) with mean minimum 19.4 ºC and maximum 22.0 ºC temperatures (Temperature Humidity Index; THI 65-71). This was followed by HOT treatment with a daily temperature range from 26.2 to 39.6 ºC (THI 76-92) for Days 6 to 8, then 23.5 to 34.6 ºC (THI 71-86) for Days 9 and 10, then 20.2 to 30.5 ºC (THI 67-80) for Days 11 and 12. Lastly a 5 day recovery period at TN with temperatures 19.8 to 21.5 ºC (THI 65-69) for Days 13 to 17. Jugular blood was collected at 0730h and plasma stored at -80ºC. Plasma concentrations of bone formation markers OCN and BALP (MicroVue, Quidel) and bone resorption marker CTX-1 (Immunodiagnostic Systems) were determined by immunoassays, and plasma mineral concentrations by standard clinical assays (IDEXX). Four steers were removed due to ill-health and results from such animals are excluded from analyses. Significant differences were determined by repeat measures ANOVA and post-hoc Tukey's comparison test.

Results & discussion Steers exposed to severe heat exhibited significant ($P<0.05$) decreases in plasma BALP and OCN concentrations, together with increased CTX-1 concentrations (Table 1). Bone mobilisation therefore appears to predominate under 3 days of severe heat stress (THI 76-92). However, with a slight reduction in heat load (THI 71-86, Days 9 and 10) and likely physiological adaptations in animals, both OCN and CTX-1 concentrations on the morning of Day 11 had returned to values similar to those observed under TN conditions. In contrast, plasma BALP concentrations remained reduced even after a return to TN conditions (Day 15), indicative of ongoing defective bone mineralisation. Plasma mineral concentrations, exhibited small but significant ($P<0.05$) changes during hot conditions (Table 1). A decrease in Ca was observed during the entire hot period, whilst a decrease in Mg was only noted on Day 9. Plasma P concentrations also increased to a peak on the morning of Day 9, but were reduced thereafter. Overall plasma mineral concentrations were within normal ranges, indicative of maintenance of mineral homeostasis.

Table 1 Plasma metabolite concentrations in steers in climate chambers.

Day in climate room	Day 5 TN	Day 7 HOT	Day 9 HOT	Day 11 HOT	Day 13 TN	Day 15 TN
BALP U/L	42.5 ± 3.3^{a}	29.7 ± 2.8^{c}	24.1 ± 2.0^{cd}	22.2 ± 2.0^{d}	24.9 ± 1.8^{c}	29.8 ± 1.9^{b}
OCN ng/ml	85.4 ± 5.0^{a}	69.3 ± 4.2^{b}	69.1 ± 4.0^{b}	86.3 ± 3.4^{a}	100.1 ± 3.8^{a}	98.9 ± 4.4^{a}
CTX-1 ng/ml	1.3 ± 0.2^{a}	1.8 ± 0.3^{b}	2.3 ± 0.4^{c}	1.5 ± 0.3^{ab}	1.6 ± 0.4^{ab}	1.7 ± 0.3^{b}
Ca mM	2.50 ± 0.01^{a}	2.33 ± 0.01^{b}	2.34 ± 0.01^{b}	2.36 ± 0.01^{b}	2.45 ± 0.01^{a}	2.46 ± 0.02^{a}
P mM	2.45 ± 0.04^{a}	2.42 ± 0.07^{a}	2.70 ± 0.06^{b}	2.33 ± 0.04^{a}	2.15 ± 0.05^{c}	2.22 ± 0.07^{c}
Mg mM	0.96 ± 0.02^{a}	0.97 ± 0.03^{a}	0.88 ± 0.01^{b}	0.98 ± 0.02^{a}	1.04 ± 0.03^{a}	1.05 ± 0.03^{a}

n=20 per treatment. Values are mean±SEM. Superscripts denote a significant ($P<0.05$) difference, Tukey's post-hoc test.

Conclusion Growing beef steers under severe heat stress exhibit decreased bone formation and increased bone resorption. Reduced BALP is a robust biomarker of heat stress in cattle, indicative of defective bone mineralisation.

Acknowledgements This study was funded by Meat and Livestock Australia.

References

Kamiya Y, Kamiya M, Tanaka M. 2006. West Japan Journal of Animal Science. 49: 25-30.
Anderson S 2017. European Society of Veterinary and Comparative Nutrition, Cirencester UK.

Effects of stocking density on lactation performance and protein quality of early lactating dairy cows

Shixin Lin, Jianxin Liu, Diming Wang

Dairy Science Institute, Hangzhou, Zhejiang, China

E-mail: wdm@zju.edu.cn

Take home message Our study recommended that a stocking density lower than 100% would be beneficial to early lactating dairy cows.

Introduction Compared with mid-lactating dairy cows, early lactating dairy cows are more sensitive to oxidative stress (Burton *et al*., 1993). However, optimized stocking density (SD) for early lactating cows is not well evaluated. Previous finding suggested that an average stall occupancy of only about 75% when stall SD arrived at 100%, indicating that a SD less than 100% would be more preferable for dairy cows (Wagnerstorch *et al*., 2003). Thus, the current study was conducted to determine the effect of SD (75% SD (75SD) *vs*. 100% SD (100SD)) on lactation performance, milk protein fractions, rumen fermentation variables, and blood variables in early lactating dairy cows.

Material & methods Thirty-two multiparous Holstein dairy cows (BW=538 kg, standard deviation 9.4; parity＝2.85, standard deviation 0.19, milk yield＝35.1 kg/d, standard deviation 0.70, days in milk =65, standard deviation 4.8) were selected for this trail, the selected cows were allocated into 16 blocks, based on the parity, DIM and milk yield before the experiment. The 16 block of cows were then randomly assigned into 2 stocking densities including 75% (20 stalls and feed bins for 16 cows) and 100% (16 stalls and feed bins for 16 cows). The ingredients and chemical composition of the TMR diet were designed according to NRC-dairy (2001). Milk samples were collected from each cow on 3rd, 4th, and 5th d of adaptation, 2nd, 4th, 6th, and 8th week to determine milk compositions. Another fresh milk sample was collected on the 4th and 8th weeks and were centrifuged at 3,000 × g for 15 min, and then stored at −20°C for the analysis of 6 major milk protein fractions including αs1-casein, β-casein, κ-casein, αs2-casein, α-lactalbumin, and β-lactoglobulin by reverse-phase HPLC. On the 4th day of 4th and 8th experimental week, blood samples were obtained from the coccygeal vein into evacuated tubes containing sodium heparin 4 h after the morning feeding to prepare plasma, and then related plasma variables were determined. On the 4th d of 4th and 8th experimental week, rumen fluid samples were collected 3 h after the morning feeding using an oral stomach tube to determine rumen fermentation variables.

Statistical analysis All data were analyzed using the PROC MIXED protocol of the SAS software system (SAS Institute, 2000). A randomized block design with repeated measurements was used with wk, treatment, block, treatments nested in block, and the treatment × wk interaction as fixed effects, and cow within treatment was used as a random effect. The statistical model was as below: $Yijk = \mu + Bi + Tj + T(B)ij + Wk + TWjk + \varepsilon ijk$, Where, Yijk=dependent variable, μ=overall mean, Bi=block effect, Tj=treatment effect, Wl=wk effect, TWjk=interaction of treatment and wk; Two error terms are T(B)ij (treatment nested into block) and εijk (the residual error). The results were reported as least squares means. The values of $P < 0.05$ were used to define statistical significance, and the values $P < 0.10$ and $P > 0.05$ were defined as statistical trends.

Results & discussion Raw and energy-corrected milk yield, and milk protein rate were greater in 75SD dairy cows than those of 100SD cows. Proportion of casein (total, κ-, and αs-1) to protein were greater, while whey to protein were lower in 75SD cows than those of 100SD animals. Compared with 100SD cows, plasma concentrations of triglyceride, very low density lipoprotein, superoxide dismutase, total anti-oxidative capacity, insulin, and insulin/glucagon were higher in those of 75SD cows. While NEFA and BHBA in the plasma were lower in 75SD animals relative to those of 100SD cattle. Propionate content in the rumen fluid was greater in 75SD cows than the 100SD cows.

The greater milk yield in 75SD cows relative to 100SD was possibly attributed to greater available propionate from the rumen and the relatively lower oxidative stress. The change of casein proportion (total, κ-, and αs-1) between cows at different SD could be induced by different hormones levels, including insulin, prolactin and cortisol between the two groups of cows. Moreover, compared with 100SD-cows, the higher VLDL and TG content in the plasma suggested better TG clearance capacity in the liver of 75SD-cows, which could be attributed to the lower oxidative stress in the low SD-animals.

Conclusion In conclusion, our study suggested that early lactating cows should be stocked under 100% to avoid the loss on milk yield, protein quality and hepatic health.

Acknowledgements This research was supported by the grants from the National Key Research and Development Program of China (2016YFD0500500) and China Agriculture Research System (CARS-37).

References

Wagnerstorch AM, Palmer RW and Kammel DW2003. Journal of Dairy Science 6, 2253-2266.
Burton JL, Mallard BA and Mowat DN. 1993. Journal of Animal Science 6, 1532-1539.

Realimentation of nutrient restricted pregnant beef cows impacts proteomes of foetal muscular and adipose tissues

Muriel Bonnet[1], Kimberly A Vonnahme[2], Ioana Molnar[1], Leticia E Camacho[2], Didier Viala[1], Brigitte Picard[1]

[1]*Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès-Champanelle, France,* [2]*Department of Animal Sciences, North Dakota State University, Fargo, ND 58108, United States Minor Outlying Islands*

E-mail: muriel.bonnet@inra.fr

Take home message Maternal nutrition during the first half of pregnancy programs the abundance of muscular and adipose proteins controlling cell cycle progression, a cellular event known to determine the number of cells.

Introduction Producing meat animals with adequate muscular and adipose masses (*i.e.* lean-to-fat ratio) is an economic challenge for the beef industry. The lean-to-fat ratio is the result of a dynamic balance between the number and size of muscular and adipose cells partly set during the foetal life because the number of muscle fibres is fixed before the end of gestation in bovine. Maternal nutrition as a lever of foetal lean-to-fat ratio has been rarely addressed in bovine (Bonnet *et al.*, 2010). We aimed to evaluate the effects of nutrient restriction during early gestation followed or not by realimentation to mid-gestation both on the molecular events related to adipose tissue (AT) and on muscle growth.

Materials & methods. Early-maturing Angus cows pregnant from 30-d were either fed at 100% nitrogen and energy recommendations (NER) for maintenance and foetal growth from 30 to 140 dpc (CC, n= 5), restricted to 60% NER until 85 dpc and re-fed to 100% NER up to 140 dpc (RC, n=5) or restricted to 60% NER from 30 to 140 dpc (RR, n= 5; Gonzalez *et al.*, 2013). Perirenal and omental AT, *Semitendinosus* and *Longissimus thoracis* muscles were sampled from foetuses at 140 days post-conception (dpc). Two-dimensional gel electrophoresis and nanoflow liquid chromatography (Taga *et al.*, 2012), have identified 98 adipose and 69 muscular proteins. Proteins with abundances modified ($p < 0.05$) by diets (CC versus RC or RR) whatever the anatomical site were targeted by a multivariate ANOVA (R software).

Results & discussion The abundances of seven muscular proteins involved in the cytoskeleton organization, glycolysis and HSP90 complexes were modified ($p < 0.05$) by maternal nutrition (Table 1). They are annotated by a term related to the regulation of G2/M transition of mitotic cell cycle, a biological process proposed to regulate the total number of fibres (Chaze *et al.*, 2008). The abundances of 12 adipose proteins were modified ($p < 0.05$) by maternal nutrition. Among them CCT3 and CCT6A are subunits of the chaperonin-containing t-complex 1 (TCP1), a complex known to be markedly enhanced at the early S phase of the cell cycle, a critical cellular step of adipogenesis. In growing foetal AT, TCP1 abundance was shown to be increased simultaneously to the number of adipocytes (Taga *et al.*, 2012). We speculate that a down-regulation of CCT3 and CCT6A in response to maternal nutrition may inhibit or delay the proliferation of adipocyte precursors, and consequently decrease the number of AT cells.

Table 1 Foetal tissue proteins with abundances modified by maternal nutrition and their corresponding annotations.

Tissues	Gene Name (% of variation in RC and RR versus CC)	Enriched (P value <1 e-04) gene ontology terms in the biological process category
Longissimus T.	C10H14ORF166 (-25, -11), PDHB (-28, -6), MYL1 (+9, +9)	Regulation of G2/M transition of mitotic cell cycle
Semitendinosus muscle	ENO1 (-2, +18), PPID (-12, +1), TUBB4B (+30, +56) TUBB5 (+30, +56)	
Perirenal AT	ACTR1B (+23, -12), ALAD (+18, -14), ANXA1 (+3, -31), CCT3 (-5, -39), CCT6A (10, -26), FGG (+74, +11), GALK1 (2, -19), HBBF (-6, +28), LDHB (34, -1), SEPT2 (-9, -35)	Positive regulation of protein localization to Cajal body, positive regulation of telomerase RNA localization to Cajal body, positive regulation of telomere maintenance via telomerase, '*de novo*' protein folding
Omental AT	ACTR1A (+3, +41), ALAD (-19,-24), GALK1 (17, 2) GRB2 (+18,+33)	

Conclusion These results are the first and were obtained in Angus, thus their transposal to continental breeds remains to be unravelled. Variation in maternal nutrition during the first mid of gestation has modified mainly the abundance of proteins involved in the regulation of the cell cycle, with potential short or long term impact on adipose or muscular cell numbers.

Acknowledgements We thank the Inra Phase division for funding proteomics assay and APIS-GENE for funding I. Molnar.

References

Bonnet M, Cassar-Malek I, Chilliard Y and Picard B 2010. Animal 4, 1093-1109.
Chaze T, Meunier B, Chambon C, Jurie C and Picard B 2008. Proteomics 8, 4236-4248
Gonzalez J M, Camacho L E, Ebarb S M, Swanson K C, Vonnahme KA, Stelzleni A M and Johnson SE 2013. Journal of Animal Science 91, 4797-4806
Taga H, Chilliard Y, Meunier B, Chambon C, Picard B, Zingaretti MC, Cinti S and Bonnet M 2012. Journal of Cellular Physiology 227, 1688-1700

Evaluation of the enteric methane emission factor for cattle in the subhumid zone of Senegal

Séga Ndao[1,2], Jonathan Vayssières[3,2], Alexandre Ickowicz[3,5], El Hadji Traoré[6], Charles-Henri Moulin[3,4], Philippe Lecomte[3,2], François Bocquier[3,4]

[1]ISRA, CRZ de Kolda, BP 53, Kolda, Senegal, [2]CIRAD, UMR SELMET, PPZS, BP 2057, Dakar, Senegal, [3]SELMET, Univ Montpellier, CIRAD, INRA, Montpellier SupAgro, Montpellier, France, [4]SupAgro, Umr Selmet, 2 place Viala, 34060, Montpellier, France, [5]CIRAD, UMR SELMET, F-34398, Montpellier, France, [6]ISRA, LNERV, BP 2057 Dakar Hann, Dakar, Senegal

E-mail: sega.ndao@supagro.fr

Take home message Taking into account seasonality of feed resource strongly reduced methane emission of Senegalese cattle.

Introduction A sensitivity analysis applied to the Tier 2 model reveals the feed energy digestibility (DE, MJ/d or %) as the most important parameter contributing to the uncertainty in the predicted annual enteric methane emission factor (AMEF, g CH_4/kg BW/yr) (Opio et *al*., 2013). In the Senegalese subhumid zone, Ickowicz and Mbaye (2001) confirmed the digestibility of organic matter (OMd, %) highly varies along the year. The aim of this work is to realistically evaluate the MEF for this type of livestock system, focusing on an improved DE estimates along seasons *i.e.*, Wet Season (WS), Early Dry Season (EDS) and Late Dry Season (LDS).

Materials & methods To use Tier 2 methodology, input parameters such as average live weight (BW, kg), average daily gain (ADG, kg/d), Milk Yield (MY, L/d), feed types and their DE were gathered. Knowledge of animal performances allowed calculation of seasonal energy requirements (NE, MJ/d) which in turn gave a reliable estimate of feed intake and thus average daily gross energy intake (GE, MJ/d) needed at different stages (e.g., maintenance, growth) and for each animal subclasses. Thanks to previous observations the necessary step of seasonal DE intakes in this area (Figure 1), the seasonal MEF (SMEF, g CH_4/kg BW/s) is then calculated from the GE which is multiplied by the methane conversion factors (Y_m) available from Kennedy and Charmley (2012).

Results & discussion Our estimated AMEF are 114.5±2.6, 99.4±3.1 and 106.1±8.9 g CH_4/kg BW/yr for juvenile, sub-adult and adult classes, respectively (Table 1). Considering all animal categories, our weighted AMEF is 105.6 g CH_4/kg BW/yr. This last value fully agrees with Lecomte *et al* (2016) who suggest 106.4 g CH_4/kg BW/yr, using F-NIR approach. Besides, our assessment is 9% and 38% lower than 116.4 g CH_4/kg BW/yr and 172.6 g CH_4/kg BW/yr reported by Herrero *et al* (2008) and IPCC (2006, Tier 1), respectively for the West Africa region.

Table 1 Estimated of SMEF and AMEF from cattle classes. SMEF were computed for each animal class, respecting seasons, hence summed to obtain AMEF. To achieve average AMEF of the herd, each SMEF was weighted, respecting the associated ratio of considered class into the herd structure.

Sex	Class	Ratio	BW	SMEF[1]			AMEF[2]
				WS	EDS	LDS	
F	J	0.06	42	39	39	39	117
	S	0.28	91	33	31	38	102
	A	0.37	202	36	34	42	112
M	J	0.06	49	38	38	37	113
	S	0.13	104	30	30	36	96
	A	0.10	250	31	31	38	100

F: female; M: male; BW: body weight

J: juvenile (0-1year); S: sub-adult (1-4year); A: adult (>4year)

[1] SMEF are expressed in g CH_4/kg BW/s (s=season)

[2] AMEF is expressed in g CH_4/kg BW/yr (yr=year)

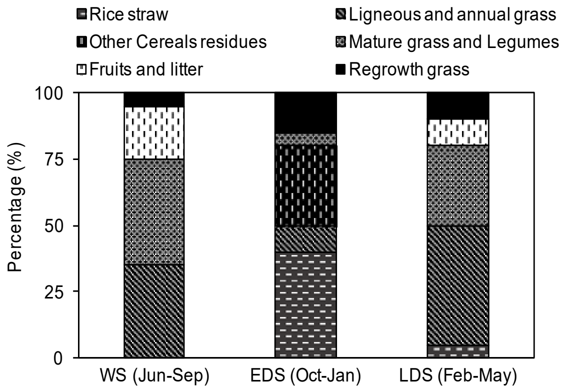


Figure 1 Average proportion of grazing-time of Ndama cattle according to season and diet availability in the rangeland.

Conclusion Due to large seasonal variations of feed availability coupled with a drastic reduction of digestibility, cattle change their feed intake thus leading to a clear reduction of methane emissions as compared with previous estimations. When expressed in standard Tropical Livestock Unit (TLU, animal of 250 kg BW) the annual methane emission factor is of 26.4 kg/TLU. In order to further reduce the uncertainty associated with the development of enteric methane emission factors, additional parameters such as the spatiotemporal variation of DE must be better described in this type of livestock system.

Acknowledgements This research was supported by grants from WAAPP and the SELMET Research Unit.

References

Herrero M, Thornton PK, Kruska R and Reid RS 2008. Agriculture, Ecosystems and Environment, 126, 122-137

IPCC 2006. 2006 IPCC Guidelines for National Greenhouse Gas Inventories

Kennedy PM and Charmley E 2012. Animal Production Science, 52, 225-239.

Lecomte P, Decruyenaere V, Eugene M, Bois B, Ndao S and Ickowicz, A 2016. 6th GGAA Conference, Melbourne, Australia.

Opio C, Gerber P, Mottet A, Falcucci A, Tempio G, MacLeod M, Vellinga T, Henderson B and Steinfeld H 2013. A global life cycle assessment. Food and Agriculture Organization of the United Nations (FAO), Rome.

Suckling dromedary calves become hyperglycemic and low insulin sensitive with age

Elena Díaz-Medina[1,2], Gerardo Caja[1], Ahmed A. K. Salama[1,5], Soraya Cabrera[2], Mohammed Alshaikh[3], Moez Ayadi[3,4], Riyadh Aljumaah[3], Maristela Rovai[5]

[1]*Group of Research in Ruminants (G2R), Department of Animal and Food Sciences, Universitat Autonoma de Barcelona, Bellaterra, Barcelona, Spain,* [2]*Oasis Park-Fuerteventura, La Lajita, Fuerteventura, Canary Islands, Spain,* [3]*Department of Animal Production, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia,* [4]*Department of Animal Biotechnology, Institut Supérieur de Biotechnologie de Beja, Université de Jendouba, Beja, Tunisia,* [5]*Department of Dairy Science and Food Science, South Dakota State University, Brookings, South Dakota, USA*

E-mail: gerardo.caja@uab.es

Take home message Dromedary calves became hyperglycemic and resistant to insulin (INS) with age. Their control of blood glucose (GLU) and secretion of INS decreased when getting older, resulting in a diabetes-like stage, useful to prevent dehydration after weaning.

Introduction Camelids have plasma GLU values twice higher than true-ruminants and similar to monogastrics (165 vs. 65 mg/100 mL; Cebra *et al.*, 2014). Plasma INS of fasted camelids is less than half of other mammals (Cebra *et al.*, 2014). Adult dromedaries are recognized by their low sensitivity to INS (Kaske *et al.*, 2001) and impaired use of INS-dependent GLU transporter (GLUT4) in muscle and fat, leading to the need of using INS-independent GLU transporters and to maintain hyperglycemia as an strategy for GLU uptake. Very little is known about GLU and INS metabolism in dromedaries and if this diabetes-like stage is congenital or acquired, which is studied here.

Materials & methods Ten suckling dromedaries (5 males and 5 females), from the Oasis Park herd (Fuerteventura, Canary Islands, Spain), were allocated in two groups by age (A, 15 ± 3 d and 39.7 ± 1.8 kg BW; B, 132 ± 19 d and 115.2 ± 5.8 kg BW, n = 5) and submitted to GLU tolerance test (GTT, 0.25 g GLU/kg BW) and INS challenge (4.6 µg INS/kg BW) after fasting overnight. Blood samples were collected at 12 time-points (min –15 to 150), processed for plasma and frozen until analysis. Plasma GLU was analyzed by hexokinase (Olympus, Krefeld, IE), NEFA by enzymatic colorimetric (Wako, Neuss, DE) and BOHB by kinetic enzymatic (Ranbut, Randox Labs, Crumlin, UK) methods in a multi-analyzer (Olympus AU400, Hamburg, DE). Plasma INS was analyzed by ELISA immunoassay for bovine-INS (Mercodia, Uppsala, SE). Surrogate indices for GLU tolerance and INS sensitivity (*i.e.*, QUICKI, RQUICKI, HOMA and HOMA-IR), were calculated by using the fasting values. Data were processed by Proc MIXED for repeated measures of SAS v.9.1.3 (SAS Inst. Inc., Cary, NC, USA).

Results & discussion Basal GLU was high and increased by age (A vs. B, 121 ± 7 vs. 147 ± 7 mg/100 mL; $P < 0.05$), peaked higher in B than A at min 5 ($P < 0.01$), and did not return to the baseline during the GTT. Basal INS was low and similar between groups (0.14 ± 0.03 ng/mL), also peaked at min 5 with an effect of age (A vs. B, 1.24 ± 0.12 vs. 0.39 ± 0.12 ng/mL; $P < 0.001$) and returned rapidly to the baseline. Basal NEFA did not differ between groups (0.68 ± 0.12 mmol/L), but decreased sharply after GLU infusion, reached a nadir at min 20, and only returned to the baseline in the A group.

Regarding the INS challenge, basal INS did not differ between groups (0.16 ± 0.09 ng/mL), as for GTT, but INS infusion decreased GLU parallel in both groups, which did not return to the baseline. Basal NEFA differed in this case between groups (A vs. B, 0.82 ± 0.06 vs. 0.56 ± 0.06 mmol/L; $P < 0.05$) but decreased similarly in A and B during GTT until min 20; from there, it increased dramatically in A camels and reached a high plateau, whereas returned to the baseline in B, indicating that older camels were able to use the hyperglycemia. Basal BOHB were low and different by age (A vs. B, 0.029 ± 0.004 vs. 0.016 ± 0.004 mmol/L; $P < 0.05$), decreased to a similar value at min 30 and reached the baseline at min 90. BOHB values steadied low and close to the baseline for the whole INS challenge in B. Surrogate indices decreased (QUICKI, 0.40 ± 0.01 vs. 0.36 ± 0.01; $P < 0.05$) and increased (HOMA, 0.53 ± 0.14 vs. 1.00 ± 0.14; $P < 0.05$) by age (A vs. B, respectively), confirming that camel calves became hyperglycemic and resistant to INS when going older.

Conclusion Camel calves became hyperglycemic and resistant to INS, at the same time that tolerance to GLU infusion and secretion of INS decreased, by age. These changes resulted in a diabetes-like stage with basal hyperglycemia and low fat mobilization during GLU or INS administration, respectively, that we suggested is related to their adaptation to desert conditions.

Acknowledgements The Oasis Park team (Fuerteventura, Spain) and the King Saud University (KSU, Riyadh, Saudi Arabia) for their support.

