



## **Inhibition of enteric methanogenesis in cows induces changes in plasma metabolome highlighting potential emission markers**

Bénédict Yanibada, Ulli Hohenester, Mélanie Pétéra, Cécile Canlet, Stéphanie Durand, Fabien Jourdan, Cécile Martin, Maguy Eugène, Diego Morgavi, Hamid Boudra

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*Alexandre Berndt  
Luiz Gustavo Pereira Ribeiro  
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# Foreword

## **GGAA history, purpose, aims and audience**

The Greenhouse Gas and Animal Agriculture Conference (GGAA) is the premier international conference summarising the collective state of scientific knowledge on greenhouse gas abatement strategies and production systems adaptation needs for the livestock sector. The gathering features leading scientists and policymakers reviewing the current state of knowledge and presenting significant new developments in policy, measurement, modelling, mitigation and adaptation efforts associated with greenhouse gases from animal agriculture. The Conference takes place every 3 years, moving from continent to continent at each edition. The first conference, GGAA2003, was held in Japan with 200 delegates from 20 countries. Five subsequent GGAA conferences have been convened: GGAA2005, Switzerland; GGAA2007, New Zealand; GGAA2010, Canada, with the biggest in Ireland, GGAA2013, attracting 460 delegates from 41 countries. The GGAA2016 in Australia received more than 300 delegates from 36 countries.

## **GGAA2019**

The GGAA2019 focused on the theme “Science supporting Practices” and happened for the first time in Latin America. Following on from the six previous GGAA conferences, presented the latest research on the measurement, modelling, mitigation of greenhouse gases, while also seeking to discuss adaptation efforts and the impact of these advances for farmers, managers and policy makers. At the GGAA2019 conference, held in Iguassu Falls, Brazil, almost 200 delegates from 39 countries gathered to participate in a program featuring 11 invited keynote speakers, 47 offered presentations and 111 poster presentations. The conference was organised into four sessions covering: Technical advances: from genomics to precision agriculture, that addressed aspects related to Measuring and Modelling GHG; Farm level low carbon initiatives, which addressed Mitigation and Adaptation strategies to provide more Resilient production systems; Regional low carbon initiatives, which considered the Landscape, Regional management and National Commitments; International low carbon initiatives, that presented examples from IPCC, NDCs, FAO, World Bank. Delegates were offered the opportunity to publish their research in this peer-reviewed Special Edition of Animal, Cambridge University Press, with all abstracts published in conference proceedings. Volume One of the GGAA2019 Special Edition will include 20 peer-reviewed papers and will be available within the last quarter of 2019. The ‘virtual’ Volume Two will present the Proceedings... in a digital way, available in the web site. Taken together, these special editions provide the latest summary of the current state of knowledge on policy developments, measurement, modelling, mitigation and adaptation efforts associated with greenhouse gases from animal agriculture.

## **VENUE**

The city of Iguassu Falls was selected to host the event for the first time in Latin America thanks to its excellent geographical location within the triborder region of Brazil, Argentina and Paraguay. The city boasts an international airport that connects to the leading international airports in South America and can easily be reached by Latin American participants via bus or car. Besides the facilitated access, Iguassu Falls is further complemented by technical and scientific attractions part of the context of the conference, such as waterfalls, a national park, bioenergy and hydroelectric complex which were included as technical visits.

Alexandre Berndt, Chair

On behalf of the GGAA2019 Local Organising Committee and  
International Scientific Committee



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# **Session 1**

**Technical advances: from genomics to  
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## 1.1 Nitrous Oxide (Oral)

### Application of edge computing for near-continuous monitoring of NH<sub>3</sub> and N<sub>2</sub>O emissions

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A facility was developed to provide near-real-time aerially-integrated field-scale measurements of NH<sub>3</sub> and N<sub>2</sub>O emitted from multiple agricultural field treatments. A scanning long path Fourier Transform Infrared Spectrometer measuring NH<sub>3</sub> and N<sub>2</sub>O was interfaced to a cloud-edge computer (EC). Data logger-controlled sequentially sampled air around and within the fields. The N<sub>2</sub>O in the air was measured by a laser-based analyzer concentrations and air sampling conditions reported to the EC. Long optical path NH<sub>3</sub> lasers, sonic anemometers and other atmospheric sensors communicated wirelessly with the EC. A remotely-viewable xymon dashboard for instrument and trailer power status checks reduced time on-site for field staff. EC programs check data quality and merge the data streams for transmission by high-speed WiFi at midnight and input into multiple inverse dispersion models running on a 12 CPU node Beowulf cluster. Resulting system provided prior-day emissions by 6 AM the following day.

Terms for indexing: High-performance computing, Dashboards, Cloud Edge computing, RF communications, WiFi

## Deriving nitrous oxide emission factors for manures applied to agricultural land in the UK

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Annual nitrous oxide (N<sub>2</sub>O) emission factors (EFs) were calculated from measurements of emissions from manures applied to UK arable crops and grassland as part of a wider research programme to reduce uncertainty in the UK national agricultural N<sub>2</sub>O inventory. Field studies were undertaken between 2011 and 2013 at 3 arable and 3 grassland sites in the UK. Nitrous oxide emissions were measured for 365d from replicated (x3) plots using the static chamber technique (5 chambers/plot) following the autumn and spring application of different manures (cattle/ pig slurry, cattle/pig farmyard manure (FYM), poultry layer manure and poultry broiler litter) at typical rates, using representative manure application and soil incorporation methods. A nil nitrogen control treatment was also included. Direct N<sub>2</sub>O EFs ranged from -1.35 to 2.84% of total nitrogen applied, with the variability driven by a range of factors including differences in manure composition, application method, soil type and climatic conditions. When data from all the site/seasons were pooled, N<sub>2</sub>O EFs from poultry manures (0.87%) were found to be greater ( $p < 0.01$ ) than from FYM (0.36%) and slurry (0.59%). Emission factors from the autumn application timings (0.83%) were significantly greater ( $p < 0.05$ ) than from the spring application timings (0.38%). No difference was found in the EF for bandspread (trailing hose/trailing shoe) compared with surface broadcast slurry.

Data from this project have been used to establish robust Tier 2 country-specific EFs for inclusion in the UK national greenhouse gas inventory.

Terms for indexing and Index terms: arable, grassland, manure type, application timing, application method, N<sub>2</sub>O inventory



## Nitrous oxide emission factors following land application of manure and livestock grazing in developing countries

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Livestock manure (including excreta deposited during grazing) is a significant source of greenhouse gas emissions, contributing 30-50% of global nitrous oxide emissions from agriculture. Several within-country studies have focused on estimating nitrous oxide emission factors following the land application of livestock manure and direct deposition of excreta during livestock grazing. 'Dataman' is an international project that aims to help refine and understand processes influencing nitrous oxide emission factors from manure by collating manure management data from multiple countries. As part of the initial data analysis, this paper focuses on data from seven developing countries spread across South America, Asia and Africa. Data from published research, theses and conferences were identified by searching different publication platforms for a combination of three keyword categories (type of manure, animal and greenhouse gas). The criteria used for identifying suitable publications were: 1) only one type of manure included in the measurements, 2) trials included a non-amended control and 3) nitrous oxide measurements were made for at least 30 days. Thirty-three studies were included in the data analysis, providing 212 observations. Brazil represented 66% of the collated data, followed by Kenya, China, Zimbabwe, Colombia, Argentina and Nicaragua. Urine was the most common nitrogen source (40% of data) followed by dung, slurry, solid manure and broiler litter. Cattle (beef and dairy) were the most common animal type (68% of data) followed by swine, sheep and poultry. We will present preliminary results from our analysis of the nitrous oxide emission factors.

**Keywords:** dung, manure management, greenhouse gas, slurry, solid manure, urine.

## Quantification of sheep urine patch N<sub>2</sub>O emission factors from extensively grazed grasslands

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Extensively grazed grasslands are understudied in terms of their contribution to greenhouse gas (GHG) emissions from livestock. Such areas are often situated on mountainous terrain and differ widely from intensively managed grasslands (e.g. acidic soils, greater organic matter content, higher rainfall, contrasting vegetation types and lower stocking density). Current IPCC default and UK country specific N<sub>2</sub>O emission factors (EFs) for excreta deposited during grazing (EF3) are based on cattle excreta applied to lowland intensively managed areas. We hypothesised sheep urine EFs on extensive grasslands would differ widely from these current IPCC values. Sheep urine EFs were quantified using an automated GHG monitoring system from two upland sites: a semi-improved grassland on an Orthic Podzol soil and an unimproved acid moorland on a highly organic (Histosol) soil type. Sheep urine N<sub>2</sub>O-N EFs were negligible across the two sites (all < 0.08 % of the N applied), with evidence to show that low nitrification rates under acidic soil conditions were limiting N<sub>2</sub>O emissions at both sites. Our results support the suggestion of disaggregating EFs based on altitude gradients or degree of management intensity. Assuming our data are typical for extensively grazed systems, utilising hill-grazing specific urine patch N<sub>2</sub>O-N EFs would reduce the annual estimate of N<sub>2</sub>O derived from UK sheep excreta deposited during grazing by ca. 43%. The results also have implications for the C-footprint of upland-reared livestock products.

## What is the best proportion of Forage peanut and Marandu grass to reduce N<sub>2</sub>O emissions?

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Inclusion of legumes in grassland has been found to be environmentally and economically beneficial for ranchers by providing more sustained forage for cattle. However, data on the impacts of proportion of legumes inclusion on nitrous oxide (N<sub>2</sub>O) emissions are lacking. The goal of this study was to evaluate effects of legume (*Arachis pintoi* cv. *mandobi*) and grass (*Urochloa brizantha* cv. *marandu*) mixture on N<sub>2</sub>O emissions. Treatments were 5 proportion of legume-grass: 100:0, 75:25, 50:50, 25:75 and 0:100 in a completely randomized design with 5 repetitions. We quantified N<sub>2</sub>O emissions using static closed chamber methodology and gas chromatography. A strong effect of legume grass proportion was observed on N<sub>2</sub>O emissions. The greatest emissions occurred in the 100% of grass litter (62.4 mg N<sub>2</sub>O plot<sup>-1</sup>), while the emissions from 100% legumes were 23.3 mg N<sub>2</sub>O plot<sup>-1</sup>. The inclusion of legume litter decreased N<sub>2</sub>O emissions in all mixed treatment and the lowest N<sub>2</sub>O emissions were found in the treatment 50:50 legume:grass that were similar to 25:75. Probably the low C and N ratio is driving the N<sub>2</sub>O losses. Previous studies have been showed that the proportion of Forage peanut with Marandu grass for animal performance range from 30-50% of legumes. Our results showed that the most beneficial proportion of the legumes to mitigate N<sub>2</sub>O emissions are in line with that is most beneficial for forage and animal production. Further studies are required to assess leaching and ammonia losses and to do a complete assessment of sustainability in terms of environmental impact.

Keywords: consorciation, N pollution, N<sub>2</sub>O mitigation, forage management

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## 1.1 Nitrous Oxide (Poster)

### Ammonia emissions from permanent dairy pastures during different seasons in Costa Rica

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Agriculture is the largest source of ammonia (**NH<sub>3</sub>**) emissions. As NH<sub>3</sub> is an indirect greenhouse gas (**GHG**), NH<sub>3</sub> measurements are crucial to improve GHG emission predictions and inventories. Moreover, NH<sub>3</sub> emissions have also wider implications for environmental and human health. This study measured NH<sub>3</sub> emissions from permanent dairy pastures in Costa Rica. Emissions were measured using a micrometeorological integrated horizontal flux mass balance method during wet, transition, and dry season [January (11.3mm rain/d), February (3.0mm rain/d), and April 2018 (1.5mm rain/d), respectively] on a commercial dairy farm located in tropical rainforest climate at 638 m.a.s.l. During each season, emissions were measured from dung and urine (excretal) deposition by cattle and from subsequent ammonium nitrate (**AN**) fertilizer applications, as it is common practice to fertilize a pasture after each grazing rotation in Costa Rica. Cattle deposited about 41.5, 36.6, and 48.6 kg of excretal N/ha during each measurement period in January, February, and April, respectively, and 14.5 kg N/ha was applied on each occasion as AN fertilizer. Measured NH<sub>3</sub> nitrogen emissions were 0.18, 1.07, and 1.81 kg/ha in January, February, and April, respectively. As expected, emissions increased with decreasing precipitation. Measured emissions were lower than estimates using IPCC Tier 1 methodology, which gave emissions (uncertainty range) of 9.7 (2.0-19.7), 8.8 (1.8-18.2), and 11.2 (1.1-11.4) kg N/ha in January, February, and April, respectively. The methodology does not capture emissions when there is no wind, thus, measurements may include a small underestimation as wind frequency was low during all periods (mean±SD = 57.8±3.2%).

## Attribution of measured pasture N<sub>2</sub>O emissions to grazing animal excreta and fertiliser applications

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**Abstract**—Fertilised and grazed pastures are considerable sources of the greenhouse gas N<sub>2</sub>O. While fertiliser applications usually lead to short emission pulses, animal excreta lead to small- scaled emission hotspots resulting in a non-homogeneous source distribution. The strong spatial and temporal source variability represents an inherent problem for the quantification of gaseous emissions from pastures with chamber techniques. The eddy covariance method, integrating emissions over a larger footprint domain, seems better suited to quantify the total N<sub>2</sub>O emissions of grazed fields. We present results of a pasture experiment with dairy cows in Switzerland where N<sub>2</sub>O fluxes were measured continuously with the eddy covariance technique during five years. The pasture was managed in an intensive rotational grazing scheme with 2-3 fertiliser applications per year. A data-based partitioning method was used to attribute the observed field-scale emissions to the main source classes (urine/dung patches, synthetic and organic fertiliser applications, and background). The attribution to urine and dung patches and to background was validated during one season by simultaneous chamber measurements. The small-scale chamber fluxes were up-scaled to match the footprint area of the eddy covariance fluxes determined by a dispersion model. This allowed a direct comparison between the two measurement methods, which showed a good agreement. Urine and dung N excretion were estimated based on animal energy demand, feed and milk N content, and digestibility. Finally the specific emission factors for the different N inputs were determined. They were significantly lower for dung compared to urine patches and for organic compared to synthetic fertiliser.

**Index terms:** nitrous oxide, eddy covariance, emission factor.

## Development of a High Throughput Screen to Identify Potential New Nitrification Inhibitors for the Mitigation of Nitrogen Loss by the Ammonia-Oxidizing Bacterium *Nitrosomonas europaea*

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Under aerobic environmental conditions, ammonia (NH<sub>3</sub>) present in agricultural fertilisers and released from urea in bovine urine patches is oxidized predominantly by ammonia-oxidizing bacteria (AOB). Key AOB mediating nitrification are *Nitrosomonas* species. While nitrification is of critical importance in the conversion of urea or fertilizer to nitrate, this biological process also represents a major loss of nitrogen (N) from pasture soils. Gaseous loss occurs through the release of nitrous oxide (N<sub>2</sub>O), a potent greenhouse gas. To overcome the challenge of N loss, research efforts have focused on finding mitigation solutions for N<sub>2</sub>O emissions and nitrate (NO<sub>3</sub><sup>-</sup>) leaching in grazing livestock systems. One approach is the use of chemical nitrification inhibitors (NIs) to reduce the microbial metabolism involved in the conversion of ammonium to NO<sub>3</sub><sup>-</sup>. However, while effective, current chemical NIs have drawbacks. For example, nitrapyrin and 3, 4-dimethylpyrazole phosphate (DMPP) have not always been suitable for use in grazed pasture systems due to their physicochemical properties (e.g. volatility) or toxicity. To discover new chemical NIs against the key AOB *Nitrosomonas europaea* we developed a high throughput assay based on the nitrite (Griess assay) assay in 96-well microtiter plates. The method was validated using well-characterised inhibitors of ammonium oxidation, and additionally, we screened the commercial Screen-Well® Natural Product Library as a proof-of-principle for our assay. The developed method provides a foundation for screening commercial compound libraries to discover novel NIs for the mitigation of N loss from grazed pasture soils.

**Keywords:** Ammonia-oxidizing bacteria, Nitrous oxide (N<sub>2</sub>O), Nitrite, Ammonium, *Nitrosomonas europaea*, Nitrification Inhibitors, Mitigation, High-throughput Screen.

## Effect of excreta type, season and tropical pasture management on $\text{NH}_3$ volatilization: an indirect source of $\text{N}_2\text{O}$

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Ammonia ( $\text{NH}_3$ ) is an important indirect source of nitrous oxide ( $\text{N}_2\text{O}$ ). According intergovernmental pannel on climate change (IPCC) 1% of N lost as  $\text{NH}_3$  is emitted as  $\text{N}_2\text{O}$ . In this work we aimed to analyze the effect of type of excreta, pasture management and season on  $\text{NH}_3$  losses from bovine excreta in tropical pasture. We used data from seven trials that measured  $\text{NH}_3$  volatilization using the semi-open static chamber methodology from 2012 and 2018 in tropical pasture (*Urochloa brizantha* cv. marandu grass). The treatments were 2 type of excreta (urine and faeces), 2 season (rainy and dry) and 3 pastures management (non-fertilized, fertilized and consorciation legume-grass). Excreta type and season affect  $\text{NH}_3$  volatilization and this effect follow the same pattern within season and pasture management. Overall  $\text{NH}_3$  losses from urine averaged 11% and from faeces 4%, which probably occur due to the N form in each excreta. During the rainy season  $\text{NH}_3$  volatilization was 6% and during dry season was 9%, maybe because in rainy season more N is leached. The IPCC default  $\text{NH}_3$  emission factor is 20% that is higher than  $\text{NH}_3$  losses observed in the 7 sevens trials here investigated. Maybe indirect  $\text{N}_2\text{O}$  emissions are overestimated due the higher EF for  $\text{NH}_3$  losses from cattle excreta. Based in the 7 experiments conducted by us in tropical pasture, desegregating the EF for  $\text{NH}_3$  volatilization from cattle excreta is recommended.

Keywords: inventories, N pollution, indirect  $\text{N}_2\text{O}$ , cattle excreta

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## Effects of Nitrification Inhibitors on $\text{NH}_3$ and $\text{N}_2\text{O}$ Emissions from Beef Cattle Urine Patches on a Semiarid Native Pasture

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We assessed the effects of two nitrification inhibitors [nitrapyrin and dicyandiamide (DCD)] on  $\text{NH}_3$  and  $\text{N}_2\text{O}$  emissions, and their emission factors (EF), from beef cattle urine patches on a semiarid native pasture with a sandy soil texture in Southern Alberta, Canada. Gas fluxes were measured over 12 months in Year One (May 26, 2017 and June 1, 2018) and only during the grazing season in Year Two (May 30 to September 19, 2018). Urine, with and without nitrification inhibitors, and a deionized water control were applied twice each year (on Days 0 and 56 of each year). Most (> 95%)  $\text{NH}_3$  emissions occurred in the four months after the first urine application, equating to 3.2-4.3% of urine-N, and  $\text{NH}_3$  emission was not affected by nitrification inhibitors in either year. Large pulses of  $\text{N}_2\text{O}$  emissions occurred nine months after the last urine application. The  $\text{N}_2\text{O}$  EF is lower with nitrapyrin (EF=0.118%) than urine alone (EF=0.218%) or urine with DCD (EF=0.235%) in Year One. In Year Two, the  $\text{N}_2\text{O}$  EF values over the grazing season ranged from 0.042 to 0.064% and were not affected by either nitrification inhibitor. Urine  $\text{N}_2\text{O}$  EFs for this native pasture were lower than the 2% IPCC default value. Nitrapyrin may reduce the amount of N lost as  $\text{N}_2\text{O}$  from urine patches in semiarid native pastures. When compiling the national greenhouse gas inventory, the contribution of cattle urine on native pastures should take climate and site conditions into consideration.

**Keywords:** greenhouse gas, ammonium, dry mixedgrass prairies eco-region, native pasture, cattle grazing, emission factor.