References

Cebra CK, Anderson D, Tibary A, Van Saun R and Johnson L 2014. Chapter 41. Hepatic, pancreatic, and metabolic disorders in Llama and Alpaca Care. Medicine, Surgery, Reproduction, Nutrition and Herd Health. Pp. 537−552 C. Cebra, ed. Elsevier, St. Louis, MO.

Kaske M, Elmahdi B, von Engelhardt W and Sallmann HP 2001. Journal of Comparative Physiology B 171, 549-546.

Quantifying the uncertainty of the cattle enteric methane emissions inventory for the dry-land regions of Mexico: a Monte Carlo approach

Octavio A. Castelán-Ortega[1], Juan C. Ku-Vera[2], Juan C. Angeles-Hernández[1], Mohammed Benaouda[1], Gloria S. Hernández-Pineda[1], Sofía V. Castelán-Jaime[1], Ana R. Praga-Ayala[1], Hugo Montelongo-pérez[1], Eduardo Cardozo-Gutiérrez[1]

[1]*Universidad Autónoma del Estado de México, Estado de México, Mexico,* [2]*Universidad Autónoma del Estado de Yucatan, Yucatán, Mexico*

E-mail: oaco2002@yahoo.com.mx

Take home message Analytical quantification of uncertainty improves the quality of inventories of methane emissions by cattle.

Introduction Most national greenhouse gases (GHG) inventories for cattle have been prepared following the IPCC 2006 guidelines. However, in many cases the uncertainty of the inventories is high because the activity data and emission factors used are not robust as they do not take into account existing knowledge gaps and they are not specific for geographic region and animal category. When the IPCC Tier 1 approach is used, the estimation of uncertainty may be more relaxed (IPCC, 2006), but this is not the case with the Tier 2 method because a more robust measure of uncertainty is required. Monte Carlo analysis (MCA) can be utilized to calculate and propagate uncertainty for the Tier 2 approach; since MCA deals with several probability density functions (PDF), and it can handle correlations of varying degrees and can also deal with complex models. The aims of the present study were: 1) to identify, categorize, and define sources of uncertainty in the inventory of methane emissions from cattle for the dry-land regions of Mexico using the Tier 2 method (IPCC, 2006), and 2) to compute and propagate the uncertainty of the input variables of the Tier 2 method using MCA.

Materials & methods A geo-spatial segregation of the national herd of cattle was carried out through the use of the existing classification of the Mexican territory into five agro-climatic regions: temperate, dry, very dry, tropical-humid and tropical sub-humid. In addition, the herd was divided into nine sub-categories: dairy cows, beef cows, dual-purpose cows, calves, young steers, young heifers, steers, heifers, and bulls; and the inventory was calculated accordingly for regions and categories. The IPCC Tier 2 inventory was calculated with specific activity data for each region and animal categories including local emission factors. Only the results of the dry land regions are presented here. Source category uncertainties were specified, which included uncertainties of the emission factors (obtained from experimental assays in respiratory chambers), activity data (surveys, census, experts' opinion and scientific literature) and parameters from principal method of Tier 2. The PDF and cross-correlations between these variables were calculated and a "bottom–up" approach was carried out to deal with uncertainty in the inventory. The PDF were defined following the recommendations of IPCC 2006 using the Culley and Frey plot and several statistics (*i.e.* Kolmovorov-Smirnov) and criteria of goodness of fit (*i.e.* AIC and BIC) using the MCA. Computation of expanded uncertainty (95 % confidence interval C.I.) was conducted by means of bootstrap simulation (10^4 simulations), employing the *fitdistplus* package (Delignette and Dutand, 2015) in R software (R core team). The propagation of uncertainty was done by the first and second Taylor derivate and MCA using the *Propagate* package (Spiess, 2017) in R software.

Results & discussion The calculated methane inventory for the 7.7 million head of cattle in the dry land region of Mexico is 607.01 Gg year^{-1} CH_4 (C.I. 485.91 – 751.9). Methane estimations showed a generalized normal distribution and an uncertainty of -19.95, +23.88 % (Figure 1). A total of 117 sources of uncertainty were identified, of which 35 were associated with emission factors and 82 with activity data. The sources of uncertainty with largest values (>40%) were associated with activity data (*i.e.* body weight, average daily gain, etc.) and the outputs of formulas of the IPCC 2006 Tier 2 main method with an exponential structure. Input variables with smallest uncertainties (<10%) were data of emission factors obtained from experimental assays (*i.e.* digestibility, methane conversion factor, etc.). Several shapes of PDF were identified (*i.e.* normal, log-normal, generalized normal, Johnson SU, etc.); however, most of the PDF belong to the normal family of distributions. There were differences in the shape and the level of uncertainty among cattle sub-categories; steers showed the largest uncertainty (-55, +82 %) and the smallest uncertainty was found in young heifers (-28, +36 %).

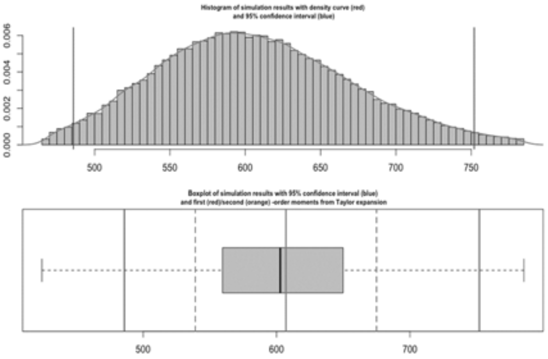


Figure 1 PDF of total of enteric methane emissions (Gg year^{-1} CH_4) of cattle in dryland regions of Mexico.

Conclusion The geo-spatial location of enteric methane emissions, categorization of cattle and the MCA are useful analytical tools for the identification of the main sources of uncertainty in the inventories when the Tier 2 method is used.

References

Delignette-Muller LM and Dutang M 2015. Journal of Statistical Software 64, 1-34
Spiess A 2017. Propagate: Propagation of Uncertainty. R package version 1.0-5.
IPCC. 2006. Emission from livestock and manure Management

Drinking behaviour of lactating Saanen and Alpine goats under mild water and heat stress episodes

Lina Jaber[1], Christine Duvaux-Ponter[2], Sylvie Giger-Reverdin[2]

[1]American University of Beirut, Faculty of Agricultural and Food Sciences, Department of Agriculture, Beirut, Lebanon, [2]INRA, AgroParisTech, Université Paris-Saclay, UMR Modélisation Systémique Appliquée aux Ruminants, Paris, France

E-mail: lj01@aub.edu.lb

Take home message Saanen and Alpine goats resort to compensatory over-drinking following water restriction and increase total water intake under mild heat stress without reducing feed intake.

Introduction Water scarcity and high environmental temperature are growing global constraints for livestock production, including in Europe that's being increasingly affected by climate change. The adaptation mechanisms of European high producing goat breeds to these constraints are rarely assessed in literature (Silanikove and Koluman, 2015). This study aimed to assess the effect of mild water and heat stress episodes on drinking behaviour in lactating Saanen and Alpine goats.

Materials & methods Four Alpine and four Saanen goats (160 DIM at the start of the experiment) were included. They were housed in metabolic crates for continuous monitoring of eating and drinking behaviour. The feed consisted of a total mixed ration (TMR) offered twice daily after milking at 3:30 p.m. and at 7:30 a.m. Respective feed and water intakes were recorded every two min, separately for each animal, with a precision of 5 g. Data were recorded during three periods (P), consisting of two consecutive days each. In P1, the animals were offered feed and water as usual and the Temperature-Humidity Index (THI) was mild (THI=63.5). In P2, water was withheld after the afternoon milking until the next morning with a THI of 66.8 and in P3, feed and water were offered as usual with a higher THI (71.9) than in P1 and P2. The assessed parameters included the total daily feed and water intake, the evening and the following morning water intake, the latency between first feeding and the following drinking event after return from milking, and the amount of water drank in the first 10 min. of the first drinking event following feeding. Data, presented as the mean of the two days in each period, were analysed using the mixed linear model approach on IBM SPSS 24 with goat and period as fixed factors. The interaction between the two factors was not significant and thus not included in the model.

Results & discussion The period had a significant effect on all the assessed parameters except total feed intake and the evening latency (Table 1). Under water restriction (P2), the animals were able to maintain their normal daily water and feed intake, however they resorted to drinking large amounts of water in the first drinking event after feeding. Under mild heat stress (P3), the goats drank more water daily as well as in the morning and the evening as compared to the control period (P1). The goat factor was also significant for most parameters except for evening water and water drank in the first 10 min after the beginning of the first drinking event following feeding.

Table 1 Effect of mild water and heat stress on drinking in lactating Saanen and Alpine goats.

	P1	P2	P3	SEM	P[1] (Period)	p (Goat)
Total water intake (L)	6.194^{b}	6.830^{b}	9.180^{a}	0.297	0.000	0.004
Water intake (afternoon) (L)	4.056^{b}	0.000^{c}	5.961^{a}	0.339	0.000	0.242
(morning) (L)	2.138^{c}	6.827^{a}	3.219^{b}	0.176	0.000	0.030
Total feed intake (Kg)	5.140^{a}	5.081^{a}	5.325^{a}	0.081	0.123	0.000
Latency[2] (afternoon) (min)	31.30^{a}	_	26.79^{a}	3.672	0.414	0.082
(morning) (min)	30.27^{a}	18.274^{b}	28.76^{a}	3.194	0.037	0.002
1st water[3](afternoon) (L)	0.719^{a}	0.000^{b}	0.673^{a}	0.088	0.000	0.239
(morning) (L)	0.623^{b}	4.078^{a}	0.575^{b}	0.161	0.000	0.208

[1] Means in a row followed by different superscripts are significantly different

[2] Latency between first feeding and the following drinking event after return from milking (afternoon or morning)

[3] Amount of water drank in the first 10 min. of the first drinking event following feeding

Conclusion Lactating Saanen and Alpine goats seem capable of coping with mild water and heat stress through adaptive drinking behaviours such as compensatory over-drinking in a short period of time following water restriction, and increased water consumption under heat stress. The individual variation in drinking behaviour warrants further study.

Acknowledgements The authors thank J. Tessier and his colleagues from MoSAR goat unit for taking care of the animals.

References

Silanikove N and Koluman N 2015. Small Ruminant Research 123, 27-34.

Inventory of enteric methane emissions by cattle in the dry-land regions of México using the IPCC 2006 Tier 2 main method

Octavio A. Castelán-Ortega[1], Juan Carlos Ku-Vera[2], Sofía V. Castelán-Jaime[1], Gloria S. Hernández-Pineda[1], Mohammed Benaouda[1], Juan Carlos Angeles-hernández[1], Ana R. Praga-Ayala[1], Hugo Montelongo-Pérez[1]

[1]Universidad Autónoma del estado de México, Estado de México, Mexico, [2]Universidad Autónoma del Estado de Yucatán, Yucatán, Mexico

E-mail: oaco2002@yahoo.com.mx

Take home message The use of the Tier 2 main method improved the accuracy of the inventory of cattle's enteric methane emission.

Introduction A precise methane (CH_4) emission inventory based on the geographical localization of the country's cattle population is useful for specific mitigation policies in order to meet the compromises established in the Paris agreement. Previous CH_4 inventories in Mexico are based on the IPCC's Tier I approach (IPCC, 2006), which is less accurate because it uses default emissions factors (EF) recommended by IPCC instead of locally generated EF. It also makes no differentiation for agro ecological zone or cattle category. The Tier 2 main method allows better estimation of CH_4 inventories because it takes into account specific activity data associated with animal performance, diet-climate characteristics and the structure of the national herd. Thus, the aim of the present work was to calculate the inventory of enteric CH_4 emissions from cattle of the dry-land regions in Mexico using the Tier 2 main method and compare the results with Tier 1 method.

Materials & methods The national herd was geo-spatially segregated using the existing classification of the Mexican territory into five agro-ecological zones: dry, very dry, tropical humid, tropical sub-humid and temperate. Only the population of the dry region is presented here. Following the IPPC (2006) recommendations, the herd was disaggregated into nine categories: dairy cows, beef cows, dual-purpose cows, bulls, heifers, steers, young heifers, young steers and calves. In order to characterise the nutritional management in the dry region, 67 cattle farms were surveyed, where a questionnaire was applied to the owners of the farms in order to know feeding systems and ingredients used. The survey data was analysed by correspondence analysis to identify the most frequent diet ingredients and their inclusion level, in order to define the one "typical diet" (TD) for each cattle category. Other activity data was obtained from scientific literature, expert's opinion and National Agricultural Census. Values of digestibility and methane conversion factor (Y_m) required for CH_4 emission calculation specific for the region (and) were obtained from four experiments with a total of 41 replicates conducted in open-circuit respiratory chambers (Benaouda 2018). The gross energy intake and total emission of each category was calculated as in IPCC Tier 2 (2006): enteric CH_4 emission Gg CH_4 $yr^{-1} = EF_{(T)}*(N_{(T)}/10^6)$. Where: $EF_{(T)}$ is the specific emission factor to each category (kg CH_4 $head^{-1}$ yr^{-1}); $N_{(T)}$ is the number of heads in the category; T is cattle category. The total CH_4 emission by cattle was calculated using Tier 1 (default values of EF) and Tier 2 approach (country specific EF) using the following formula: Total $CH_{4\ \text{enteric}} = \sum_I E_i$. Where: Total $CH_{4\ \text{enteric}}$ is total methane emission from enteric fermentation (Gg CH_4 yr^{-1}); E_i is the emission for the i^{th} category. The uncertainty of the inventory was calculated for each category using the bootstrap method in the *fitdistrplus* package (Delignette and Dutand, 2015) of the R software.

Results & discussion Using the Tier 2 approach the total methane emission originated from the 7,738,218 heads of cattle in the dry-land region of Mexico was 607.01±67.92 Gg $year^{-1}$ CH_4, which was higher in comparison with Tier 1 estimation of 454.43 Gg $year^{-1}$ CH_4. Thus, the Tier 1 method underestimates the inventory in 25.1%. Differences in the amount of CH_4 emissions for Tier 2 were found between categories (Figure 1); cows (dairy, beef and dual purpose) contributed with 79.8 % of the total CH_4 production, where dairy cows showed the highest emission factor (137.5±31.84 kg CH_4 $head^{-1}$ $year^{-1}$). In contrast, calves showed the lowest emission factor (8.56±2.3 kg CH_4 $head^{-1}$ $year^{-1}$). Results showed that cattle in this region contributes with 36% of the total emissions based on the national inventory calculated by Castelán *et al.* (2014) and represents 17.8% of the population of the national herd. The uncertainty of the inventory using Tier 2 approach was -19.95, +23.88 %.

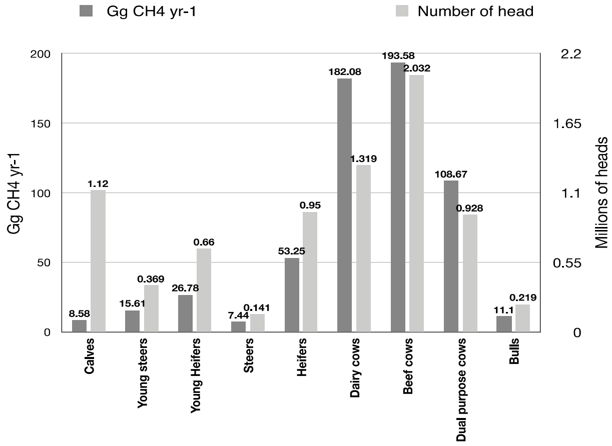


Figure 1 Methane emissions and population by cattle category.

Conclusion The use of the IPCC 2006 Tier 2 method allowed better differentiation and quantification of the enteric CH_4 emission for each category of cattle in the total region's inventory than the Tier 1 approach.

References

Castelán-Ortega OA,. Ku-Vera JC and Estrada-Flores J 2014. Atmósfera, 27, 185-191.
Delignette-Muller L.M., Dutang M. 2015. Journal of Statistical Software, 64, 1-34.
IPCC 2006. Emission from livestock and manure Management. Guidelines for National Greenhouse Gas Inventories.

The feed availability for dairy cows during dry and wet seasons in the main dairy areas of Yogyakarta – Indonesia

Budi Widyobroto, Rochijan Rochijan, Cuk Noviandi, Andriyani Astuti

Universitas Gadjah Mada, Yogyakarta, Indonesia

E-mail: budi_widyobroto@ugm.ac.id

Take home message Continuity and availability of forages and other feedstuffs is important to reduce the dependency of dairy smallholder farmers on off-area's support.

Introduction As the main feed for ruminants, forages need to meet the nutrient requirements, both in quality and quantity. However, due to the difference in conditions between the dry and wet seasons, it is difficult to provide high-quality forage in sufficient quantities throughout the year in tropical regions such as Indonesia (Handayanta *et al*., 2014). Therefore, it is necessary to understand the availability and nutrient contents of forages and other natural resources during dry and wet seasons in order to improve the productivity of dairy cows raised by smallholder farmers in Yogyakarta.

Materials & methods The method used was surveys (interviews, questionnaires, field observations, and feed samplings) at the cooperative units, farmers, dairy farmers, and dairy farmer groups in one of the areas of dairy cooperation in Yogyakarta, Indonesia. Feed samples were analysed in the Laboratory of Dairy Science and Milk Industry, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta. The data were descriptively analysed with Statistical Program for Social Science version 16.0.

Results & discussion Supplies of forages, agricultural by-products, feedstuffs, and commercial concentrates were available throughout the year, although the number was limited. Most of dairy smallholders had greater access to forages and agricultural by-products during wet season than the dry season (Table 1); this resulted directly in a better productivity of dairy cows during the wet season (Widyobroto *et al*., 2017). In both seasons, farmers brought commercial concentrates from other areas to support the cows feed requirements. In addition, farmers rarely store forages using feed technology, such as silage, to cope with feed scarcity during the dry season.

Table 1 Feed availability during dry and wet seasons in Yogyakarta.

Feed Type	Dry season			Wet season		
	Early	Mid	End	Early	Mid	End
Diet types:						
Grasses	3	2	2	3	4	3
Legumes	2	2	2	2	2	2
Weeds	2	2	2	2	3	3
Agricultural by-products	3	2	2	3	3	3
Average feed availability	3	2	2	3	3	3

0 = No access to feed; 1 = <25% farmers has access to feed; 2 = 25 – 50% farmers has access to feed; 3 = 51 – 75% farmers has access to feed; 4 = >76% farmers has access to feed.

Conclusion Farmers have better access to forages and agricultural by-products in the wet season than the dry season, but they still have to buy commercial concentrates from outside areas in both seasons. Assuring the continuity and availability of forages and concentrate in this area is needed to reduce the dependency of support from outside of the region.

Acknowledgements This study was supported by PTUPT Grant 2018 from RISTEKDIKTI and Universitas Gadjah Mada.

References

Handayanta E, Ifar S, Hartutik and Kusmartono 2014. Botanical composition and quality of ruminant feed resources in the dry land farming areas in Yogyakarta, Indonesia. Journal of Biology, Agriculture, and Healthcare 4, 26-33.

Widyobroto BP, Rochijan, Noviandi CT and Astuti A 2017. Dairy cows productivity and socio-economic profile of dairy smallholder's communities in Daerah Istimewa Yogyakarta, Indonesia. IOP Conference Series: Earth and Environmental Science 119, 012060.

Kinetic of gas production *Moringa oleifera* Lam collected in tropical dry forest areas from Colombia

Indira Isis Garcia[1,2], Jairo Mora-Delgado[1,2]

[1]*Universidad del Tolima, Ibague, Colombia,* [2]*Grupo de Investigación Sistemas Agroforestales Pecuarios, Ibague, Colombia*

E-mail: jrmora@ut.edu.co

Take home message The difference in gas production is associated with the bromatological quality of each of the accessions of *Moringa oleifera*.

Introduction *Moringa oleifera* Lam, is considered as an alternative to improve the availability and quality of forage for feeding ruminants in tropical dry forest. Nutritional quality and gas production three ecotypes of M. oleifera in pre flowering was evaluated.

Materials & methods Plant samples (leaves and stems thin) from three crops, located in the municipalities of Ar Armero (Tolima), Flanders Fl (Tolima) and Pl Palmira (Valle) were taken during dry season. In the laboratory of Animal Nutrition at the National University of Colombia - Palmira, neutral detergent fiber (NDF), fiber in acid detergent (ADF), acid detergent lignin (ADL), hemicellulose, and cellulose, crude protein (CP), ether extract (EE), total dry matter (DM), metabolizable energy (ME) determined in calorimetry chambers. Gas production was determined using the Gas Pressure Traducer Technique GTT (Theodorou *et al.*, 1994). Incubation times were 0, 2, 5, 8, 12, 16, 20, 24, 48 and 72 hours. The values obtained were used to run a Gompertz model building kinetic curves gas production. We used a completely randomized design with three replications. To compare the mean test Tukey HSD ($P < 0.05$) was applied.

Results & discussion Results suggest that the percentage of fibers for Pl, Fl and Ar ecotypes was: NDF (35.7, 41.9, 27.5%); ADF (22.3, 24.9, 15.0%); ADL (4.0, 4.9, 2.5%), respectively. For hemicellulose, no significant differences between the three ecotypes (13.4, 17.0, 12.4%) were found and the Fl and Ar cellulose ecotypes differ relative to Pl (18,2, 20,0, 12, 6). The time to inflection point (HIP, hours), was 134,5 for Fl, 15.80 to 24.0 for Ar and 19.3 to Pl. The volume of gas at the inflection point (GIP ml), was higher in Ar eco-type (78,8 ml) and lower to Fl (61.3 ml)). The maximum gas production rate (**MGPR**, ml / h) was found to Ar (10,7). The lower lag phase (LP or microbial establishment, h) was 12.1 h for Pl eco-type (Table 1).

Table 1 Gas production and parameters of the logistic model for the fermentation kinetics of three eco-types of *Moringa oleifera*.

Eco-type	a	b	c	R^2	PIH horas	GIP ml	MGPR ml/h	LP h
Flandes (Fl)	166.5	7.2	0.1	96.4	134.5	61.3	6.1	62.4
Armero (Ar)	214.2	3.1	0.1	98	24.0	78.8	10.1	16.2
Palmira (Pl)	190.2	2.7	0.1	99.1	19.3	69.9	9.7	12.1

HPI = Time to the inflection point (h); GIP = gas at the inflection point (ml); MGPR = maximum gas production rate (ml. h^{-1}); LP = lag phase (h).

There is a negative correlation between the NDF content of a substrate and the magnitude of gas production obtained in fermentation (Njidda and Nasiru, 2010). It has been reported that the microbial efficiency and the maximum speed of gas production, for Moringa was higher than for the other species (Rodriguez, *et al.*, 2014). The performance of these parameters, in the case of Moringa, could be related to their higher content of easily fermented carbohydrates (Akinfemi *et al.*, 2009).

Conclusion Bromatological quality of M. oleifera and gas production differs according to agronomic conditions of cultivation.

Acknowledgements to Universidad del Tolima and Universidad de Caldas by facilitating this research work.

References

Akinfemi A Adesanya AO and Aya VE 2009. American-Eurasian Journal of Scientific Research 4, 240
Njidda AA and Nasiru A 2010. Pakistan Jounal of Nutrition 9, 60
Rodríguez R, González N Alonso J, Domínguez M and Sarduy L. 2014 Revista Cubana de Ciencia Agrícola, 48, 4

A suppressed metabolic endocrine state in growing cattle under high heat load

Gene Wijffels[1], Megan Sullivan[2], Sally Stockwell[1], Susan Briscoe[1], Cinita de Melo Costa[1,3], Russell McCulloch[1], Judy Cawdell-Smith[2], Joesph Olm[2], Stephen Anderson[2], John Gaughan[2]

[1]CSIRO Agriculture and Food, Qld, Australia, [2]The University of Queensland, Qld, Australia, [3]UNESP, Sao Paulo, Brazil

E-mail: gene.wijffels@csiro.au

Take home message Many metabolic hormones are reduced in beef cattle exposed to high heat load.

Introduction Hot climatic conditions are associated with reduced productivity of feedlot beef cattle. Major heat waves further disrupt the growth trajectory of feedlot cattle due to reduced feed intake, concomitant weight loss and reduced feed efficiency. This often is followed by a prolonged recovery phase. While the metabolic perturbations at the systemic level which are induced by moderate to severe heat stress in cattle are becoming understood, especially in dairy cows, the endocrine milieu regulating metabolism is less well described. In attempting to establish a scenario realistic to Australian conditions, heavy late summer feedlot steers were thermally challenged with a '7-day heatwave' in climate chambers and monitored during the challenge, through recovery and on return to outdoor pens.

Materials & methods Two replicates (n=12 each) of ~600 kg Black Angus steers backgrounded on a feedlot finisher ration were housed in climate-controlled rooms (The University of Queensland). The steers were subjected to four climatic periods with daily cyclic temperature and humidity regimes: preHOT (5 d thermoneutral, daily THImax of 71); HOT (7 d with 2 d steps down of THImax at 92, 86 and 80): Recovery (5 d, daily THImax of 69); and return to outdoor PENS for three weeks (late summer). Plasma was collected every 48 h during preHOT and Recovery, with daily bleeds during HOT, and bleeds at weeks 1 and 3 were undertaken after return to PENS. In-house ELISAs were used to measure plasma leptin, adiponectin, prolactin, T4 and T3 concentrations. Plasma insulin was determined by a commercial IRMA (DiaSource). One-way ANOVAs and multiple t-tests indicated significant differences between treatment periods. Data from four animals were excluded from the analyses.