## Evidence that condensed tannin may mitigate N<sub>2</sub>O emission from bovine urine

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Phytogenic additives can affect the population of microorganisms and consequently the emission of nitrous oxide (N<sub>2</sub>O). The objective of this study was to quantify the urine N<sub>2</sub>O emissions of Nelore bulls with increasing additions of condensed tannin. A completely randomized design incubation with three treatments and five replications was performed. The treatments were tannin doses in the proportion of 0, 0.5 and 1% added to the urine. The N<sub>2</sub>O production was quantified using the static chamber methodology and determination of the N<sub>2</sub>O concentration by gas chromatography. N<sub>2</sub>O emission differed between treatments and weeks. In the first week the averages were -0.41, 126.18 and 89.15 µg of N-N<sub>2</sub>O m<sup>-2</sup> h<sup>-1</sup> for 0, 0.5 and 1%, respectively. In the second week the averages were 1.18, 1168.13 and 772.32 µg of N-N<sub>2</sub>O m<sup>-2</sup> h<sup>-1</sup> for zero, moderate and high doses, respectively. In the third week the averages were 339, 559 and 520 µg of N-N<sub>2</sub>O m<sup>-2</sup> h<sup>-1</sup> for zero, moderate and high tannin dose, respectively. In the fourth week the averages were 138, 18 and 24 µg of N-N<sub>2</sub>O m<sup>-2</sup> h<sup>-1</sup> for zero, moderate and high, respectively. Initially the addition of condensed tannin stimulates N<sub>2</sub>O production, this effect ceased after 15 days of application, probably due to the action of the tannin on the N-organic, facilitating N<sub>2</sub>O production. However, when analyzing the data of the whole period of evaluation, N<sub>2</sub>O emissions decreased with high dose of tannin.

Keywords: Phytogenic Additive, N<sub>2</sub>O Mitigation, Climate Change, Animal Supplementation.

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## Greenhouse gas emissions from pen surfaces at a Texas High Plains beef cattle feedyard

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Nitrous oxide (N<sub>2</sub>O) and methane (CH<sub>4</sub>), are produced by manure management systems at beef cattle feedlots. In April and August 2018 trials, six automated Licor Long-Term chambers were set up on a grid within a recently vacated feedyard pen in semiarid West Texas (average annual rainfall 500 mm). Chambers closed hourly with headspace gas concentrations measured by Los Gatos Enhanced Performance analyzers. The pen surface condition was dry and dusty with friable material overlaying compacted manure of variable thickness during both trials. The temperature during the trials averaged 12.1°C and 23.4°C, respectively. Fluxes were monitored for 48 hours before 12.7 mm of simulated rainfall was added to each chamber base to mimic the most significant, flux perturbing factor. Monitoring continued for a further 48 hours in spring and 96 hours in summer. Rainfall increased N<sub>2</sub>O emissions but decreased CH<sub>4</sub> emissions. There was considerable temporal and spatial variation in fluxes. The average N<sub>2</sub>O-N flux before the simulated rainfall event during the trials was 0.198 and 2.68 mg m<sup>-2</sup> h<sup>-1</sup> respectively. In the 2 days post rainfall, the average N<sub>2</sub>O-N fluxes were 0.919 and 4.47 mg m<sup>-2</sup> h<sup>-1</sup>, respectively. The average CH<sub>4</sub> fluxes during the trials were 2.022 and 1.56 mg m<sup>-2</sup> h<sup>-1</sup>, respectively before the simulated rainfall event. In the post rainfall period, the average CH<sub>4</sub> fluxes were 0.771 and 0.629 mg m<sup>-2</sup> h<sup>-1</sup>, respectively. Data from this and other field and laboratory trials will be used to inform emissions inventories and to enhance the process-based understanding of these emission processes.

Index Terms: nitrous oxide, methane, manure.

## N<sub>2</sub>O emissions in tropical grassland was affect by N source but not by N rates

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Grasslands cover extensive areas in the tropical areas of the Earth. Nitrogen fertilization of grassland can lead to N<sub>2</sub>O emissions a potent greenhouse gas. We aimed to quantify N<sub>2</sub>O emissions from different sources of nitrogen fertilizer (urea, ammonium nitrate and ammonium sulphate applied at different rates (0, 90, 180 and 270 kg N ha<sup>-1</sup>). The experimental design was randomized block with four repetitions. The fertilizers were applied to marandu-grass (*Urochloa brizantha*) during the grass growing season split in 3 times. The static closed chambers methodology was used to quantify N<sub>2</sub>O production and gas chromatography to measure N<sub>2</sub>O concentrations (minimal detection limit  $\pm 0.03\mu\text{g}$ ). The evaluations occurred for 365 days. Only N source affect N<sub>2</sub>O emissions ( $P=0.007$ ), while N rate ( $P=0.63$ ) and interactions ( $P=0.23$ ) did not affected. The annual emissions averaged -11.31, 9.28 and 8.95  $\mu\text{g N-N}_2\text{O m}^{-2} \text{ h}^{-1}$  for urea, ammonium nitrate and ammonium sulphate, respectively. Over the year N<sub>2</sub>O consumption occurred in all treatments, mainly in the urea treatment. The calculated EFs, only for the growing season, were 0.01, 0.44 and 0.45% for urea, ammonium nitrate and ammonium sulphate, respectively. These emissions factors are lower than that of 1% used for national inventories of N<sub>2</sub>O. Urea appears to be an option to mitigate N<sub>2</sub>O emissions.

Keywords: Nitrogen fertilization, tropical grassland, N<sub>2</sub>O consumption, grassland management.

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## Potential N<sub>2</sub>O producer located in dairy manure compost surface

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Compost surface is the important source of the greenhouse gas nitrous oxide (N<sub>2</sub>O) in the dairy manure composting system. Here we observed N<sub>2</sub>O, nitric oxide (NO) and oxygen profile determined by microsensor measurement. Also, we performed the incubation experiments with enhanced (by NO<sub>2</sub> addition) and suppressed (with antibiotics treatment) N<sub>2</sub>O production to identify the bacterial group which is responsible for N<sub>2</sub>O production by tracking the change of the bacterial community with 16S rRNA gene amplicon sequencing. Isotopic analysis of N<sub>2</sub>O was also performed to understand its source. Significant N<sub>2</sub>O and NO concentrations (up to 645.3 ppm and 8.4 μM) were observed, which clearly shows that significant nitrification/denitrification is occurring *in situ*. Isotopic signature implies that these N<sub>2</sub>O coming from bacterial denitrification pathway. The incubation experiment revealed that the bacteria which belong to classes *Sphingobacteria* and *Flavobacteria* seems to be responsible for denitrification and significant N<sub>2</sub>O production which occurs just after the pile turning events.

Keywords: N<sub>2</sub>O, compost, denitrifying community, microsensor, isotopocule.

## Targeting farm scale features for nitrification inhibitor application: an effective N<sub>2</sub>O mitigation strategy?

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The movement and behaviour of grazing livestock can influence the fate of nitrogen at the farm scale. Features such as gateways, shaded areas, laneways and soil areas around drinking troughs are potential hotspots of emissions of the powerful greenhouse gas, nitrous oxide (N<sub>2</sub>O). Here, elevated excretal loads can supply substrates (N-rich urine and C-rich dung) which fuel N<sub>2</sub>O production processes, with elevated compaction also creating favourable conditions for denitrification. Nitrification inhibitors are a potential technology to reduce N losses from dairy pastures, but their effectiveness in reducing both N<sub>2</sub>O and N<sub>2</sub> emissions from features where livestock congregate have not been widely studied. Utilising an automated chamber system N<sub>2</sub>O emissions were monitored from a Vertosol soil at a dairy pasture in subtropical Australia, with the following treatments: i) control (0 kg N ha<sup>-1</sup>), ii) urine (344 kg N ha<sup>-1</sup>) applied to standard pasture, iii) urine applied near a gateway, and iv) urine + DMPP (1L ha<sup>-1</sup>) applied near a gateway. Overall N<sub>2</sub>O emissions were slightly higher from the gateway (391 ± 102 mg N<sub>2</sub>O-N m<sup>-2</sup>) than the standard pasture (324 ± 40 mg N<sub>2</sub>O-N m<sup>-2</sup>), however, DMPP did not significantly reduce cumulative N<sub>2</sub>O-N (413 ± 34 mg N<sub>2</sub>O-N m<sup>-2</sup>) or NO<sub>3</sub><sup>-</sup> accumulation from urine applied to soil around a gateway. In conclusion, under the conditions of this study, DMPP was not found to be an effective N<sub>2</sub>O mitigation strategy for soils highly frequented by livestock.

## 1.2 Methane (Oral)

### Breeding for lowered methane emissions in New Zealand Sheep\*

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New Zealand is heavily reliant on pastoral based agriculture and has a national sheep flock of ~18.5 million breeding ewes. Maternal sheep production is reliant on the ewe overwintering and successfully rearing at least one lamb. The sustainability and profitability of this system, however, is facing a new challenge as awareness grows of the magnitude and impact of ruminant methane emissions on the environment. Ruminant enteric methane emissions are responsible for a third of New Zealand's total greenhouse gas emissions. Strategies limiting methane from ruminants have been put forward to protect the environment and to maintain global food security. Independent breeding strategies exist for increased production and for reduced methane emissions but, to date there has been no data to show whether these breeding objectives might be synergistic, neutral or antagonistic. Ten years ago, a divergent flock of sheep was created to evaluate selection for methane and to monitor the effects of this selection. Initially, sheep were ranked for breeding using methane measures from respiration chambers. Subsequently, a number of proxies have been investigated and effects of selection on methane emissions, production traits, feed intake, carcass and milk quality have been evaluated. Here we describe the main results and describe the flock divergence over the ten-year period. Low methane animals have ~12% less methane than sheep from the high line, appear economically favourable, grow more wool, have different microbiomes and differ in fatty acid profiles in muscle.

Terms for Indexing: genomics, portable accumulation chambers, methane yield, microbiomes, ovine, ruminant emissions.

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## Development of a bovine rumen-specific amplicon sequencing standard

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In recent years, amplicon sequencing has been widely adopted to identify rumen microbes that generate approximately 40% of global agriculture's greenhouse gas emissions. This has enabled unprecedented insight into these highly complex and dynamic communities which are largely recalcitrant to in vitro culture. However, rumen NGS analyses are currently often conducted with no validation of the complex laboratory and computational methods that are employed. Surprisingly, many such studies are still published without the inclusion of internal positive controls. While commercial internal microbial mock communities (either cellular and DNA-based) are available for human bacterial and yeast pathogens, controls should be specific to the environment under investigation in order to evaluate biases in library preparation and whether the databases that are used in the analyses are accurate, appropriate and comprehensive. Through the international collaboration *RumenPredict*, a rumen-specific amplicon sequencing standard, comprised of 16S/18S/ITS synthetic sequences from common rumen microbes, has been developed. This standard was sequenced in 3 separate 16S rRNA amplicon Illumina MiSeq sequencing runs. Sequence analysis was conducted using DADA2 to generate amplicon sequence variants. Four 16S rRNA databases (RefSeq, SILVA, RDP and Genome Taxonomy Database) were assessed for the accurate taxonomic assignments. The greatest accuracy of assignments was achieved with the RefSeq database. This allowed correct identification of all 3 of the archaea sequences and 12 of the 13 bacteria to the correct genus level. Good concordance ( $r_s0.88-0.92$ ) was observed between the taxonomy profiles that were generated by the 3 separate sequencing runs.

Index terms: bos taurus, next generation sequencing, rumen microbiome.

## Enteric methane emission and lactational performance of dairy cows fed 3-nitrooxypropanol

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This study evaluated effects of 3-nitrooxypropanol (3-NOP), an investigational product, on enteric methane emission and lactational performance of Holstein cows. Forty multi- and primiparous cows (118 ± 28 days in milk) were blocked and fed a control diet or a diet containing 60 mg 3-NOP/kg feed dry matter for 15 consecutive weeks. Enteric gaseous emissions were measured using the GreenFeed system. Data were analyzed using PROC MIXED of SAS as repeated measures. Compared with control, 3-NOP decreased ( $P < 0.001$ ) daily methane emission (411 vs. 302 g/d; SEM = 6.16), yield (16.4 vs. 11.9 g/kg dry matter intake; SEM = 0.25), and intensity (11.5 vs. 8.2 g/kg energy corrected milk; SEM = 0.28). Treatment increased ( $P < 0.001$ ) hydrogen emission (0.44 vs. 2.55 g/cow/d; SEM = 0.119) and had no effect on carbon dioxide emission ( $P = 0.27$ ). Dry matter intake, milk yield, and cow body weight change were not affected ( $P \geq 0.45$ ) by 3-NOP (mean ± SEM: 25.6 ± 0.38 kg/d, 38.4 ± 0.63 kg/d, and 458 ± 50.9 g/d, respectively). Treatment increased ( $P = 0.01$ ) milk fat concentration (3.83 vs. 4.08%; SEM = 0.064), tended to increase ( $P = 0.07$ ) fat yield, increased ( $P = 0.04$ ) milk urea nitrogen (11.6 vs. 12.1 mg/dL; SEM = 0.175) and had no other effects on milk components. In this experiment, 3-NOP decreased daily enteric methane emission by 26% and methane yield (27%) and intensity (29%) without affecting feed intake and milk yield and increased milk fat in lactating dairy cows.

**Keywords:** dairy cattle, 3-nitrooxypropanol, enteric methane, milk fat.

## Heat production and methane emission measured using a face mask method in growing dairy heifers

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The aim of this study was to estimate heat production (HP) and methane emission (CH<sub>4</sub>) from growing dairy heifers that were fed different nutritional plans (NP). Thirty-six heifers (12 Holstein, 12 Gyr and 12 Holstein x Gyr crossbred) were distributed into 2 blocks in a 3x3 factorial arrangement (3 NP x 3 genotypes). Heifers were randomly distributed to one of three different NP: 1) 1.0x maintenance, 2) 1.5x maintenance and 3) 2.0x maintenance. Heifers were fed a diet consisting of 85.0% corn silage and 15.0% concentrate mixture on a dry matter basis. The HP and CH<sub>4</sub> were estimated using the face mask method. Dry matter intake and metabolizable energy intake were lower ( $p < 0.05$ ) for Gyr heifers (3.88 kg/d and 9.41 Mcal/d) compared to the others. Body weight (BW) was affected ( $p < 0.05$ ) by genotype, and Gyr heifers also had less BW (240.2 kg) compared to the Holstein-Gyr heifers. The HP, expressed in Mcal per metabolic body weight (Mcal/BW<sup>0.75</sup>), was affected ( $p < 0.05$ ) by genotype, and Gyr heifers presented reduced HP (163.2) compared to Holstein heifers (201.0). An interaction ( $p < 0.05$ ) between the genotype and the NP was found in CH<sub>4</sub> emission in grams per day and grams per kg of BW<sup>0.75</sup>. Our findings demonstrate that only in the 1.5x and 2.0x maintenance level Gyr heifers CH<sub>4</sub> emissions were lower compared to Holstein and Holstein x Gyr, respectively. No differences between genotypes and NP were found in the results of the CH<sub>4</sub> yield (CH<sub>4</sub> in g/kg DMI).

Index terms: cattle, genotype, sustainability

## Inhibition of enteric methanogenesis in cows induces changes in plasma metabolome highlighting potential emission markers<sup>1</sup>

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There is scarce information on whether inhibition of rumen methanogenesis induce metabolic changes on the host ruminant. Understanding these possible changes is important for the acceptance of methane-reducing practices by producers. In this study we explored the changes in plasma profiles associated with the reduction of methane emissions. Plasma samples were collected from lactating primiparous Holstein cows fed the same corn silage-based diet with (Treated, n=12) or without (Control, n=13) an anti-methanogenic feed additive for six weeks. We used a specific anti-methanogenic compound that inhibits the last step of methanogenesis and has no known effect on the host animal metabolism. Methane emissions (g CH<sub>4</sub>/d), measured in open-circuit respiration chambers in week five, were reduced by 23% in the Treated group with no changes in milk production, feed intake, body weight, and health status. Plasma metabolome analyses were performed using untargeted (nuclear magnetic resonance and liquid chromatography-mass spectrometry (LC-MS)) and targeted (LC-MS/MS) approaches. We identified 47 discriminant metabolites. Some metabolites, mainly of microbial origin, can be related to rumen methanogenesis and can potentially be used as markers. The other discriminant metabolites are produced by the host or have a mixed microbial-host origin. These metabolites, which increased in treated cows, belong to general pathways of amino acids and energy metabolism suggesting a systemic effect on the animal.

Index terms: *Bos taurus*, methane, metabolomics, NMR, LC-MS

## Machine learning ensemble algorithms in predictive analytics of dairy cattle methane emission using imputed *versus* non-imputed datasets

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The use of machine learning (ML) algorithms in predictive modelling of CH<sub>4</sub> emission from heterogeneous across-country datasets containing missing or imputed data-points is rarely reported. The objective was to use ML ensemble algorithms to predict CH<sub>4</sub> output and assess the effects of imputation on within and between-herd prediction accuracies. Data was from dairy cattle CH<sub>4</sub> production and related proxies from 16 herds across 10 European countries. Two datasets were used: the first had no dry matter intake (DMI) whilst in the second DMI imputed using non-parametric k-nearest neighbor approach was included. The final dataset contained 48,804 observations from 2,391 cows. Milk yield, fat and protein, days in milk (DIM), DMI, herd, country, CH<sub>4</sub> measurement method and breed were the proxies in the prediction model. For the predictive analysis, ML ensemble algorithm Random Forest (RF) was used. The predictive ability of RF models was estimated using three different metrics. Feature importance, in terms of percent increase in node purity showed that DMI, milk, DIM, fat, body weight, protein, herd, CH<sub>4</sub> measurement method and breed were important proxies. When DMI was imputed, normalized discounted cumulative gain increased by 0.02 – 0.48 points indicating improved prediction accuracies. Overall, RF model prediction accuracy was 0.82 and 0.81 for with and without imputed DMI, respectively. In predictive modelling involving heterogeneous across-country data ML algorithms proved robust. Traits based on such information could be used to monitor emissions in national, regional inventories as well as farm management tools assisting informed herd improvement decisions.

Terms for indexing: enteric methane, ruminants, random forest, prediction accuracy

## Measurement of the enteric emission factor of methane of dairy goats in Taiwan

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It is aimed to establish the country values of GHG emission from livestock sector as the mitigation basis. Four stages of Alpine female goats, nursing, growing, dry, and lactating, were measured sequentially. Their numbers, age and BW were 15 head, 3-mon-old and 15 kg; 8, 8 and 33; 5, 26 and 44; and 8, 38, 54 and 3.1 kg milk yield. Nursing goats were fed on milk replacer and grain mixture. Totally mixed ration for the other three groups included corn silage, alfalfa hay, wet brewer's grains, soybean hull pellet, and grain mixture. After adapted to diet, goats were moved into the ventilation-controlled stainless chamber, with 23.4 m<sup>3</sup> volume, for one to three days. Twelve pairs of gas samples taken from air inlet and exhaust tube in 24 hrs were analyzed for CH<sub>4</sub> and CO<sub>2</sub>. Difference of the gas content, air velocity, temperature, and gas recovery of the chamber were calculate for the daily CH<sub>4</sub> and CO<sub>2</sub> emission factors. They are 6.4, 18.4, 26.6, and 57.4 g per head for CH<sub>4</sub> for four groups, respectively. By times the percentage of each group in the herd, the weighted CH<sub>4</sub> emission factor for dairy goat in Taiwan is 12.03 kg a head a year. The CO<sub>2</sub> emission factor is 374.8 kg.