Results & discussion At the onset of the thermal challenge, DMI fell ~50% relative to the PreHOT period to 5.2 kg/head/day. During Recovery and over the three weeks in PENS, mean DMIs were 7.8 and 9.5 kg/head/day respectively. With DMI at these levels during HOT and Recovery, ADG was -0.04 kg/head/day. The plasma concentration of most hormones fell 20-50% during the HOT period between relative to preHOT baseline levels (Table 1). Insulin and prolactin were reduced to a lesser extent. Plasma glucose concentration was 14% below the PreHOT concentration and there was clear indication of fatty acid oxidation during the HOT and Recovery periods with plasma β-hydroxybutyrate concentrations rising 35 and 17% respectively (over that of the PreHOT interval). During the Recovery period hormones generally remained inhibited. Insulin concentration was at its lowest during Recovery, despite the resumption of feed intake and rapidly increasing blood glucose. However plasma prolactin and T3 levels return to near PreHOT concentrations. There were transient increases in adiponectin and T4 levels during Recovery yet remained lower than the PreHOT baseline concentrations. Return to PENS saw a diversity of responses as ADG rose to 3.75 kg/head/day. Plasma leptin remained reduced despite weight gain and presumably fat deposition. The insulin concentration increased, possibly reflecting insulin insensitivity often seen in long fed beef cattle. Plasma T3 concentration was the most responsive hormone in Recovery and PENS, however, plasma T4 remained lower suggesting that T3 levels are supported despite reduced T4 availability.

Table 1. Mean % change and direction of change relative to baseline concentrations (PreHOT)

Plasma analyte	HOT	Recovery	PENS	One way ANOVA p-value
Adiponectin	36% ↓[c]	24% ↓[a]	45% ↓[c]	<0.0001
Leptin	47% ↓[c]	46% ↓[b]	46% ↓[a]	0.0004
Prolactin	15% ↓[b]	4% ↓[a]	6% ↓	0.0145
Insulin	12% ↓	23% ↓[b]	27% ↑[b]	<0.0001
Insulin: glucose ratio	3% ↓	18% ↓[a]	16% ↑	0.0038
T4	35% ↓[c]	18% ↓[c]	36% ↓[c]	<0.0001
T3	24% ↓[c]	6% ↓	9% ↑	<0.0001

Superscripts indicate levels of significance: a, $p<0.05$; b, $p<0.005$; c, $p<0.0005$.

Conclusion Beef cattle near market weight that are exposed to high heat loads experience major and persistent perturbations of hormones which regulate metabolic activity and appetite. It is feasible, that as an adaption, metabolic and endocrine set points are altered to ensure resilience in the face of future heat waves.

Acknowledgements This study was funded by Meat and Livestock Australia.

Forage for cattle with woody species: a proposal to reduce the density of shrubs in silvopastoral systems in dry areas

Nelson Pérez[1,2], Jairo Mora-Delgado[2], Sonia Ospina[1]

[1]*Corpoica, El Espinal, Colombia,* [2]*Universidad del Tolima, Ibagué, Colombia*

E-mail: jrmora@ut.edu.co

Take home message Woody species of high production and quality forage, at 45 days of age can be used with low densities per hectare, decreasing establishment costs and improving the comfort and nutrition of bovines in dry areas.

Introduction Scientific research shows a limited number of woody species recognized and valued for silvopastoral systems, especially those used to feed cattle in direct browsing or cutting. However, the recommendation of high density of sowing, above 8,000 shrubs per hectare, does not have technical criteria that relate forage production with the requirements of bovines. This study sought to adjust the times of forage production and quality in each evaluated species, to determine combinations of species that help to diminish the densities of the shrubs in the silvopastoral systems adjusted with the vegetative cycle of the pastures in zones of the tropical dry forest.

Materials & methods 10 *in situ* forage tree species were evaluated in the forage species collection of Corpoica Espinal, Tolima. No water or fertilizers were supplied to simulate real conditions of the farmers. A randomized complete block design (RCBD) was used, with three cut-off periods at 30, 45 and 60 days. Each treatment had 270 individuals per species, distributed in three rows of 90 m. Biomass production and bromatological quality were determined by the AOAC 984.13.

Results & discussion The results show that at 45 days the best response in forage production and nutritional quality was obtained (Figure 1). At this age, the forage of Cordia alba showed the best productive performance ($p < 0.0002$) under the described conditions (450g of MS and 20% protein), followed by *Tithonia diversifolia* (230g of MS and 15% protein). This is superior to many species recognized by technicians and ranchers, for example, *L. leucocephala, G. sepium, G. ulmifolia and M. oleifera*. It has been reported that *T. diversifolia* is the only species that can be used for cattle consumption between 30 and 35 days, but it should be used only for cutting and carrying because of its characteristics and structural fragility. In addition, there are few reported species that support high planting densities in browsing, such as *Leucaena leucocephala*, in densities above 10,000 plants / ha (Uribe *et al*., 2011).

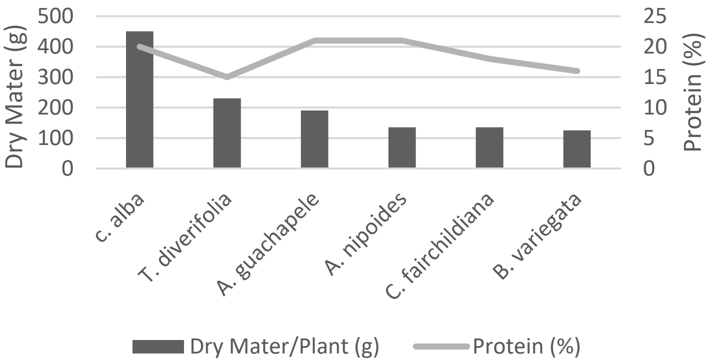


Figure 1 Edible fodder and nutritional quality of woody species.

Conclusion These species show excellent productive response capacity in environments with water scarcity. Knowing the production and quality forage of the shrub species and the nutritional requirements, mainly PC and NDT, of cattle, it was estimated that the density of shrubs / ha, does not need to be greater than 1,500. With this density you can adjust the animal load capacity and balance the diet in the field, to manage three bovines of 400 kg of live weight average, during 60 days, in direct browsing.

Acknowledgements To Ministerio de Agricultura y Desarrollo Rural and CORPOICA from Colombia.

References

Uribe F, Zuluaga AF, Valencia L, Murgueitio E, Zapata A, Solarte L, Cuartas C, Naranjo J, Galindo W, Gonzalez J, Sinisterra J, Gomez J, Molina C, Molina E, Galindo A, Galindo C and Soto R 2011. Establecimiento y manejo de sistemas silvopastoriles. Manual 1, Proyecto Ganadería Colombiana Sostenible. GEF, BANCO MUNDIAL, FEDEGAN, CIPAV, FONDO ACCION, TNC. Bogotá, Colombia. 78p.

Betaine supplementation during the fattening period mitigates hyperthermia in heat-stressed, gradually hypohydrated Awassi ram lambs

Hosam Al-Tamimi, Bilal Obeidat, Kamel Mahmoud, Mousa Daradka

Jordan University of Science and Technology, Irbid, Jordan

E-mail: hjaltamimi@just.edu.jo

Take home message Daily dietary supplementation of betaine at 8 g/head during fattening period mitigates heightened solar radiation-induced hyperthermia in Awassi ram lambs.

Introduction Fattening lambs in desert areas often experience disturbances in thermoregulatory abilities during the summer season, mainly manifested as hyperthermia. Betaine is an osmolyte that was consistently shown (in our laboratory with rats and broiler chicks) to alleviate symptoms of heat stress. The present project was carried out to examine the potential of daily betaine supplementation at 8 grams/head in modulating thermoregulation in fattening Awassi ram lambs (over a 56 days period), followed by exposure to excessive summer time solar radiation, superimposed onto a gradual hypohydration challenge protocol.

Materials & methods Thirty six 60-days old weaned Awassi ram lambs were allotted to four treatment groups. Following a fattening period of 56 days, all animals underwent minor surgical procedures to implant miniature thermologgers (iButton DS1921 H, Maxim Integrated Products, CA, USA) at different body locations to measure temperatures of the intraperitoneal cavity (T_{ip}), subcutaneous (T_{sq}) and intra-scrotal cavity (T_{sc}), as previously described by Al-Tamimi *et al.* (2013). Finally, an inertly coated logger was inserted - by gavage - to reside into the reticulo-rumen for the same purpose (T_{rum}). The thermologgers were pre-synchronized with an outer meteorological station [measuring light intensity (L), solar radiation (SR), ambient temperature (T_a) and percent relative humidity (RH%)]. After a one week-recovery post-surgical period, treatments commenced. Half of the lambs ingested daily dietary betaine (BET; top spread) at 8 g/head throughout the fattening and trial period (total of 77 days), while the remaining half acted as controls (CON; BET-free). All animals were initially kept in shaded (SHD) individual pens for a period of seven days. Then, nine lambs (n=9) from each treatment group were moved to unshaded (SUN) individual pens, and exposed to summer solstice direct SR (daytime values exceeding 885 W/m^2).

Results & discussion Shifting from SHD to SUN housing resulted in pronounced rises ($P < 0.01$) of T_{ip} (Figure 1.) and T_{sq} (Figure 2.) - but not T_{rum} - in both treatments, with BET being responsible for lower degree of displayed hyperthermia than CON (an interaction; $P = 0.02$). The upsurges in T_{ip} and T_{sq} were 0.29 and 0.30 ± 0.03 °C, for CON over BET counterparts (when switched to SUN; $P < 0.05$, but not maintained in SHD), suggesting an improved osmoregulation potential by BET, and hence an enhanced resistance to hyperthermia. Regardless of BET and/or SUN treatments, all lambs similarly ($P > 0.70$) maintained a pooled mean daily gradient of T_{sc} below T_c by 3.71-4.93 ± 0.02 °C. This was not unanticipated, due to the vitality of spermatogenesis and the evolutionary trend of species survival. Interestingly, T_{rum} did not vary among treatment groups. Consistently, based upon mean daily values, T_{rum} exceeded T_c by 0.33 and 0.50 ± 0.05 °C, in SHD and SUN exposure, respectively. This latter demarcation strongly suggests more involvement of cardiovascular and respiratory mechanisms than metabolic pathways within the gastrointestinal tract in thermoregulation by young Awassi lambs. Betaine inclusion in lamb diets is a promising tool in mitigating eminent hyperthermia often experienced in young lambs in desert climates.

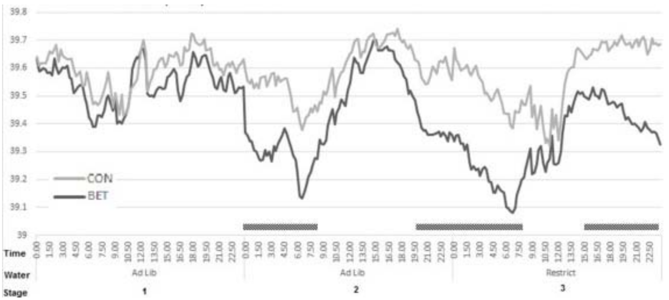


Figure 1 Core body temperature means of betaine-treated (BET) or untreated (CON) Awassi lambs. Horizontal dshed bars indicate time*treatment interaction (*p*<0.05).

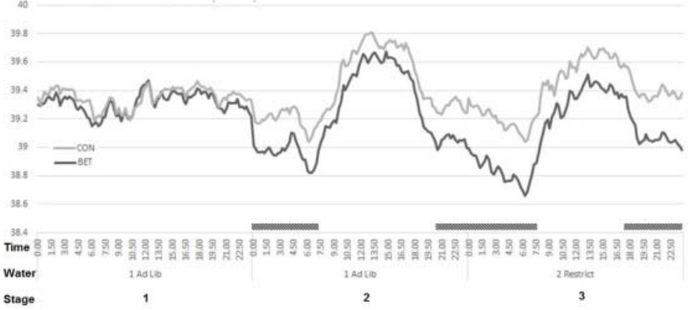


Figure 2 Subcutaneous body temperature means of betaine-treated (BET) or untreated (CON) Awassi lambs. Horizontal dshed bars indicate time*treatment interaction (*p*<0.05).

Conclusion In desert areas, excessive summer heat (mainly contributed by solar radiation) results in hyperthermia in young growing lambs and was evinced in the present trial. Effects of this thermal challenge was primarily displayed as rises of the core and peripheral body sites. Dietary BET showed a pronounced hyperthermia-alleviating effect, without significant shifts in ruminal temperatures. Scrotal (and hence, testicular) temperatures receive more careful temperature tuning than the other measured body sites, as evidenced by the scrotal cooling mechanisms shown, for optimizing spermatogenesis.

Acknowledgements This project was generously funded by the Research Deanship at the Jordan University of Science and Technology.

References

Al-Tamimi HJ, Obeidat BS, Abdullah AY and Atiyat RM 2013. Small Ruminant Research 109, 1-8.

Effects of prenatal heat stress on postnatal performances of dairy goats

Wellington N. Coloma[1], Nabil Mehaba[1], Ahmed A. K. Salama[1,2], Xavier Such[1], Gerardo Caja[1]

[1]Universitat Autonoma de Barcelona, Bellaterra, Barcelona, Spain, [2]South Dakota State University, Brookings, South Dakota, USA

E-mail: gerardo.caja@uab.es

Take home message Short-term heat stress at early pregnancy negatively affected dairy goats and kids.

Introduction There is evidence that the environmental conditions affecting mothers during pregnancy modify fetal programming through physiological and epigenetic changes (Viltart & Vanbesien-Mailliot, 2007) which permanently modify the behaviour, health and productivity of their offspring. Studies in animals have shown that episodes of stress during pregnancy have negative effects on the postnatal life of the offspring such as reduction of the duration of pregnancy and birth weight (Schroeder & Weller, 2009) and alteration of the immune system (Tuchscherer *et al*., 2002).

Materials & methods Lactating Murciano-Granadina dairy goats (n = 30; 41.8 ± 5.7 kg BW) were divided in two balanced groups and housed in six pens (5 × 2.5 m) of five goats each. After two weeks of adaptation, goats were synchronized by sponges (12 d) and naturally mated by the same buck at 2-d intervals in May. Groups were assigned to two climatic conditions (night-day, 12-12 h): thermo-neutral (TN; 15-20°C; n = 15) and heat stress (HS; 30-37ºC; n = 15) that were maintained before mating (d 1 to 12) and early pregnancy (d 13 to 57). Feed was provided *ad libitum* as a total mixed ration (70% alfalfa hay and 30% concentrate) and water was freely available at room temperature. Room temperature and humidity were recorded every 10 min throughout the experiment by data loggers. Rectal temperature and respiration rate were recorded thrice-a-week at 0800, 1200 and 1700 h. Feed and water intake were recorded daily. Pregnancy check was done by trans-rectal ultrasound at d 21 and 45 after mating. After d 45 of pregnancy all the goats were joined in a single group and managed under semi-intensive conditions (grazing 6 h/d and complemented in shelter). Two weeks before the expected date of parturition, the goats were weighed and moved to kidding pens for permanent surveillance and parturition assistance. Pregnancy length, litter size and weight of the doe and kids were recorded after parturition. Colostrum was milked and fed to the kids that were reared with a milk replacer until d 35 of age. Data were analysed by the PROCMIXED for repeated measurements of SAS (v.9.1.3).

Results & discussion During the period of thermal treatment (d 1 to 57) the HS showed higher rectal temperature and respiration rate (+0.68ºC and +76 breaths/min; $P < 0.01$) than TN does, but lower feed intake (−15% DM; $P < 0.001$). Performances at parturition are shown in Table 1. Pregnancy of HS does decreased in 3 d ($P < 0.01$) and birth weight of their kids tended to decrease by 7% lower ($P < 0.10$). No effects of treatments were detected on litter size, birth weight and weight of 35-d kids.

Table 1. Performance and reproductive parameters in goats and goat kids at parturition and early postpartum period.

Variables	TN	HS	RMSE[1]	*P* value	
				Treatment	Litter size
Litter size	2.31	2.23	0.79	0.806	-
Litter weight (kg)	5.40	4.71	0.71	0.061	0.001
Duration of pregnancy (d)	146	143	2.0	0.006	0.915
Birth weight of kids (kg)	2.34	2.18	0.38	0.122	-
Weight of 35-d-old kids (kg)	7.88	7.64	1.34	0.520	-

[1] Root means standard error.

Conclusions HS during the period of mating until the first 45 d of pregnancy, reduced the duration of pregnancy with probable effects on the weight of the kids.

Acknowledgements Funded by the Ministry of Economy and Competitiveness of Spain (Project AGL2013-44061-R).

References

Hamzaoui S, Salama AAK, Albanell E, Such X and Caja G 2013. Journal of Dairy Science 96, 6355–6365.
Schroeder M and Weller A 2009. Development Psychobiology 55, 176-192.
Viltart O and Vanbesien-Mailliot CCA 2007. The Scientific World Journal 7, 1493–1537.
Tuchscherer M, Kanitz E, Otten W and Tuchscherer A 2002. Veterinary Immunology Immunopathology 86, 195-203.

Long-term effects of early life inoculation with rumen fluid on rumen and colon fermentation of goats

Alejandro Belanche, Juan Manuel Palma-Hidalgo, Ibtissam Nejjam, Rosa Serrano, Elisabeth Jiménez, A. Ignacio Martín-García, David Rafael Yáñez-Ruiz

Estación Experimental del Zaidín (CSIC), Granada, Spain

E-mail: a.belanche@csic.es

Take home message Inoculation of newborn goats with rumen fluid had positive long-term effects on the rumen function.

Introduction Rumen microbiome in the adult animals is highly redundant and resilient, making it difficult to impair a permanent effect using conventional interventions (*e.g.* prebiotics and probiotics); on the contrary, the developing rumen in newborn animals, due to its greater plasticity, represents an opportunity as it provides a unique environment for manipulation of such a complex ecosystem with potential long–term effects (Yáñez-Ruiz *et al*., 2015). Although the rumen is home to a highly diverse microbiota, only few members of this community have been explored for their potential as probiotics to be used in young ruminants. A companion paper (Belanche *et al*., 2018) noted positive short-term effects of early life inoculation with a complex rumen microbial community on the rumen development. This study aimed to investigate the long-term effects of such inoculation on the foregut and hindgut fermentations and microbial abundances.

Materials & methods A total of 36 newborn goats kids were randomly distributed into four groups. Kids were daily inoculated from birth up to 11 weeks of age with autoclaved rumen fluid as prebiotics (PRE), fresh rumen liquid adapted to concentrate (RLC), fresh rumen liquid adapted to forage (RLF) or absence of inoculation (CTL). Rumen inoculum was obtained at 3h after feeding from adult rumen-cannulated goats fed either concentrate (33:67 F:C ratio) or 100% forage diets. Inoculum was filtrated and orally inoculated to the young animals (1 mL/kg BW). Kids received milk replacer for 7 weeks. Experimental groups were kept physically separated and had *ad libitum* access to commercial concentrate and oat hay. Animals were euthanized at 5 months of age and colon (diluted with 33% of PBS) and rumen contents were filtrated to determine pH, ammonia and volatile fatty acids (VFA) concentrations. DNA was extracted from lyophilized samples and the main microbial groups were determined by qPCR. Data were analysed by ANOVA using SPSS software.

Results & discussion The inoculation with either fresh or autoclaved rumen fluid promoted a higher concentration of rumen protozoa (P<0.001) which may explain the higher levels of ammonia-N (P=0.023) and butyrate (P=0.010) observed in inoculated vs CTL animals. Moreover, inoculation of fresh rumen fluid as probiotics (RLC and RLF groups) boosted the rumen fermentation leading to higher levels of total VFA (P<0.001) and methanogens (P=0.047) than CTL and PRE groups. On the contrary, minor effects were noted on the colon fermentation pattern (pH and VFA) which remained fairly constant across treatments. However, inoculation process tended to increase the levels of methanogens (P=0.053) and decreased that of iso-valearte (P=0.079) and anaerobic fungi (P<0.001), possibly as a result of a shift towards a hindgut compensatory fermentation in CTL animals (Clauss et al., 2010).

Table 1 Effect of early life inoculation with rumen fluid on the rumen and colon fermentation in adult goats.

Location	Rumen						Colon					
Treatment	CTL	PRE	RLC	RLF	SED	*P*	CTL	PRE	RLC	RLF	SED	*P*
NH_3-N (mg/dL)	5.61^{b}	13.3^{a}	13.1^{a}	15.1^{a}	2.668	*						
pH	5.87	6.31	6.07	5.93	0.182	ns	6.16	6.22	6.18	6.16	0.128	ns
Total VFA (mM)	74.1^{b}	75.6^{b}	96.7^{a}	99.0^{a}	6.480	***	102	99	101	101	9.230	ns
Molar proportion (%)												
Acetate	55.4	54.5	54.7	56.3	2.484	ns	72.7	68.4	69.2	70.9	2.293	ns
Propionate	33.8	30.7	31.3	26.7	3.060	ns	13.8	15.2	13.4	13.9	0.885	ns
Butyrate	7.35^{b}	10.1^{b}	10.2^{b}	13.4^{a}	1.487	*	10.2	13.4	15.0	12.6	2.291	ns
Iso-valerate	1.03^{ab}	1.63^{a}	0.86^{b}	0.97^{b}	0.263	*	1.23	0.84	0.69	0.67	0.203	†
Microbes (gene copies/g FM)												
Bacteria	9.22	9.04	9.34	9.04	0.208	ns	10.4	10.2	10.2	10.2	0.146	ns
Methanogens	4.80^{b}	4.83^{b}	5.46^{a}	4.99^{ab}	0.242	*	6.49	6.98	6.89	6.95	0.163	†
Anaerobic fungi	4.79	4.74	4.59	4.78	0.278	ns	3.80^{a}	3.50^{b}	3.37^{b}	3.48^{b}	0.087	***
Protozoa	2.96^{b}	6.83^{a}	7.30^{a}	7.20^{a}	0.270	***	5.01	4.77	4.72	4.68	0.185	ns

*** P<0.001; ** P<0.01, * P<0.05, † P<0.1, ns not significant (*n*=9)

Conclusion Our findings suggested that inoculation of young animals with fresh rumen fluid as probiotics had some positive long lasting effects on the rumen fermentation. These effects were less obvious when autoclaved rumen fluid was used as inoculum. These results open the possibility of rumen microbial programing in early life.

Acknowledgements This study was funded by the Economy and Competitiveness Spanish Ministry (AGL2017-86938).

References

Belanche, A, J.M. Palma-Hidalgo, E. Jimenez, Martín-García A.I, and D.R. Yáñez-Ruiz. 2018. Proceedings ISNH
Clauss, M., I. Hume, and J. Hummel. 2010. Animal 4, 979-992.
Yáñez-Ruiz DR, Abecia L and Newbold CJ 2015. Frontiers in Microbiology 6, 1133

Goat grazing or pine-tree removal in after-fire management of rocky slopes in Mediterranean forests

Yan Landau, Noa Cohen, Tova Deutsch, Yagil Osem

Agricultural Research Organization, Rishon LeZion, Israel

E-mail: vclandau@agri.gov.il

Take home message High-density stands of pine-trees (*Pinus halepensis*) germinated after wildfires represent a major hazard of future fire propagation. Goat grazing, applied six years after a fire, did not decrease significantly pinetree density but resulted in low, cone-less trees and low cover of flammable biomass. Pine mortality was higher in control than in grazed plots and pine-trees adapted to grazing by growing in the summer, while control counterparts were inactive. Selective pine-tree removal did not succeed in eradicating the stand. Pine-trees can constitute up to 17% of goats' diets.

Introduction A violent forest fire has claimed 44 lives and put 3500 hectares of natural and planted pine forests and natural oak woodland to ashes in the Carmel National Park in December 2010. Crown driven fire in adult pine-trees represent the most dangerous way of fire-expansion. The cycle of fires in pine forests is initiated by the post-fire large-scale germination of pine-tree seedlings resulting from fire-triggered aperture of the cones. Therefore, early control of pine-tree seedlings density in fire-prone zones is germane to the prevention of fire in the pine forests. Mechanical clearance of the vegetation in rocky and steep wadi slopes is practically impossible. The effect of goat grazing on pine-trees germinated after-fire has not been studied in a holistic way, encompassing the nutrition of goats and the effects of grazing on the development of pine-trees and their associated species, *Cistus spp* and lentisk - *Pistacia lentiscus*. We hypothesized that selective pine removal and goat grazing would eradicate stands of pine-tree seedlings.

Materials & methods Six plots of 0.1 ha were allotted to three treatments: control (C), selective pine-tree removal (PR) or goat grazing (GG) in March 2016. Vegetation volume was measured along two transects in each plot for two consecutive years and individual pine-tree seedling development and ecophysiology were monitored. Goat grazing was applied in late spring at high stocking rates (1380 and 2760 grazing days/ha in 2016 and 2017, respectively). A fecal NIRS calibration (N=48) was established to elucidate the proportions of lentisk, pine tree and cistus in the diets selected by goats in 2017, as explained in Glasser *et al.* (2008) and feces were NIR-scanned to elucidate dietary botanical composition. Tree Leaf Area Index (LAI)), an indicator of total vegetation biomass, and stomata aperture were estimated using a SunScan apparatus with 64 sensors and with a ΔT pressure cell, respectively.

Results & discussion Vegetation cover was 89, 81, and 58%, *i.e.*, 35.% lower in GG plots than in controls throughout, PR being intermediate. Pine-tree cover, which did not differ before grazing, represented 34, 17, and 46% after grazing, in PR, GG, and C, respectively. The leaf to stem ratio of pines was 0.4 in GG, compared with 1.4 in C or PR (SEM=0.09). Neither PR nor GG resulted in eradication of the pine stand: pine-tree density stabilized at 2000 in PR (down from 10000) and ca. 4000 (down from 6000) per hectare in GG and C. Stem diameter increment did not differ between GG and C pine-trees, but height increased in C (and PR), but not in GG trees. Pine-trees adapted to grazing by growing new leaves and transpiring during the summer. Finally, LAI was 0.5 in GG and 2.0 in the other groups. In other words, combustible biomass was four-fold higher under C and PR than under GG management. Fecal NIRS performance was excellent (Table 1) and pine-trees represented 9-17% of ingested DM (Figure 1). On average, goats ingested ca. 200 g/day of DM from pine-trees.