Keywords: Alpine dairy goats, carbon dioxide, chamber, methane

## Methane emissions and post-ruminal protein supply of tropical pasture *Brachiaria hybrid* cv. Cayman diets with inclusion of *Leucaena*

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Tropical forages with high content of tannins (e.g., *Leucaena* genus), have the capacity to modify rumen fermentation parameters. However, the concentration and activity of tannins varies between sources and interact with other characteristics of the substrate available for fermentation. This study evaluated the effects of tannins presented in *Leucaena leucocephala* (LI) and *Leucaena diversifolia* (Ld) in methane emissions and post-ruminal protein supply. For this, LI and Ld were incubated using the Hohenheim Gas Test, in two proportions (25% and 50%) with *Brachiaria* grass hybrid cv. Cayman with and without polyethylenglycol (PEG). Results showed that inclusion of PEG increased gas production in LI and Ld treatments confirming the activity of tannins. Greatest increment in gas production (GP) were observed for LI indicating a greater activity of tannins in this forage legume. Overall, LI showed higher GP than Ld and GP decreased with increasing levels of legume (50%). Methane production was lower for treatments including LI and Ld, with active tannins, than for the LI and Ld+PEG, highlighting the inhibitory effect of tannins on CH<sub>4</sub> production. Major CH<sub>4</sub> mitigation was observed for treatments with 25% of inclusion Ld compared with the ones with LI (15.8 vs 18.5 mg of CH<sub>4</sub>/g DM incubated). The total amount of nitrogen in undegraded feed+microbial mass after the *in vitro* incubation increases in treatments containing tannins from LI and Ld, indicating an increase in protein degradation. These results suggest that the activity of tannins can modify ruminal fermentation, improving the energy use efficiency for cattle.

Keywords: Condensed tannins, ruminal fermentation, tropical forages, greenhouse gases.



## Selective breeding as a mitigation tool for methane intensity of dairy cattle

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The global livestock sector, particularly ruminants, contribute substantially to the total anthropogenic greenhouse gases. Management and diet solutions to reduce enteric methane (CH<sub>4</sub>) emissions are extensively researched. Animal breeding that exploits natural variation in CH<sub>4</sub> emissions is an additional mitigation solution that is cost-effective, permanent, and cumulative. We quantified the effect of including CH<sub>4</sub> production in the Dutch breeding goal using selection index theory. The current Dutch national index contains 15 traits, related to milk yield, longevity, health, fertility, conformation and feed efficiency. From literature we obtained a heritability of 0.21 for enteric CH<sub>4</sub> production, and genetic correlations with milk lactose, protein, fat and dry-matter intake of 0.43, 0.37, 0.77 and 0.42, respectively. Correlations between enteric CH<sub>4</sub> production and other traits in the breeding goal were set to zero. When including CH<sub>4</sub> production in the current breeding goal with a zero economic value, CH<sub>4</sub> production increases each year with 1.5 g/d as a correlated response. When extrapolating this, the average daily CH<sub>4</sub> production of 392 g/d in 2018 will increase to 440 g/d in 2050 (+12%). However, in the same period the CH<sub>4</sub> intensity (g CH<sub>4</sub>/kg milk) will reduce by 13%. By putting an economic weight on CH<sub>4</sub> production in the breeding goal, selective breeding can reduce the CH<sub>4</sub> intensity even by 27% in 2050. This shows that breeding is a valuable contribution to the whole set of mitigation strategies that could be applied in order to achieve the mitigation goals for 2050 set by the EU.

Keywords: *Bos taurus*, genetics, genomics, selection index theory.

## The SF<sub>6</sub>-technique is a feasible method to estimate the methane emissions from sheep in Norway

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An annual sheep production system in Norway incorporates grazing natural pastures for five months. Yet no measurements of enteric methane emissions from grazing sheep have been performed under Norwegian conditions. A pilot study was conducted with 12 Norwegian White ewes to test the feasibility of using the sulphur hexafluoride (SF<sub>6</sub>) tracer technique under Norwegian conditions. The ewes were split onto one of two pastures, mixed grass (*Bromus inermis* dominated, *Poa pratensis*, *Festuca pratensis*) or white clover (*Trifolium repens*). After five days adaptation to pastures, breathe samples were collected into evacuated one L PVC canisters over three consecutive days where capillary tubes (0.38 to 0.42 mL/min initial flow) restricted flow. Gas samples were analysed by gas chromatography. Feed intakes were not recorded. Average live weights were 64 kg and 66 kg for ewes on grass and clover pastures, respectively. Methane emissions were 23.9 g/d from sheep on mixed grass and 28.2 g/d for sheep on white clover. Methane emissions were not correlated ( $p=0.33$ ) to ewe live weight. One explanation for the 15% difference in methane emission could be differences in feed intake. The few days of sample collection could also explain some of the observed difference since the technique is recommended to be used over five days to reduce errors in observations. This pilot study shows that using the SF<sub>6</sub>-technique is a feasible method for future research. More experiments measuring enteric methane emission over a longer period from grazing sheep and cattle under Norwegian conditions are planned for the future.

Keywords: Pasture, clover, mixed grass, grazing.

## Uncertainties in enteric methane inventories and measurement techniques<sup>1</sup>

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There are large uncertainties in national and global livestock enteric methane inventories, including animal numbers, feed dry matter intake (DMI) data, ingredient and chemical composition of animal diets, and methane emission factors. Globally, atmospheric mole fraction of methane has continuously increased since 2006 but there is no consensus about the major drivers for this increase and there is a considerable disagreement regarding the contribution of livestock to global methane emissions. Accurate prediction of methane emissions, for various animal categories and production systems, is important for both inventory and mitigation purposes. Complex prediction models have the disadvantage of requiring inputs that may not be uniformly available or may be inaccurate. Therefore, prediction models based on feed-related data, such as DMI, will likely have wider practical application. Meta-analyses of individual dairy and beef cattle data have shown that models based on DMI alone or DMI and limited feed- or animal-related inputs can

predict average methane emission with a similar accuracy as more complex empirical models. There is also uncertainty associated with enteric methane measurements. The most commonly used techniques for measuring enteric methane emissions are respiration chambers, the sulfur hexafluoride tracer technique, and the automated head chamber system (AHCS). Meta-analyses, however, have shown poor relationship between DMI and methane emission for the latter two techniques. In the case of AHCS, the relationship likely depends on the time of measurement relative to time of feeding and can be improved by matching diurnal DMI and methane emission patterns.

Keywords: enteric methane, uncertainty, dry matter intake, cattle.

<sup>1</sup>This study is part of the Joint Programming Initiative on Agriculture, Food Security and Climate Change (FACCE-JPI)'s GLOBAL NETWORK project (<https://globalresearchalliance.org/research/livestock/collaborative-activities/global-research-project>).

## 1.2 Methane (Poster)

### Cattle ranked in terms of methane intensity have reduced methane output and heavier carcass weight

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Selecting cattle based on methane intensity (MI), methane output corrected for animal product produced, provides an opportunity to sustainably produce animal protein, without negatively affecting performance. The objective of this study was to investigate differences in animal performance of beef cattle ranked in terms MI. Thirty LIMX steers consuming a TMR of 10kg concentrates and 3kg of hay, underwent measurements of feed intake, feed conversion ratio (FCR) and methane output. Methane output was estimated over a 3 week period, using the GreenFeed System. Cattle were ranked in terms of MI with the 10 highest (H; n=10) and lowest (L; n=10) cattle in terms of MI selected for comparison. Statistical analysis was carried out in SAS using the PROC TTEST function. MI (g CH<sub>4</sub>/ kg carcass weight) was significantly lower in the L compared to H group (0.43 vs. 0.66; P 0<0.001) as was daily methane production (165.0 vs. 228.3g/ day; P< 0.001). No significant differences in FCR, DMI, ADG or methane measurement weight were detected between the groups. However, cattle with a lower MI produced a significantly greater carcass weight (347.4 vs. 380.7 kg; P<0.02) and had a greater kill out (%) (54.47 vs. 57.22%; P0<.01). Selecting cattle for improved MI therefore has potential to decrease methane output while maintaining, and potentially increasing, meat yield.

Index terms: bos taurus, enteric fermentation, animal breeding, beef production.

## Commercial-scale evaluation of fed 3-NOP in reducing methane from cattle feedlots using micrometeorological methods

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It is highly desirable to use agricultural emission mitigation strategies on a whole-farm scale to ensure all aspects of management and production operations are included in the treatment response. Working at a commercial beef cattle feedlot in southern Alberta, Canada, we compared the cattle enteric methane emissions using the Concentration Ratio Method and an Inverse Dispersion Method for a control and treatment block of pens containing a total of 1500 cattle. There was clear reduction in methane emission reduction of 70% for cattle fed the compound 3-nitrooxypropanol (3-NOP) compared to cattle fed just the basal diet. Both micrometeorological methods measured an increase in the concentration ratio (a decrease in the emission rate) during study. There was also a diel fluctuation in methane emission that coincide with feeding in the early morning. The simplicity and sensitivity of the Concentration Ratio Method is expected to have applications for evaluating other mitigation strategies at large commercial scales, such as the application of manure additives to pen floors to reduce odours and ammonia emissions.

Keywords: emissions, concentration, ruminant, techniques, inhibitor

## Database construction to predict enteric methane emission from ruminants under different nutritional mitigation strategies

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The objective of this work was the construction of individual database to estimate enteric methane (CH<sub>4</sub>) emissions from ruminants considering the mitigating strategy used in the diet. Individual animal records of daily CH<sub>4</sub> emissions were collected (n=729) from 20 studies conducted at INRA, since 2003. The homogenization of data and analysis of outliers were performed and missing information of dietary chemical composition were completed using INRA feed tables (2017). The database contained CH<sub>4</sub> emissions data obtained with the SF<sub>6</sub> technique (80.5%) and respiration chambers (19.5%) on dairy cattle (n data; n studies= 373; 11), beef cattle (181; 4) and sheep (175; 5). According to the purpose of each study in the database, data were coded and classified into 5 CH<sub>4</sub> mitigating strategies: **(A)** starch supplementation, **(B)** enhancing forage quality, **(C)** lipid supplementation, **(D)** plant extract supplementation, and **(E)** other additives. The inclusion of lipid and starch in the diets ranged between 19.0 and 80.1 g ether extract/kg DM and between 25 and 472 g starch/kg DM, respectively. The CH<sub>4</sub> emissions averaged 305 and 203 g/d for dairy and beef cattle, respectively, and 23 g/d for sheep. The large ranges of dietary lipid and starch content showed in the meta-design will allow analyzing the variation of CH<sub>4</sub> emission under different levels of lipid and starch supplementations. However, few direct comparisons of the source of lipid or starch were made. Next step will consist in using this database to evaluate the performance of models on CH<sub>4</sub> emissions.

Index terms: Meta-design, prediction equation, methane, nutritional practices



## Effects of an essential oils blend on productivity and methane emissions in dairy cows: meta-analysis

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There is growing pressure to identify feed additives which increase productivity and decrease methane emissions without compromising animal health. Essential oils (EO) have shown positive effects on the ruminal fermentation *in vitro*, but there are few *in vivo* studies describing animal responses and results are often inconsistent. This study described the effects of supplementing a specific EO blend (Agolin<sup>®</sup> Ruminant) at 1g/d per cow in comparison to non-treated animals. A total of 20 *in vivo* studies and farm trials were identified and a meta-analysis was performed to determine the standardized effect size on milk yield, rumen fermentation and methane emissions. Results indicated that an adaptation period of at least 4 weeks of treatment is required. Whereas short-term studies showed minor and inconsistent effects of EO, long-term trials ( $\geq 4$  weeks) revealed that EO supplementation increases milk yield (+3.8%,  $P<0.001$ ), fat and protein corrected milk (+4.5%,  $P<0.001$ ) and feed efficiency (+3.3%,  $P<0.001$ ) without further changes in milk composition or feed intake. Long-term treatment also decreased methane production per day (-9.6%,  $P<0.001$ ), per DM intake (-13.6,  $P=0.001$ ) and per milk yield (-12.7%,  $P=0.042$ ) without having negative effects on the body condition score, fertility or health. In conclusion, although the mode of action is still unclear, this study suggested that Agolin supplementation may promote a subtle modulation of the rumen fermentation and protozoal levels which favoured the efficiency of the cow. Despite the small number of studies considered, these findings show that Agolin represents an encouraging alternative to improve productivity in dairy cattle.

Index terms: Bos taurus, essential oils, meta-analysis, methane, milk yield

## Enteric gas emissions and lactational performance of dairy cows fed 3-nitrooxypropanol: A meta-analysis

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A meta-analysis was performed to examine the effect of the investigational product 3-nitrooxypropanol (3-NOP) on enteric gaseous emissions (methane, carbon dioxide, and hydrogen) and lactational performance of dairy cows. Data from four randomized complete block design experiments (a total of 185 cows; duration of 31 to 105 days) conducted at The Pennsylvania State University were used in the analysis. Cows received a control diet or a diet containing 3-NOP at 40 to 200 mg/kg feed dry matter. Enteric gas emissions were measured using the GreenFeed system. Addition of 3-NOP decreased ( $P < 0.001$ ) daily methane emission [effect size (ES)  $\pm$  SE:  $-101 \pm 8.05$  g/d], emission yield ( $-1.95 \pm 0.198$  g/kg dry matter intake), and emission intensity ( $-1.30 \pm 0.179$  g/kg energy corrected milk). Treatment increased hydrogen emission ( $+2.23 \pm 0.211$  g/d;  $P < 0.001$ ) and had no effect on carbon dioxide emission ( $P = 0.34$ ). Dry matter intake, milk yield, and cow body weight were not affected ( $P \geq 0.72$ ) by 3-NOP (mean  $\pm$  SE:  $25.4 \pm 1.01$  kg/d,  $42.8 \pm 1.69$  kg/d, and  $631 \pm 16.3$  kg, respectively). Treatment increased milk fat and milk urea nitrogen concentrations (ES  $\pm$  SE:  $+0.33 \pm 0.167$  % and  $+0.38 \pm 0.166$  mg/dL, respectively;  $P \leq 0.05$ ) but had no other effects on milk components. In this meta-analysis, 3-NOP decreased daily enteric methane emission by 24% and both methane yield and intensity by 25% without affecting dry matter intake and milk yield and increased milk fat concentration in lactating dairy cows.

Keywords: dairy cattle, enteric methane, milk production, milk fat

## Enteric methane emission from sheep fed diets including red macroalgae

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Edible seaweed biomass is a valuable alternative feed ingredient for livestock. The composition of seaweeds is highly variable, with large differences in proteins, minerals, lipids and carbohydrates. Species, season, harvesting year, habitat, and prevailing proximate environmental conditions account for this variation. Using seaweeds as a protein source for production animals is of interest. Studies suggest that some seaweed species may have bioactive compounds with antimethanogenic properties. To investigate the effect of the red seaweed *Porphyra* *ssp* on enteric methane produced by sheep an *in vivo* study was carried out with 24 Norwegian White ewes. The ewes were allocated into four groups receiving a control diet or one of three supplemented diets. All groups were fed grass silage *ad libitum*, crushed oat and mineral pellets; the three supplemented diets included a protein source where dried and powdered *Porphyra* *ssp* was compared with white clover silage or pelleted soybean meal. The ewes were fed their respective diets for a two-week adaptation period and a 72-h experimental period during which methane was measured individually using open-circuit respiration chambers. Weight changes and methane production (L CH<sub>4</sub>/kg DM intake) were analysed using the GLM procedure with diet as fixed effect. No differences in weight changes and methane production between diets were found. Diet did not affect weight changes and methane production but DM intake was higher ( $P < 0.001$ ) for diets including soybean and macroalgae than white clover. Feeding red macroalgae showed no reduction in enteric methane production compared to the control diet.

Keywords: *porphyra* *ssp*, protein, feed intake, methane

## Evaluation of interval time and calculation method to quantify methane emissions measured by respiration chamber

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The present study was designed to evaluate the effects of interval time of measurement and calculation method on the quantification of enteric CH<sub>4</sub> emissions measured by respiration chamber. Methane concentrations were measured at every 0.5 min to obtain the 23-h kinetics of CH<sub>4</sub> emissions of ten growing Chinese Holstein dairy heifers. Two calculation methods include using mean value of measuring period (Mean method) and the nearest value of measurement just before chamber opening (Nearest method). The results showed that the increment of interval time decreased maximum rate of CH<sub>4</sub> emission and increased minimum rate of CH<sub>4</sub> emission. Interval time of measurement caused a less 5% of difference in measuring CH<sub>4</sub> emissions. Although mean method had greater estimated daily CH<sub>4</sub> emission than nearest method, the difference was within 3%. In conclusion, both interval time of measurement and calculation method causes a less 5% of difference in measuring enteric CH<sub>4</sub> emissions.

Keywords: Methane emission, Respiratory chamber, Interval time, Calculation method

## Evaluation of low-cost carbon dioxide and methane sensors to determine methane/carbon dioxide ratio in the breath of cows during milking

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For the purpose of genetic selection, a method to determine methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>) concentration in the breath of cows during automatic milking and to estimate enteric CH<sub>4</sub> emission using CH<sub>4</sub>/CO<sub>2</sub> ratio has been established. Of equipment comprised in gas collection apparatus, gas analyzer is the most expensive one. The objective is to evaluate low-cost gas sensors as an alternative to high-spec nondispersive infrared (NDIR) gas analyzer. Gas around feed bin in the automatic milking robot was collected with air-pump via air-filter and dryer. A part of collected gas was sent to test sensors of NDIR CO<sub>2</sub> sensor and semiconductor CH<sub>4</sub> sensor, and another part was sent to high-spec NDIR CO<sub>2</sub> and CH<sub>4</sub> analyzers as a reference. Gas collection was made with 91 visits of Holstein lactating cows during 6 days. Output voltage from CO<sub>2</sub> test sensor linearly correlated with reference CO<sub>2</sub> concentration, and was stable through a measurement period, while output from CH<sub>4</sub> test sensor curvilinearly related with reference CH<sub>4</sub> concentration. The calibration curve for CH<sub>4</sub> concentration was obtained every day from varying voltage outputs and reference CH<sub>4</sub> concentrations during one cattle visit. Average CO<sub>2</sub> and CH<sub>4</sub> concentration (Y) during one visit obtained from test sensor correlated with each reference gas (X);  $Y=0.89X+0.02$  ( $R^2=0.97$ ;  $n=91$ ) for CO<sub>2</sub>, and  $Y=1.02X$  ( $R^2=0.95$ ;  $n=91$ ) for CH<sub>4</sub>, respectively. Average CH<sub>4</sub>/CO<sub>2</sub> ratio during one visit obtained from test sensors correlated with that from reference gases (X);  $Y=1.01X$  ( $R^2=0.84$ ).

Key words: Automatic milking system, Holstein cows, NDIR sensor, semiconductor sensor

## Extreme concentrate level decrease enteric methane significantly, and more in Holstein than in Jersey cows.

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The aim of the study was to investigate the effect on methane emission when feeding three diets with increasing levels of concentrate to 12 Holstein and 12 Jersey lactating cows. Concentrate constituted 49, 70 or 91 % of dry matter intake (DMI), forage (grass-clover and maize silage 1:1, DM basis) constituted 50, 25 or 0 % of DMI, whereas the remaining 1, 5 or 9 % of DMI was barley straw. Concentrate consisted of sugar beet pulp, barley, rape seed meal, soy bean meal, dried distillers grain, NaOH treated wheat, molasses, minerals and vitamins. Diets were isoenergetic. Increasing level of concentrate gave a slight increase in crude protein and starch, and a slight decrease in NDF content in DM. Diets were thoroughly mixed after adding water to a DM content of 40 %. Methane emission was measured in respiration chambers during three days. The enteric methane emission was 565, 505 and 295 L/d for Holstein for the 49, 70 and 91 % concentrate diets, respectively. For Jersey the emission was 478, 450 and 422 L/d. Daily DMI was 22.5, 23.9 and 22.4 kg/d for Holstein, and 16.5, 18.9 and 19.0 kg/d for Jersey. Methane emission per kg DMI for the three diets was 25.3, 21.1 and 13.2 L/kg for Holstein and 29.0, 23.8 and 22.4 L/kg for Jersey. In conclusion, methane emission per kg DMI could be reduced by 48 % for Holstein when no forage and 91 % concentrate was fed, but only 23 % for Jersey.