Table 1 Calibration performance of fecal NIRS.

Consti-tuent	Mea n	SD	SE C	R2ca l	SEva l	R2va l
Cistus	5.5	10.0	2.4	0.94	3.5	0.88
Lentisk	31.4	28.0	3.4	0.99	5.5	0.96
Pine	24.6	29.3	2.6	0.99	4.1	0.98
Conc.	17.6	23.0	4.0	0.97	5.4	0.94

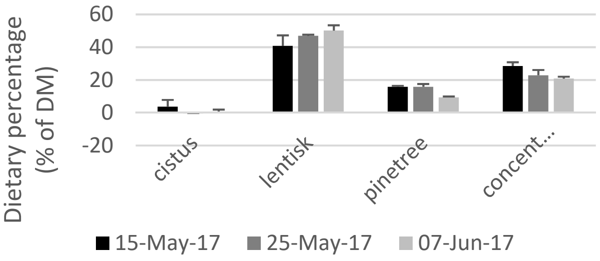


Figure 1 Dietary components (means of two plots and SE).

Conclusion Goat grazing at high stocking rate is instrumental in decreasing total brush cover and impairing the development of pine-tree seedling germinated after forest fires but does not eradicate the stand.

Acknowledgements Funding from the Israel Ministry of Environment is acknowledged.

References

Glasser T1, Landau S, Ungar ED, Perevolotsky A, Dvash L, Muklada H, Kababya D and Walker JW 2008 Journal of Animal Science 86 1345-1356.

Performance and cost evaluation of West African Dwarf bucks managed under five housing patterns

Magnus Anya[1], Glory Eyoh[2], A.Ausaji Ayuk[1]

[1]University of Calabar, Calabar, Cross River, Nigeria, [2]Akwa Ibom State University, Obiokpa, Akwa Ibom, Nigeria

E-mail: yinsaj@yahoo.com

Take home message Bamboo housing is ideal for raising goats compared to other housing patterns in Akwa Ibom state.

Introduction The West African Dwarf (WAD) goat is the most prevalent small ruminant in the hot humid forest zone of southern Nigeria. Its relative tolerance to excessive humidity and trypanosomiasis singled it out as a breed of choice (Odoemelam, 2012) to improve daily protein intake of Nigerians. However, the lack of proper housing coupled with the problem of inadequate nutrition leads to undulating growth patterns of these animals. Therefore, we evaluated the effect of five housing patterns on the performance and cost of production of the WAD goats.

Materials & methods Five different types of housing patterns (T_1CH – Concrete house, T_2ZH - Zinc house, T_3MH – Mud house, T_4BH – Bamboo house, T_5TH – Thatch house) were built to accommodate the experimental animals. Twenty (20), five months old weaner WAD goats of mixed sexes (10 bucks and 10 does) of average initial body weight of 8.63kg and 10.25kg respectively, were randomly assigned to each of the five housing units in a Randomized Complete Block Design (RCBD) experiment. Four forages were combined in the ratio 1:1:1:1 and offered to the animals in their respective housing units. These forages included *Andropogon tectorum, Aspilia africana, Panicum maximum,* and *Gmelina arborea*. Clean fresh water and salt licks (TANLICK®) were provided *ad libitum.* Feed offered and left over were recorded daily. The cost of each housing pattern and average weekly weight gains were computed. The data collected were subjected to analysis of variance procedures. Significant means were separated using Duncan's New Multiple Range Test (Duncan, 1955).

Results & discussion The results showed significant ($P<0.05$) differences for performance parameters such as final weight gain, daily feed intake and feed conversion ratio (Table 1). Bamboo house recorded the highest value for final weight (11625.00g) followed by zinc house, thatch house and mud house while concrete house had the least value (9675.00g). The cost of production was least for Bamboo house T4 while Zinc house T_2 recorded the highest value (Figure 1).

Table 1 Growth Performance of West African dwarf goats under five housing patterns.

Parameters	T_1 (CH)	T_2(ZH)	T_3(MH)	T_4(BH)	T_5(TH)	SEM
Initial weight(g)	8625.00	10000.00	10125.00	10250.00	975. 00	630.81
Final weight (g)	9675.00^{c}	11575.00^{a}	11125.00^{ab}	11625.00^{a}	11150.00^{b}	566.86
Average total weight (g)	1300.00^{c}	1575.00^{b}	1000.00^{d}	1625.00^{ab}	1650.0^{a}	316.36
Total feed intake (g)	108.90^{c}	109.60^{c}	250.20^{ab}	227.90^{b}	272.40^{a}	62.78
Daily feed intake (g)	1945.00^{d}	1957.00^{c}	56400.00^{a}	56400.00^{a}	56100.00^{b}	351.67
Av. total weight gain (g)	172.77^{bc}	206.44^{a}	207.59^{a}	207.58^{a}	199.11^{b}	0.00
FCR	11.48	9.58	9.86	9.38	9.77	0.57

$^{a-d}$ means in same row with different superscripts are significantly different ($P<0.05$).
SEM: Standard error of mean, T_1 (CH) – Concrete house, T_2 (ZH) – Zinc house, T_3 (MH) – Mud house T_4 (BH) – Bamboo house, T_5 (TH) – Thatch house

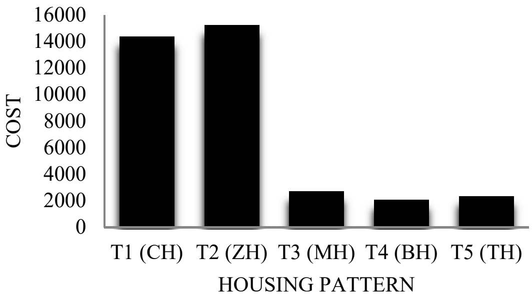


Figure 1. Cost implication of West African Dwarf goats in five housing patterns

Conclusion It can be concluded that bamboo housing pattern had the highest weight gain and the least cost of production. Therefore, it should be adopted as the most productive and affordable housing for West African Dwarf goats.

References

Duncan DB 1955. Biometrics. 11, 1-42.
Odoemelam VU 2012. Ph.D dissertation. Umudike Nigeria

ASKBILL: Regional flystrike forecasts to improve the wellbeing and productivity of sheep

Brian Horton[2], Johan Boshoff[1,3], Lewis Kahn[1,3], James Rowe[3]

[1]*Faculty of Science, Agriculture, Business and Law, University of New England, Armidale, NSW, Australia,* [2]*Tasmanian Institute of Agriculture, University of Tasmania, Launceston, Tasmania, Australia,* [3]*Cooperative Research Centre for Sheep Industry Innovation, Armidale, NSW, Australia*

E-mail: lkahn3@une.edu.au

Take home message The ASKBILL forecast of flystrike will improve decisions about the need for future treatments.

Introduction Sheep producers rely heavily on their experience from past events and current visual signs of their animals as the basis for the management of their sheep flocks. Uncertainty is a feature of grazing systems, as is the understanding that negative impacts for wellbeing and productivity of livestock occur before the appearance of visual signs. Under these conditions the use of heuristic principles to guide decisions about future events is prone to systematic errors (Kahneman and Tversky 1982) and forecasts provided through decision support such as ASKBILL (Kahn *et al*. 2017) provide valuable information to help decision makers towards a better outcome. The risk of flystrike is determined by a combination of climatic variables, and the actual incidence of flystrike can be accurately predicted (r=0.77) by computer modelling based on actual climate data (Wardhaugh *et al*. 2007). The accuracy of regional near-term forecasts for the ASKBILL flystrike model (based on Horton and Hogan, 2010) is described in this paper.

Materials & methods ASKBILL is a web-based application developed by the Sheep CRC in Australia to provide forecasts to help sheep producers better manage their flocks. The application contains a number of biophysical models adjusted on a daily time-step in response to: user inputs; Australian sheep breeding values; and weather forecast data. Weather forecasts are updated daily from the Australian Bureau of Meteorology based on near term dynamical deterministic records as well as seasonal outlook dynamical probabilistic records on a 5x5 km grid for Australia. Total flystrike risk (predictions exceeding 25 strikes/week/10,000 sheep) for ewes and lambs obtained from the ASKBILL flystrike model using weather hindcasts (forecasts of past events) for 20 different starting dates chosen at random from the period 1990-2012 for 21 locations around Australia, were compared with outputs from actual weather for the 5-day duration of the hindcasts. For each starting date there were 11 ensemble hindcasts which included minimum and maximum temperature and rainfall. Humidity was not available at the time of the comparison, so an average 9am relative humidity of 71% was used. In addition to this comparison, model outputs were aggregated to provide Australia-wide forecasts at a resolution of 5x5 km.

Results & discussion The number of days where the prediction of total flystrike risk from hindcasts exceeded the threshold incidence rate of 25 strikes/week/10,000 sheep closely matched the predictions based on actual weather. The correlations of predictions based on hindcast and actual weather over the 5-day period were in the range 0.85-0.86 for ewes and 0.82-0.94 for lambs. Correlations for day 1 forecasts were higher than for day 5 forecasts. Aggregating flystrike risk in a national map can provide a visual means of identifying regional risk (Figure 1).

Figure 1 Regional total flystrike risk across Australia at the scale of 5x5 km. Increasing risk is indicated by a darker shade of grey. Example for 20 January 2018.

Conclusion The forecast of near-term risk of flystrike can be used as a reliable guide to whether or not the next five days will be a period of unusually low or high risk and assist in deciding whether protective treatment should be applied.

Acknowledgements We acknowledge Alister Hawksford, Australian Government Bureau of Meteorology.

References

Horton,B, Hogan L 2010. Animal Production Science 50, 1069–1076
Kahn LP, Johnson I, Rowe JB, Hogan L and Boshoff J 2017. Animal Production Science 57, 2257–2262.
Kahneman D and Tversky A 1982. Cognition, 11, 123-141.
Wardhaugh KG Morton R Bedo D Horton BJ and Mahon RJ 2007. Medical and Veterinary Entomology 21, 153-167

Compact total mixed ration – effects on feed intake, rumen pH and milk production in dairy cows

Mikaela Lindberg, Cecilia Kronqvist

Swedish University of Agricultural Sciences, Uppsala, Sweden

E-mail: mikaela.lindberg@slu.se

Take home message Decreased sorting possibilities of a total mixed ration (TMR) by increased mixing time and addition of water resulted in decreased feed intake but did not alter the milk production or rumen pH of lactating dairy cows.

Introduction When feed is distributed on a feed table with 2-3 cows per meter feeding space, there is a risk that high-ranked cows fed TMR sort and eat the more palatable concentrate parts to a higher extent than the roughage, leaving a less energy dense mix to the low-ranked cows. A way to handle this risk is to make the feed mix more homogenous and dense, by soaking the concentrates and mixing the diet more thoroughly. However, as this results in a decreased particle size, it could possibly result in decreased pH in the rumen. The aim of this study was to evaluate the effects of increased mixing and reduced dry matter (DM) content in a TMR on feed intake, milk production and rumen pH. The hypotheses were that increased mixing and reduced DM would decrease particle size, decrease rumen pH and thus reduce feed intake and milk yield in dairy cows compared with cows fed a traditional TMR.

Materials & methods Two dietary treatments were tested in 40 mid-lactation dairy cows, including four rumen cannulated cows, in a change-over experiment with randomized block-design. The two experimental periods consisted of two weeks for adaptation and one week for measurements. The cows were housed in a free-stall barn and milked twice a day. The dietary treatments were; TMR or compact mixed ration (cTMR). Both treatments were mixed and distributed twice daily using the same silage and concentrate with the difference that approximately 30% water was added to the cTMR and it was mixed for 60 minutes whereas the TMR was mixed for 10 minutes. The increased mixing time in cTMR was performed to decrease particle size and increase the homogeneity of the feed. The silage:concentrate ratio was 60:40 on DM basis. The DM content of the silage was 42% and the diet contained (/kg DM) 12.5 MJ metabolizable energy (ME), 187 g crude protein (CP) and 367 g neutral detergent fibre (NDF). The cows had *ad libitum* access to the feed and individual intake of feed and water was recorded using feed mangers on weight scales, with three animals per manger, and water meters in water cups (Biocontrol A/S, Rakkestad, Norway). Particle size was evaluated using the Penn State particle separator (Lammers *et al*., 1996) with 2 sieves and a bottom pan. Rumen pH was measured manually (MP 125 Mettler Toledo, Schwerzenbach, Switzerland) in the cannulated cows every hour covering a 24h period. Data were analysed with mixed models including block, treatment and period as fixed effects, cow as random effect, and repeated measures within cow modelled by spatial power covariance structures. Data on rumen pH were analysed both as absolute values and as time below pH 5.8.

Results & discussion The increased mixing of the cTMR-diet decreased particle size markedly, and the addition of water decreased dry matter from 52 % to 37 %. Total dry matter intake (DMI) was higher in cows fed TMR compared to cows fed cTMR (Table 1). However, milk production was not different between the two diets. Even if intake of drinking water was higher in cows fed TMR, total intake of water was higher in the cows fed cTMR, and the water content of the diet may have been a limiting factor for DMI. Rumen pH or time below pH 5.8 did not differ between the treatments, indicating that the decreased particle size did not reduce buffering capacity in the rumen.

Table 1 Results of the change-over experiment on 40 lactating cows. Data presented as least squares means.

Item	TMR	cTMR	SEM	p-value
DM in top sieve (> 19 mm, %)	32	6	1.7	0.001
Dry matter intake (kg/day)	28.6	26.8	0.62	0.001
Total water intake (kg/day)	136.3	144.3	3.04	0.001
Milk yield (kg/day)	36.4	36.2	0.87	0.395
Rumen pH	5.8	5.7	0.11	0.638
Time below pH 5.8 (hours/day)	14.6	14.9	1.60	0.902

Conclusion Increased mixing time and addition of water to a TMR consisting of grass silage and concentrate (60:40) resulted in decreased particle size of the diet and decreased feed intake but did not have a significant effect on rumen pH or milk production.

Acknowledgements The research project was funded by SLU EkoForsk.

References

Lammers BP, Buckmaster DR and Heinrichs AJ 1996. Journal of Dairy Science 79, 922-928.

The management of herbage allowance improves beef cattle productivity in Campos grazing systems

Martin Do Carmo[1], Pablo Soca[2], Geronimo Cardozo[3]

[1]Universidad de la República, Montevideo, Uruguay, [2]Universidad de la República, Paysandu, Uruguay, [3]Instituto Nacional de Investigación Agropecuaria, Treinta y Tres, Uruguay

E-mail: martindocarmocorujo@gmail.com

Take home message Herbage allowance management improves individual and/or per ha animal productivity in Campos grassland.

Introduction Herbage allowance (HA, kg DM/kg BW) has proved to be an effective tool for management to increase beef cattle productivity in Campos grassland (Soares *et al*. 2005, Mezzalira *et al*. 2012, Claramunt *et al*. 2016, Do Carmo *et al*. 2018). However validation at commercial systems has to be evaluated, and a project was carried out in two commercial farms to validate coefficients of HA proposed by the research results.

Materials & methods Based on data from experimental results of HA and its effect on ADG (average daily gain) for growing steers and heifers and weaning rate and calf weight for cattle, coefficients of HA that maximize the individual and per ha animal productivity were summarized in a guideline (Table 1). In Farm 1 (2200 ha) after evaluation of herbage height (15 to 45 cm) on all the paddocks and the average stocking rate (360 kg/ha on average) we identified that productivity per ha in the fattening process can be increased by diminishing herbage allowance, increasing stocking rate but maintaining individual animal productivity (100 to 150 kg/head/year). In Farm 2 (260 ha), after evaluation of main process (breeding) and its historical pregnancy rate (70%) and herbage height on the paddocks, we focused on how to increase pregnancy rate by increasing herbage allowance. Herbage mass was evaluated by comparative yield method (Haydock and Shaw 1975). In Farm 2, one restriction was imposed by the owner, stocking rate cannot be modified on average, in the short-term.

Results & discussion On Farm 1, we challenged the manager to give us one paddock where we started our management in January 2013 and a paddock next to with manager treatment, after 26 days, ADG was 1,87 kg/head/day in both paddocks, but stocking rate were 957 kg/ha in our paddock and 260 kg/ha in the manager management. After another month with equal ADG (0.18 kg/day) the manager started to change the traditional management for fattening steers and we increased the area under our management up to 406 ha of the farm (2200 ha). Also paddocks assigned to categories changed, *e.g.* paddocks with fattening process from spring to autumn were used with beef cows during winter to remove dry accumulated herbage with a HA coefficient of 3 as proposed in the guideline. Stocking rate for paddocks under our management increased to 560 kg/ha (55% greater than average, for 2.5 years), and ADG was maintained on 0.25 kg/day annual average. Herbage mass decreased from 5500 kg/ha to 2500 kg/ha. On Farm 2, on contrary to Farm 1, HA was increased from 4 to 6 kg DM/kg BW on the biggest paddock (160 ha), decreasing the HA for the categories with lower energy requirements (pregnant cows, empty cows, fat cows, and growing heifers, Table 2). Herbage mass increased from 978 to 1800 kg DM/ha (average of the last year). Suckling restriction was coupled with HA at the beginning of breeding season.

Table 1 Recommended herbage allowance coefficients for management of stocking rate for different animal categories Su = summer, Sp = spring, Au = autumn and Wi = winter

Cattle category	Su	Sp	Au	Wi
Cow	6 or +	6 or +	6 or +	3 or +
Heifer-Steer up 350 kg	3	2	3	3
Steer up 550 kg	5-6	5-7	6-8	7-10

Table 2 Results on Farm 2 for pregnancy rate and calf weight at weaning in May of each year.

Response variable	Years or time for comparison		
	Historical	2013/14	2014/15
Pregnancy rate (%)	70	88	90
Regional pregnancy rate (%)	---	81	72
Calf weight (kg)	---	150	180

Conclusions Herbage allowance was an effective tool to increase individual and/or per ha productivity in both cases, with differences on the implementation due to different restrictions and different resources to deal with. Spatial arrangement of categories and HA coefficients in combination with animal categories need further investigation to improve farm productivity and profitability.

References

Claramunt M, Fernandez A and Soca P 2017. Animal Production Science doi.org/10.1071/AN16601
Do Carmo M, Sollenberger LE, Carriquiry M and Soca P 2018. Professional Animal Scientist 34, 32-41.
Haydock KP and Shaw NH 1975. Australian Journal of Experimental Agriculture and Animal Husbandry 15, 663-670.
Mezzalira JC, Carvalho PCF, da Trindade JK, Bremm C, Fonseca L, do Amaral MF and Reffatti MV, 2015 Ciência Rural 42, 1264-1270.
Soares AB, Carvalho PCF, Nabinger C, Semmelmann C, da Trindade JK, Guerra E, de Freitas TS, Pinto CE, Fontoura JA and Frizzo A 2005. Ciência Rural 35, 1148-1154.

The INRA 2018 updated calculation of fermentable organic matter intake improves the prediction of net portal appearance of volatile fatty acids in ruminants

Christelle Loncke[1], Isabelle Ortigues-Marty[2], Daniel Sauvant[1], Jean Vernet[2], Pierre Nozière[2]

[1]*UMR Modélisation Systémique Appliquée aux Ruminants, INRA, AgroParisTech, Université Paris Saclay, 75005, Paris, France,* [2]*INRA-Vetagrosup, UMR1213 Herbivores, 63122, Saint Genès Champanelle, France*

E-mail: christelle.loncke@agroparistech.fr

Take home message The INRA 2018 updated calculation of fermented organic matter (FOM) in the rumen, which takes into account digestive interactions, improves the prediction of net portal flow of volatile fatty acids (VFA) in ruminants.

Introduction The FOM in the rumen is a key predictor of the production of VFA, the main energy nutrients for ruminants (Loncke *et al*., 2009). Until recently, two calculation methods were used in France (INRA, 1978, 2007). In 2016, the calculation was largely revised (Sauvant and Nozière, 2016). It now takes into account digestive interactions due to level of intake, proportion of concentrate and rumen protein balance. It also better integrates partition of digestion between rumen and intestines for protein and starch considering that the outflow rate of particles and liquid changes with level of intake and proportion of concentrate. This revised FOM is now included in the new INRA (2018) feeding system. The aim of the present study is to compare the ability of the three calculations to predict the net portal appearance of VFA.

Materials & methods Meta-analyses (Sauvant *et al*., 2008) were applied on published data compiled from the INRA Flora database (Vernet and Ortigues-Marty, 2006), which gathers published results on net portal appearance of nutrients in ruminants. The FOM of diets was calculated according to INRA 1978 (FOM_1978), INRA 2007 (FOM_2007) and INRA 2018 (FOM_2018) using the systoolweb.fr application (Chapoutot *et al*., 2015). The FLORA database is totally independent from databases used to establish the INRA 2018 feeding system. Relationships between NPA of total VFA and the three expressions of the FOM were studied by intra-experiment covariate models.

Results & discussion For a wide range of diets containing 25 ± 31% concentrate and ingested at a dry matter intake level of 1.86 ± 0.65 %BW, the net portal appearance of total VFA was significantly predicted by all of the three expressions of the FOM (Table 1). Adjustment was, however, better for the FOM_2018 model than for the other two models. Overall it had smaller RMSE and higher adjusted R². The standard error on the slope was also smaller (0.0338 = 13% of the slope, vs. 15% for FOM_2007 and 16% for FOM_1978). The intercept was not significantly different from 0, suggesting no VFA production at zero intake. Finally, level of intake and diet composition did not influence the parameters of the FOM_2018 model. By contrast, dietary starch content was an interfering factor on residues and individual slopes, and the proportion of concentrate was interfering on residues of the FOM_1978 model. Altogether, this suggests that the 2018 updated calculation of the FOM better reflects the outcomes of ruminal digestion. The slope of 0.265 corresponds to 6.36 mmol/d/kg BW, which is equal to 76% of total VFA produced in the rumen as calculated by the new INRA 2018 system (8.35 mol/d/kg FOM).

Table 1 Linear intra experiment response equations for the relationships between fermented organic matter intake (FOM, g/d/kg BW) calculated from INRA (1978, 2007 and 2018) and the net portal appearance of total volatile fatty acids (mmol/h/kg BW)

Variable	Mean ± SD	nexp	ntr	intercept	se	slope	se	RMSE	Adjusted R²
FOM_1978	11.03 ± 3.98	24	54	0.734*	0.411	0.182***	0.0291	0.424	0.879
FOM_2007	9.68 ± 3.74	24	54	0.579*	0.333	0.225***	0.0341	0.411	0.886
FOM_2018	9.39 ± 3.14	24	54	0.256^{NS}	0.321	0.265***	0.0338	0.369	0.909

Probability of significance: ^{NS} P > 0.1 ; *P<0.05; ***P<0.001; nexp : number of experiments ; ntr : number of treatments

Conclusion The FOM of the new INRA system improves the prediction of net portal appearance of total VFA.

References

Chapoutot P, Martin O,Nozière P and Sauvant D. 2015. Annual Meeting of the European Federation of Animal Science
INRA 1978. Alimentation des Ruminants. INRA Publications, Versailles, 597 pp
INRA 2007. Tables INRA 2007, Versailles, 307 pp.
INRA 2018. INRA feeding system for ruminants. Wageningen Academic Publisher, Wageningen, 640 pp
Loncke C, Ortigues-Marty I, Vernet J, Lapierre H, Sauvant D and Nozière P 2009. Journal of Animal Science 87, 253-268.
Sauvant D and Nozière P 2016. Animal 10, 775-770
Sauvant D, Schmidely P, Daudin JJ and St-Pierre NR 2008. Animal 2, 1203-1214.
Vernet J and Ortigues-Marty I 2006. Reproduction Nutrition Development 5, 257-546

Induced copper deficiency in sheep with application of alternative lime amendment to soil

Robert Van Saun[1], John Spargo[1,2], Marvin Hall[1]

[1]*Pennsylvania State University, University Park, Pennsylvania, USA,* [2]*Agricultural Analytical Services Laboratory, University Park, USA*

E-mail: rjv10@psu.edu

Take home message Plant tissue molybdenum (Mo) content was increased in a dose-dependent response from application of an alternative lime product to amend soil pH that contained high Mo.

Introduction A clinical situation occurred where a sheep flock experienced high death rate (24 of 25) in only their two year-old ewes and high stillborn and neonatal deaths (27%). All diagnostic information on lamb and ewe necropsies showed no infectious agent and low liver Cu concentration. The interaction of molybdenum (Mo), sulphur, and copper (Cu) in ruminant animals has been well described (Suttle, 2010). The Cu deficiency was believed to be induced by application of an alternative lime (slag) product being applied to the farm's land for the previous two years resulting in high forage Mo content. This slag was found to contain between 50 and 80 mg/kg Mo where cautionary labels are required for fertilizers containing >10 mg/kg Mo. Over a 4-year period following the initial application of the slag product, home raised forages fed to the sheep had 196% higher Mo, no difference in Cu and a 46% reduction in Cu:Mo ratio compared to previous forage data. The objective of this study was to determine if the alternative lime product could increase plant tissue Mo content.