**Keywords:** Cattle, greenhouse gasses, high concentrate diet, low forage diet

## Feed efficiency and enteric methane emission of Nellore cattle<sup>1</sup>

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The aim of the study was to calculate the residual feed intake (RFI), average daily weight gain (ADG), dry matter intake (DMI) and metabolic weight (MW) and to estimate the correlation with enteric methane emission. The influence of animal class RFI on the enteric methane emission was also verified. Seventy-two Nellore cattle with mean weight of  $273 \pm 43.8$  kg and age of  $350 \pm 26.2$  days were evaluated. The animals remained in collective pens equipped with automated troughs (GrowSafe) for recording individual daily intake. The feed efficiency test had a duration of 84 days. Animals were weighed every 21 days. Animals were classified as lower RFI with mean of  $-0.697 \pm 0.30$  kg/day ( $n = 24$ ), medium RFI with mean of  $-0.023 \pm 0.15$  kg/day ( $n = 25$ ) and high RFI with mean of  $0.752 \pm 0.31$  kg/day ( $n = 23$ ). Enteric methane was measured by tracer gas technique sulfur hexafluoride. The results were analyzed using the GLM procedure and Pearson correlation coefficients were determined between variables. Average enteric methane emission was  $199 \pm 35.9$  g/day, and showed low correlation with RFI ( $r = 0.069$ ), differently from those found with ADG (0.671), DMI (0.610) and MW (0.634). The RFI classes did not influence the enteric methane emission ( $P = 0.7035$ ). Enteric methane emission showed low correlation with RFI, and was not influenced by its classes. There isn't evidence that high-efficiency animals emit less enteric methane, even with lower dry matter intake and same performance than their inefficient counterpart.

Keywords: correlation, Nellore cattle, residual feed intake, SF<sub>6</sub> technique.

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## Feed intake level on enteric methane emission from growing dairy heifers

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It was aimed to determinate methane emission using SF<sub>6</sub> tracer technique from growing dairy heifers fed at three different nutritional plans (NP). Thirty-six heifers, 12 Holstein, 12 Gyr and 12 F1 Holstein-Gyr, 10 months old, initial body weight 174.8±42.6 kg, 123.5±44.4 kg and 201.5±44.6 kg to Holstein, Gyr and F1 Holstein-Gyr respectively, were distributed in multiples latin squares in a 3x3 factorial arrangement (3 nutritional plans x 3 genotypes). Within each latin square, heifers were randomly distributed in 3 different nutritional plans (NP) predicted by NRC (2001), which were (1) 1.0x maintenance; (2) 1.5x maintenance and (3) 2.0x maintenance. Heifers were fed a diet consisting of 85.0% corn silage and 15.0% concentrate on dry matter basis. Dry matter intake and nutrients showed interaction ( $p<0.05$ ) among genotype and nutritional plan. Gyr heifers present greatest ( $p<0.05$ ) crude protein digestibility than other heifers. Average daily gain (ADG) also had interaction between genotype and NP, which was lower to Gyr heifers. Methane emission in grams per day was influenced ( $p<0.05$ ) by intake level and genotype. When heifers increased their intakes, CH<sub>4</sub> emission expressed in animal basis (g/animal/d) increased, but when expressed by unit of daily gain (g/kg of ADG) declined, and this was associated with a dilution on CH<sub>4</sub> production by live weight gain. This suggests that an important benefit of high feed intake level is a reduction on emission intensity as CH<sub>4</sub>/product unit.

Index terms: genotype, milk, sustainability

## Freezing and thawing permeation tubes does not alter their subsequent rate of SF<sub>6</sub> release

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Permeation tubes are used in the measurement of enteric methane emissions, but they cannot be manufactured with a narrow range of sulphur hexafluoride (SF<sub>6</sub>) release rate, a key requirement of the SF<sub>6</sub> technique. One way to achieve a small range in release rate is to make a large batch of permeation tubes, order them by release rate, then select a subset for each experiment. The remainder are then stored at -80°C until required since there is no release of SF<sub>6</sub> at this temperature. The release rate of SF<sub>6</sub> from freshly made permeation tubes follow Michaelis-Menten kinetics, but it is not known if freezing then thawing permeation tubes has any effect on the subsequent pattern of SF<sub>6</sub> release rate. Twenty permeation tubes had their initial release rate measured over 3 weeks. Ten of the permeation tubes were stored at -80°C ('frozen'), with the remaining 10 tubes maintained at 39°C ('control') and their masses measured monthly for the next 3 years. After 365 days at -80°C, the frozen permeation tubes were returned to 39°C and their masses measured monthly for three years. The permeation tubes did not lose any mass while frozen. Excluding the period when permeation tubes were in the freezer ('frozen adjusted'), the release rate and patterns of SF<sub>6</sub> release were identical to those of control permeation tubes (Fig. 1). Individual permeation tubes can be stored at -80°C until required with no change in release rate or pattern of SF<sub>6</sub> release.

Terms for indexing / Index terms: sulphur hexafluoride, Michaelis-Menten kinetics

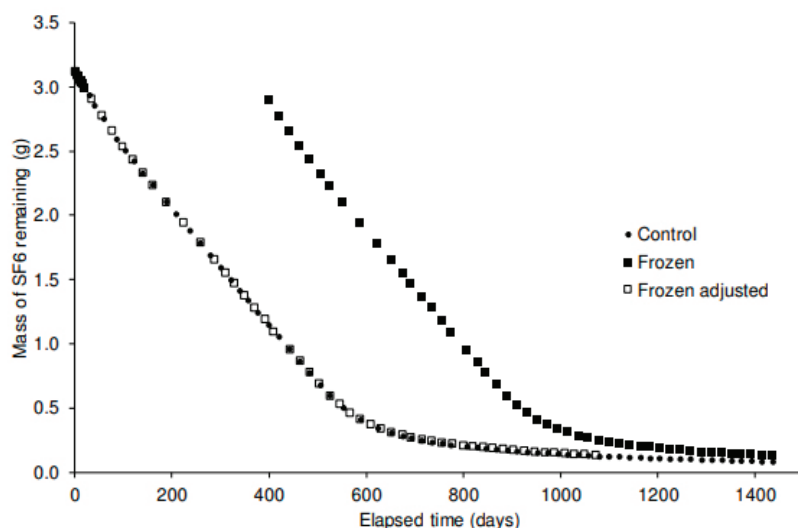


Fig. 1 Pattern of SF<sub>6</sub> release from control and frozen permeation tubes.

## Gaseous emissions in split sex fattening of heavy pigs

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Swine intensive rearing systems produce emissions of several air pollutants, such as greenhouse gases (GHG) and ammonia (NH<sub>3</sub>). This work aims to evaluate the effect of split sex fattening on carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), nitrous oxide (N<sub>2</sub>O) and NH<sub>3</sub> emissions in finishing pig farms from piggery and slurry storage. An experimental trial has been carried out in a commercial swine farm, by growing three groups of 500 pigs each, grouped according to gender: female (F), male castrated (M), and mixed (MF). The three groups were fed similar diet, fitted to different protein and energy requirement of each group. During the trial the emissions from housing facilities, as well as live and slaughtering animal performances were assessed. In addition, manure samples were collected during the rearing period to evaluate at a laboratory-scale NH<sub>3</sub> and GHG emission potential during the subsequent phase of slurry storage prior to land application. The results showed that split sex cannot be considered as a valid strategy for reducing gaseous emissions from pig houses, as MF showed always the lowest emission ( $P < 0.01$ ). Specifically, the average NH<sub>3</sub> and GHG emissions from MF resulted, respectively, 15.9% and 28.8% lower than M, and respectively 24.0% and 38.2% lower than F. Live performances and the overall productivity were not affected by split sex, whereas higher dressing percentage ( $P < 0.05$ ) and back fat depth ( $P < 0.01$ ) were found in MF. Laboratory-scale experiment revealed no significant ( $P > 0.05$ ) effect of split sex on NH<sub>3</sub> and GHG emis

## Grassland nitrogen fertilization did not affect enteric methane emission from young Nellore bull

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The synchronism between protein and energy in bovine diet can increase the efficiency of energy usage and may lead to a reduction in the enteric methane production that is a way to reduce  $H^+$  concentrations in the rumen. Tropical grasses are poor in protein and nitrogen fertilizer can increase forage N content. We aimed to quantify enteric methane production from young Nellore bulls during the rearing phase in grassland fertilized with different N rates. Experimental design was completely randomized with four treatments: 0, 90, 180 and 270 kg N ha<sup>-1</sup> and six replications. Enteric methane production was quantified using the technique of gas tracer (SF<sub>6</sub>) and gas determination by gas chromatography. Nitrogen application did not affect daily CH<sub>4</sub> emissions, CH<sub>4</sub> per kg of DM ingested, CH<sub>4</sub> per kg of gain and methane conversion rate (Y<sub>m</sub>). Methane production average 167 g CH<sub>4</sub> animal<sup>-1</sup> day<sup>-1</sup>, 28.6 g CH<sub>4</sub> kg<sup>-1</sup> DM and the Y<sub>m</sub> was 8.74%. Our data is similar to that default for beef cattle reported in the 2006 IPCC guidelines for national inventories. Increases in N application rate do not increase enteric CH<sub>4</sub> production from young Nellore bull during the rearing phase in tropical pastures.

**Keywords:** pasture management, beef cattle, N pollution.

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## Methane emissions in sheep infected with parasitic nematodes and supplemented with *Acacia mearnsii*

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Our objective was to evaluate the effects of *Acacia mearnsii* bark extract (PAB) on methane (CH<sub>4</sub>) production in sheep infected with *Trichostrongylus colubriformis* and *Haemonchus contortus*. Twenty 10 month-old Santa Inês lambs were used in a 50-day trial. Four treatment groups were formed: two control groups uninfected, one without PAB (C-) (n=4) and one with PAB (C+) (n=4); and two infected groups, one without PAB (I-) (n=6) and another receiving PAB (I+) (n= 6). Animals were kept in individual pens for 40 days, and received ad libitum chopped tifton 85 hay (*Cynodon* spp.) and 210 g/animal/day of concentrate (PAB supplementation: 15 g/animal/day – in concentrate). Animals were reared indoors to avoid helminthic infections (faecal egg count (FEC) was monthly controlled). At day 0, infected groups were artificially infected with L3 larvae of *T. colubriformis* (7500) and *H. contortus* (5000). After 28 days post-infection, establishment of infections was confirmed by FEC: I-: 1150 + 530.1 and I+: 567 + 544.6 eggs/gram of feces. After 40 days of trial (during which other parameters were evaluated - data not shown), CH<sub>4</sub> emissions in chambers were measured and variance analysis (proc GLM, SAS®) showed no PAB effects on methanogenesis (p > 0.05), however, both infected groups had higher CH<sub>4</sub> emissions than the control groups: C-: 16.7 + 10.91; C+: 23.7 + 12.88; I-: 43.1 + 6.37; I+: 40.6 + 6.02 (g CH<sub>4</sub>/kg dry matter intake) (p < 0.05). The methanogenic effect of parasitic infections should be explored in studies addressing sustainability of animal production.

Keywords: Black wattle, *Haemonchus contortus*, Small chamber, *Trichostrongylus colubriformis*, Tannins

## Methane mitigation trial from Vietnam local cattle production system

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Methane emission from cattle production is a critical problem for global warming issue. Mitigation option for CH<sub>4</sub> emission in south-east Asian countries is important since the demand of beef tend to increase in this region. We tested the effect of cashew nuts shell liquid (CNSL) feeding (8 g/100 kgBW) on CH<sub>4</sub> emission from four Vietnam local cattle (Lai sind). Head box chamber system was used for CH<sub>4</sub> measurement. Rumen fluid was sampled and microbial community shift with CNSL feeding was monitored by 16S rRNA gene amplicon sequencing. Results showed that a range of 20.2 to 23.4% of CH<sub>4</sub> emission was mitigated through CNSL feeding ( $P < 0.05$ ), while no adverse effect was observed on feed intake or feed digestibility ( $P > 0.1$ ). Also, rumen fluid analysis showed that the propionate proportion in total VFA significantly increased (6.9 to 9.5%,  $P < 0.05$ ), while no significant effect was observed on acetate proportion (76.3 to 73.6%,  $P > 0.1$ ). Relative abundance of Methanobacteriales significantly decreased (3.1% to 1.4%,  $P < 0.05$ ), indicating that direct inhibiting effect of CNSL on methanogens. Relative abundance of orders Clostridiales and species Prevotella significantly increased ( $P < 0.05$ ), indicates the increase of hydrogen production activity and propionate production pathway activity.  $\delta^{13}\text{C}$  of CH<sub>4</sub> ranged from -74.2 to -66.6‰. From the results above it was clearly proved that CNSL feeding can mitigate CH<sub>4</sub> emission from local cattle production system of Vietnam. Although further understanding of mechanism behind is needed, CNSL feeding would be a promising way to mitigate CH<sub>4</sub> emission from cattle production in SE Asia through expanding the local CNSL supply system.

Keywords: CH<sub>4</sub>, cattle rumen, cashew nuts shell liquid, 16S rRNA gene sequencing

## **Methane production from an extensive beef herd grazing natural veld over a complete production cycle.**

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The need for accurate estimates of enteric methane production in ruminants in different production and grazing systems has been emphasized in a number of reports. Techniques monitoring methane at individual animal level under extensive conditions is crucial to better monitor methane mitigation alternatives while improving production efficiency. This study was aimed to measure methane production with a Laser Methane Detector under extensive beef production conditions. Twenty-six Bonsmara heifers were allocated to the project after weaning at the Agricultural Research Council Roodeplaat campus in Gauteng South Africa, which is described as a Savannah region. Heifers were extensively managed and mated naturally at 24 months over a three month breeding season (January 2018 – March 2018) and calved between October 2018 and December 2018. All calves were weaned July 2019. Enteric methane production was measured every three months for 10 consecutive days at dawn, until these heifers weaned their first calves to include a full production cycle. Due to the Laser Methane Detector measuring in parts per million per meter, results were reworked by means of a deterministic model to methane produced in grams per day. It can be concluded that significantly less methane was produced during spring 2017 (76g/day) with average heifer weight of 283kg and summer 2018 (88g/day) with average heifer weight of 347kg due to higher grazing quality when rangeland was in a vegetative growth state. The highest methane production was obtained in autumn (123g/day) with average heifer weight of 397kg and winter (131g/day) with average heifer weight of 420kg.

**Keywords:** Enteric methane production, extensive beef cattle



## Milk fatty acids can predict enteric methane emissions from dairy cows fed various diets<sup>(1)</sup>

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Proxies are needed to develop genetic selection of low methane (CH<sub>4</sub>) emitting ruminants and to evaluate at large scale, CH<sub>4</sub> mitigating strategies on farm. Milk fatty acids (**MFA**) have been used as proxies to predict CH<sub>4</sub> emissions from dairy cows because of common rumen biochemical pathways between the two processes. The objectives of the study were (1) to construct a set of empirical models to predict CH<sub>4</sub> emissions using MFA records of individual lactating dairy cows (n =825) fed a wide range of diets, (2) to increase the representativeness of the models by including additional independent variables such as dietary chemical composition [organic matter (**OM**); neutral detergent fiber (**NDF**); crude protein (**CP**); starch; ether extract (**EE**)], milk yield and composition, and animal characteristics [days in milk (**DIM**) or body weight (**BW**)], and (3) to evaluate the performance of the developed models on two independent datasets (individual measurements and treatment means). Prediction equations based only on MFA [C10:0, *iso* C17:0 + *trans*-9 C16:1, *cis*-11 C18:1, and *trans*-11, *cis*-15 C18:2 for CH<sub>4</sub> production (g/d); *iso* C16:0, *cis*-11 C18:1, *trans*-10 C18:1, and *cis*-9, *cis*-12 C18:2 for CH<sub>4</sub> yield (g/kg of dry matter intake (**DMI**)); *iso* C16:0, *cis*-15 C18:1, and *trans*-10+*trans*-11 C18:1 for CH<sub>4</sub> intensity (g/kg of milk)] have root mean square error of 58.6 g/d, 2.8 g/kg DMI and 3.7 g/kg milk, respectively. The models including DMI, dietary nutrient contents (NDF, EE, starch), and BW had lower root mean square errors of 42.8 g/d, 2.5 g/kg DMI and 3.3 g/kg milk, respectively.

Index terms: cattle, methane equation, milk fatty acids

<sup>(1)</sup>This study is part of collaborative project led by INRA and funded by 11 institutes and private companies: Adisseo France SAS (Antony, France), Agrial (Caen, France), APIS-GENE (Paris, France), Deltavit (Janzé, France), DSM Nutritional Products AG (Kaiseraugst, Switzerland), Institut de l'Élevage (Paris, France), Lallemand (Blagnac, France), Moy Park Beef Orléans (Fleury-les-Aubrais, France), Neovia (Saint-Nolff, France), Techna France Nutrition (Couëron, France), Valorex (Comboursillé, France).

## Mitigation of enteric methane using chestnut bark in Llamas (*Lama glama*) pastored in Andean grasslands

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The objective of the present study was to measure the emission of enteric methane in Llamas and the use of tannins as a mitigation strategy. The study was carried out from August to September 2016 at the Experimental Center IVITA-Marangani-Cusco-Peru (4200 masl), 20 young female llamas were used, with an average live weight of 104.5 kg. They were divided into two treatments (Control and Tannin); Intake was calculated from excretion of organic matter and digestibility in feces, methane emissions were determined during seven continuous days, using the SF6 technique, while grazing in a native pasture with dominance of *Festuca rigida* and *Calamagrostis amoena*; Tannin group was provided chestnut tannin (*Castanea* sp.) with a dose of 11 g orally/day before grazing. The enteric methane emission for Control was  $27.35 \pm 10.47$  g/day and for Tannin it was  $19.32 \pm 6.84$  g/day ( $t < 0.001$ ); showing the same tendency when the emission of CH<sub>4</sub> was compared according to the live weight of the animal that for Control was  $0.24 \pm 0.10$  g / Kg PV / day and for Tannin it was  $0.17 \pm 0.07$  g / Kg PV / day ( $t < 0.001$ ); Intake for Control was 1494.03 g / day and for Tannin it was 1514.71 ( $t > 0.05$ ) and the weight gain was similar ( $t > 0.05$ ) for both groups (5.7 kg and 5.2 kg for Control and Tannin, respectively). It is concluded that the use of tannins in the present study reduced the emission of methane by approximately 30% without affecting intake.

Keywords: Tannins, dosification, intake, *Castanea* sp.

## Non-linear effect of N fertilizers doses on CH<sub>4</sub> oxidation in tropical grassland

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Recently was suggested that tropical grassland could be great sink of methane (CH<sub>4</sub>). Approximately 16% of greenhouse gases emissions came from CH<sub>4</sub> production, which requires mitigation strategies. Thus, grassland nitrogen fertilization can increase CH<sub>4</sub> oxidation. Our goal in this research was to quantify the effect of N fertilization level on CH<sub>4</sub> production and oxidation. The treatments were 4 doses of N (0, 90, 180 and 270 kg N ha<sup>-1</sup>) in completely randomized design with four replications. The nitrogen fertilization was split in 3 times during the growing season of marandu-grass managed at continuous stocking at 25 cm sward height and put-and-take methodology. Gaseous production was quantified using the static closed methodology and CH<sub>4</sub> quantification by gas chromatography. Nitrogen fertilization induced CH<sub>4</sub> oxidation. In the background treatment CH<sub>4</sub> emissions was, 79.7 µg CH<sub>4</sub> m<sup>-2</sup> h<sup>-1</sup>, while in the treatments the oxidation averaged: -50.5, -60.6 and -45.1 µg CH<sub>4</sub> m<sup>-2</sup> h<sup>-1</sup> for 90, 180 and 270 kg N ha<sup>-1</sup>, respectively. Methane oxidation was affected by N doses (P<0.001) and presented a non-linear response. A relative low N dose can be used as strategy to mitigate CH<sub>4</sub> emissions in tropical grassland. Our study confirms that tropical grassland can be a sink of CH<sub>4</sub> production.

Keywords: CH<sub>4</sub> mitigation, nitrogen fertilization, grassland management, CH<sub>4</sub> oxidation.

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## Nutritional evaluation and ruminal parameters in sheep fed the tropical legume *Stizolobium aterrimum*

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Based on the hypothesis that condensed tannins (CT) commonly found in legumes may affect ruminal fermentation, we aimed to evaluate the effects of the legume *Stizolobium aterrimum* (mucuna-preta - MUC) (5.6 g/kg dry matter) on total gas, methane (CH<sub>4</sub>) production and fermentation parameters in lambs, by using both, in vitro and in vivo assays. Initially, ground (1 mm) MUC hay samples (0.5 g) were incubated with or without polyethylene glycol (PEG) in an in vitro semi-automatic system. Increased total gas, ammonia nitrogen and lower pH were observed in samples incubated with PEG ( $p < 0.05$ ). After, during 24 days (d) of experimental period (18 d to adaptation and 6 d to collections), in vivo CH<sub>4</sub> emissions and fermentation parameters from Santa Inês lambs ( $n = 18$ ; 24 months-old;  $47 \pm 7.8$  kg) fed on this legume were measured by using chambers system. Diets were: chopped Panicum maximum cv. Aruana hay (CON), CON supplemented with MUC (MWO) or CON supplemented with MUC plus PEG (MWP). No effects on CH<sub>4</sub> emissions were observed ( $p > 0.05$ ) but reduced production of short-chain fatty acids (SCFA) and propionate were observed for CON and both groups fed MUC showed reduced acetate:propionate (A:P) ratio while animals fed MWP had reduced protozoa count ( $p < 0.05$ ). Based on the results, CT from MUC showed to be capable of reducing in vitro degradability of substrates, while in vivo, it did not affect CH<sub>4</sub> production, but quality of fermentation end-products (i.e. A:P ratio) was improved when feeding this legume.

Keywords: Forages, In vitro gas production, Mucuna-preta, Polyethylene glycol, Small chambers.

## Perspective on the Methane Production of Primary Beef Production Systems in South Africa

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Livestock is important to mankind since most of the world's vegetation biomass is rich in fibre. Only ruminants can convert this high fibre vegetation into high quality protein sources (e.g. meat and milk) for human consumption. Despite this important role, they are specifically targeted and singled out as producers of large quantities of GHG that contribute to climate change. A simulation study was conducted to estimate the methane footprint of primary beef production, using the national average weights for beef cattle, grazing extensively in South Africa. The principle of Large Stock Units (LSU) were used as a reference, with the guideline that a LSU produce 94kg of methane per year in South Africa. Thus, a LSU produce approximately 260g (0.26kg) of methane per day. Both a weaner calf production system and an 18 month ox production system were simulated. Furthermore, weaning percentages of 60% and 80% were simulated and a 20% replacement rate was assumed. Using the simulations described, it was estimated that the methane footprint of a weaner calf of 220kg was 248kg of methane at a weaning percentage of 60% and it decreases to 196kg at a weaning percentage of 80%. In an ox production system (marketing at 18 months of age), the methane footprint of a 350kg ox was 332kg and 280kg of methane for weaning percentage of 60% and 80% respectively. The effects of production system, weaning percentage and replacement rate all influence the methane production of beef.

**Keywords:** Greenhouse gas, Large Stock Units, Weaning percentage, replacement rate

## Prediction of enteric methane emissions from cattle raised in tropical conditions

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A dataset containing diet composition, intake, digestibility and CH<sub>4</sub> emissions (717 observations, 153 treatments means, 34 studies) obtained in Brazil was used to: *i*) evaluate 41 equations available in the literature; *ii*) develop equations to predict yield (%GEI) and production (Mj/d) of CH<sub>4</sub>. Methane measurements were obtained by open- circuit respirometric chambers (n=430), SF<sub>6</sub> technique (n=200), or GreenFeed<sup>®</sup> system (n=87). For general (n=717) and lactating cows (n=284) datasets, the best fit equation showed a significant mean bias (-0.59MJ/d and -0.62MJ/d, respectively). For growing cattle and non-lactating cows dataset (n=433) the equation that best fit did not present significant mean slope bias. The IPCC Tier 2 method resulted in an over prediction of 0.55, 0.62 and 0.50MJ/d of CH<sub>4</sub> for general, lactating cows, and growing cattle and non- lactating cows datasets, respectively. None of the equations from literature precisely predicted CH<sub>4</sub>. Multiple regression equations were developed using mixed models and the best-fit equations were: general dataset, CH<sub>4</sub>(Mj/d)=- 3.7(±1.56)+0.7(±0.08)×DMI+0.01(±0.002)×BW-0.03(±0.01)×EE+0.005(±0.002)×GE<sub>cd</sub> [RMSE%<sub>obs</sub>=12.62; R<sup>2</sup>=0.92] and CH<sub>4</sub>(%GEI)=28.9(±11.32)-1.8(±0.48)×DMI+0.07(±0.01)×BW-0.29(±0.12)×EE+0.04(±0.01)×GE<sub>cd</sub> [RMSE%<sub>obs</sub>=10.65; R<sup>2</sup>=0.77]; lactating cows, CH<sub>4</sub>(Mj/d)=3.1(±1.69) +0.05(±0.007)×GEI(Mcal) [RMSE%<sub>obs</sub>=12.58; R<sup>2</sup>=0.69] and CH<sub>4</sub>(%GEI)=38.03(±21.15)+5.02(±2.67)×DMI-0.23(±0.08)×DMI<sup>2</sup> [RMSE%<sub>obs</sub>=12.43; R<sup>2</sup>=0.26]; Growing cattle and non-lactating cows, CH<sub>4</sub>(Mj/d)=-3.6(±1.32)+2.0(±0.27)×DMI-0.05(±0.02)×DMI<sup>2</sup>-0.18(±0.04)×DMI×BW-0.04(±0.01)×EE+0.007(±0.002)×GE<sub>cd</sub> [RMSE%<sub>obs</sub>=9.11; R<sup>2</sup>=0.93] and CH<sub>4</sub>(%GEI)=43.2(±8.29)-1.6(±0.45)×DMI-0.3(±0.10)×EE+0.05(±0.01)×GE<sub>cd</sub> [RMSE%<sub>obs</sub>=10.32; R<sup>2</sup>=0.82]. The equations developed in the present study can be useful for proper estimation of the CH<sub>4</sub> emissions in Brazil, as well as to provide a better understanding of the dietary and animal characteristics that influence the yield and production of enteric CH<sub>4</sub> in tropical feeding systems.

Keywords: Beef, Dairy, Greenhouse gas, Livestock

Acknowledgment: Embrapa, Pecu/RumenGases, CNPq, CAPES, FAPEMIG and INCT/Ciência Animal

## Relationship between rumen archaea diversity and methane production in three breed composition and nutritional plans of dairy heifers<sup>1</sup>

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The aim of this study was to evaluate the effect of breed composition and nutritional plans on the archaeal community and the correlation of the archaeal population with CH<sub>4</sub> emissions, animal performance and fermentative variables. Data were derived from twenty-seven heifers of different breeds (9 Gyr, 9 Holstein x Gyr and 9 Holstein) that were randomly allocated to three nutritional plans: intake of 11, 14 and 19 g/kg body weight (90 days trial). Diets consisted of corn silage and concentrate (70.7% and 29.3% dry matter basis). The production and yield of enteric CH<sub>4</sub> were measured by open-circuit respiration chambers for two consecutive 24-h periods. The phylogenetic diversity of the ruminal archaeal community was assessed by sequencing the V6-V8 region of the 16S rRNA gene. Breeds composition influenced the relative abundances of the genera *Methanobrevibacter* and *Methanosphaera*, and the species *Methanobrevibacter acididurans*. *Methanosphaera* was more abundant in Holstein, while *Methanobrevibacter* and *M. acididurans* were more abundant in Gyr. The regularized canonical correlation analysis showed positive correlation between *M. acididurans* and acetate: propionate ratio, and a negative correlation was observed between *Methanosphaera* ISO3-F5 and acetate: propionate ratio. The results of this study support the hypothesis that there is a contribution of host genetics to the composition of the ruminal microbiome, and no correlation was confirmed between the diversity of the archaea community and CH<sub>4</sub> emissions, however, it was possible to observe a strong correlation between the diversity of the archaea community and the acetate: propionate ratio.

Keywords: rumen microbiome, dairy heifer, zebu cattle.

## Relative quantification of methanogenic bacteria from qPCR assays in bovine treated with natural additive.

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The aim of this work was quantify the population of the methanogenic bacteria by the quantitative PCR assay (qPCR), from the ruminal liquid sample collected from bovines treated with additive natural. This additive is compound by mix of aminoacids, probiotics, essential fatty acid (omega 3 and omega 6), organic minerals and surfactants. The experiment were realized using 4 animals donor of ruminal liquid under pasture during the transition from drought to rainy season, receiving daily 5 g of additive Fator P® and negative control. After of the 15 days of the adaptation of ruminal microbiota, the samples were collected in five times (0, 2, 8, 16 and 24 hours) after the treatment, and in the end of experiment, was prepared the pool of each time of each treatments. The DNA were extracted using the kit QIAamp® DNA (Qiagen 51504) followed by qPCR reaction in triplicate of each time of each treatments, previous standardized in four concentrations (100nM, 200nM, 300nM e 400nM), using primers sequence of methanogenic bacteria as described in the literature. The mean of results of the duplicate of the five times of each treatment, showed the reduce of 64,8% ( $p=0,0009$ ) in population of total methanogenic bacteria for the animals treated with the additive natural. The resulted showed the importance of presence of the additive for modulation of ruminal flora in improving the use of diet of animals, and in reduction of the enteric methane gas emissions from bovine breeding.

**Keywords:** Methanogenic, Ruminal Metabolism, Additives, Bovine Breeding, Molecular Biology.



## Repeatability of CH<sub>4</sub> measurements by the SF<sub>6</sub> tracer gas technique in Nellore steers in feedlot system<sup>1</sup>

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The aim of this study was to verify the repeatability and the mean values of 3, 4 and 5 days of measurement of enteric methane (CH<sub>4</sub>, g/day) in Nellore steers using the tracer gas sulfur hexafluoride (SF<sub>6</sub>). CH<sub>4</sub> emissions were evaluated using collection cylinders on the back of 70 male Nellore bulls in feedlot system. Permeation tubes were calibrated (average daily emission of  $3.132 \pm 0.25$  mg/day) and supplied to the animals. The initial vacuum of the cylinders was around -13.600 psi and the capillary tubes were calibrated to absorb  $50 \pm 3\%$  of the sample inside the cylinder in 24 hours. During five consecutive days, the cylinders were changed for another every day at the same time, totaling five samples per animal. The repeatability of dry matter intake (DMI) during the collection days was 0.49. DMI and CH<sub>4</sub> variance components were estimated using MIXED procedure and the CH<sub>4</sub> means were compared using Tukey test ( $P < 0.05$ ). Repeatability of CH<sub>4</sub> measurement were 0.78, 0.80 and 0.82 for 5, 4 and 3 days, respectively. The average of CH<sub>4</sub> emission obtained in 5, 4 and 3 days did not differ (199.5, 198.6 and 196.0 g/day). The results showed high repeatability for CH<sub>4</sub> measurement using this technique. More research using a larger number of animals should be performed to assure the reliability in measuring the emission of enteric methane in 4 or 3 days of collection through the SF<sub>6</sub> tracer gas technique.

Keywords: enteric methane, cattle, cylinder, sulfur hexafluoride.

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## Ruminal fluid metabolomics associated with diet and environmental impact of Nelore cattle: initial results

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The objective of this study was to apply metabolomics tool (untargeted metabolite analysis) to improve our understanding of how feeding aim to reduces greenhouse gas emissions of bovine. A population of 52 Nelore animals was submitted to different nutritional interventions (conventional n = 26 and by-product-based diet n = 26) for 105 days in collective pens equipped with automated feeding (GrowSafe®), where methane emissions were measured. After slaughter, ruminal fluid samples were collected and submitted to metabolomics analysis performed by nuclear magnetic resonance spectroscopy (<sup>1</sup>H NMR) in a 14 T Bruker Avance III spectrometer. Principal component analysis (PCA), Partial least squares discriminant analysis (PLS-DA) and Variable importance in the projection (VIP score) were conducted by MetaboAnalyst 4.0 software to identify differentially expressed metabolites (p<0.05) between the nutritional interventions. The by-product-based diet affected the rumen fermentation and decreased methane production compared to conventional nutrition. PLS-DA revealed the formation of two clusters of metabolic profiles for ruminal fluid samples and were correlated to the two different feeding systems. Comparative analysis based on VIP score values led to the identification of differentially expressed metabolites, of which the top 15 metabolites presented VIP score > 1.8 and the compounds belonging to the by-product-based diet showed higher intensity, when compared to conventional diet. Thus, the results of this study demonstrated that <sup>1</sup>H NMR metabolomics combined with multivariate analysis may be capable of distinguishing the rumen fluid derived from animals on different feeding systems and may help to understand the relation between feeding and methane production.

Index terms: *Bos indicus*, bovine metabolome, metabolites, nuclear magnetic resonance

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## Ruminal metabolism and methane emission in a dual-flow continuous culture system fed different protein levels.

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The objective of this study was to evaluate the effects of increased dietary CP levels on ruminal nutrient digestibility, ruminal fermentation, N metabolism, microbial efficiency and methane emission in dairy cattle diets using a dual-flow continuous culture system. Three fermenters ( $1,550 \pm 32$  mL) were used in a 3 x 3 Latin square design with periods lasting 10 d each (7 d for adaptation and 3 d for sampling). Experimental diets were composed, on dry matter basis, of 26.6% lucerne hay, 30.8% maize silage and 42.6% concentrate based on maize grain (MG) and soybean meal (SBM). Proportions of MG and SBM were such to result in diets with 13, 16 and 19% crude protein contents. Fermenters were fed 90 g/d and solid and liquid dilution rates were adjusted to 5 and 10%/h, respectively. Data were analyzed using the MIXED procedure in SAS with  $\alpha = 0.05$ . Apparent and true digestibility's, total volatile fatty acids (VFA) concentration and molar proportions of acetate, propionate and butyrate were not affected ( $P > 0.05$ ) by increasing dietary CP.  $\text{NH}_3\text{-N}$  concentration and total flows of N,  $\text{NH}_3\text{-N}$  and dietary undegraded protein increased linearly ( $P < 0.01$ ) in response to increasing dietary CP, while efficiency of bacterial N synthesis decreased linearly ( $P < 0.01$ ). Methane production and microbial population (total and methanogenic) were not affected by treatments. At the lower CP levels, a low level of  $\text{NH}_3\text{-N}$  in the rumen may have a slightly improved ruminal function. Therefore, diets with 13% and 16% of CP may be the best strategy for improving N utilization in the rumen, without affecting methane output.

**Keywords:** ruminants, nitrogen metabolism, methane emission, rumen microorganisms.

## Ruminal Methanogen Community Changes in Response to the Rumen Development and the Addition of Rhubarb

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This study was performed to investigate the initial colonization of metabolically active methanogens and subsequent changes in four fractions: the rumen solid-phase (RS), liquid-phase (RL), protozoa-associated (RP), and epithelium-associated (RE) from 1 to 60 d after birth, and manipulate methanogen community by early weaning on 40 d and supplementing rhubarb from 40 to 60 d in black goats. The RNA-based real-time quantitative PCR and 16S rRNA amplicon sequencing were employed to indicate the metabolically active methanogens. Results showed that active methanogens colonized in RL and RE on 1 d after birth. RP and RE contained the highest and lowest density of methanogens, respectively. *Methanobrevibacter*, *Candidatus Methanomethylophilus*, and *Methanosphaera* were the top three genera. The methanogen communities before weaning differed from those post weaning and the structure of the methanogen community in RE was distinct from those in the other three fractions. The discrepancies in the distribution of methanogens across four fractions, and various fluctuations in abundances among four fractions according to age were observed. The addition of rhubarb significantly ( $P < 0.05$ ) reduced the abundances of *Methanimicrococcus* spp. in four fractions on 50 d, but did not change the methanogen community composition on 60 d.

**Keywords:** Microbial programming, 16S rRNA amplicon sequencing, Rumen solid, Rumen liquid, Rumen protozoa, Rumen epithelium

## Ruminal microbiota change and methane emission in cattle receiving nitrate in diet using the SF<sub>6</sub> tracer technique.

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The aim of this study was to evaluate the impact of nitrate inclusion in the diet on methane emission and ruminal microbiota. Eighteen Holstein heifers and steer (214±13.5 kg live weight) were allocated to two dietary treatments (isonitrogenated and isoenergetic). Control diet (CD) was corn, urea and soybean mix (80%) and grass hay (20%); nitrate diet (ND) was the CD (98.7%) plus 1.3% calcium nitrate. The trial included a 30-d adaptation to the diet, followed by 7-d feed intake measurement, 5-d measurement of methane emission and a 3-d period of sampling of ruminal contents. The ND decreased the total methane emissions (178 vs 242 g/d, P=0.009) and per unit of dry matter intake (24 vs 31g/kg; P=0.050). Monitoring of ruminal microbiota (Log<sub>10</sub> copies/g) in the ND revealed that total bacteria, *Rumicoccus albus* and *Ruminococcus flavefaciens* were reduced (P<0.05) in pre-feeding (11.5 vs 11.9; 6.6 vs 7.8 and 7.4 vs 9.1, respectively), while for methanogens no differences were found (8.5 vs 8.5). Conversely, *F. succinogenes* was higher in the ND than CD (7.2 vs 6.4; P<0.05). At 4 and 8 h post-feeding the methanogens were reduced in ND (8.1 vs 8.4; 7.9 vs 8.3, respectively; P<0.05), while total bacteria, *albus* and *R. flavefaciens* were maintained in a lower concentration. However, *F. succinogenes* remained in a higher concentration in ND than CD. The inclusion of nitrate reduced absolute methane emissions by 27% without affecting feed intake, and it could be related to the reduction in methanogen populations.

Keywords: SF<sub>6</sub> tracer technique, Dairy cattle, methanogens.

## Selection for lower methane emission in dairy cattle

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Over the last decade concerted research thrust has been directed at methane mitigation strategies. Many animal science disciplines have been involved including: nutrition and physiology, microbiology, genetic management and engineering. There is a fundamental need for research within the area of methane mitigation strategies and indeed research across disciplines is needed. Different approaches to obtain data needed for genetic analysis have been performed and results all come to the same conclusion. Methane emission is under some genetic control ( $h^2=0.2$ ) but by far more controlled by other effects in the surrounding environment. Genetic selection for a trait has the potential to make changes because the effect is cumulative and lasting. The effect is persistent over days and the inherited effect is cumulative from generation to generation. Often discussions are related to how methane should be placed in a breeding goal. Selection never will be put on methane alone. It will always be part of the existing total merit index and given a proper weight to ensure balanced breeding. Therefore beginning to select for decreased methane emission will not lead to cows that will not digest roughage or cows that select concentrate in their diet, because this would have enormous consequences for many other traits in the existing total merit index and therefore bulls that produce such offspring will not be selected as superior bulls. No significant correlations has been estimated so selection for reduced methane emission will likely have minimal consequences on other traits.