Materials & methods To test the hypothesis of the slag lime directly increasing forage Mo content a controlled greenhouse study was performed with a factorial split plot experimental design. Two common agronomic forage plants, Orchardgrass (*Dactylis glomerata*) and alfalfa (*Medicago sativa*), were used to mimic common pasture plants for the region. Seeds (10 each, 20 per pot) were planted into soil (5 mm depth) that was treated with dolomitic lime or slag at four different rates of application (0, 0.5, 1.0 and 2.0x) based on recommended calcium carbonate equivalents (CCE) required to amend soil pH to a final value of 7.0 (8.96 Mg CCE/ha). Soil used in pots was matched in composition and pH to that of the region of the farm. Soil mineral content was determined prior to the study to ensure no biases. Pots were fertilized with phosphorus and potash and provided 16 hr daylight. Upon emergence, plants in each pot were reduced to five of each species. Plants were harvested at 150 mm height when alfalfa bloomed and plant material separated into species and dried for compositional analysis. Nitrogen fertilization was applied following the first harvest based on browning of the orchardgrass suggesting soil nitrogen depletion. A total of three harvest cuttings were collected and analysed separately and cumulatively as a weighted average. Analysis of variance (ANOVA) and regression modelling were applied to the data. Independent variables in the ANOVA model performed by plant species with model effects of treatment (lime vs slag) and dose (4 levels) and their interactions on plant tissue Mo and Cu content and calculated Cu:Mo ratio.

Results & discussion Both lime and slag effectively increased soil pH ($P<0.0001$), though the slag product was more effective ($P=0.009$) at an equivalent CCE. Addition of the slag product to soil increased ($P<0.0001$) plant tissue Mo concentration in both orchardgrass and alfalfa for all three harvests in a dose-dependent response from a single soil application to amend pH. There was no effect of either lime or slag on plant Cu content. Rate of application ($P=0.041$) and slag by rate interaction ($P<0.0001$) reduced plant tissue Cu:Mo ratio for both plant species. Measured changes in Mo and Cu:Mo ratio in this study reflect the observed changes in forage on the affected sheep farm. Weighted average cumulative data showed a similar slag effect across all harvests on increasing ($P<0.0001$) plant Mo content (Figure 1). Contrary to what was expected, orchardgrass had higher Mo content compared to alfalfa when it is often assumed legumes accumulate Mo better than grasses. Weighted average biomass Cu:Mo ratio was fitted to a three parameter decay model. Regression analysis to determine Mo uptake coefficient (UC = mg Mo kg^{-1} forage/ kg Mo ha^{-1}) showed much higher UC (5.58 orchardgrass; 4.32 alfalfa) than what has been previously estimated (2.25) for Mo.

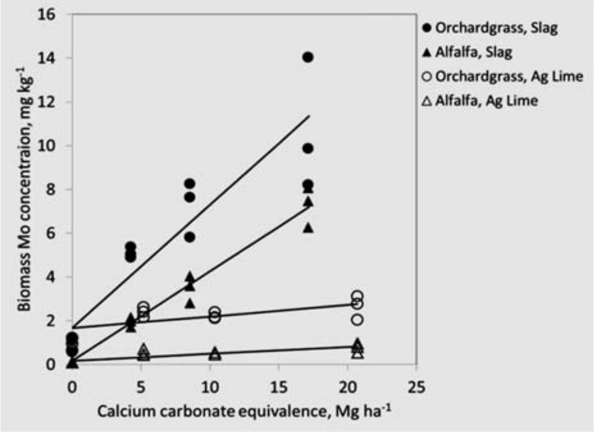


Figure 1 Cumulative orchardgrass or alfalfa plant tissue Mo content following treatment with lime or slag at four different dosing rates.

Conclusion Molybdenum in an alternative lime product seems readily available to growing plants as evidenced by increased plant tissue content in a dose-dependent manner. Plant Mo content remained high in all three harvests without any further addition of lime product. The elevation in plant Mo was consistent with observed Mo content in the case study and suggests the slag product was responsible for the sheep flock's health issues related to Cu deficiency.

Acknowledgements This research was funded by a grant from the Pennsylvania Department of Agriculture.

References

Suttle N.F. 2010. The Mineral Nutrition of Livestock, 4th ed. CABI, Wallingford, Oxfordshire, UK

Suckling of dairy calves by their dams: consequences of a welfare-friendly practice on milk yield, milk composition and growth of the calves

Dominique Pomiès[1], Bruno Martin[1], Matthieu Bouchon[2], Isabelle Veissier[1]

[1]*Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès-Champanelle, France,* [2]*Herbipôle, INRA, F-63122 Saint-Genès-Champanelle, France*

E-mail: dominique.pomies@inra.fr

Take home message Innovative ways to allow dairy calves to suckle their dam must be designed to obtain a good compromise between calves welfare and growth, and milk sold.

Introduction Dairy calves are often separated from their dam at birth, and fed artificial or bulk milk until 10-12 weeks. Such an early separation questions calves' welfare (EFSA, 2006). Some farmers, particularly in organic farming, consider maintaining calves with their dam until weaning. Consequences on behaviour or welfare have been addressed but little is known on cow milk yield and milk quality (Johnsen *et al*., 2016), and on how to carry out this management on-farm.

Materials & methods At INRA experimental farm 'Herbipôle', we tested two practices allowing calves to suckle their dam until weaning (12 weeks), and compared them to the conventional practice. We measured milk yield, milk composition, calves growth and milk consumption estimated by weighing calves before and after suckling. Three groups of 14 dam-calf couples (Holstein or Montbéliarde) were followed for 15 weeks after calving. In control group, the calves were separated from their dam at birth and fed bulk milk by an automatic feeder. Calves of the other groups joined their dam for suckling, twice a day for 2 weeks, then once a day (at morning milking) until weaning. In Group 'Before', the dams and the calves were grouped into a pen facing the milking parlour, for 20 min before milking. In Group 'After' they were grouped in the dams cowshed, for 2 h after milking. We used the Mixed procedure of SAS to compare the three groups, taking into account calving date, breed, parity and dairy index (for cows), and sex and birth weight (for calves) as covariates.

Results & discussion The weak growth of calves 'After' (375 g/d) that consumed half the milk of calves in the other two treatments (Table 1) led to a premature end of this experimental treatment after eight weeks. Conversely, the growth of calves 'Before' was faster than that of controls (832 vs. 644 g/d until Week 8), in line with their higher milk consumption. We collected less milk at the milking parlour from cows 'Before' and 'After' than from controls (Figure 1). Logically, the highest difference was observed at the morning milking for group 'Before', when suckling was just before milking (-11.2 kg/cow; Table 1). Average milk collected from group 'Before' increased after weaning, but gradually because some calves still suckled other dams of the group; on Week 15 the difference with control cows was only -5.2 kg/d ($P<0.001$) while it was -12.4 kg/d before any calf was weaned. Due to the relative order between milking and suckling, the average milk fat content from the groups 'Before' or 'After' was significantly different from control (+2.7 and -5.1 g/kg). Milk somatic cell count (SCC) was not significantly different between the three groups, despite high SCC of 2-3 cows from groups 'Before' and 'After'.

Table 1 Milk consumption by calf, milk collected from cow and its average composition; mean values by group adjusted by the mixed model, and group effect *(Week 1 to 8 after calving).*

	Control	'Before'	'After'	P
Milk consumed (kg/d)	6.7* ^{a}	7.6 ^{b}	3.2 ^{c}	<0.001
Morning milking (kg/d)	14.9 ^{a}	3.7 ^{b}	11.6 ^{c}	<0.001
Evening milking (kg/d)	10.0 ^{a}	8.5 ^{b}	5.9 ^{c}	<0.001
Milk fat content (g/kg)	36.3 ^{a}	39.0 ^{b}	31.2 ^{c}	<0.001
Milk SCC (×10^3/mL)	69	100	120	0.412

* bulk milk from the automatic feeder

$^{a, b, c}$: values with no common letter significantly differ ($P<0.05$

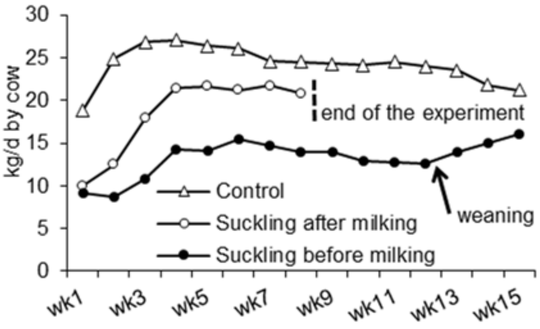


Figure 1 Average milk collected by group at milking parlour; raw data *(Week 1 to 15 after calving).*

Conclusion Suckling their dam after morning milking did not allow calves to grow enough. By contrast, suckling before milking was profitable to calves, but was time-consuming for farmers and decreased the amount of milk sold. Alternative ways of suckling, less constraining for farmers and allowing a prolonged dam-calf contact will be investigated.

Acknowledgements The authors acknowledge O. Troquier and the staff of the INRA experimental farm 'Herbipôle' for taking care of the animals, and the INRA-PHASE Division for funding the study.

References

EFSA 2006. AFSA Journal 366, 1-36.

Johnsen JF, Zipp KA, Kälber T, Passillé AM de, Knierim U, Barth K and Mejdell CM 2016. Applied Animal Behaviour Science 181, 1–11.

Effect of feeding BAIF's VIMICON mineral mixture over chelated mineral mixture on productive performance of breeding bulls

Manojkumar Aware, Amol Ghavate, Surekha Kale, Narayan Yan Phadke, Jayant Khadse, Ashok Pande

Baif Development Research Foundation, Central Research Station, Pune, Maharashtra, India

E-mail: manojkumar.aware@baif.org.in

Take home message BAIF's VIMICON mineral mixture enriched with vitamins increased the semen volume of Holstein Friesian breeding bulls by 6.5% without affecting semen quality (sperm concentration and motility).

Introduction Minerals are essential for proper metabolic functions in breeding bulls. Under poor feeding management conditions, when animals are fed diets with high proportions of roughages, bull semen quality can depend on his mineral status (Stephen, 2013). Good management practices and feeding balanced ration are critical for developing and maintaining bull fertility. Reproductive performance may indeed be greatly affected if Zinc, Copper, Manganese or Selenium levels are deficient. Mineral requirements are based, for the most part, on bodyweight but there are multipliers related to breed type, production stage, dry matter intake or environmental factors. Reproductive performance of animals is the reflection of many factors, individually and in combination, springing from different aspects namely genetics, nutrition, management and environment (Kumar 2003). The sources of minerals and vitamins influence the productive performance of the breeding bulls. For instance, vitamin A deficiency is associated with degeneration of testicular germinal epithelium, resulting in either reduction or cessation of spermatogenesis, depending upon the severity of the deficiency (Maynard *et al*., 1979). Vitamin E deficiency has a deleterious effect on germ cell proliferation. Effect of vitamin E occurs directly or indirectly on the regulation of intra-testicular factors which regulate specific steps of germ cell development (Cooper *et al*., 1987). Giving proper attention to the mineral component of their nutritional program helps insuring breeding bull growth and optimal performance during the breeding stage. Here we aim to compare the performance of breeding bulls fed with BAIF's VIMICON mineral mixture containing different trace elements fortified with vitamins against chelated mineral mixture.

Materials & methods Study was conducted at BAIF's Central Research Station, Uruli Kanchan, Pune, Maharashtra, India. Twenty Holstein Friesian breeding bulls, 6-yr-old on average were selected and divided in two groups to balance average body weight (796.2±13.0 kg). Uniform balanced diet was provided to these groups including concentrate mixture (3.2 kg), green fodder (Maize 34 kg, Lucerne 5 kg), and dry fodder (4 kg). BAIF's VIMICON mineral mixture or chelated mineral mixture were used for supplementation with a daily dose of 60g per day during 270 days. BAIF's VIMICON mineral mixture and Chelated mineral mixtures contained the same amounts of different macro (calcium, phosphorous) and micro elements (except chelated sources of copper, cobalt, zinc, iodine, manganese, iron in chelated mineral mixture), and BAIF's VIMICON is fortified with vitamins like Vit.A (20,00,000 I.U./kg), Vit.D3 (4,00,000 I.U./kg), Vit.E (300 mg/kg), Vit.B2 (800 mg/kg), Vit.B12 (2.4 mg/kg), Vit.K (400 mg/kg) with supplementation of niacinamide (4g/kg), choline chloride (60 g/kg) and calcium D pantothenate (1200 mg/kg). Daily data was recorded for different semen traits like total number of ejaculates, average semen volume per ejaculate, initial motility (in %), sperm concentration (in millions/ml), number of straws per ejaculate, and post thaw motility (%).

Results & discussion Feeding of BAIF's VIMICON Mineral Mixture to breeding bulls have shown highly significant (P<0.01) positive effect on average semen volume per ejaculate and average number of straws frozen per ejaculation as compared to Chelated Mineral Mixture (Table 1). There was no significant difference between the groups in terms of initial sperm motility in neat semen and post thaw motility.

Table 1 Semen traits and their values in the two groups.

Parameters	BAIF's VIMICON	Chelated mineral mixture	Statistical difference[1]
Total number of ejaculates	1270	1127	
Semen volume / ejaculate (ml)	6.89	6.47	**
Initial motility (%)	78.9	78.9	NS
Sperm concentration (millions/ml)	1251	1250	NS
Number of straws / ejaculate	418	391	**
Post thaw motility (%)	55.7	55.6	NS

[1] Comparison with Student's t-test; **: P<0.01

Conclusion A study of feeding two different mineral mixtures on performance of breeding bulls reveals that BAIF's VIMICON mineral mixture is superior for breeding bulls for semen quality in terms of an increase by 6.5% of semen volume per ejaculate, and of 6.9% of the total number of straws produced per ejaculate. Semen quality (sperm concentration, motility) was unaffected. This mixture (VIMICON) has been commercialized by BAIF for three decades and has been made available to about 1 million smallholder dairy families from 13 Indian states as part of a Research-Development program. Success of this program is revealed under field condition by the better conception rate achieved: 48% for farmers using BAIF semen *vs* 35% national average conception rate.

References

Cooper DR, Kling OR and Carpenter MP 1987. Endocrinology 120, 83-90

Maynard LA, Loosli JK, Hintz HF and Warner RG 1979. Reproduction.Animal Nutrition 16, 472.

Stephen B.2013 The Brahman Journal 41, 40-46.

Effect of energy allowance to triplet-bearing ewes of Norwegian White Sheep in late gestation on ewe and lamb performance

Ingjerd Dønnem[1], Erik Georg Granquist[2], Åshild T. Randby[1]

[1]*Norwegian University of Life Sciences, Faculty of Biosciences, Department of Animal and Aquacultural Sciences, Ås, Norway,* [2]*Norwegian University of Life Sciences, Faculty of Veterinary Medicine, Department of Production Animal Clinical Sciences, Oslo, Norway*

E-mail: ingjerd.donnem@nmbu.no

Take home message Increasing plane of nutrition to ewes in late gestation may have an impact on growth and weaning weight of lambs reared as triplets.

Introduction The Norwegian white sheep is a productive and prolific breed for meat production. Amongst ewes lambing in 2016, 44% of the adult ewes (older than 1 year) gave birth to litters containing three or more lambs (Animalia, 2016). In the last trimester of gestation, nutrient requirements for mature ewes bearing triplets are greater than that of the single- and twin-bearing ewes (NRC, 2007). In this period the growth of approximately 70% of the fetus occurs (Robinson, 1990). The plane of nutrition in late gestation may influence birth weight, colostrum production, and milk production with potential subsequent effect on lamb weaning weight (McGovern *et al*., 2015). The aim of this work was to study the effect of different energy levels to mature, triplet bearing ewes in late gestation on ewe performance and on growth of lambs reared as triplets.

Materials & methods The experiment was performed with 27 multiparous ewes of the breed Norwegian White Sheep. All ewes carried three foetuses, and they were placed in individual pens. Until gestation day 96 (G96) they were fed at maintenance level, and at G96 the ewes were distributed to three groups, each of 9 ewes. The three groups were randomly allocated to dietary energy treatment based on INRA requirements (Jarrige, 1989): 1) 85% of predicted net energy lactation (NEL) requirement; 2) 100% of predicted NEL requirement; and 3) 120% of predicted NEL requirement, on individual body weight basis. The ewes were offered a restricted portion of early harvested grass silage (6.7 NEL/ kg DM) and an adjusted level of concentrate in all treatments. After lambing, ewes in all groups were offered grass silage *ad libitum* and supplemented with concentrate according to the number of suckling lambs. Ewes were weighed and assessed for body condition score (BCS) at G104, G135, four days after lambing and when turning out on pasture about 4 weeks after lambing. The lambs were weighed at birth and weekly before turning out on pasture, and at weaning (mean age at weaning=116 days).

Results & discussion Ewe daily live weight gain (±SEM) from G104 to G135 increased ($P<0.001$) with increased NEL allowance; 227, 325 and 416 (±27.6) g/day for 85%, 100% and 120% NEL diet. The ewes offered the 120% NEL diet gained ($P=0.002$) BCS between G104 and G135, while the ewes offered 85 and 100% NEL diet lost ($P=0.04$) BCS in this period. The ewes offered the 120% NEL diet lost more body condition (-0.22 units) in early lactation when compared with the 100% NEL group (0.13 units) ($P=0.01$). There was no effect of ewe nutritional treatment on lamb weight at birth or at turnout to pasture (Table 1). At weaning, the number of triplet litters was 3, 5 and 7 in the 85%, 100% and 120% NEL diet, and this was due to stillbirths (4 lambs), neonatal deaths, life-saving bottle-feeding of lambs and one dead ewe. When regarding triplet litters only, the 120% NEL lambs were heavier at weaning and had a higher live weight gain from birth to weaning than the 100% NEL lambs. The 85% NEL lambs were intermediate, which may be due to the low number of lambs left in this group.

Table 1 Live weight (LW) and live weight gain from birth to weaning (LWG) of lambs. Number of lambs in parenthesis.

	Treatment 85% NEL	100 % NEL	120 % NEL	SEM	P-value
LW at birth, kg	4.41 (27)	4.37 (27)	4.69 (27)	0.166	0.34
LW at turnout, kg	12.8 (21)	13.1 (25)	13.3 (27)	0.637	0.69
LW at weaning, kg	39.5 (20)	40.2 (21)	40.8 (25)	1.88	0.77
LW at weaning, triplets, kg	36.4^{ab} (9)	33.5^{a} (15)	37.3^{b} (21)	0.837	0.02
LWG, triplets, g/d	271^{ab} (9)	246^{a} (15)	279^{b} (21)	11.53	0.02

Conclusion Increasing the level of energy to the ewe in late gestation altered the pattern of body reserve mobilization during late gestation and early lactation, but there was no observed variations in lamb performance during this time. However, it seemed to be an impact on growth and weaning weight of lambs still in complete triplet litters at weaning.

References

Animalia, 2016. https://www.animalia.no/no/Dyr/husdyrkontrollene/sauekontrollen/arsmeldinger/
NRC, 2007. The National Academic Press, Washington DC.
Robinson J 1990. Nutrition Research Review 3, 253-276.
McGovern FM, Campion FP, Lott S and Boland, T.M. 2015. Journal of Animal Science 10, 4860-4872.
Jarrige R 1989. Institute National de la Recherche Agronomic, Paris.

Bred well fed well: one-day practical workshop delivers behavioural change and improved marking percentage in Australia.

Serina Hancock[1], Sarah Blumer[1], Jason Trompf[2]

[1]*Murdoch University, Western Australia, Australia,* [2]*J.T. Agri-source Pty Ltd, Victoria, Australia*

E-mail: s.hancock@murdoch.edu.au

Take home message Bred Well Fed Well (BWFW) is a one-day practical workshop delivered to sheep producers in Australia to improve reproductive performance by combining genetics and feed management. Since 2011 the workshop has been delivered to over 4,000 producers, of which almost 1400 (34%) believe BWFW has improved their management and lamb marking rates. Among those 1400 participants the average gain in marking rate is 9%, which equates to 287,000 extra lambs weaned per year as a result of BWFW.

Introduction Lamb mortality costs the Australian sheep industry approximately AUD$1 billion per annum through production losses (Young *et al.* 2013). Supported learning extension programs designed to improve producer understanding and develop the skills and confidence to improve ewe management have delivered significant changes in producer behaviours and have increased lamb weaning percentages by about 10% (Trompf *et al.* 2011). However, some producers prefer attending shorter one-day workshops than year-long extension programs. BWFW is a one-day 'hands-on' workshop delivered to sheep producers in Australia to improve breeding and nutritional management of their ewe flock. This paper reports the effectiveness of BWFW to communicate key principles and change management practices and weaning rates.

Materials & methods Bred Well Fed Well is a practical, one-day workshop which highlights the key production benefits of genetics, plus feed management and husbandry for improved reproduction. The workshop commenced in 2011 and since then a total number of 4080 participants have attended 173 workshops across Australia (Nov 2011 to Jan 1 2018). To date there have been four continuous phases to BWFW, each targeting around 1000 producers.The workshops evaluate in real-time the reactions of producers including change in knowledge, decision making, skills and attitudes, satisfaction and intended practice change through the use of an audience response system. A post-workshop survey was conducted on participating producers 9-12 months after attending the workshop. The data presented in this paper is based on surveys conducted on phase 2 and 3 participants between 2013 and 2015 (n=61) to evaluate attitudes, on-farm changes and emerging impacts of change.

Results & discussion During 2013/2014 and 2014/2015 34% of participants surveyed believed that BWFW had improved their management and lamb marking rates, and those who had applied the principles of BWFW increased weaning rates by about 9%. Across all participants, who on average manage 2300 ewes, adoption of skills and practices from attending the BWFW workshop delivered an extra 287,000 lambs at weaning. The significant changes in marking percentage were delivered mainly through understanding of appropriate condition score targets and the need for increased and targeted supplementary feeding during the different stages of the reproductive cycle (Table 1).

Table 1 Changes in marking percentage for BWFW attendees that did not adopt specific management practices (NN), that did adopt these practice changes (NY) or that had already adopted these practices (YY). NN and YY have been combined in the analysis as these two groups have not adopted practice due to BWFW.

Practice	NN/YY (%)	NY (%)	P value
Condition score	2.54	5.08	0.061
Manage to CS targets	2.52	5.98	0.020
Pregnancy scan wet/dry	3.20	5.18	0.269
Pregnancy scan for multiples	3.19	5.17	0.268
Separate twins	3.08	6.04	0.099
Supplementary feeding	2.78	6.65	0.043
Feed budget for ewes	3.45	4.12	0.696
Allocate mobs to paddocks based on FOO and nutritional needs	3.31	5.42	0.317

Conclusion The impact of these workshops has delivered a small increase to the overall national weaning percentages (0.17%), with ewe and lamb losses reduced by an average of 44,000 for each year/phase and a predicted value to industry of approximately 1 million AUD per annum. The key components of best practice reproductive management for sheep are detailed and complicated. While these tools are best delivered in repeated interactions within a small group (as per Lifetime Ewe Management), short format workshops do have the potential to deliver change. Understanding which tools are integrated into producer management may help future workshop design to increase uptake of reproductive management tools.

Acknowledgements The Bred Well Fed Well workshops are supported by producer levies via Meat and Livestock Australia and Australian Wool Innovation.

References

Trompf J, Gordon D, Behrendt R, Curnow M, Kildey L and Thompson A. 2011. Animal Production Science 51, 866-872.

Sustainable and efficient production of growing steers in the tropical pastures

André Pastori D'Aurea, Luis Eduardo Ferreira, Lauriston Bertelli Fernandes

Premix Company, Patrocinio Paulista, São Paulo, Brazil

E-mail: andre.daurea@premix.com.br

Take home message Ensure productivity, profitability and sustainability in the production of beef cattle on the pasture during the dry season.

Introduction The nutrition during the growing phase is critical to the development of the animal so that it can express the most of their genetic potential. The animals at this stage of development have greater potential for growth, where nutrition is the main influential factor. In Brazil, during the winter (dry season) there is a reduction in the quantity and quality of forage. The production of tropical forages in the lower winter due to the decrease in rainfall indices, lower temperature and lower photoperiod. Thus, supplementation strategies are fundamental to the success of cattle production in pasture and keep good production rates during the winter is one of the biggest challenges of Brazilian beef cattle. The objective of this pasture production system is to maintain beef cattle productivity in winter and ensure the sustainability of beef cattle production on pasture.

Materials & methods This production system has been applied 2016 /2107 in two Brazilian states (São Paulo and Minas Gerais). The Research Center of Premix Company and partners' farms were used to develop this production system. Two thousand beef cattle (steers) were evaluated during one year. The parameters used were daily average gain and gain per area. At the beginning of the project it was necessary to analyse the soil for possible corrections and design the fertilizations. The diet was based on forage and protein-energetic supplements (0.3% of live weight). The supplement used was added with natural additive (Fator P® – Premix Company). Fator P® is an additive composed by amino acids (lysine, methionine and tyrosine, 16400, 2980 and 3000 mg.kg-1 respectively), minerals, probiotics (Saccharomyces cerevisae, 7 x 108 CFU.kg-1) and essential fatty acids (linoleic and oleic acids, 108.9 and 99g.kg-1 respectively). In winter, it was supplemented with corn silage. It was offered 1% of live weight in silage corn and 0.3% of the live weight in protein-energetic supplements with additive Fator P ®. At the end of the period, the productivity was calculated by summing the individual gains for the area used.

Results & discussion The average daily gain of the animals was 620 grams per day and were produced 950 kg / hectare / year. This production is about four times higher than the average of the Brazilian national production. To succeed in this project it is necessary to increase the gains per area, so it is necessary to increase the capacity per area. In this way the use of animals in the growth phase is ideal. The response to supplementation is greater and more animals are used per area. Thus, the productive principles of this system are:

- Increased individual gains - better supplementation;
- Increase of productive units (light animals);
- Better forage quality;
- Forage conservation (silage or hay) for winter;
- Use the natural additives.

The system is based on the principle of soil-plant-animal. The pasture management is very important to the success of the project where the reference is the quantity and quality of the forage. Use of integrated systems (agriculture-livestock) is important in pasture reforms and the environment. After one year the steers are close to 450 kilograms live weight, with approximately 20 months of age. They are ready for termination in feedlot, where they will remain about 100 days. Guaranteeing productivity and sustainability in the production of cattle to pasture, favoring the use of land and use of natural resources.

Conclusion The sustainable production of beef cattle on pasture can be ensured with better use of forage, young steers, efficient supplementation and additional roughage in the dry season.

Acknowledgements Premix company**.**

References

Lobato JFP, Freitas, AK, Devincenzi T, Cardoso LL, Tarouco JU, Vieira RM, Dillenburg DR and Castro I 2014. Meat Science 98, 336-345.
Jouany J-P and Morgavi DP 2007. Animal 1, 1443-1466.

Full copies of the following keynotes will be published online in the journal ANIMAL

Review: Domestic herbivores and food security: current contribution, trends and challenges for a sustainable development

A. Mottet[1], F. Teillard [1], P. Boettcher[1], G. De' Besi[1], B. Besbes[1]

[1]*Animal Production and Health Division, FAO, Viale delle Terme di Caracalla, 00153, Rome, Italy*

E-mail: Anne.Mottet@fao.org

Abstract

Herbivores are found in a variety of ecosystems all over the world. Permanent pastures and meadows cover about 25% of global land. We currently count one domesticated herbivore for 2 people in the world and the number is growing. Production systems and products are highly diverse. This high diversity is the result of thousands of years of natural selection and human-controlled breeding, as well as migration and trade. Because of the high diversity of domestic herbivore genetic resources, herders have been able to live in regions where no alternative for income generation exists. Meat and milk from domestic herbivores provide 16% and 8% of the global protein and kilocalorie consumption respectively. They also provide of a variety of essential micronutrients but can contribute to overweight and obesity when consumed in excess. Domestic herbivores also make significant contribution to food security through the production of manure, draught power and transport and the generation of income at household and national level. They have a key role to play in women's empowerment and gender equality, both in rural and urban areas. Demand for meat and milk is increasing because of population growth, rising incomes and urbanisation. This trend is expected to continue, especially in Latin America, South Asia and China. The sustainable development of domestic herbivore production needs to address the feed/food and the efficiency of herbivores in turning forages into protein. It also needs to address the contribution of herbivores to greenhouse gas emissions, especially of ruminants through enteric fermentation, and their mitigation potential, including through carbon sequestration. Animal genetic resources have a key role to play in mitigating and adapting to climate change. The role of ruminants in the circular bioeconomy needs to be enhanced, promoting the use of by-products and waste as livestock feed and the recycling of manure for energy and nutrients. Finally, the role of domestic herbivores in providing secure livelihoods and economic opportunities for millions of smallholder farmers and pastoralists needs to be enhanced. The sustainable development of the sector therefore requires adequate policies, and there are already a variety of mechanisms available, including regulations, cross-compliance systems, payments for environmental services and research and development. Priority areas for policy makers should be aligned with the global framework of the Sustainable Development Goals and include: (i) food security and nutrition, (ii) economic development and livelihoods, (iii) animal and human health and finally, (iv) environment, climate and natural resources.

Review: Role of herbivores in sustainable agriculture in sub-Saharan Africa

A.A. Ayantunde[1], A.J. Duncan[2], M.T. van Wijk[3], P. Thorne[2]

[1]International Livestock Research Institute (ILRI), 01 BP 1496, Ouagadougou, Burkina Faso; [2]International Livestock Research Institute (ILRI), PO Box 5689 Addis Ababa, Ethiopia; [3]International Livestock Research Institute (ILRI), PO Box 30709, 00100 Nairobi, Kenya

E-mail: A.Ayantunde@cgiar.org

Abstract

The role of herbivorous livestock in supporting the sustainability of the farming systems in which they are found is complex and sometimes conflicting. In sub-Saharan Africa (SSA), the integration of livestock into farming systems is important for sustainable agriculture as the recycling of nutrients for crop production through returns of animal manure is a central element of the dominant mixed crop-livestock systems. Sustainable agriculture has been widely advocated as the main practical pathway to address the challenge of meeting the food needs of the rapidly growing population in SSA while safeguarding the needs of future generations. The objective of this paper is to review the state of knowledge of the role of herbivores in sustainable intensification of key farming systems in SSA. The pathways to sustainable agriculture in SSA include intensification of production and livelihood diversification. Sustainable agricultural practices in SSA have focused on intensification practices which aim to increase the output: input ratio through increasing use of inputs, introduction of new inputs or use of existing inputs in a new way. Intensification of livestock production can occur through increased and improved fodder availability, genetic production gains, improved crop residue use and better nutrient recycling of manure. Livestock deliver many "goods" in smallholder farming systems in SSA including improving food and nutrition security, increased recycling of organic matter and nutrients and the associated soil fertility amendments, adding value to crop residues by turning them into nutrient-rich foods, income generation and animal traction. Narratives on livestock "bads" or negative environmental consequences have been largely shaped by the production conditions in the Global North but livestock production in SSA is a different story. In SSA, livestock are an integral component of mixed farming systems and they play key roles in supporting the livelihoods of much of the rural population. None-the-less, the environmental consequences of livestock production on the continent cannot be ignored. To enhance agricultural sustainability in SSA, the challenge is to optimize livestock's role in the farming systems by maximizing livestock "goods" while minimizing the "bads". This can be through better integration of livestock into the farming systems, efficient nutrient management systems, and provision of necessary policy and institutional support.

Review: Make ruminants green again - How can sustainable intensification and agroecology converge for a better future?

B. Dumont[1], J.C.J. Groot[2], M. Tichit[3]

[1]*Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, 63122 Saint-Genès-Champanelle, France*
[2]*Farming System Ecology Group, Wageningen University & Research, Wageningen 6700 AK, The Netherlands*
[3]*UMR SAD-APT, INRA, AgroParisTech, Université Paris-Saclay, 75000 Paris, France*

E-mail: bertrand.dumont@inra.fr

Abstract

Livestock farming systems provide multiple benefits to humans: protein-rich diets that contribute to food security, employment and rural economies, capital stock and draught power in many developing countries, and cultural landscape all around the world. Despite these positive contributions to society, livestock is also the centre of many controversies as regards to its environmental impacts, animal welfare and health outcomes related to excessive meat consumption. Here, we review the potentials of sustainable intensification (SI) and agroecology (AE) in the design of sustainable ruminant farming systems. We analyse the two frameworks in an historical perspective and show that they are underpinned by different values and worldviews about food consumption patterns, the role of technology and our relationship with nature. Proponents of SI see the increase in animal protein demand as inevitable and therefore aim at increasing production from existing farmland to limit further encroachment into remaining natural ecosystems. Sustainable intensification can thus be seen as an efficiency-oriented framework that benefits from all forms of technological development. Proponents of agroecology appear more open to dietary shifts towards less animal protein consumption to rebalance the whole food system. Agroecology promotes system redesign, benefits from functional diversity and aims at providing regulating and cultural services. We analyse the main criticisms of the two frameworks: Is SI sustainable? How much can AE contribute to feeding the world? Indeed, in SI, social justice has long lacked attention notably with respect to resource allocation within and between generations. It is only recently that some of its proponents have indicated that there is room to include more diversified systems and food-system transformation perspectives, and to build socially fair governance systems. As no space is available for agricultural land expansion in many areas, agroecological approaches that emphasise the importance of local production should also focus more on yield increases from agricultural land. Our view is that new technologies and strict certifications offer opportunities for scaling-up agroecological systems. We stress that the key issue for making digital science part of the agroecological transition is that it remains at a low cost and is thus accessible to smallholder farmers. We conclude that SI and AE could converge for a better future by adopting transformative approaches in the search for ecologically benign, socially fair and economically viable ruminant farming systems.

Review: The compositional variation of the rumen microbiome and its effect on host performance and methane emission

I. Mizrahi[1] and E. Jami[2]

[1] *Department of Life Sciences and the National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer-Sheva, Israel*
[2] *Department of Ruminant Sciences, Agricultural Research Organization - the Volcani Center, 68 HaMaccabim Road, P.O.B 15159 Rishon LeZion 7505101, Israel*

E-mail: imizrahi@bgu.ac.il

Abstract

The rumen microbiome has the important task of supplying ruminants with most of their dietary requirements and is responsible for up to 90% of their metabolic needs. This tremendous feat is possible due to the large diversity of microorganisms in the rumen. The rumen is considered one of the most diverse ecosystems on the planet in terms of species diversity and functional richness. From the moment the feed is ingested, it enters a vast cascade in which specialized microorganisms degrade specific components of the feed turning them into molecules which in turn are utilized as anabolic precursors and energy sources for the animal. The output of this degradation process not only affects the animal, but also has an extensive impact on the environment. Some of the byproducts that are emitted as waste from this process, such as methane, act as greenhouse gases which greatly contribute to global warming. Recent technological advances developed to study this community enabled a larger overview of its vast taxonomic and functional diversity, thus leading to a better understanding of its ecology and function. This deeper understanding of the forces affecting the microbiome includes the forces that shape composition, the variation among animals, the stability of its key components, the processes of succession on a short and long-time scales such as primary colonization and diurnal oscillations. These collective understandings have helped provide insights into the potential effects that these forces have on the outputs observed from the animal itself. Over recent years, there has been a growing body of evidence demonstrating the link between the microbiome and its effect on productivity of the host animals and the environment, which has placed rumen microbiome studies in the forefront of animal agricultural research. In this review, we focus on the natural variations in community composition, which are not the results of different management or feed, but rather intrinsic features of animals. We characterize the rumen microbiome, its potential impact on its host, as well as the barriers in implementing the current knowledge to modulate the microbiome and point towards potential avenues to overcome these hurdles.

Review: The application of omics to rumen microbiota function

S. E. Denman[1], D. P. Morgavi[2] and C. S. McSweeney[1]

[1] *CSIRO, Agriculture and Food, St. Lucia, QLD, Australia*
[2] *Institute National dela Recherche Agronomique, UMR1213 Herbivores, Clermont Université, VetAgroSup, UMR Herbivores, Clermont-Ferrand, France*

E-mail: Stuart.Denman@csiro.au

Abstract

Rumen microbiome profiling uses 16S rRNA (18S rRNA, ITS) gene sequencing, a method that usually sequences a small portion of a single gene and is often biased and varies between different laboratories. Functional information can be inferred from this data, but only for those that are closely related to known annotated species, and even then may not truly reflect the function performed within the environment being studied. Genome sequencing of isolates and metagenome-assembled genomes (MAG) has now reached a stage where representation of the majority of rumen bacterial genera are covered. The creation of a microbial genome (bins) database with associated functional annotations will provide a consistent reference to allow mapping of RNA-Seq reads for functional gene analysis from within the rumen microbiome. The integration of multiple omic analytics is linking functional gene activity, metabolic pathways, and rumen metabolites with the responsible microbiota, supporting our biological understanding of the rumen system. The application of these techniques has advanced our understanding of the major microbial populations and functional pathways that are used in relation to lower methane emissions, higher feed efficiencies, and responses to different feeding regimes. Continued and more precise use of these tools will lead to a detailed and comprehensive understanding of compositional and functional capacity and design of techniques for the directed intervention and manipulation of the rumen microbiota towards a desired state.

Review: Precision nutrition of herbivores: approaches, challenges and potential gains

L. A. González[1], I. Kyriazakis[2], and L. O. Tedeschi[3]

[1] *Sydney Institute of Agriculture, School of Life and Environmental Sciences, The University of Sydney, 380 Werombi Rd, Camden, NSW, Australia*
[2] *Agriculture, School of Natural and Environmental Science, Newcastle University, Newcastle upon Tyne, NE1 7RU, United Kingdom*
[3] *Department of Animal Science, Texas A&M University, 230 Kleberg Center, College Station, TX 77843-2471, United States of America*

E-mail: luciano.gonzalez@sydney.edu.au

Abstract

A plethora of sensors and information technologies with applications to the precision nutrition of herbivores have been developed and continue to be developed. The nutritional processes start outside of the animal body with the available feed (quantity and quality) and continue inside it once the feed is consumed, degraded in the gastrointestinal tract and metabolised by organs and tissues. Finally, some nutrients are wasted via urination, defecation and gaseous emissions through breathing and belching while remaining nutrients ensure maintenance and production. Nowadays, several processes can be monitored in real-time using new technologies, but although these provide valuable data 'as is', further gains could be obtained using this information as inputs to nutrition simulation models to predict unmeasurable variables in real-time and to forecast outcomes of interest. Data provided by sensors can create synergies with simulation models and this approach has the potential to expand current applications. In addition, data provided by sensors could be used with advanced analytical techniques such as data fusion, optimisation techniques and machine learning to improve their value for applications in precision animal nutrition. The present paper reviews technologies that can monitor different nutritional processes relevant to animal production, profitability, environmental management and welfare. We discussed the model-data fusion approach in which data provided by sensor technologies can be used as input of nutrition simulation models in near-real time to produce more accurate, certain, and timely predictions. We also discuss some examples that have taken this model-data fusion approach to complement the capabilities of both models and sensor data, and provided examples such as predicting feed intake and methane emissions. Challenges with automatizing the nutritional management of individual animals include monitoring and predicting of the flow of nutrients including nutrient intake, quantity and composition of body growth and milk production, gestation, maintenance and physical activities at the individual animal level. We concluded that the livestock industries are already seeing benefits from the development of sensor and information technologies, and this benefit is expected to grow exponentially soon with the integration of nutrition simulation models and techniques for big data analysis. However, this approach may need re-evaluating or performing new empirical research in both fields of animal nutrition and simulation modelling to accommodate a new type of data provided by the sensor technologies.

Review: Grass based dairy systems, data, and precision technologies

L. Shalloo[1], M. O 'Donovan[1] , L. Leso[3], J. Werner[4], E. Ruelle[1], A. Geoghegan[1], L. Delaby[2] and N. O'Leary[1]

[1] *Animal & Pastureland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland*
[2]*Animal et les Systèmes d'Elevage, Domaine de la Prise, 35590 Saint Gilles, France.*
[3] *University of Florence, Department of Agricultural, Food and Forestry Systems, 50145 Firenze, Italy*
[4] *Institute for Agricultural Engineering, University of Hohenheim, 70599 Stuttgart, Germany*

E-mail: Laurence.Shalloo@Teagasc.ie

Abstract

Precision technologies and data have had relatively modest impacts in grass-based livestock ruminant production systems compared to other agricultural sectors such as arable. Precision technologies promise increased efficiency, reduced environmental impact, improved animal health, welfare and product quality. The benefits of precision technologies have, however, been relatively slow to be realised on pasture based farms. Though there is significant overlap with indoor systems, implementing technology in grass based dairying bring unique opportunities and challenges. The large areas animals roam and graze in pasture based systems and the associated connectivity challenges may, in part at least, explain the comparatively reduced use of such technologies in such systems. There are thus few technologies designed specifically to increase pasture utilisation with the exception of sensor and Bluetooth enabled plate meters. Terrestrial and satellite-based spectral analysis of pasture biomass and quality is still in the development phase. One of the key drivers of efficiency in pasture based systems has thus only been marginally impacted by Precision technologies. In contrast, technological development in the area of fertility and heat detection has been significant and offers significant potential value to dairy farmers, including those in pasture based systems. A past review of sensors in health management for dairy farms concluded that while the collection of accurate data was generally achieved, the processing, integration and presentation of the resulting information and decision-support applications were inadequate. The technologies value to farming systems is thus unclear. As a result, it is unclear if farm management is being sufficiently improved to justify widespread adoption of precision technologies. We argue for a user need-driven development of technologies and for a focus on how outputs arising from Precision technologies and resulting in decision support applications are delivered to users to maximise their value. Further cost/benefit analysis is required to determine the efficacy of investing in specific PTs, likely taking account of as yet to ascertained farm specific variables.

Review: Modulating ruminal lipid metabolism to improve the fatty acid composition of meat and milk from ruminants. Challenges and opportunities.

P. G. Toral[1], F. J. Monahan[2], G. Hervás[1], P. Frutos[1] and A. P. Moloney[3]

[1]*Instituto de Ganadería de Montaña, CSIC-Universidad de León, Finca Marzanas s/n, 24346 Grulleros, León, Spain*
[2]*UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland*
[3]*Teagasc, Animal and Grassland Research and Innovation Centre, Grange, Dunsany Co., Meath, Ireland*

E-mail: pablo.toral@csic.es

Abstract

Growth in demand for foods with potentially beneficial effects on consumer health has motivated increased interest in developing strategies for improving the nutritional quality of ruminant-derived products. Manipulation of the rumen environment offers the opportunity to modify the lipid composition of milk and meat by changing the availability of fatty acids (FA) for mammary and intramuscular lipid uptake. Dietary supplementation with marine lipids, plant secondary compounds and direct-fed microbials has shown promising results. In this review, we have compiled information about their effects on the concentration of putative desirable FA (e.g., *c*9*t*11-conjugated linoleic acid and vaccenic, oleic, linoleic and linolenic acids) in ruminal digesta, milk and intramuscular fat. Marine lipids rich in very long-chain n-3 polyunsaturated fatty acids (PUFA) efficiently inhibit the last step of C18 FA biohydrogenation (BH) in the bovine, ovine and caprine, increasing the outflow of *t*11-18:1 from the rumen and improving the concentration of *c*9*t*11-18:2 in the final products, but increments in *t*10-18:1 are also often found due to shifts toward alternative BH pathways. Direct-fed microbials appear to favourably modify rumen lipid metabolism but information is still very limited, whereas a wide variety of plant secondary compounds, including tannins, polyphenol oxidase, essential oils, oxygenated FA and saponins, has been examined with varying success. For example, the effectiveness of tannins and essential oils is as yet controversial, with some studies showing no effects and others a positive impact on inhibiting the first step of BH of PUFA or, less commonly, the final step. Further investigation is required to unravel the causes of inconsistent results, which may be due to the diversity in active components, ruminant species, dosage, basal diet composition and time on treatments. Likewise, research must continue to address ways to mitigate negative side-effects of some supplements on animal performance (particularly, milk fat depression) and product quality (e.g., altered oxidative stability and shelf-life).

Review: Nutrigenomics of marbling and fatty acid profile in ruminant meat

M. M. Ladeira[1], J. P. Schoonmaker[2], K. C. Swanson[3], S. K. Ducket[4], M. P. Gionbelli[1], L. M. Rodrigues[1], P. D. Teixeira[1]

[1]*Department of Animal Science, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil, 37.200-000*
[2]*Department of Animal Science, Purdue University, West Lafayette, Indiana, United States, 47907*
[3]*Department of Animal Science, North Dakota State University, Fargo, North Dakota, United States, 58108-6050*
[4]*Animal and Veterinary Sciences Department, Clemson University, Clemson, South Carolina, 29634-0311*

E-mail: mladeira@dzo.ufla.br

Abstract

The present review will present recent published results and discuss the main effects of nutrients, mainly fatty acids, on the expression of genes involved in lipid metabolism. In this sense, the review focus in two phases: prenatal life and finishing phase, showing how nutrients can modulate gene expression affecting marbling and fatty acid profile in meat from ruminants. Adiposity in ruminants starts to be affected by nutrients during prenatal life when maternal nutrition affects differentiation and proliferation of adipose cells enhancing the marbling potential. Therefore, several fetal programming studies were carried out in the last 2 decades in order to better understand how nutrients affect long-term expression of genes involved in adipogenesis and lipogenesis. In addition, during the finishing phase, marbling becomes largely dependent on starch digestion and glucose metabolism, being important to create alternatives to increase these metabolic processes, and modulate gene expression is one of them. Different lipid sources and their fatty acids may also influence the expression of genes responsible to encode enzymes involved in fat tissue deposition, influencing meat quality. In conclusion, the knowledge shows that gene expression is a metabolic factor affecting marbling and fatty acid profile in ruminant meat and diets and their nutrients have direct effect on how these genes are expressed.

Review: Alternative and novel feeds for ruminants - nutritive value, product quality and environmental aspects

A. Halmemies-Beauchet-Filleau[1], M. Rinne[2], M. Lamminen[1,3], C. Mapato[4], T. Ampapon[4], M. Wanapat[4] and A. Vanhatalo[1,3]

[1]*Department of Agricultural Sciences, FI-00014 University of Helsinki, Finland*
[2]*Production Systems, Natural Resources Institute Finland (Luke), FI-31600 Jokioinen, Finland*
[3]*Helsinki Institute of Sustainability Science, FI-00014 University of Helsinki, Finland*
[4]*Tropical Feed Resources Research and Development Center, Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand*

E-mail: anni.halmemies@helsinki.fi

Abstract

Ruminant-based food production faces currently multiple challenges such as environmental emissions, climate change and accelerating food-feed-fuel competition for arable land. Therefore, more sustainable feed production is needed together with the exploitation of novel resources. In addition to numerous food industry (milling, sugar, starch, alcohol or plant oil) side streams already in use, new ones such as vegetable and fruit residues are explored, but their conservation is challenging and production often seasonal. In the temperate zones, lipid-rich camelina (*Camelina sativa*) expeller as an example of oilseed by-products has potential to enrich ruminant milk and meat fat with bioactive *trans*-11 18:1 and *cis*-9,*trans*-11 18:2 fatty acids and mitigate methane emissions. Regardless of the lower methionine content of alternative grain legume protein relative to soybean meal (*Glycine max*), the lactation performance or the growth of ruminants fed faba beans (*Vicia faba*), peas (*Pisum sativum*) and lupins (*Lupinus* sp.) are comparable. Wood is the most abundant carbohydrate worldwide, but agroforestry approaches in ruminant nutrition are not common in the temperate areas. Untreated wood is poorly utilised by ruminants because of linkages between cellulose and lignin, but the utilisability can be improved by various processing methods. In the tropics, the leaves of fodder trees and shrubs (e.g. cassava (*Manihot esculenta*), *Leucaena* sp., *Flemingia* sp.) are good protein supplements for ruminants. A food-feed production system integrates the leaves and the by-products of on-farm food production to grass production in ruminant feeding. It can improve animal performance sustainably at smallholder farms. For larger-scale animal production, detoxified jatropha (*Jatropha* sp.) meal is a noteworthy alternative protein source. Globally, the advantages of single-cell protein (bacteria, yeast, fungi, microalgae) and aquatic biomass (seaweed, duckweed) over land crops are the independence of production from arable land and weather. The chemical composition of these feeds varies widely depending on the species and growth conditions. Microalgae have shown good potential both as lipid (e.g. *Schizochytrium* sp.) and protein supplements (e.g. *Spirulina platensis*) for ruminants. To conclude, various novel or underexploited feeds have potential to replace or supplement the traditional crops in ruminant rations. In the short-term, N-fixing grain legumes, oilseeds such as camelina and increased use of food and/or fuel industry by-products have the greatest potential to replace or supplement the traditional crops especially in the temperate zones. In the long-term, microalgae and duckweed of high yield potential as well as wood industry by-products may become economically competitive feed options worldwide.

Multi-criteria evaluation of dairy cattle feed resources and animal characteristics for nutritive and environmental impacts

H.J. van Lingen[1], J.G. Fadel[1], A. Bannink[2], J. Dijkstra[3], J.M. Tricarico[4], D. Pacheco[5], D.P. Casper[6] and E. Kebreab[1]

[1] *Department of Animal Science, University of California, Davis, CA 95616, USA*
[2] *Wageningen Livestock Research, Wageningen University & Research, PO Box 338, 6700 AH, Wageningen, the Netherlands*
[3] *Animal Nutrition Group, Wageningen University & Research, PO Box 338, 6700 AH, Wageningen, the Netherlands*
[4] *DMI Innovation Center for US Dairy, Rosemont, IL 60018, USA*
[5] *AgResearch Limited, Grasslands Research Centre, Private Bag 11008, Palmerston North 4442, New Zealand*
[6] *Furst McNess Company, Freeport, IL, 61032, USA*

E-mail: ekebreab@ucdavis.edu

Abstract

On-farm nutrition and management interventions to reduce enteric CH_4 (eCH_4) emission, the most abundant greenhouse gas from cattle, may also affect volatile solids and N excretion. The objective was to jointly quantify eCH_4 emissions, digestible volatile solids (dVS) excretion and N excretion from dairy cattle, based on dietary variables and animal characteristics, and to evaluate relationships between these emissions and excreta. Univariate and Bayesian multivariate mixed-effects models fitted to 520 individual North American dairy cow records indicated DM intake and dietary ADF and CP to be the main predictors for production of eCH_4 emissions and dVS and N excreta (g/d). Yields (g/kg DM intake) of eCH_4 emissions and dVS and N excreta were best predicted by dietary ADF, dietary CP, milk yield and milk fat content. Intensities (g/kg fat- and protein-corrected milk) of eCH_4, dVS and N excreta were best predicted by dietary ADF, dietary CP, days in milk and BW. A *K*-fold cross-validation indicated that eCH_4 and urinary N variables had larger root mean square prediction error (RMSPE; % of observed mean) than dVS, fecal N and total N production (on average 24.3% and 26.5% *vs.* 16.7%, 15.5% and 16.2%, respectively), while intensity variables had larger RMSPE than production and yields (29.4%, 14.7% and 14.6%, respectively). Univariate and multivariate equations performed relatively similar (18.8% *vs.* 19.3% RMSPE). Mutual correlations indicated a trade-off for eCH_4 *vs.* dVS yield. The multivariate model indicated a trade-off between eCH_4 and dVS *vs.* total N production, yield and intensity induced by dietary CP content.