## Strategies for enteric methane mitigation in ruminants fed low-quality tropical forages

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Methane is a greenhouse gas which is produced and eructated to the atmosphere in large volumes by ruminants grazing tropical forages. Tropical grasses have been claimed to give rise to higher emissions of methane than temperate grasses when fed to ruminants. Fourteen experiments were performed in open-circuit respiration chambers and head-boxes with crossbred cattle and hair sheep respectively, to assess the mitigation potential of foliages and pods of tropical and temperate plants, as well as nitrates and oils in practical rations. On the basis of 125 individual determinations of methane carried out in respiration chambers, it can be asserted that the average methane yield for cattle fed (>70% ration DM) low-quality tropical grasses is 17.1 g CH<sub>4</sub>/kg DMI. When foliages and ground pods of tropical trees and shrubs were incorporated into the ration of cattle and sheep, methane yield (g CH<sub>4</sub>/kg DMI) was decreased in the general range of 15 to 25%, depending on plant species and level of inclusion. Incorporation of nitrates and oils in the ration also decreased methane yield. It is concluded that condensed tannins and saponins, as well

as starch contained in foliages and pods of tropical and temperate trees and shrubs can be fed to cattle and sheep to mitigate enteric methane emissions under practical feeding conditions with smallholders. Strategies of enteric methane mitigation in ruminants fed low-quality tropical forages in Latin America and the Caribbean can effectively increase productivity while decreasing methane emissions per unit of product (meat/milk), thus reducing the contribution of livestock to greenhouse gas emissions and therefore to climate change.

Keywords: Tropical grasses, legumes, rumen fermentation, methane production, greenhouse gases



## The recovery rate of methane, carbon dioxide and hydrogen at different flow levels in respiration chambers.

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During recent years, there has been an increased focus on the quality of data generated from systems measuring the enteric methane emissions, as data e.g. are used for country specific methane emissions inventories. The aim of this study was to investigate the recoveries of methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>) and hydrogen (H<sub>2</sub>) at four different air flowrates for the respiration chambers at Aarhus University. The system has four identical chambers, with adjustable airflow. The air flowrates tested were 755±15, 1523±30, 1910±30 and 3074±27 liters per minute (45, 65, 75 and 100% of maximum flow). For each test CO<sub>2</sub> recovery was measured in all chambers, CH<sub>4</sub> recovery in two chambers and H<sub>2</sub> recovery in one chamber. At each air flowrate four repetitions were performed. The data was analysed with PROC GLM in SAS with airflow, gas, chamber and two-way interactions as fixed effects. The recovery was affected by gas, airflow and interaction between chamber and airflow. For the main effect gas recoveries were 98.7, 98.4 and 95.0 for CH<sub>4</sub>, CO<sub>2</sub> and H<sub>2</sub>. The recovery of H<sub>2</sub> was significantly lower than of CH<sub>4</sub> and CO<sub>2</sub>. For the main effect airflow the recoveries were 95.1, 98.3, 99.3 and 96.8% at 45, 65, 75 and 100% of max airflow. The recoveries were similar at 65 and 75% of max airflow. Within each air flowrate H<sub>2</sub> always had the lowest recovery, and CH<sub>4</sub> were highest except for air flowrate 75%. In conclusion, it is important to use gas and flowrate specific recoveries.

Index terms: chambers, test, quality

## Towards the application of 3-nitrooxypropanol in pastoral farming systems

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The investigational product 3-nitrooxypropanol (3-NOP) is an effective methane inhibitor that has been extensively evaluated mixed into rations in non-pastoral dairy and beef systems. The effects of 3-NOP when used in fresh-pasture rations were evaluated in three studies with cattle in respiration chambers (n=4 per treatment). In trial 1, compared to controls, there was a 52% reduction ( $P < 0.01$ ) in methane yield when 3-NOP (2.5 g/d per cow) was mixed into fresh cut pasture fed in two daily allocations. Methane yield was decreased by 39% ( $P = 0.03$ ) when 3-NOP was mixed in a supplement offered twice daily immediately before allowing access to fresh-cut pasture meals. In trial 2, no significant effect of 3-NOP was detected when 3-NOP was dosed at 2.5 g/d per cow in a supplement during milking but access to pasture was withheld for 1 h to simulate the delay in time from milking to grazing ( $P = 0.20$ ). Feed intake was not affected by 3-NOP. In both trials and 3-NOP had no effect on milk production in Trial 2. These results indicate that the time between 3-NOP administration and access to feed needs to be shortened or its longevity in the rumen extended. In trial 3, new slow release formulations of 3-NOP were tested and showed potential to extend the time that 3-NOP is active in the rumen, based on gas emission profiles from cows. Further studies are planned to refine promising formulations and to establish their methane reduction potential for pasture fed cattle.

Index Terms: methane mitigation, dairy cattle, temperate pastures, pastoral systems

## Variability in the relationship between enteric methane emission and dry matter intake in dairy cows

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We examined the relationship of enteric methane emission, measured using the GreenFeed system (GF), and dry matter intake (DMI) in a 15-week experiment with lactating dairy cows receiving a control diet or a diet containing the investigational product 3-nitrooxypropanol (3-NOP at 60 mg/kg feed DM). Daily methane emission and DMI were clustered into 12 time-slots of 2 h each. Methane emission and DMI were the lowest 2-h before feeding and highest within 6 h after feeding. The overall (24 h) relationship between methane emission and DMI was poor ( $R^2 = 0.01$ ). The relationship for the control (but not 3-NOP) cows was improved ( $R^2 = 0.31$ ;  $P < 0.001$ ) when DMI was allocated to time-slots and was strongest ( $R^2 = 0.53$ ;  $P < 0.001$ ) 8-10 h after feeding. Analysis of the 3-NOP emission data showed marked differences in the mitigation effect of this proven methane inhibitor with time (overall 27%). There was a lack of effect in the 2-h time-slot before feeding, the mitigation effect was highest (45%) immediately after feeding, persisted at around 32-39% within 10 h after feeding, and decreased to 13%, 4-h before feeding. These trends were clearly related to DMI (i.e., 3-NOP intake). This analysis showed that the relationship of enteric methane emission, measured using GF, and DMI in dairy cows depends on the time of measurement relative to time of feeding and can be as high as determined in respiration chambers. The methane mitigation effect of 3-NOP is highest immediately after feeding and lowest before feeding.

Keywords: cattle, measurement technique, 3-nitrooxypropanol

## LivestockPlus: Supporting low emissions development in the Latin American cattle sector.

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After three years of successful operation, the LivestockPlus project enabled the development and the first steps for implementation of Nationally Appropriate Mitigation Actions (NAMAs) for the cattle sector in Costa Rica and Colombia. It provided technical support and generated critical information and guidelines allowing the identification of options by quantifying changes in fluxes of greenhouse gases, soil carbon stocks, and the productivity of cattle-based systems. This initiative helped to determine technical options for low emissions pasture development in Latin America, and how these can be scaled up using NAMAs and other environmental policies in Colombia and Costa Rica. Efforts are now expanded beyond these two countries across the region as applicable. The project facilitated synergies that enabled overcoming barriers to the adoption of improved management practices and, consequently, the achievement of low emission development by: (i) Fostering partnerships among relevant stakeholders; (ii) Identifying and evaluating best-fit mitigation options; (iii) Evaluating measurement reporting and verification (MRV) systems for the cattle sector in the target countries. Concrete examples will be shown on testing different forage-based diets *in vitro* and *in vivo* for their potential to reduce enteric methane emissions, potential of improved grasses to reduce soil nitrification and nitrous oxide emissions, the influence of improved forages in carbon stock accumulation in soil, Life Cycle Analysis of livestock farms with different production systems, definition of MRV guidelines for silvopastoral systems, as well as improvement of GHG national inventories by the validation of methane predicting models.

Index terms: Nationally Appropriate Mitigation Actions, Nationally Determined Contributions, Colombia, Costa Rica, Mitigation of climate change.

## 1.3 Modelling (Oral)

### A disaggregated analysis of global N<sub>2</sub>O emissions from livestock supply chains

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The disruption of the nitrogen (N) cycle pose a threat to the environment and human life. Hence, analyzing N flows and losses from livestock is essential to understand the sector's contribution to the transgression of the planetary boundaries, in particular, climate change. Our study assessed the contribution of global livestock supply chains to anthropogenic N losses and flows on different spatial scales, using an updated version of the Global Livestock Environmental Assessment Model (GLEAM), which analyzed N inputs, N outputs, N loss by compounds and N recycled along the chain. The results show that global livestock N<sub>2</sub>O emissions are about 815 Tg CO<sub>2</sub> eq. y<sup>-1</sup>. In terms of N losses, N<sub>2</sub>O emissions are equivalent to 1.7 Tg N y<sup>-1</sup> and account for 3% of the total N losses from the livestock sector. Feed production is the largest source of N<sub>2</sub>O emissions, accounting for a 75%, while the remaining 25% is from manure management. Whereas N<sub>2</sub>O is an important greenhouse gas, climate change discussions should also consider the impacts of other forms of N. The current study provides a detailed disaggregated analysis of the magnitude, sources and pathways of N and insights on how to manage N<sub>2</sub>O emissions in the context of a wider N cycle that can support evidence-based decisions to shape the future of livestock sector.

Keywords: livestock, animal, nitrous oxide, nitrogen, climate change, GLEAM

## Evaluation of mathematical models to predict methane emissions from ruminants under different dietary mitigation strategies <sup>(1)</sup>

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This study evaluated the ability of published models to predict enteric methane (CH<sub>4</sub>) emissions from dairy cattle, beef cattle, sheep and dairy goat, using a large database (3183 individual animal data). Models for each animal subcategory and CH<sub>4</sub> dietary mitigating strategies of lipid or starch supplementation and of diet quality (described by organic matter digestibility and neutral-detergent fiber digestibility) were assessed. Models were ranked according to root mean square prediction error (RMSPE; % of observed mean) to standard deviation of observed values ratio (RSR) and RMSPE, using all data within each animal subcategory. For dairy cattle, CH<sub>4</sub> emissions (g/day) were predicted with the smallest RSR using the model based on feeding level [dry matter intake (DMI)/body weight (BW)], digestibility of feed gross energy (dGE) and dietary ether extract (EE) content (RSR=0.66, RMSPE=15.6%). For beef cattle, the smallest RSR was obtained using GE intake, BW, forage and EE content (RSR=0.83, RMSPE=27.2%). For sheep and goat, there were limited published models; the smallest RSR was observed for a sheep model based on digestible energy intake (RSR = 0.61, RMSPE = 19.2%). IPCC Tier 2 models (1997; 2006) had low predictive ability for variation in dietary

EE content, neutral detergent fiber content and organic matter digestibility (RMSPE 14.3-30.5% and 23.0-40.5% for dairy and beef cattle, respectively). No model predicted CH<sub>4</sub> emissions accurately under all dietary mitigation strategies. Some models gave satisfactory predictions and for improved prediction, models should include feed intake, digestibility and information on dietary chemical composition.

Index terms: Prediction equation, enteric methane, livestock, nutritional practices.

<sup>(1)</sup>This study is part of the Joint Programming Initiative on Agriculture, Food Security and Climate Change (FACCE-JPI)'s "GLOBAL NETWORK" project and the "Feeding and Nutrition Network" (<http://animalscience.psu.edu/fnn>) of the Livestock Research Group within the Global Research Alliance for Agricultural Greenhouse Gases ([www.globalresearchalliance.org](http://www.globalresearchalliance.org)).

## Prediction of enteric methane can be improved by using the NorFor feed evaluation system for dairy cows.

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NorFor is a semi-mechanistic, static and science-based feed evaluation system that predicts nutrients supply and requirements for cattle. The aim of the study was to investigate whether variables in the NorFor feed evaluation system could be used to predict the enteric methane emissions. A dataset was compiled from 11 studies, comprising 43 diets and 243 observations. Chemical composition of feedstuffs, live weight and dry matter intake (DMI) of individual cows were used in the NorFor model to estimate 134 variables describing nutrient intake, flows and digestion. The highest correlation was found between methane (L/d) and intake of fill units (0.77). Fill units describes the fill of feedstuffs and forms the basis for the feed intake system in NorFor. The fill value is fixed for concentrate, but for roughage it depends on organic matter (OM) digestibility and neutral detergent fibre (NDF) concentration. The correlations between methane and recorded DMI and NorFor estimated rumen digested OM and total digested OM were 0.74, 0.73 and 0.70, respectively. A prediction model for methane (L/d) was formulated using an automatic selection tool (PROC GLMselect in SAS). The following variables were selected; intake of fill units (no unit), intake of indigestible NDF from concentrate (g/day), rumen passage rate of roughage (%/h) and NDF in concentrate (%/h), sum of rumen degraded starch and sugar (g/day), and digestibility of crude fat (%). Methane emissions predicted were improved using a model based on NorFor estimates with a correlation of 0.89 between predicted and measured.

Index terms: dry matter intake, fill units, rumen passage



## Variability in greenhouse gas emissions intensity of farm-scale suckler cow beef production systems

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The variability in greenhouse gas emission intensities were estimated from 27 beef farms in Norway with Angus, Hereford, and Charolais cattle, using the whole-farm model HolosNorBeef. HolosNorBeef is an empirical model based on the HolosNor model and the methodology of the Intergovernmental Panel on Climate Change with modifications to Norwegian conditions. HolosNorBeef considers direct emissions of methane (CH<sub>4</sub>), nitrous oxide (N<sub>2</sub>O) and carbon dioxide (CO<sub>2</sub>) from on-farm livestock production and indirect N<sub>2</sub>O and CO<sub>2</sub> emissions associated with inputs used on the farm. The corresponding soil carbon (C) emissions are estimated using the implemented Introductory Carbon Balance Model (ICBM). The farms were distributed across Norway with varying climate, natural resource base, feeding and management practices. Estimated emission intensities ranged from 20.9 to 44.2 CO<sub>2</sub> equivalents (eq) (kg carcass)<sup>-1</sup> when C-balance was included. Enteric CH<sub>4</sub> was the largest contributing source of GHG accounting for 50% of the total emissions on average dependent on animal requirement and diet composition. Variation in the estimated soil C emissions contributed the most to variation (average 6 %) of total emissions between individual farms. Differences in GHG intensity among farms were reduced when soil C balance was excluded, giving emission intensities from 20.5 to 30.3 CO<sub>2</sub> eq (kg carcass)<sup>-1</sup>. High C losses from soils with high initial soil C content suggest that modelling of C balance in soils with high initial C contents might be encumbered with methodical deficiencies.

Keywords: whole-farm, modelling, methane, soil carbon, resource base

## 1.3 Modelling (Poster)

### Environmental footprinting of upland lamb production

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The environmental impacts of agricultural systems, particularly those associated with livestock production, are receiving increasing attention worldwide. This study will explore the emissions (nitrous oxide (N<sub>2</sub>O) from urine/dung and enteric methane (CH<sub>4</sub>)) and other environmental impacts associated with lamb production in UK upland areas. Measured and modelled data will be used to determine improved emission factors for upland systems, allowing development of a subsequent environmental footprint using Life Cycle Assessment (LCA) approaches. The study will also explore upland management scenarios including mitigation options and environmental trade-offs between land uses. Methodologies include measuring ruminant methane emissions (using a GreenFeed system), initially in cut and carry experiments for grass harvested from pastures at different altitudes (commencing summer 2019), followed by field grazing trials. Mobile soil gas measurement systems will be used to measure N<sub>2</sub>O emissions from sheep urine/dung. LCA techniques will be applied to determine the environmental footprint of lamb production and draw comparisons between upland and lowland systems. We expect differences between the systems due to environmental context and management differences. Lower urine N<sub>2</sub>O emissions factors are expected for upland vs. lowland pastures, however ruminant CH<sub>4</sub> emission may be higher due to poorer pasture quality. Overall, the environmental impacts of upland systems may compare favourably against lowland systems, particularly when wider ecosystem services delivered by extensive livestock management in upland areas are considered. This work is intended to support policy decision making for the future of these nationally significant areas and aid in improving the environmental sustainability of the sheep industry.

Index Terms: ruminants, agriculture, life cycle assessment, climate change, upland management

## Life Cycle Assessment of small, medium and large dairy cattle farms in Colombia<sup>(1)</sup>

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In Colombia, cattle production is responsible for 31% of greenhouse gas (GHG) emissions from the agricultural sector. Dairy farms account for 15% of the Colombian cattle herd. Life Cycle Assessment (LCA) of GHG emissions from these farms are not common, and when performed, are based on a small number of farms. LCA is important because the identification of appropriate GHG mitigation actions requires larger data sets. This study quantified the carbon footprints (CF) on 192 dairy farms in Colombia by using LCA methodology. Farms were classified according to their herd size: small (0-30 animals; n=94), medium (31-50; n=32), large (>50; n=66). Emissions were estimated using the GHG emission factors reported in the 2006 IPCC Guidelines for National Greenhouse Gas Inventories, and locally estimated emission factors. A “cradle to farm gate” system boundary was established, and a physical allocation method proposed by the International Dairy Federation, was used to distribute GHG emissions between co-products. The functional units were 1 kg fat and protein corrected milk (FPCM) and 1 kg live weight gain (LWG). The CF (kg CO<sub>2</sub>-eq kg<sup>-1</sup>FPCM/LWG) for medium (1.6 FPCM and 13.8 LWG) and large farmers (1.6 FPCM and 13.1 LWG) were the lowest among the categories. The medium and large farm categories were characterized by implementing better pasture and herd management practices, by the highest milk productivity (3240.8 and 3645.6 kgFPCM Cow<sup>-1</sup>Year<sup>-1</sup>) and stocking rate (4.5 AU ha<sup>-1</sup>; 4.4 AU ha<sup>-1</sup>). It is possible to improve the environmental performance of farms by increasing productivity and adopting better agricultural practices.

**Keywords:** carbon footprint (CF), global warming potential (GWP), greenhouse gases (GHG), livestock production systems.

<sup>(1)</sup>Carried out with support from CGIAR Fund Donors and through bilateral funding agreements and COLCIENCIAS Doctoral Study Program in Colombia (call 727 of 2015).

## Predicted performance of Afrikaner, Bonsmara and Nguni dams and top-crosses sired by Angus and Simmental

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Crossbreeding systems allow for use of favourable effects of heterosis and the opportunity to use breeds whose additive effects offset each other's weaknesses. This study predicts levels of cow-calf production that's achieved through top-cross, two-breed rotation, and terminal sire crossbreeding systems. The data originated from cows that were from the Vaalharts Research Station. Calves, sired by Afrikaner (AF), Bonsmara (BN), Nguni (NG), Angus and Simmental bulls and out of AF, BN, and NG dams, were weaned from 2015 to 2017. Predicted performance of the various crossbreeding systems followed standard regression theory. Overall, the straightbred system was least efficient, followed by the criss-cross system (+2%) and the terminal sire system that utilized Simmental (+4%), with the terminal sire system utilizing Angus being on average most efficient (+8%). The severe drought and extreme heat of the 2015/2016 summer season had a marked effect on the pre-weaning performance of the calves (preceding year ADG =  $879 \pm 27$  g/d., 2015/2016 ADG =  $687 \pm 28$  g/d, following year ADG =  $770 \pm 28$  g/d). The Nguni calves birth weights were less and also grew slower from birth to weaning than Bonsmara calves. As a consequence, Bonsmara calves were heavier at weaning than either Afrikaner or Nguni calves. Calves sired by Afrikaner bulls were less affected by the year effects than the Bonsmara, Nguni, Angus and Simmentaler sired calves. Despite the relatively small magnitude of observed genetic effects, advantages of crossbreeding that captures substantial heterosis effects became evident.