Review: Biological determinants of between-animal variation in feed efficiency of growing beef cattle

G. Cantalapiedra-Hijar[1], M. Abo-Ismail[2], G. E. Carstens[3], L. L. Guan[2], R. Hegarty[4], D. A. Kenny[5], M. McGee[6], G. Plastow[2], A. Relling[7], I. Ortigues-Marty[1]

[1]*Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès-Champanelle, France*
[2]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, T6G 2P5, Canada*
[3]*Department of Animal Science, Texas A&M University, College Station 77843, USA*
[4]*University of New England, Armidale, NSW, 2350, Australia.*
[5]*Animal and Bioscience Research Department, Animal & Grassland Research and Innovation Centre, Teagasc, Grange, Dunsany, Co. Meath, Ireland*
[6]*Livestock Systems Research Department, Animal & Grassland Research and Innovation Centre, Teagasc, Grange, Dunsany, Co. Meath, Ireland*
[7]*Department of Animal Sciences, The Ohio State University, 1680 Madison Ave., Wooster, OH, 44691.USA*

E-mail: gonzalo.cantalapiedra@inra.fr

Abstract

Animal's feed efficiency in growing cattle (i.e. the animal ability to reach a market or adult body weight with the least amount of feed intake), is a key factor in the beef cattle industry. Feeding systems have made huge progress to understand dietary factors influencing the average animal feed efficiency. However, there exists a considerable amount of animal-to-animal variation around the average feed efficiency observed in beef cattle reared in similar conditions, which is still far from being understood. This review aims to identify biological determinants and molecular pathways involved in the between-animal variation in feed efficiency with particular reference to growing beef cattle phenotyped for residual feed intake (RFI). Moreover, the review attempts to distinguish true potential determinants from those revealed through simple associations or indirectly linked to RFI through their association with feed intake. Most representative and studied biological processes which seem to be connected to feed efficiency were reviewed, such as feeding behaviour, digestion and methane production, rumen microbiome structure and functioning, energy metabolism at the whole body and cellular levels, protein turnover, hormone regulation and body composition. In addition, an overall molecular network analysis was conducted for unravelling networks and their linked functions involved in between-animal variation in feed efficiency. The results from this review suggest that feeding and digestive-related mechanisms could be associated with RFI mainly because they co-vary with feed intake. Although much more research is warranted, especially with high-forage diets, the role of feeding and digestive related mechanisms as true determinants of animal variability in feed efficiency could be minor. Concerning the metabolic-related mechanisms, despite the scarcity of studies using reference methods it seems that feed efficient animals have a significantly lower energy metabolic rate independent of the associated intake reduction. This lower heat production in feed efficient animals may result from a decreased protein turnover and a higher efficiency of ATP production in mitochondria, both mechanisms also identified in the molecular network analysis. In contrast, hormones and body composition could not be conclusively related to animal-to-animal variation in feed efficiency. The analysis of potential biological networks underlying RFI variations highlighted other significant pathways such as lipid metabolism and immunity and stress response. Finally, emerging knowledge suggests that metabolic functions underlying genetic variation in feed efficiency could be associated with other important traits in animal production. This emphasises the relevance of understanding the biological basis of relevant animal traits to better define future balanced breeding programs.

Review: Selecting for improved feed efficiency and reduced methane emissions in dairy cattle

P. Løvendahl[1], G.F. Difford[1,2], B. Li[1,3], M.G.G. Chagunda[4], P. Huhtanen[5], M.H. Lidauer[6], J. Lassen[1,7], P. Lund[8]

[1]*Dept. Molecular Biology and Genetics, Aarhus University, DK 8830 Tjele, Denmark*
[2]*Wageningen University and Research, NL 6708 Wageningen, The Netherlands*
[3]*Swedish University of Agricultural Sciences, SE 75007 Uppsala, Sweden*
[4]*University of Hohenheim, DE 70599 Stuttgart, Germany*[5]*Swedish University of Agricultural Sciences, SE 90183 Umeå, Sweden*
[6]*Natural Resources Institute Finland (Luke), SF 31600 Jokioinen, Finland*
[7]*Viking Genetics, DK 8900 Randers, Denmark*
[8]*Dept. Animal Science, Aarhus University, DK 8830 Tjele, Denmark*

E-mail: peter.lovendahl@mbg.au.dk

Abstract

It may be possible for dairy farms to improve profitability and reduce environmental impacts by selecting for higher feed efficiency and lower methane (CH_4) emission traits. It remains to be clarified how CH_4 emission and feed efficiency traits are related to each other which will require direct and accurate measurements of both of these traits in large numbers of animals under the conditions in which they are expected to perform. The ranking of animals for feed efficiency and CH_4 emission traits can differ depending upon the type and duration of measurement used, the trait definitions and calculations used, the period in lactation examined, and the production system, as well as interactions among these factors. Because the correlation values obtained between feed efficiency and CH_4 emission data are likely to be biased when either or both are expressed as ratios, therefore researchers would be well advised to maintain weighted components of the ratios in the selection index. Nutrition studies indicate that selecting low emitting animals may result in reduced efficiency of cell wall digestion, that is neutral detergent fibre (NDF), a key ruminant characteristic in human food production. Moreover, many interacting biological factors that are not measured directly, including digestion rate, passage rate, the rumen microbiome, and rumen fermentation, may influence feed efficiency and CH_4 emission. Elucidating these mechanisms may improve dairy farmers ability to select for feed efficiency and reduced CH_4 emission.

Review: The influence of genotype x environment interactions on the robustness of dairy cows for grazing systems

J. R. Roche[1,2], D. P. Berry[3], L. Delaby[4], P. G. Dillon[3], B. Horan[3], K. A. Macdonald[1], and M. Neal[1]

[1]*DairyNZ, Hamilton 3240, New Zealand.*
[2]*School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand.*
[3]*Teagasc Moorepark, Fermoy, Co. Cork, P61 C996, Ireland.*
[4]*INRA, Rennes, France*

E-mail: John.Roche@mpi.govt.nz

Abstract

Although food from grazed animals is increasingly sought by consumers because of perceived animal welfare advantages, grazing systems provide the farmer and the animal with unique challenges. The system is dependent almost daily on the climate for feed supply, with the importation of large amounts of feed from off farm, and associated labour and mechanisation costs, sometimes reducing economic viability. Furthermore, the cow may have to walk long distances and be able to harvest feed efficiently in a highly competitive environment because of the need for high levels of pasture utilisation. She must, also, be 1) highly fertile, with a requirement for pregnancy within 83 days post-calving; 2) 'easy care', because of the need for the management of large herds with limited labour; 3) able to walk long distances; and 4) resilient to changes in feed supply and quality, so that short-term nutritional insults do not unduly influence her production and reproduction cycles. These are very different and are in addition to demands placed on cows in housed systems offered pre-made mixed rations. Furthermore, additional demands in environmental sustainability and animal welfare, in conjunction with the need for greater system-level biological efficiency (i.e., 'sustainable intensification'), will add to the 'robustness' requirements of cows in the future. Increasingly, there is evidence that certain genotypes of cows perform better or worse in grazing systems, indicating a genotype x environment interaction. This has led to the development of tailored breeding objectives within countries for important heritable traits to maximise the profitability and sustainability of their production system. To date, these breeding objectives have focussed on the more easily measured traits and those of highest relative economic importance. In the future, there will be greater emphasis on more difficult to measure traits that are important to the quality of life of the animal in each production system and to reduce the system's environmental footprint.

Review: Epigenetics, developmental programming and nutrition in herbivores

P. Chavatte-Palmer [1], M. A. Velazquez [2], H. Jammes [1] and V. Duranthon [1]

[1] *UMR BDR, INRA, ENVA, Université Paris Saclay, 78350, Jouy en Josas, France*
[2] *School of Natural and Environmental Sciences, Newcastle University, NE1 7RU,* United Kingdom

E-mail: pascale.chavatte-palmer@inra.fr

Abstract

Epidemiological studies in humans and animal models (including ruminants and horses) have highlighted the critical role of nutrition on developmental programming. Indeed, it has been demonstrated that the nutritional environment during the periconceptional period and fetal development can altered the postnatal performance of the resultant offspring. This nutritional programming can be exerted by maternal and paternal lineages and can affect offspring beyond the F1 generation. Alterations in epigenetic mechanisms has been proposed as the causative link behind the programming trajectories observed in the offspring. Although a clear cause-effect relationship between epigenetic modifications during early development and later offspring phenotype has not been demonstrated in livestock species, strong associations have been reported for some epigenetic marks (e.g. miRNA) that are worth exploring as possible predictors of future offspring phenotype. In this review we shortly describe the main epigenetic mechanisms studied so far in mammals (i.e. mainly in the mouse) thought to be associated with developmental programing, and discuss the few studies available in mammalian herbivores (e.g. cattle) showing the effect of nutrition on epigenetic marks and the associated phenotype. Clearly, there is a need to develop research on nutritional strategies capable of modulating the epigenetic machinery with positive influence on the phenotype of livestock herbivores. This type of research is needed to alleviate the challenges currently faced by the livestock industry (e.g. impaired fertility of high-yielding dairy cows). This in turn will have a positive influence on animal welfare and productivity of livestock enterprises.

Review: Livestock production increasingly influences wildlife across the globe

I.J. Gordon[1,2,3]

[1] *Division of Tropical Environments & Societies, James Cook University, 1 James Cook Drive, Townsville, Queensland 4811, Australia*
[2] *James Hutton Institute, Craigiebuckler, Aberdeen, AB15 8QH, UK.*
[3] *Fenner School of Environment & Society, Australian National University, Canberra, ACT 2601, Australia*

E-mail: iain.gordon@jcu.edu.au

Abstract

With the growing human population, and their improving wealth, it is predicted that there will be significant increases in demand for livestock products (mainly meat and milk). Recent years have demonstrated that the growth in livestock production has generally had significant impacts on wildlife worldwide; and these are, usually, negative. Here I review the interactions between livestock and wildlife and assess the mechanisms through which these interactions occur. The review is framed within the context of the socio-ecological system whereby people are as much a part of the interaction between livestock and wildlife as the animal species themselves. I highlight areas of interaction that are mediated through effects on the forage supply (vegetation) - neutral, positive and negative - however, the review broadly analyses the impacts of livestock production activities. The evidence suggests that it is not the interaction between the species themselves but the ancillary activities associated with livestock production (e.g. land use change, removal of predators, provision of water points) that are the major factors affecting the outcome for wildlife. So in future, there are two key issues that need to be addressed – firstly, we need to intensify livestock production in areas of "intensive" livestock production in order to reduce the pressure for land use change to meet the demand for meat (land sparing). And secondly, if wildlife is to survive in areas where livestock production dominates, it will have to be the people part of the socio-ecological system that sees the benefits of having wildlife co-exist with livestock on farming lands (land sharing and win-win).

Review: Using physiologically-based models to predict population responses to phytochemicals by wild vertebrate herbivores

J.S. Forbey[1], R. Liu[2], T.T. Caughlin[1], M.D. Matocq[3], J.A. Vucetich[4], K.D. Kohl[5], M.D. Dearing[6], A.M. Felton[7]

[1]*Deptartment of Biological Sciences, Boise State University, Boise, ID, USA.*
[2]*Department of Mathematics, University of Wyoming, Laramie, Wyoming, USA*
[3]*Program in Ecology, Evolution, and Conservation Biology, Department of Natural Resources and Environmental Science, University of Nevada, Reno, Nevada 89557, USA*
[4]*School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931, USA*
[5]*Department of Biological Sciences, University of Pittsburgh, 4249 Fifth Ave., Pittsburgh, PA, 15260, USA*
[6]*Department of Biology, School of Biological Sciences, University of Utah, 257 South 1400 East, Salt Lake City, UT, 84112, USA*
[7]*Southern Swedish Forest Research Centre, Faculty of Forest Sciences, Swedish University of Agricultural Sciences, P.O. Box 49, Alnarp SE-230 53 Sweden*

E-mail: jenniferforbey@boisestate.edu, annika.felton@slu.se

Abstract

To understand how foraging decisions impact individual fitness of herbivores, nutritional ecologists must consider the complex *in vivo* dynamics of nutrient-nutrient interactions and nutrient-toxin interactions. Mathematical modeling has long been used to make foraging predictions (e.g., optimal foraging theory) but has largely been restricted to considering a single currency (e.g., energy) or using simple indices of nutrition (e.g., fecal nitrogen) without full consideration of physiologically-based interactions among numerous co-ingested phytochemicals. Here, we describe a physiological approach to more accurately identify the underlying drivers of foraging decisions based on how both toxic and nutritional food constituents are metabolically processed, to predict individual fitness, and ultimately, population trajectories. We draw on the rich mechanistic understanding of how chemicals are processed by the human body and integrate mechanistic physiologically-based models into the geometric framework of nutrition that links multidimensional nutrient intake and post-absorptive processes to individual fitness. The novel component of our integrated models is that we allow food intake of an herbivore to be instantaneously influenced by concentrations of toxic and nutritional phytochemicals that are in excess of what herbivores can tolerate based on known physiological capacity of an animal. The physiologically-based methods will improve our ability to understand the foraging decisions of vertebrate herbivores and may help identify key behavioral and physiological mechanisms that underlie diet-based ecological adaptations.

Review: Enhancing gastrointestinal health in dairy cows

J.C. Plaizier[1], M. Danesh Mesgaran[2], H. Derakhshani[1], H. Golder[3], E. Khafipour[1], J. Kleen[4], I. Lean[3], J.J. Loor[5], G. Penner[6], Q. Zebeli[7].

[1]*Department of Animal Science, University of Manitoba, MB, Canada*
[2]*Department of Animal Science, Ferdowsi University of Mashhad, Mashhad, Iran*
[3]*Scibus, Camden, NSW, Australia*
[4]*Cowconsult, Coldinne, Germany*
[5]*Department of Animal Sciences, UIUC, Urbana, IL, USA*
[6]*Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada*
[7]*Department for Farm Animals and Veterinary Public Health, Vienna University of Veterinary Medicine, Vienna, Austria*

E-mail: Kees.Plaizier@umanitoba.ca

Abstract

Due to their high energy requirements, high-yielding dairy cows receive high-grain diets. This commonly jeopardizes their gastrointestinal health by causing subacute ruminal acidosis (SARA) and hindgut acidosis. These disorders can disrupt nutrient utilizations, impair the functionalities of gastrointestinal microbiota, and reduce the absorptive and barrier capacities of gastrointestinal epithelia. They can also trigger inflammatory responses. The symptoms of SARA are not only due to a depressed rumen pH. Hence, the diagnosis of this disorder based solely on reticulo-rumen pH values is inaccurate. An accurate diagnosis requires a combination of clinical examinations of cows, including blood, milk, urine, and feces parameters, as well as analyses of herd management and feed quality, including the dietary contents of NDF, starch and physical effective NDF. Grain-induced SARA increases acidity and shifts availabilities of substrates for microorganisms in the reticulo-rumen and hindgut and can result in a dysbiotic microbiota that are characterized by low richness, diversity, and functionality. Also, amylolytic microorganisms become more dominant at the expense of proteolytic and fibrolytic ones. Opportunistic microorganisms can take advantage of newly available niches, which, combined with reduced functionalities of epithelia, can contribute to an overall reduction in nutrient utilization and increasing endotoxins and pathogens in digesta and feces. The reduced barrier function of epithelia increases translocation of these endotoxins and other immunogenic compounds out of the digestive tract, which may be the cause of inflammations. This needs to be confirmed by determining the toxicity of these compounds. Cows differ in their susceptibility to poor gastrointestinal health, due to variations in genetics, feeding history, diet adaptation, gastrointestinal microbiota, metabolic adaptation, stress, and infections. These differences may also offer opportunities for the management of gastrointestinal health. Strategies to prevent SARA include balancing the diet for physical effective fibre, non-fibre carbohydrates and starch, managing the different fractions of non-fibre carbohydrates, and consideration of the type and processing of grain and forage digestibility. Gastrointestinal health disorders due to high grain feeding may be attenuated by a variety of feed supplements and additives, including buffers, antibiotics, probiotics/direct fed microbials, and yeast products. However, the efficacy of strategies to prevent these disorders must be improved. This requires a better understanding of the mechanisms through which these strategies affect the functionality of gastrointestinal microbiota and epithelia, and the immunity, inflammation and "gastrointestinal-health robustness" of cows. More representative models to induce SARA are also needed.

Review: Individual variability in feeding behaviour of domesticated ruminants

H. W. Neave [1], D. M. Weary [1], M. A. G. von Keyserlingk [1]

[1] *Animal Welfare Program, Faculty of Land and Food Systems, University of British Columbia, 2357 Main Mall, Vancouver, British Columbia, Canada V6T 1Z4*

E-mail: nina@mail.ubc.ca

Abstract

Individual animals behave differently from one another, especially when confronting challenges such as changes in diet (e.g. weaning), environment (e.g. moving from pasture to feedlot), and social grouping (e.g. movement to lactating group after parturition). Each of these challenges involves some element of novelty, impacting the welfare and productivity of the animal. Indeed, the large individual variability in the development and expression of feeding behaviour cannot be fully explained by differences in genetics, management practices, body size, or growth rate. In this review we outline evidence that individual variability in feeding behaviour is associated with the personality of the individual. We focus on three key personality traits: exploration, fear or reactivity, and sociability. Individuals differ in how much they explore their feeding environment, with more exploratory individuals being less reactive to novel situations. Feeding behaviour can be impaired in individuals that are especially reactive to a change in their environment, change in diet or handling or restraint by humans. The social environment is also a major factor affecting how individuals express their behaviour. Sociability of the individual, including dominant-subordinate and affiliative relationships, affects how individuals make foraging decisions, gain access to feed, and adopt particular social strategies to maintain or adjust feeding patterns when the social environment changes. Personality traits such as exploration, boldness and sociability also affect the use of social information when learning where, how or what to eat. Our review highlights the implications of feeding behaviour variability for the welfare and productivity of the individual, and how an understanding of personality can help tailor management to the needs of the individual.

Review: Adaptation of animals to heat stress

V. Sejian[1], R. Bhatta[1], J. Gaughan[2], F. R. Dunshea[3] and N. Lacetera[4]

[1]*ICAR- National Institute of Animal Nutrition and Physiology, Bangalore, State: Karnataka, India*
[2]*School of Agriculture and Food Sciences, The University of Queensland, Gatton, Queensland, Australia*
[3]*Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Australia*
[4]*Department of Agriculture and Forestry Science, Tuscia University, Viterbo, Italy*

E-mail: drsejian@gmail.com

Abstract

Livestock plays an important role in the global economy. Climate change effects are not only limited to crop production, but also affect livestock production e.g. reduced milk yields and milk quality, reduced meat production and reduced fertility. Therefore, livestock-based food security is threatened in many parts of the world. Further, multiple stressors are a common phenomenon in many environments, and are likely to increase due to climate change. Among these stresses, heat stress appears to be the major factor which negatively influences livestock production. Hence, it is critical to identify agro-ecological zone specific climate resilient thermo-tolerant animals to sustain livestock production. Livestock respond to the changing environments by altering their phenotypic and physiological characters. Therefore, survivability of the animal often depends on its ability to cope with or adapt to the existing conditions. So to sustain livestock production in an environment challenged by climate change, the animals must be genetically suitable and have the ability to survive in diversified environments. Biological markers or biomarkers indicate the biological states or alterations in expression pattern of genes or state of protein that serve as a reference point in breeding for the genetic improvement of livestock. Conventionally, identification of animals with superior genetic traits that were economically beneficial was the fundamental reason for identifying biomarkers in animals. Further, compared to the behavioural, morphological or physiological responses in animals, the genetic markers are important because of the possibility of finding a solution to animal adaptability to climate change.

Review: Adaptation of ruminant livestock production systems to climate changes

B. K. Henry[1], R. J. Eckard[2] and K. A. Beauchemin[3]

[1]*Science and Engineering Faculty, Queensland University of Technology, Brisbane, Qld 4001, Australia*
[2]*Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, VIC, 3010, Australia*
[3]*Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre, 5403 1st Avenue South, Lethbridge, Alberta, Canada*

E-mail: beverley.henry@qut.edu.au

Abstract

There is growing evidence on the extent to which projected changes in climate, including increases in atmospheric levels of carbon dioxide, higher temperatures, changes in amount, seasonality and variability of precipitation and increases in extreme weather events, may affect future availability of ruminant animal products. Elements of climate change affect livestock systems through direct impacts on animal physiology, behaviour, production and welfare and indirectly through feed availability, composition, and quality. These impacts may be positive or negative and will vary across geographical regions, animal species, and with adaptive capacity. However, adverse impacts are likely to be greatest in tropical and sub-tropical regions including countries where both current need and future growth in demand for nutrition is greatest. The complexity of effects means that effective adaptation strategies to mitigate negative impacts on ruminant production systems to climate changes will need to be multi-dimensional. While predictions of future climate, particularly on regional and local scales, have a degree of uncertainty, adaptation planning is starting to be informed by changes already being observed and adjustments in management being made by farmers to maintain productivity and profitability. Regional case studies illustrate the benefits and limitations of adaptive management: potential mitigation through heightened awareness of heat stress related mortality in French cattle; evidence of a drop in milk production in south-eastern Australian dairies during a January 2014 heat wave, from the theoretical potential of 53% to only 10% across the state; and limitations in response options to climate-induced thermal, nutritional and water stress for sheep and goat farmers in northern Ethiopia. Review of research on climate change impacts on ruminant livestock and effective adaptation together with evidence of practical adaptive management provide insights into potential strategies and gaps in knowledge to address challenges and improve future decisions.

Review: Converting nutritional knowledge into feeding practices: a case study comparing different protein feeding systems for dairy cows

H. Lapierre[1], M. Larsen[2], D. Sauvant[3], M. E. Van Amburgh[4] and G. Van Duinkerken[5]

[1]*Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada J0B 1M0*
[2] *Department of Animal Science, Aarhus University, DK-8830 Tjele, Denmark*
[3]*Inra, AgroParisTech, Université Paris-Saclay, 75005, Paris, France*
[4]*Department of Animal Science, Cornell University, Ithaca, NY 14850 USA*
[5]*Wageningen University & Research, Wageningen Livestock Research, 6708WD, Wageningen, The Netherlands*

E-mail: Helene.Lapierre@AGR.GC.CA

Abstract

Improving milk nitrogen efficiency through a reduction of CP supply without detrimental effect on productivity requires usage of feeding systems estimating both the flows of digestible protein, the exported true proteins and from these predict milk protein yield (MPY). Five feeding systems were compared in their ability to predict MPY vs. observed MPY in two studies where either protein supply or protein and energy supply were changed. The five feedings systems were: Cornell Net Carbohydrate and Protein System (v6.5.5), Dutch protein evaluation system (1991 and 2007), INRA (2018), National Research Council (2001), and NorFor (2011). The key characteristic of the systems with the best predicted MPY was the inclusion of a variable efficiency of utilization of protein supply taking into account the supply of both protein and energy. The systems still using a fixed efficiency had the highest slope bias in their prediction of MPY. Therefore, the development of new feeding systems or improvement of existing systems should include a variable efficiency of utilization of the protein related to both the protein and energy supply. The limitation of the current comparison did not allow determining if additional factors, as used in INRA (2018), were beneficial. This concept should also probably be transferred to essential amino acids.

Summaries of the invited speakers
during the
acidosis & rumen health satellite conference

Mining the omics data for clues on host-microbial interactions contributing to the promise to improve rumen health

L. Guan

Faculty of Agricultural, Life and Environmental Sciences, University of Alberta, Canada

Email: leluo.guan@ualberta.ca

Take home message: Rumen health is essential for achieving the high productivity. The application of advanced omics approaches has led to the enhanced understanding on the microbiome (composition and function) and its interaction with rumen epithelial transcriptome, which regulates the rumen function. This knowledge can help industry to develop more selective management to improve the animal productivity through maintaining and/or enhancing the rumen health.

The recent advances in next generation sequencing technology has accelerated the research in studying host-microbial interactions using high throughput sequencing based omics including transcriptomics, metagenomics and metatranscriptomics. The genome wide transcriptomics can reveal potential functional changes in tissues and/or cells under different metabolic and physiological conditions at molecular level (Wickramasinghe et al., 2014), while metagenomics and metatranscriptomics could capture the whole genome (DNA) and transcriptome (RNA) repertoire of the microbiome, providing both taxonomic and functional information of microbiota (Franzosa et al., 2014; Schwab et al., 2014).

In the last decade, the rumen microbiome has been widely explored on its diversities, composition, and functional characteristics, especially with the advancement of molecular biology techniques. To date, hundreds to thousands of microbial phylotypes have been identified from various rumen samples using sequencing of marker gene amplicons, revealing that that the rumen microbiota is complex and consists of up to 1000 phylotypes including bacteria, archaea, fungi, ciliated protozoa, and viruses (Henderson et al., 2015). At the same time, genomic information of rumen microorganisms has rapidly accumulated through genome sequencing of microbial cultures, particularly promoted by the recent Hungate 1000 project (http://www.rmgnetwork.org/hungate1000.html). Based on the genomic information, underlying functions of each sequenced microbial phylotype can be predicted. However, due to the lack of available genomes for many important but uncultivable rumen microorganisms (such as members belonging to *Bacteroidetes*), there is still a large gap in accurate prediction of functional profiles of rumen microbiome. Therefore, metagenomics and metatranscriptomics are useful tools to globally catalogue microbial gene/transcript profiles and to predict overall metabolic functions of rumen microbiome.

In this presentation, a general workflow to conduct rumen metagenomics and metatranscriptomics will be introduced, aiming to provide a useful guide for the future application of these omics approaches to understand the role of rumen microbiome in ruminal acidosis.