Keywords: Crossbreeding, Vaalharts, heat, top-cross, pre-weaning

## Variability in ammonia emissions from an open-lot dairy

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Manure on dairies is the second largest agricultural source of ammonia (NH<sub>3</sub>) emissions in the USA. Ammonia emissions were measured for multiple weeks at a time for most seasons over two years at an open-lot dairy using a backward Lagrangian Stochastic model in combination with long-path gas concentration and turbulence measurements. Background concentrations (C<sub>bg</sub>) were estimated: emissions decreased with increasing C<sub>bg</sub>. Mean daytime emissions were twice that of nighttime emissions. Half-hourly emissions variability was best explained by air temperature, with wind speed and saturation vapor density deficit explaining only 2-5% more of the variability. Daily emissions variability was best explained by the saturation vapor density deficit with air temperature and wind speed contributing 5% more each to the explained variability. The mean annual daily NH<sub>3</sub> emissions at the farm was estimated at 82 g NH<sub>3</sub> d<sup>-1</sup> per animal with mean emission during the summer of 124 g NH<sub>3</sub> d<sup>-1</sup> per animal.

Terms for indexing: *Bos taurus*, saturation vapor density, backward Lagrangian modeling, dry environment

# **Session 2**

**Farm level low carbon initiatives, measuring  
and modelling GHG**

## 2.1 Mitigation (Oral)

### **A molecular pattern recognition receptor signalling compound (Biozest) presents an economically advantageous emissions reduction solution**

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A biogenic compound trademarked Biozest enables pasture to synthesise more simple sugars and bioactive molecules; phenylpropanoids. Phenylpropanoids help pasture overcome pest, disease and environmental stress to increase pasture quality and yield. When consumed by ruminants phenylpropanoids can protect proteins from rapid degradation and improve the conversion efficiency of pasture protein to milk and meat. The effect of the compound on both pasture and ruminant efficiency was evaluated via split block trials followed by full-scale, full life-cycle on-farm trials. Aspects measured include pasture resilience and productivity, livestock productivity (milk production, live-weight) and urea excretion. Biozest was found to improve pasture resilience, quality and yield (by over 100%). The higher content of phenylpropanoids in Biozest treated pasture form conjugates with pasture protein. This resulted in reduced urea excretion (20–48%) and increased milk and meat production (30%). Methane production is expected to be reduced because phenylpropanoid/protein conjugates cannot be deaminated to ammonia and methane; instead they are converted to milk or meat. During carbohydrate digestion, the higher content of simple sugars in treated pasture (brix increased by 18%) is expected to favour production of propionate rather than methane, acetate and butyrate. Urea excretion is reduced so it follows that nitrous oxide production from urine and dung patches will be reduced. Future work may include quantification of nitrous oxide and methane emissions reductions. However, the productivity and urea benefits of Biozest have been clearly established via this series of trials proving the compound to be an economically smart and climate-smart choice.

## An early life methane inhibitor treatment reduced methane emissions in dairy calves

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Recent evidence suggests that changes in colonization of the rumen prior to weaning may influence the rumen microbiome and modify phenotypes later in life. The influence of dietary manipulation from birth on growth, methane production, and gastrointestinal microbial ecology in dairy calves was investigated. At birth, 18 female Holstein and Montbéliarde calves were randomly assigned to either the investigational product, 3-Nitrooxypropanol (3-NOP; 3 mg/ kg BW, n=10) or control (CONT, n=8). Treatment was administered daily from birth until three weeks post-weaning (week 14). Samples of rumen fluid were collected at weeks 11, 14, 23, and 60 of life. Enteric methane emissions from the calves were measured using GreenFeed systems (week 11-23 and week 56-60 of life). BW, ADG and individual VFA were similar across groups throughout the trial. Treated calves showed a persistent reduction (11.6%,  $P = .03$ ) in methane emissions (g CH<sub>4</sub>/d) throughout the post-weaning period, despite treatment ceasing three weeks post-weaning. CONT calves aged 60 weeks showed greater archaeal richness than 3-NOP calves. Correspondingly, beta-diversity differed ( $P \leq 0.036$ ) between CONT and 3-NOP calves in week 60. No clear trends emerged in microbes' relative abundance as a result of 3-NOP treatment. However, patterns of co-occurrence of ruminal archaea, bacteria and anaerobic fungi indicated a more inter-dependant relationship in 3-NOP calves. Despite only minimal modifications to the microbiota composition, the reductive effect of the treatment on methane production persisted following cessation of the treatment period, perhaps indicating that microbial activity or a host response was programmed by the treatment.

Keywords: Bovine, Microbial, Rumen, 3-nitrooxypropanol



## Combining brewers' grains with rapeseed meal in dairy cows reduces feed carbon footprint and methane emissions

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Enteric methane mitigation in ruminants is necessary to tackle climate change. Replacing soybean meal (SBM) by a combination of brewers' grains (BG) and rapeseed meal (RSM) might improve the carbon footprint of the diet. Furthermore, a preliminary trial showed the enteric methane (CH<sub>4</sub>) mitigation potential of such a diet. In a 15 week trial with three groups of 10 high-productive cows, the impact on CH<sub>4</sub>, milk production and carbon footprint of BG alone and in combination with RSM (BG-RSM) was compared with a reference diet (REF). The first two weeks all cows received REF (62% maize silage, 38% grass silage and SBM). In week 3 BG and BG-RSM switched to 53% maize silage, 32% grass silage, 15% BG, less SBM for BG and no SBM but RSM for BG-RSM. Throughout the trial, CH<sub>4</sub> was measured with a GreenFeed unit. Dry matter intake increased with 7% for both BG-RSM and BG, milk production was not affected. BG-RSM significantly reduced CH<sub>4</sub> compared to REF: 14% for CH<sub>4</sub>/kg DMI; 10% for CH<sub>4</sub>/kg FPCM. BG alone did not affect CH<sub>4</sub> parameters. Including BG alone or with RSM in the diet reduced the feed carbon footprint by 13% and 43% respectively compared with REF. Together BG and BG-RSM showed a reduction in total carbon footprint (feed and CH<sub>4</sub>) of 9% and 33%, respectively. Replacing SBM by alternative protein sources in smart combinations allows to reduce the carbon footprint of dairy production systems at feed and enteric CH<sub>4</sub> level.

Index terms: *Bos taurus*, protein source, methane mitigation, feeding strategy

## Desmanthus: A tropical legume for mitigating methane emissions in Northern Australian beef cattle

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The aim of this study was to examine enteric CH<sub>4</sub> emissions in beef cattle in response to incremental supplementation with 2 species of *Desmanthus* (*D. leptophyllus* and *D. bicornutus*) – a tropically adapted legume for grazing in the drier parts of Northern Australia. Seven Droughtmaster steers were allocated to each of the 2 legume cultivars. The animals were fed basal Rhodes Grass (*Chloris gayana*) plus fresh *Desmanthus* at 0, 12, 24, 36 and 48% of DM in each period. Every period lasted 21 days and CH<sub>4</sub> production was measured by open-circuit gas exchange in the last 2 days of every period. Results showed no differences in CH<sub>4</sub> emissions between *Desmanthus* cultivars. Yet, a significant decrease in CH<sub>4</sub> emissions with increasing level of *Desmanthus* ( $p=0.014$ ) was observed. The reduction followed a linear pattern and could be predicted ( $R^2=0.76$ ) with the equation: CH<sub>4</sub> emissions (g/kg DMI)=18.46– 0.037xDes. level (%). The study showed that *Desmanthus* can reduce *in vivo* CH<sub>4</sub> emissions from cattle.

Terms for indexing: *Chloris gayana*, *Desmanthus leptophyllus*, *Desmanthus bicornutus*.

## Economic analysis of silvopastoral systems eligible for the Carbon Neutral Brazilian Beef protocol <sup>(1)</sup>

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Economic analyses of two silvopastoral systems eligible for the Carbon Neutral Brazilian Beef (CNBB) protocol and one typical monoculture sown pasture (PM) were undertaken. Experiments were established in the Brazilian Savanna (Quartzarenic Neossol) in 2015. The trees (*Eucalyptus urograndis*) arrangements were 28x2 m (single lines) with 178 trees/ha (SP-178) and 28x2x3 m (triple lines) with 441 trees/ha (SP-441). Charcoal and timber production estimates were 16.5 and 65.9 m<sup>3</sup>, respectively, for SP-178, and 62.6 and 75.1 m<sup>3</sup> for SP-441. Rearing Nellore steers were fattened on *Urochloa brizantha* (BRS Piatã) between rows. Fertilization with 20-00-20 occurred every four years (cycles), resulting in decreasing beef production throughout cycles, with an annual average of 241, 223 and 291 kg of live weight for SP-178, SP-441 and PM, respectively. Investment analysis based on a 12-year cash-flow was undertaken, using 2018 average prices, experimental and estimated data. SP-441 was economically better than SP-178, which, in turn, was better than PM. The net present value (US\$) and the payback period (years) were 1,062 and 11.3, 1,126 and 11.3, and 632 and 5.7 for SP-178, SP-441 and PM, respectively. CO<sub>2</sub>eq estimated balance was positive for SPs systems, but negative for PM, with accumulated CO<sub>2</sub>eq sequestrations by the trunk of 6.5 and 2.8 t ha<sup>-1</sup> year<sup>-1</sup> for SP-441 and SP-178, respectively, over four years. Combined economic-environmental analysis suggests both SP systems are eligible for CNBB. SP-441 showed higher potential for CNBB than SP-178, but the latter might be more appealing for beef farmers and should be further investigated.

Index Terms: economic viability; *Eucalyptus urograndis*; integrated farming systems; low carbon livestock

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## Emissions and carbon sequestration in silvopastoral cattle systems

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Goal was to evaluate enteric methane emissions and carbon sequestration under silvopastoral systems (SP), compared to monoculture sown pastures (PM) aiming enhanced sustainability. The trial was established in December 2015 at a Quatzarenic Neossol in the Savanna biome, Brazil, using *Urochloa brizantha* cv. BRS Piatã and *Eucalyptus urograndis* (clone I144). Evaluations took place in 2017-2018. Nellore steers grazed for 463 days (growing and finishing), following the Carbon Neutral Brazilian Beef (CNBB) protocol. Experimental design was random blocks with five treatments (PM, SP with 178, 238, 357 and 441 trees/ha) and two replicates. Growing animals were fed protein-energy supplement at 0.2% live weight (LW) raised to 1.2% LW for finishing. Dry matter intake (DMI), was predicted through the equation:  $DMI\ (kg/day) = -1.912 + 0.900 \times DMIs + 0.094 \times LW^{0.75} + 1.070 \times ADG - 1.395 \times ADG^2$ . Where: DMIs = supplement intake (kg/day), LW (kg), ADG = average daily weight gain (kg/day). Enteric methane (CH<sub>4</sub>) emission was calculated through:  $CH_4\ (kg/day) = -0.1011 + 0.02062 \times DMI + 0.001648 \times NDF$ . Where: NDF = neutral detergent fiber (%). There was effect of system for CO<sub>2</sub>eq emissions (kg/ha), associated to stocking rates (animal-unit, AU/ha). PM showed highest values (3,357 and 1.86), followed by SP-357 (2,784 and 1.55) and SP-178 (2,561 and 1.42). Lowest values were found in SP-238 and SSP-441, with average 2,288 and 1.27. CO<sub>2</sub>eq sequestration by trees (in the trunk) for SP-441, SP-178, SP-238 and SP-357 was: 13.9, 18.8, 32.5, and 28.5 ton/ha, respectively. CO<sub>2</sub>eq balance for SP systems was positive. However, when estimating timber-based sequestration, only SP-238 and SP-357 are able to neutralize all CO<sub>2</sub>eq generated by livestock for CNBB production.

Index Terms: Carbon Neutral Brazilian Beef, eucalyptus, stocking rate, timber.

## Engineered biocarbon does not decrease enteric methane emissions, however it does alter the rumen microbiome

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The objective of this study was to examine the effect of engineered biocarbon (EB) on rumen fermentation, apparent total tract digestibility, methane emissions, and the rumen microbiome of beef heifers. The experiment was a replicated 4 × 4 Latin square using 8 ruminally cannulated heifers (565 ± 35 kg initial BW). The basal diet contained 60% barley silage, 35% barley grain and 5% mineral supplement with EB added at 0%, 0.5%, 1.0%, or 2.0% (DM basis). Samples for profiling of the microbiome in rumen liquid, solids and feces were collected on d 15 before feeding. Rumen samples for fermentation characterization were taken at 0, 3, 6, and 12 h post feeding. Total collection of urine and feces was conducted from d 18 to 22. Heifers were housed in open-circuit respiratory chambers on d 26 to 28 to estimate CH<sub>4</sub> emissions. Dry matter intake was similar across treatments ( $P = 0.21$ ). NH<sub>3</sub>-N concentration and protozoa counts responded quadratically ( $P = 0.01$ ) to EB. Minimum pH was increased ( $P = 0.04$ ), and variation of pH was decreased ( $P = 0.03$ ) by 2.0% EB. Total tract digestibility, N balance and methane production were not affected ( $P \geq 0.17$ ) by EB. EB decreased the relative abundance of *Fibrobacter* ( $P = 0.05$ ) and *Tenericutes* ( $P = 0.01$ ), and increased the relative abundance of *Spirochaetaes* ( $P = 0.01$ ), *Verrucomicrobia* ( $P = 0.02$ ), and *Elusimicrobia* ( $P = 0.02$ ). Results suggest that at the examined concentrations, EB was ineffective at decreasing enteric CH<sub>4</sub> emissions; however, it altered the rumen microbiome.

Index terms: biochar, enteric

## Greenhouse gas emissions and profitability of dairy systems aimed at reducing nitrate leaching

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Dairy systems are being developed in New Zealand (NZ) that can maintain or increase profitability while reducing environmental impacts. Using research 'farmlets' in three regions over at least three years, we assessed the impacts of 'low nitrate leaching' systems on total greenhouse gas (GHG) emissions, milk production and profitability. Annual average GHG emissions were calculated based on the NZ inventory methodology and included off-platform feeding and imported supplements. Milk production was determined from daily volumes and weekly milk compositions, while known physical inputs and outputs were used with actual milk prices and actual or regional average costs for estimating profitability. In the Waikato, the 'low nitrate leaching' system reduced GHG emissions by 2.2 t CO<sub>2</sub>-eq/ha (16%). However, averaged over five farming seasons, milk production was reduced by 50 kg milksolids (MS)/ha (4%) and profitability by NZ\$280/ha (13%). In Canterbury, the 'low nitrate leaching' system reduced GHG by 5 t CO<sub>2</sub>-eq/ha (24%) with milk production (542 kg MS/ha, 24%) and profit (\$358/ha, 9%) also lower. In Otago, 'low nitrate leaching' systems (one with a barn and one that optimised feeding) reduced GHG emissions by between 0.3 and 1.1 t CO<sub>2</sub>-eq/ha (3-9%). Decreases in production were small (2-3%). However, the profitability of the system that included the barn was significantly lower (NZ\$600/ha), mainly due to capital and maintenance costs. In summary, 'low nitrate leaching' systems reduced N leaching by 22-45%. These lower-input, lower-stocked dairy systems reduced GHG emissions by between 3 and 24% at an average cost of approximately NZ\$100/t CO<sub>2</sub>.

Index terms: milk production, stocking rate, mitigation cost

## High by-product inclusion rate to pasture diets reduces methane production and alters the rumen microbiome

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The objective of this study was to investigate the effects of By Product (BP) inclusion rate to pasture diet on methane production, fermentation pattern and rumen microbiome composition using the rumen stimulation technique (RUSITEC). *In vitro* diets consisted of 50:50 perennial ryegrass (DM=21.6%, CP=25.01%, GE=16.97 MJ/ kg DM) and one of two concentrates, differing in their proportion of BP (BP35=35% BP DM=90.5%, CP=16.85%, GE= 16.92 MJ/ kg DM; BP95=95% BP DM=92.1%, CP=16.73%, GE=17.73 MJ/ kg DM).. Sixteen vessels (n=8/treatment) were incubated over 14 days and inoculated with 500ml of rumen fluid, obtained from rumen cannulated heifers consuming a 50:50 diet of grass silage and concentrates, and 350ml of artificial saliva. Fluid samples were collected on day 13 for microbial analysis. Rumen microbial DNA was extracted with 16S rRNA amplicon libraries generated and sequenced on the Illumina MiSeq. Sequences were analysed with DADA2 with sample functional pathway analysis predicted using CowPI. BP35 had a greater dry and organic matter digestibility ( $P<0.0001$ ) coinciding with an increased production of methane ( $P<0.0001$ ). At the phylum level, an increased relative abundance of Firmicutes and Proteobacteria in the BP35 (63.45 vs. 58.16%; adj  $P <0.01$ ) and BP95 (0.96 vs. 2.01%; adj  $P <0.01$ ) groups was observed. Methane metabolism predicted pathways tended to mirror methane output, with a greater proportion of reads associated with methane metabolism (adj  $P=0.197$ ) in the low BP group. The formulation of high BP based concentrate diets therefore has the potential to alter the rumen microbiome in favour of a reduction in methanogenesis.

Index terms: enteric fermentation, rumen microbiome, in vitro

## Improved cow-calf efficiency is the key to reduce the carbon footprint of beef production

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An effective way to reduce the carbon footprint from beef production is to increase the production per animal unit. Increased productivity generates less greenhouse gas (GHG) emission per unit of product. In beef cattle, the parent-offspring production cycle is responsible for approximately 72% of the energy consumed from conception to slaughter. In this study cow-calf efficiency is defined as (weaning rate [deducted from inter-calving period] x 205 day calf weaning weight) / cow Large Stock Unit (LSU)); the component traits being calf weaning weight, cow weight (from which LSU is estimated) and inter-calving period. In South Africa, the enteric methane emission factor (MEF<sub>enteric</sub>) of a LSU is 94 kg methane/year for beef cattle. It was estimated that the MEF<sub>enteric</sub> of the indigenous Afrikaner, Bonsmara, Drakensberger and Nguni breeds decreased by 12.0%, 6.6%, 9.3% and 6.8% respectively, because of an increase in cow-efficiency over a period of 33 years. The challenge is to define breeding objectives that will improve cow-calf efficiency and not just increase production. A possible breeding objective is kg of calf weaned per Large Stock Unit mated (cow-calf efficiency). However, a ratio has challenges. It is thus proposed to evaluate the total contribution of the following on the “methane budget/balance” of the cow-calf production system; longevity of a cow, fertility (number of calves born over a lifetime), size of her calves, feed efficiency and maintenance cost of the cow. This can then be combined in a selection index for a cow-calf “methane budget/balance”.

**Keywords:** Breeding objective, enteric methane, indigenous breeds, methane budget



## Improving the dairy farm efficiency with a milk low carbon action plan

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The French Livestock Institute (Institut de l'Elevage), in association with key players in the French dairy sector i.e. dairy advisory enterprises and French dairy board, has launched the LIFE CARBON DAIRY project with the main objective to reduce the milk carbon footprint at dairy farm level by 20% over 10 years. A national Life Cycle Assessment tool named CAP'2ER<sup>®</sup> was developed to measure the milk carbon footprint. We characterized and calculated the carbon footprint of 4,150 dairy farms and performed multivariate analysis to assess the contribution of production parameters to the carbon footprint. The average carbon footprint is 1 kg CO<sub>2</sub>e per liter Fat and Protein Corrected Milk. The top-performing farms have carbon footprints 18% lower than the average. Practices with the largest impact are milk yield, age at first calving, quantity of concentrate, N-fertilizer used and fuel consumed. The results also show that the farms can improve economic performance while also reducing carbon footprint. The top-performing group's gross margin was 30€/1000 l higher than the bottom-performing carbon footprint group. In parallel, a sample of 366 farms carried out two CAP'2ER<sup>®</sup> and gross margin analysis during the project. The farms which decrease the most their carbon footprint within three years (-21%) reduce the variable costs by 27€/1000 l as the average decrease by 17€/1000 l. These results encourage now the dairy sector to implement the Low Carbon Dairy Farm initiative at a national level for the 60 000 dairy producers with the objective to engaged all of them in 2028.