In the meantime, this presentation will also summarize the current findings on identifying the associations between the rumen microbiome and host metabolic dysfunction using omics approaches, mainly how host gene expression in the rumen epithelium may contribute to the differences in ruminal acidosis, using a transcriptome profiling based approach. As one of the key factors, ruminal epithelial metabolic functions including nutrient absorption, cell proliferation, energy metabolism which can directly impact on the rumen environment, such as pH, and short chain fatty acid concentrations. Such rumen environment can also directly affect rumen microbiota and potentially its activity.

The understanding of the transcriptome changes of the rumen epithelial tissue under different dietary conditions can provide fundamental understanding on how the function of the rumen epithelial wall is regulated and how it contributes to the healthier rumen environment. In addition, large evidences have revealed the existence of the ruminal epithelial adherent microbiota, but, its function is largely unidentified. Therefore, the understanding of the rumen epithelial function and its associated microbiota will provide more comprehensive knowledge on host-microbial interactions in the rumen, which directly affect the rumen metabolic function and health. Improvement of rumen health has been recognized as one of the common goal by research and industrial communities. The information from above can help us better understand mechanisms of the rumen function, which is vital for improving nutrient utilization and animal productivity.

References

Wickramasinghe S, Cánovas A, Rincón G, and Medrano JF 2014. Livestock Science 166: 206-216.

Franzosa EA, Morgan XC, Segata N, Waldron L, Reyes J, Earl AM, Giannoukos G, Boylan MR, Ciulla D, Gevers D, Izard J, Garrett WS, Chan AT, and Huttenhower C 2014. Proc Natl Acad Sci U S A 111, E2329-2338.

Schwab C, Berry D, Rauch I, Rennisch I, Ramesmayer J, Hainzl E, Heider S, Decker T, Kenner L, Müller M, Strobl B, Wagner M, Schleper C, Loy A, and Urich T 2014. ISME Journal 8, 1101-1114.

Henderson G, Cox F, Ganesh S, Jonker A, Young W, and Janssen PH 2015. Sci Rep 5, 14567. doi: 10.1038/srep14567.

New tools based on non-invasive indicators to improve sub-acute ruminal acidosis diagnosis in dairy cows

C. Villot

Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès-Champanelle, France

Email: villot.clothilde@gmail.com

Take home Message: Relative pH indicators measured by rumen sensors and combinations of rumen peripheral parameters are non-invasive indicators of Sub-Acute Ruminal Acidosis (SARA) and would be relevant for its diagnosis on-farm.

In ruminants, SARA is a common digestive disorder usually induced by a nutritional overload of rapidly fermentable carbohydrates. This disorder is characterized by abnormal and intermittent drops in rumen pH that alter animal production and health, and consequently increase economic losses for the farmer (Martin et al., 2006). Up to now, no pH threshold is admitted to identify SARA in dairy cows due to the inaccuracy of measurement techniques (Duffield et al., 2004) and the high rumen pH variability between animals (Penner et al., 2007).

The widespread adoption of pH boluses measuring continuously rumen pH gives new opportunities to calculate more accurate pH indicators with a standardized method of measurement. At this date, it is important to anticipate the interpretation of the large volume of data generated by this new technology.

In this context, we developed a specific mathematical approach to interpret the high resolution pH kinetic in order to calculate more universal and accurate pH indicators. We propose new relative indicators, calculated daily (kinetic normalised on 0, NpH), *i.e.:* time spent under NpH < -0.3, NpH standard deviation and NpH range. Relative NpH indicators overcome sensor's drift and noise as well as inter-animal variability of rumen pH. Therefore those relative indicators can improve SARA detection and be used for different animals and farms. A daily range of rumen NpH under 0.8 unit and a longer time than 50 min/d spent under a relative pH of -0.3 NpH unit are good indicators of SARA induced experimentally.

Even if an altered rumen pH is the first step of SARA, it is well documented that numerous but nonspecific clinical signs can also emerge depending on each animal susceptibility to the disease (Nasrollahi et al., 2017). Negative consequences on production and health in dairy cows, such as loss of milk yield, milk fat depression, laminitis and liver abscesses can be induced by rumen dysfunction. Therefore, we screened a total of 136 parameters (milk fatty acids, biomarkers of metabolism, inflammation…) in dairy cows with SARA induced experimentally in order to highlight potential combinations of rumen peripheral parameters for SARA detection.

Twenty multi-parametric models of 2 or 3 parameters measured directly on the animal (behaviour indicators) or coming from different biological matrices (blood, faeces, saliva, milk, urine) were selected with specificity and sensitivity above 90% and 75% respectively for SARA detection induced experimentally. The combination of milk urea, blood bicarbonate, and feed intake had the highest specificity and sensitivity for SARA diagnosis in our experimental conditions. Other parameters like the number of drinking acts, fat:protein in milk, saliva and faecal pH were the most frequently used in the proposed combinations. The present results were obtained with SARA conditions induced experimentally on a limited number of animals. To cope with the important variability related to field conditions, it is likely that it will be necessary to add more parameters to the models in order to obtain a good sensitivity and specificity for SARA detection on-farm.

SARA is a widespread nutritional disorder in dairy industry and new non-invasive tools could be deployed on-farm in order to have a systematic and early detection of the digestive dysfunction to prevent low milk production and subsequent diseases.

Acknowledgements

This research was part of Clothilde Villot PhD (INRA) supported with a grant from ANRT (Association Nationale Recherche Technologie), and animal nutrition companies Lallemand, Terrena and Valorex.

References

Duffield, T., J. C. Plaizier, A. Fairfield, R. Bagg, G. Vessie, P. Dick, J. Wilson, J. Aramini, and B. McBride. 2004. J Dairy Sci 87(1):59-66.
Martin, C., L. Brossard, and M. Doreau. 2006. INRA Productions Animales 19(2):93-108.
Nasrollahi, S., A. Zali, G. Ghorbani, M. M. Shahrbabak, and M. H. S. Abadi. 2017. Animal Feed Science and Technology 228:72-82.
Penner, G., K. Beauchemin, and T. Mutsvangwa. 2007. J Dairy Sci 90(1):365-375.

Feeding management of cattle and rumen health: risk factors

A. Bach

IRCEA (Institucio Catalana de Recerca i Estudis Avancats,) at the Department of Ruminant Production of IRTA, Spain

Email: alex.bach@icrea.cat

Take home Message: The main risk factors for rumen acidosis are related to rapid and large changes in starch and concentrate consumption by cattle. Other factors include particle size, moisture content, feed bunk management.

The main goal of any profitable dairy and beef enterprise should be to maximizing profit margins by increasing the conversion efficiency of dietary nutrients into milk or meat using means that are acceptable to consumers. The rumen is a complex environment inhabited by a number of microorganisms that live in an orchestrated consortium, and during the different production stages its equilibrium is challenged.

Ruminal acidosis is one of the most common nutritional disorders in both feedlot and dairy cattle. The reduction of rumen pH may alter rumen microbiota and cause some dysfunctions such as alterations in feeding and rumination patterns and total feed intake, and reductions in milk fat content. A crucial time in dairy production when the rumen equilibrium is challenged coincides with the transition from the dry to the lactating period. Dry cows generally receive diets with high inclusion levels of forage and low contents of concentrate, which typically result in slow fermentation rates in the rumen, but after calving, cows are fed rations with low inclusion levels of forage and high proportions of concentrate, which commonly have fast fermentation rates. In addition, feed intake increases rapidly and progressively after calving which may not only affect the microbiome but also the rumen and intestine epithelia.The provision of high-concentrate diets, coupled with high intakes (as typically seen in dairy cattle) may also affect the integrity of the intestine as the amount of unfermented starch that leaves the rumen can be substantial. These relatively large amounts of unfermented starch might not be completely digested in the small intestine and they are ultimately fermented in the hindgut. This fermentation in the hindgut can also cause some problems to the animal and pH in that compartment may also decrease. Bach et al. (2018) have reported using a novel technique involving an endoscope to obtain samples from both the rumen and the hindgut, marked changes in the rumen wall and more modest changes in the colon when transitioning from a dry to a lactation ration. Similarly, in beef cattle, the transition from growing to fattening poses a high risk of rumen acidosis as animals move from rations that contain 10-20% to >70% concentrate.

Other risk factors of rumen acidosis include the particle size of the ration, which contrary to what is commonly thought, long particle sizes tend to be more problematic than shorter ones (due to sorting). Another risk factor is moisture of the ration, and most commonly, changes in the moisture content of main ingredients (typically silages) that are not frequently checked and thus the amount of nutrients (and fiber) provided from these ingredients may be different than originally planned if mixed without adjusting for moisture content. Also, the buffering capacity of the ingredients in the ration are a risk factor for rumen acidosis. Two rations formulated with the same nutrients but using ingredients with different buffering capacity will have different risks of rumen acidosis. There are also risk factors associated with social behavior of cows. For example, feeding cows in automatic milking systems is challenging because fluctuations in milking and eating patterns, especially under guided-traffic conditions, make it difficult for the cow to maintain a constant proportion of forage to concentrate in the total diet. Similarly, the absence of left-overs may cause cows to slug-feeding and compete for feed. A similar situation is found when stocking density is excessive.

Nevertheless, both dairy and beef cattle are fairly effective in controlling rumen health and when they consume rations that lead to rumen upsets the first reaction is to decrease feed intake, which in most instances, solves the problem of rumen acidosis but of course hampers productivity of cows and it may also lead to metabolic disorders due to insufficient uptake of nutrients (especially energy). There are several feeding strategies to minimize rumen upsets and optimize rumen function. The most important one involves properly balancing a diet, not only for the cow but also for the rumen microorganisms. The physical form of the ration is of pivotal importance as it will not only influence sorting behavior (and thus the amount and type of nutrients consumed by the cows) but also the rate of fermentation. In some occasions the use of additives may help to overcome challenges and stabilize rumen fermentation.

References

Bach A, Guasch I, Elcoso G, Chaucheyras-Durand F, Castex M, Fabregas F, Garcia-Fruitos E, and Aris A 2018. Journal of Dairy Science, 101:1-10.
Thrune M, Bach A, Ruiz Moreno M, Stern MD, and Linn JG 2009. Livestock Science, 124:261–265.
Chaucheyras-Durand F, Walker ND, and Bach A 2008. Animal Feed Science and Technology, 145:5–26.
Poppy GD, Rabiee AR, Lean IJ, Sanchez WK, Dorton KL, and Morley PS 2012. Journal of. Dairy Science,95:6027–6041.

Current strategies to limit risk factors for rumen health: managing substrate, use of antimicrobials, and alternatives to antimicrobials

H. Golder

Scibus, Camden, NSW, Australia

Email: heleng@scibus.com.au

Take home Message: Control strategies to limit risk factors for rumen health are likely to vary between herds and individuals may respond differently.

Developing effective control strategies to maintain optimal rumen function and prevent disorders such as ruminal acidosis may be challenging as individual cattle appear to have unique ruminal responses to changes in diet for several reasons that are largely based on the type of substrate fed and feeding strategy, but also genetic variation. The base approach for maintaining good rumen function is to provide and properly manage a balanced diet containing adequate energy, forage, and fibre. However, rumen function can also be modified by the inclusion of antimicrobial and non-antimicrobial feed additives. The focus of this brief review is on limiting risk factors for rumen health by management of substrate, use of antimicrobials, and use of alternatives to antimicrobials. These strategies should be used in the context of other control methods i.e. ensuring careful adaptation to diets, well processed and integrated TMR diets, adequate physically effective neutral detergent fibre, and limiting pre-formed lactic acid (Lean et al., 2014).

Substrate Different risks have been identified for starch- and sugar-based dietary challenges to rumen stability. It is evident that dietary sugars should be controlled and not all sugars, grains, or starches have the same effect on the rumen. The following maximum percentages for TMR have been suggested: 42% total NFC, 24% starch, and 8% sugar or not exceeding approximately 0.35% of bodyweight (Lean and Golder, 2018). Observations that acidotic cattle have low rumen concentrations of ammonia (Bramley et al., 2008) and a reduction in the incidence and prevalence of acidosis with increased nitrogen in the diet (Golder et al.,2014b) suggest that microbial protein is a significant sink for hydrogen in the rumen and that energy spilling i.e. an inability of bacteria to reproduce, hence produce more VFA, may be an important part of the pathogenesis of acidosis (Lean and Golder, 2018).

Antimicrobials There is strong evidence that many of the antibiotics used in the ruminant industries such as monensin, lasalocid, tylosin, bambermycin, and virginiamycin can modify the rumen and control acidosis (Lean et al., 2014). However, the use of antibiotics as feed additives in animal nutrition is subject to regulatory change, with most banned in the European Union. There is consumer pressure to reduce overall use of antibiotics. Prudent use strategies of approved feed additives are important for countries where antimicrobial agents are available; however, animal variation suggests that no single feed additive will be capable of controlling disorders such as ruminal acidosis in all cattle (Lean et al., 2014). Different feed additives may need to be used depending on feed substrates.

Alternatives to antimicrobials include buffers and neutralising agents, yeasts, direct-fed microbials (DFM), and enzymes. A buffer, by definition, reduces the decrease in pH without causing an increase in pH. There are positive interactions for the buffer, sodium bicarbonate with the alkalising agent, magnesium oxide and their combination had similar effects as virginiamycin in controlling cyclic eating behaviour in cattle during adaptation to a diet high in grain and containing fructose (Golder et al., 2014a). There is increasing evidence that yeast additives may have a role in stabilising rumen function. Actions that have been identified with live yeasts include increases in rumen pH, reductions in lactic acid, enhanced fibre digestion, and small increases in VFA production (Lean and Golder, 2018). Responses to DFM have been inconsistent but have included a decrease in the area below ruminal pH defined for subacute ruminal acidosis, an increase in propionate concentrations, increased protozoa count, and altered counts of lactate producing and utilising bacteria (Lean et al., 2014). Exogeneous enzymes are primarily fibrolytic and improve production by increasing dry matter and neutral detergent fibre digestibility.

Use of custom-designed DFM or feed additive combinations, along with genetic selection for animals with higher acidosis resistance and favourable rumen health traits may be avenues for improved rumen health and acidosis control.

References

Bramley E, Lean IJ, Fulkerson WJ, Stevenson MA, Rabiee AR and Costa ND 2008. Journal of Dairy Science 91, 308-321.

Golder HM, Celi P, Rabiee AR and Lean IJ 2014a. Journal of Dairy Science 97, 985-1004.

Golder HM, Denman SE, McSweeney C, Wales WJ, Auldist MJ, Wright MM, Marett LC, Greenwood JS, Hannah MC, Celi P, Bramley E and Lean IJ 2014b. Journal of Dairy Science 97, 5763-5785.

Lean IJ and Golder HM 2018. Florida Ruminant Nutrition Symposium 123-141.

Lean IJ, Golder HM and Hall MB 2014. Veterinary Clinics of North America: Food Animal Practice 30, 539-575.

Interaction of rumen health with cattle behaviour

T.J. DeVries

Department of Animal Biosciences, University of Guelph, Canada

Email: tdevries@kemptvillec.uoguelph.ca

Take home message: The behaviour of cattle may interact directly with nutrition and management to affect the risk of poor rumen health. Maintaining a healthy rumen is important for consistent, high dry matter intake, good production, and optimal feed efficiency. This not only has financial benefits for producers, but also helps to promote good animal welfare. Promotion of stable rumen pH and avoiding bouts of ruminal acidosis are key to preservation of rumen health.

Nutrition and management practices that cause cattle to eat fewer and larger meals, more quickly, have been associated with an increased incidence of ruminal acidosis. Indeed, ruminal pH declines following meals, and the rate of pH decline increases as meal size increases and as dietary effective fiber concentration decreases. Further, as cattle spend less overall time feeding, and increase their rate of feed consumption, daily salivary secretion is reduced, decreasing the buffering capacity of the rumen and reducing rumen pH.

Rumen health may not only be related to how cattle eat, but also what they consume within their ration (Miller-Cushon and DeVries, 2017). Both dairy and beef cattle typically preferentially select (sort) mixed diets. They often sort for the shorter, grain components of the diet, and discriminate against longer forage components. Such sorting can result in the ration consumed by cattle being greater in fermentable carbohydrates than intended and lesser in effective fiber, thereby increasing the risk of depressed rumen pH. In dairy cattle, research has linked such behaviour with producing milk with lower fat percentage. Imbalanced nutrient intake and altered rumen fermentation, as result of sorting, also has the potential to impact the efficiency of digestion and production. Sorting may also reduce the nutritive value of the feed remaining in the feed bunk, particularly in the later hours past the time of feed delivery. For group-fed cattle, this may be detrimental for animals that do not have access to feed, at the time when it is delivered, for example when there is high competition at the feed bunk. In such cases, these cattle may not be able to maintain adequate nutrient intake needed for high levels of production.

After feed is consumed, there is another behaviour which may influence rumen health: rumination. Rumination contributes to the breakdown of feed into smaller particles, which then increases feed surface area and decreases the amount of time it takes feed to be fermented in the rumen, thus increasing the rate of digestion of that feed. In addition, the saliva produced during chewing and bolus formation in rumination not only aids in re-swallowing of that bolus, but also acts to maintain rumen pH through its buffering abilities, thus helping avoid ruminal acidosis. Related to this, there is research to suggest that monitoring of rumination behaviour, at a herd level, may provide some insight into rumen health.

To promote behavioural patterns that optimize rumen health, diets provided to cattle must have sufficient physically-effective forage, which will promote a slower consumption of feed, in smaller, more frequent meals per day. Such diets are also sorted to a lesser degree and, as result of greater fibre content and particle size, ruminated more. Despite this, the tendency in the cattle industries is to provide diets that contain moderate (dairy) or low (beef) levels of forage, which in itself tends to be chopped moderately in length. This is done in effort to maximize intake and digestibility. Given this, other opportunities to modify the eating behaviour of cows on such rations need to be explored.

There is evidence that use of feed additives (e.g. yeast supplements, ionophores), that positively impact the rumen, have concurrent benefits for feeding behaviour (DeVries and Chevaux, 2014). A common thread in these studies is an association between favourable meal patterns, greater rumination, and a reduction in ruminal pH variation. Whereas meal patterning may in itself affect ruminal pH, it is likely that feed additives that have the potential to stabilize ruminal pH and fermentation affect meal patterning as a secondary effect. Specifically, a more consistent fermentation pattern should result in less variation in volatile fatty acid production, improved fiber digestibility, and quicker return to eating.

In addition to dietary factors, management and housings strategies, which influence behavioural patterns, may impact rumen health. For example, by maintaining continual feed access, by frequent delivery of fresh feed, we promote the frequent, slow consumption of feed in small meals throughout the day. Similarly, limiting competition for resources (feed and lying space), may only promote healthy feed consumption patterns, but also result in greater rumination time.

Finally, not only do behavioural patterns influence the risk of poor rumen health, but may also change in response to changes in the rumen environment. Research data indicate that cattle will either change feed preferences or begin to sort in favour of long forage particles in mixed ration in response to experience of a bout of acidosis (DeVries et al., 2014). Further, there is evidence that the degree to which they change their sorting is directly related to the degree of acidosis incurred. From a practical standpoint, this means that obvious changes in the diet selection patterns of cattle within a herd may indicate that they are experiencing some disruption to normal rumen function.

References

Miller-Cushon, E. K., and T. J. DeVries. 2017. J. Dairy Sci. 100:4172-4183.

DeVries, T. J., and E. Chevaux. 2014. J. Dairy Sci. 97:6499-6510.

DeVries, T. J., T. Schwaiger, K. A. Beauchemin , and G. B. Penner. 2014. J. Anim. Sci. 92:1728-1737.

Rumen health and beyond

G. Penner, C. Kent-Dennis, Q. Zebeli, J.R. Aschenbach

Department of Animal and Poultry Science, University of Saskatchewan, Canada

Email: greg.penner@usask.ca

Take home Message: Improved rumen health is crucial to enhance overall health and productivity in cattle

Feeding rapidly fermentable diets, as a strategy to meet nutrient demands for beef and dairy cattle, can result in a fast rate and high extent of fermentation yielding large quantities short-chain fatty acids (SCFA) and supporting rapid microbial growth. While the previously mentioned outcomes could be considered to be positive from a nutritional perspective, if the rate of SCFA production exceeds that of SCFA removal from the rumen, ruminal pH will decrease. In addition, rapid increases in dry matter intake, even without dietary change, can induce low ruminal pH. While a consensus for the duration of time and magnitude of the depression for pH has not been achieved, sustained reductions (e.g. pH < 5.6 for >3 h/d) are used to denote exposure to ruminal acidosis. It is important to note that ruminal acidosis involves more than low ruminal pH as increased ruminal fluid osmolality, changes in the microbial community structure, and increased concentration of microbial associated molecular patterns (MAMPS) have been reported.

Cattle can regulate ruminal pH by modulating feeding behavior and regulating SCFA absorption. Feeding behavior dictates the consumption patterns and quantity of fermentable material available for fermentation; while, SCFA absorption across the ruminal epithelium (RE) regulates ruminal pH (Penner et al., 2009; Aschenbach et al., 2011), osmolality, and the risk for ruminal acidosis (Humer et al., 2018). Moreover, efficient SCFA absorption is instrumental in supplying large amounts of energy substrates to the host (Aschenbach et al., 2011) supporting high milk yields for dairy cattle and rapid rates of gain for feedlot cattle. While SCFA absorption is critical for ruminal pH regulation and energy supply, cattle must ensure that SCFA absorption across the RE does not lead to the acidification of cells within the RE or excessive transfer of SCFA and lactate into portal circulation that could lead to metabolic acidosis. Moreover, barrier function of the RE must be regulated to ensure that MAMPS and microbes themselves do not enter portal circulation.

As a consequence of ruminal acidosis, SCFA absorption decreases and excessive exposure to low pH, driven by high SCFA concentrations, challenge barrier function properties of the RE. Compromised barrier function of the RE can lead to local immune activation within the RE and a systemic acute phase protein response (Plaizier et al., 2012; Humer et al., 2018). Additionally, ruminal acidosis has been shown to reduce digesta pH in the large intestine and it has been speculated that the systemic inflammation observed with ruminal acidosis may partially originate from the intestine (Gressley et al., 2011). Induction of a systemic immune response has been shown to markedly increase both amino acid and glucose utilization. While the goal of feeding highly fermentable diets is to increase nutrient supply, if such practices induce ruminal acidosis, the benefit arising from increased nutrient supply will be marginalized.

This presentation will focus on challenges to meet the energy demand for beef and dairy cattle while maintaining rumen and gut health. Concepts presented will focus on the maintenance of acid-base regulation in the rumen and how pathways for SCFA absorption change with nutritional scenarios. Factors affecting RE barrier function and barrier function across the gastrointestinal tract will be discussed along with the stimulation of luminally-mediated local and systemic inflammation. As such, the concepts presented will link ruminal and intestinal function as factors influencing the risk for systemic inflammation and associated disorders arising from ruminal acidosis. Finally, this presentation will introduce the concept of altered nutrient utilization associated with the onset of ruminal acidosis-induced inflammation.

References

Aschenbach, J.R., Penner, G.B., Stumpff, F., Gäbel, G. 2011. Journal of Animal Science 89, 1092-1107.

Humer, E, Petri, R.M., Aschenbach. J.R., Bradford. B.J., Penner. G.B., Tafaj, M., Südekum, K.-H., Zebeli, Q. 2018. Journal of Dairy Science doi: 10.3168/jds.2017-13191.

Gressley, T.F., Hall, M.B., Armentano, L.E. 2011. Journal of Animal Science 89, 1120-1130.

Plaizier, J.C., Khafipour, E., Li, S., Gozho, G.N., Krause, D.O. 2012. Animal Feed Science and Technology 172, 9-21.

Penner, G.B., J.R. Aschenbach, G. Gäbel, R. Rackwitz, and M. Oba. 2009. Journal of Nutrition 139:1714-1720.

Index of authors

With the support of our sponsors

INRA
SCIENCE & IMPACT

Phytosynthese
GROUPE LEHNING

La Région
Auvergne-Rhône-Alpes

VILLE DE
CLERMONT
FERRAND

LALLEMAND
LALLEMAND ANIMAL NUTRITION

idena

Valorex
ICI, LA NATURE INNOVE

VetAgro Sup

APIS-GENE
investir Innover Valoriser

neovia

Désialis

Moy park
BEEF ORLEANS

SmartCow
an integrated infrastructure for increased research capability and innovation in the European cattle sector

Cniel
Centre national interprofessionnel de l'économie laitière

DSM
BRIGHT SCIENCE. BRIGHTER LIVING.

GROUPE TECHNA

casden
BANQUE POPULAIRE

BANQUE POPULAIRE
BANQUE POPULAIRE AUVERGNE RHÔNE ALPES

INVESTISSEMENTS D'AVENIR

UCA
UNIVERSITÉ
Clermont
Auvergne

CAP 20-25
I-SITE
CLERMONT
Clermont Auvergne Project

Volvic
EAU MINÉRALE NATURELLE

apave
mission de confiance

Herbivore nutrition supporting
sustainable intensification and
agro-ecological approaches

SEPTEMBER 2018 VOLUME 9 ISSUE 3

Current and Upcoming Conferences in *Advances in Animal Biosciences* and *Animal*

British Society of Animal Science *(Published April 2018)*
International Bull Fertility *(Published May 2018)*
International Symposium on the Nutrition of Herbivores
EU Cost Action IPEMA
Biology of Lactation in Farm Animals
International Symposium on Digestive Physiology of Pigs
British Society of Animal Science
International Symposium on Ruminant Physiology

animal

advances in
animal
biosciences