Terms for indexing: milk production, global warming potential, greenhouse gases, life cycle assessment, farm efficiency

## Life Beef Carbon: Commitment of beef farms from 4 European countries to reduce carbon footprint.

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Public concerns regarding climate change is pressuring the beef sector to find strategies to reduce greenhouse gas (GHG) emissions. The sector is responding to this challenge through the EU founded “LIFE BEEF CARBON” project coordinated by IDELE. LIFE BEEF CARBON’s goal is to reduce GHG emissions by 15% within 10 years across 172 volunteer beef farms in France, Ireland, Italy and Spain. Three tools are utilized to estimate emissions: CAP’2ER® for France and Italy, Carbon Audit for Ireland and Bovid-CO<sub>2</sub> for Spain. The system boundaries attempt to include all beef farming processes. The functional unit is 1 kg of Live Weight Gain (LWG). On the various beef production systems, (suckler to weanling 6-8 months, suckler to store, suckler to finish, and fattening, the carbon footprint ranges from 5 to 25 kg CO<sub>2</sub>/kg LWG, corresponding to fattening and suckler to weanling at 6 months systems, respectively. The mitigation strategies implemented by farmers are: i. improving animal welfare to increase growth, enhance reproductive performance and health; ii. reducing enteric methane emissions through feeding techniques; iii. using chemical fertilisers and manure efficiently; developing anaerobic digestion; v. preserving grassland; vi. planting hedgerows/trees and minimizing tillage. Three years after applying mitigation practices, the lowest reduction (7%) was observed in the fattening systems in France and Italy and the greatest reductions were obtained in Irish and Spanish suckler to store and suckler to weanling production systems (15%). These results exclude carbon sequestration. Farms combining more than one strategy have obtained the best results.

Keywords: carbon footprint, beef production, mitigation strategies

## Low emission, sustainable beef cattle production on mixed *Brachiaria*-grass/forage-peanut pastures

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Mixed grass/legume pastures are an alternative for increased beef cattle production in warm-season climates. The objective of this study was to evaluate the productivity of beef cattle grazing a mixed pasture of *Brachiaria brizantha* cv. Marandu and forage peanut (*Arachis pintoi*) or Marandu monoculture pasture under rotational stocking. Five trials, total duration of nine years, were conducted in northeastern Brazil, where two types of pasture were assessed: Mixed pasture of Marandu and forage peanut, and monoculture of Marandu with 120 kg ha<sup>-1</sup> yr<sup>-1</sup> of nitrogen. The stocking rate was adjusted to maintain forage allowance at 4 % of body weight day<sup>-1</sup> of forage mass. Sward structure (forage, grass and legume mass) and animal performance (average daily gain, stocking rate and area gain) were measured. A block design was used with four replicates, and the warm and cool seasons within each trial were considered using repeated measurements over time. In the warm season the forage mass in the mixed pastures was 17% greater ( $P = 0.049$ ). The stocking rate, average daily gain and area gain were 16.4, 20.0 and 28.7% greater in the mixed pasture than in the grass monoculture in the warm season ( $P = 0.056$ ,  $P < 0.001$  and  $P < 0.001$ , respectively). Average daily gain showed a positive linear relationship with botanical composition ( $P < 0.001$ ). The mixed pasture of forage peanut with Marandu sustained significantly greater beef cattle production compared to the grass monoculture fertilized with 120 kg N ha<sup>-1</sup>, the avoided emission for fertilizer manufacture alone amounted to ~500 kg CO<sub>2</sub> ha<sup>-1</sup>.

Keywords: *Arachis pintoi*, Beef cattle, *Brachiaria*, Mixed pasture, Nitrogen fertilization, Tropical legume

## Methane emission from dairy cows in cultivated and native pastures in High Andes of Peru

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The objective of the study was to determine the enteric methane emissions from lactating cows under grazing conditions in prevalent high Andean systems of Peru based on cultivated (CULP) or native (NATP) pastures during two seasons of the year. In CULP, brown Swiss cattle pastured alfalfa/oats with supplementation of with 1 kg concentrate daily per cow. Conversely, in NATP, crossbred cows pastured to native pastures without supplementation. The sulfur hexafluoride (SF<sub>6</sub>) tracer technique was used to measure CH<sub>4</sub> emissions. In CULP, CH<sub>4</sub> emission was higher ( $P < 0.001$ ) in the rainy season (322 g/d) than the dry season (274 g/d), but, in NATP this trend was opposite (213 vs. 257 g/d) ( $P < 0.01$ ). In PCUL, CH<sub>4</sub> emission per unit of intake was lower ( $P < 0.001$ ) in the rainy season (24.3 g/kg DMI) than in the dry season (28.2 g/kg DMI), but in PNAT the results were similar ( $P = 0.18$ ) between seasons (23.2 vs. 25.0 g/kg DMI). The intensity of emission in CULP was lower ( $P < 0.01$ ) in rainy than in dry season (24.6 and 27.5 g/kg ECM). In NATP, the intensity of emission was also lower ( $P < 0.001$ ) in rainy (68.9 g/kg ECM) than in dry season (131.2 g/kg ECM). Emission intensity differences between systems were remarkable, being greater in the NATP both in rainy and dry season.

Keywords: lactating cows, grazing, sulfur hexafluoride.

## Nitrous oxide emission intensity from grazed pastures of *Brachiaria brizantha* in monoculture or mixed with *Desmodium ovalifolium*<sup>(1)</sup>

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Mixed grass/legume pasture systems can be a mitigation strategy for N<sub>2</sub>O emissions. Hence, this study aimed at evaluating animal performance and monitoring N<sub>2</sub>O emissions from cattle excreta, fertilizers and plant residues of tropical pastures of *Brachiaria brizantha* cv Marandu either mixed with *Desmodium ovalifolium* or fertilized with 150 kg N ha<sup>-1</sup> year<sup>-1</sup> during three years. Using static-closed chambers gas measurements, the estimated N<sub>2</sub>O emission factors (EFs) for each year as an average of pasture types were, respectively, 0.337%; 0.579% and 0.275% for urine, year 2 being greater than year 3 (Fisher l.s.d test, p = 0.05). The N-fertilized pasture presented a mean EF of three years for urine of 0.345%, which was not different from the EF of 0.449% for the mixed pasture. An EF of 0.051% was calculated for feces from fertilized pasture and 0.030% from the mixed pasture. These were means of the three years, without statistical differences for years and pasture type. Soil N<sub>2</sub>O emissions induced by urea were also below 1% with no detectable effect of pasture litter on the background emission of this gas. The mixed pasture presented greater live weight gains (LWG) and also higher stocking rates (Fisher l.s.d test, p = 0.05). In a 16-month period the LWG was 1.7 kg ha<sup>-1</sup> day<sup>-1</sup> (0.51 kg AU<sup>-1</sup> day<sup>-1</sup>) and 2.01 kg ha<sup>-1</sup> day<sup>-1</sup> (0.56 kg AU<sup>-1</sup> day<sup>-1</sup>) for the N- fertilized and mixed pastures, respectively. Therefore, mixed grass/legume pasture presented itself as a strategy for increasing productivity, with reduced emission intensity of N<sub>2</sub>O.

Keywords: Pasture, N<sub>2</sub>O, forage legume

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## Persistency of the 3-nitrooxypropanol effect on methane and hydrogen emissions in postpartum Holstein-Friesian dairy cows

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The aim of this study was to determine the methane mitigation potential of 3-nitrooxypropanol (3-NOP) and its persistency when fed to dairy cows in early lactation. Sixteen Holstein-Friesian cows (parity 2.3

$\pm 0.48$ , average  $\pm$  SD) were blocked in pairs and randomly allocated to 1 of 2 dietary treatments; a diet including 52 mg 3-NOP/kg DM (3-NOP) and a diet including a placebo at the same concentration (control). Diets consisted of 35% grass silage, 25% corn silage, and 40% concentrates (on DM basis). Cows received the diets from 3d post-calving up to 115 days in milk. Every 4 weeks, the cows were housed in climate respiration chambers for 5 consecutive days to measure production characteristics and gas emissions. Data were subjected to repeated measures ANOVA using the MIXED procedure in SAS with dietary treatment, days in lactation, and their interaction as fixed effect and block and cow as random effects. The results indicate that dietary 3-NOP supplementation did not affect DM intake, milk yield, and fat-and protein-corrected milk. These characteristics, as well as hydrogen and methane emission, changed over time ( $P < 0.038$ ) following the expected pattern of advancing days in lactation. On average, feeding 3-NOP persistently increased ( $P < 0.001$ ) hydrogen emissions 11-fold and decreased ( $P < 0.014$ ) methane emission by 16% (e.g., 21.0 vs. 17.7 g methane /kg DM intake for 3-NOP and control diet, respectively). In conclusion, feeding 3-NOP persistently decreases methane emission in early lactation Holstein-Friesian cows without affecting animal production characteristics.

Terms for indexing: Methane mitigation; feed additive; gas emissions; production performance.

## Sprouting barley grain reduces the methane yield of cattle fed barley-based rations

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Sprouting barley prior to feeding is promoted as a means of increasing feed supply. In this study, the quantity of DM remaining after allowing barley to sprout for 5d was only 90.4% of that present before sprouting commenced. Sprouting for 5d reduced the starch content from 62.6% to 11.6% of DM and increased protein and fat contents of DM, relative to the unsprouted grain. Ten Aberdeen Angus heifers ( $348 \pm 16.1$  kg), were allocated into two diet treatments ( $n = 5$ ) in a completely randomised design lasting 42 d. Treatments were 5.97 kg/d of DM, offered as either 5-day sprouted barley fodder (25 kg as fed) plus 2 kg wheaten chaff as fed, or an equal total quantity of DM fed as 6.8 kg of commercial mix feedlot finisher (75% barley), based on the same barley. Cattle fed the sprouted barley-based diet instead of feedlot finisher exhibited a higher total VFA concentration and lower ruminal pH, ammonia and protozoal population density than cattle fed the feedlot ration. The sprouted barley-based ration also supported a significantly lower daily methane production ( $124$  v  $175$  g CH<sub>4</sub>/d;  $P=0.001$ ) and a lower methane yield ( $16.0$  v  $21.2$  g CH<sub>4</sub>/kg DM intake;  $P=0.004$ ) than did the feedlot ration. This study confirms that sprouting for 5d reduces the quantities of DM and of starch in barley, and that cattle fed a ration based on sprouted barley have a lower methane yield than cattle fed a feedlot ration based on the same barley.

Terms for indexing: hordeum vulgare, sprouts, rumen fermentation, greenhouse gas

## Supplementing the diet of dairy cows with fat or tannin reduces methane yield

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Addition of fat to the diets of ruminants is known to result in a reduction in enteric methane yield. Tannins have also been used to reduce methane yield but with mixed success. The effect of feeding fat in combination with tannin is unknown. Eight ruminally-cannulated Holstein cows were offered a basal diet of 6.0 kg DM concentrate and 20 kg DM of lucerne (*Medicago sativa*) hay with one of four dietary supplements in a double Latin-square, full crossover sequence. The supplements, 800 ml/d of water (CON), 800 g/d of cottonseed oil (FAT), 400 g/d of tannin (TAN) extracted from black wattyl (*Acacia mearnsii*), and FAT plus TAN (FPT), were administered via ruminal cannula. Methane was measured using open-circuit respiration chambers. Intakes of basal diets were not different between treatments but cows fed FAT or FPT had greater total DMI due to the addition of these ingredients to the diets. Milk yield of cows fed FAT, TAN or FPT were not different to those from cows fed CON (33.3 kg/d). However, cows fed FAT or FPT produced more milk (34.9 kg/d) than cows fed TAN (31.1 kg/d). There were no differences between treatments in energy corrected milk. Compared to cows fed CON, methane yield of cows fed FAT was reduced by 14%, TAN by 11% and those fed FPT by 20%. We conclude that both fat and tannin reduce methane, and that this reduction is mostly additive when fat and tannin are fed together.

Index terms: ruminants, methane yield, *Acacia mearnsii*, cottonseed oil, respiration chambers



## Sustained Decrease in Enteric Methane Production by Beef Cattle in a Commercial Feedlot Fed a Forage-based Backgrounding Diet Supplemented with Increasing Doses of 3- Nitrooxypropanol

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Effects of the investigational inhibitor 3-nitrooxypropanol (3-NOP) on dry matter intake (DMI) and enteric methane (CH<sub>4</sub>) production of beef cattle fed a backgrounding grower diet were evaluated in a commercial feedlot. Fifty crossbred steers (body weight = 328 ± 29 kg) were assigned in a completely randomized design to one of two treatment pens (n = 25/treatment): control and 3-NOP. The treatment pen received increasing doses of 3-NOP to adapt to the final level: 150 (low), 175 (medium) and 200 (high, final dose) mg/kg dry matter (DM), with each dose fed for 28 days. 3-NOP was mixed daily into the high-forage diet (70% corn or barley silage, DM basis). DMI of individual animals was monitored with the GrowSafe system and CH<sub>4</sub> was measured using the GreenFeed system. Over the 84 d study, compared with control (11.4 kg/d), DMI was decreased by 6.3% (P = 0.008) with 3-NOP supplementation, and this reduction was similar among the 3-NOP doses (5.8 to 6.4% decrease). On average, addition of 3-NOP lowered (P ≤ 0.002) CH<sub>4</sub> emissions (g/d) by 26% and yield (g CH<sub>4</sub>/kg DMI) by 19%. Compared with control, the greatest decrease in CH<sub>4</sub> yield occurred with the medium dose (-23%), followed by high (-19%) and low (-15%) doses. In conclusion, regardless of dose, supplementation of 3- NOP lowered enteric CH<sub>4</sub> production from commercial feedlot cattle fed a forage-based diet, with the decrease maintained over the entire study. A concurrent large pen study at the same feedlot will evaluate the effects of 3-NOP on animal performance.

**Keywords:** Beef steers, Commercial feedlot, GreenFeed, Methane.

## What are the system changes required to reduce environmental impacts of Tanzanian livestock farms?

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The Global Livestock Environmental Assessment Model (GLEAM) was used to evaluate the impacts of improved management strategies on greenhouse gas emissions from mixed dairy, pastoralist and backyard chicken farms in Tanzania. Emissions intensity i.e. emissions produced per unit of product are reported as a final output in kilogram carbon dioxide equivalents (kg CO<sub>2</sub>e). The intervention options for each production system consist of a combination of improvements in genetics, feeding, milk production, herd structure and animal health and mortality, which were compared with a baseline. The emissions intensities in improved cases reduced from 60.4 kg to 40.4 kg CO<sub>2</sub>e/kg live weight in pastoralist systems; from 8.1 kg to 7.2 kg CO<sub>2</sub>e/kg for eggs and from 12.2 kg to 11 kg CO<sub>2</sub>e/kg live weight for backyard chicken; and from 24 kg to 17 kg CO<sub>2</sub>e/kg milk, and from 78 kg to 29 kg CO<sub>2</sub>e /kg live weight in mixed dairy production systems. Note that a 10-fold increase in annual milk yield from 240 kg to 2400 kg/cow and simultaneous replacement of grazed grass with fresh cut grass would in fact result in emission intensities as low as 3 kg CO<sub>2</sub>e/kg milk. In the latter scenario for dairy systems, ammonia volatilised and nitrate leached almost halve compared to the baseline. The mitigation measures proposed here align with the intended interventions to reduce greenhouse gas emissions in Tanzania. The sector development programs are expected to facilitate the implementation; however, adapting alternative feeds and herd structure in Tanzania face potential institutional, infrastructural and societal challenges.

Terms for indexing: Africa, greenhouse gas, livestock, production efficiency

## 2.1 Mitigation (Poster)

### 3-nitrooxypropanol mitigates enteric methane with a minimal impact on the feedprint

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The investigational product 3-nitrooxypropanol (3-NOP) showed clear reductions in enteric methane emissions in cattle in studies worldwide. However, a mitigation strategy will only be effective in abating the global warming if there is a net reduction in greenhouse gas emissions. In this study the net abatement potential of 3-NOP in dairy cattle was assessed by performing a life cycle assessment (data 2016). There were three treatments: REF (no 3-NOP), NOP<sub>bas</sub> (3-NOP mixed in with the basal diet, the roughage) and NOP<sub>conc</sub> (3-NOP incorporated in a pelleted balanced compound feed). The system boundaries were: the life cycle of the feed and the feed additive from the “cradle”, i.e. resource extraction from the natural environment, to the production of milk at the farm. The measured enteric CH<sub>4</sub> emissions in CO<sub>2</sub> equivalents were added to the carbon footprint of the consumed feed to calculate a combined carbon footprint. The functional unit was defined as 1 kg fat-and-protein-corrected milk (FPCM). The combined carbon footprint of REF was 1.14 kg of CO<sub>2</sub>-eq./kg of FPCM, compared to 1.06 for NOP<sub>bas</sub> (P=0.15) and 1.04 for NOP<sub>conc</sub> (P=0.02). The enteric methane production was 23 and 21% lower for NOP<sub>bas</sub> (P<0.01) and NOP<sub>conc</sub> (P<0.01), respectively, whereas the contribution of 3-NOP in the combined carbon footprint of NOP<sub>bas</sub> and NOP<sub>conc</sub> was only 0.32% and 0.34%, respectively. Feed supplementation with 3-NOP is definitely a successful strategy for abating impact of dairy on climate, even when pre-farm emissions for feed and 3-NOP are taken into account.

Index terms: *Bos taurus*, feed additive, methane mitigation, carbon footprint

## Carbon capture by silvopastoral arrangements under contrasting stocking rates in the northern coast of Colombia

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Use of silvopastoral systems (SS) for cattle production has proven to be useful to increase productivity and generate ecosystem services, including carbon fixation through soil and vegetation, as tools to mitigate climate change pressures from the cattle sector and guide it towards environmental sustainability. Evaluate the carbon capture potential of cattle SS under two different stocking rates (SR) in the town of San Sebastian de Buena Vista, northern coast of Colombia. The SS was composed of timber trees *Eucalyptus tereticornis* planted in stripes (6m) with 3 lines of trees each (2mx2m), associated with *Megathyrus maximus* in 30m wide pasture stripes, alternating pasture and tree stripes. Treatments were: T1=High SR(3,5UA/Ha), T2=Low SR(2,0UA/Ha), with 1UA=150Kg live weight. Average SR for the area is 1UA/Ha. Both treatments were managed under 6 paddocks (7 days occupation and 42 days rest) for 2 years of paddock rotation, adjusting SR through “Put and take” method. A full random block design was used with 6 replicates (Animals) per treatment. The soil carbon stock was similar ( $P>0.05$ ) between treatments (T1=65,98 vs. T2=68,08 TCO<sub>2</sub>/Ha). Pasture carbon stock (aerial + radicular portions) was higher ( $P<0.05$ ) for T2=12,80 than T1=12,09 TCO<sub>2</sub>/Ha. The carbon captured by the trees (aerial + radicular portions) was the same ( $P>0.05$ ) for both treatments (T1=78,71; T2=81.07 TCO<sub>2</sub>/Ha). The carbon stock summation of the different silvopastoral system compartments was similar for both treatments ( $P>0.05$ ) with T1=156,75 vs T2=161,95 TCO<sub>2</sub>/Ha. Use of contrasting stocking rates had no significant effect on the carbon capture capacity of the production systems evaluated.

Keywords: Carbon immobilization, Trees, pastures, *Eucalyptus tereticornis*, *Megathyrus maximus*, silvopastoralism, bovine

## Effect of starch substitution by rapeseed oil on enteric methane emission and performance in dairy cows fed grass-silage based diets<sup>(1)</sup>

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This work aimed to study the effect of energy source supplementation on methane emission and performance in dairy cows fed grass-silage based diets. Eight multiparous lactating Holstein cows were used in a replicated 4 x 4 Latin square design with 4 periods of 28 days. Animals were limited fed (95% ad libitum) with 4 grass silage based diets supplemented with 4 concentrates in which starch was gradually substituted by increasing amounts of rapeseed oil (1.5, 3.0 and 4.5% on a dry matter basis). Diets were formulated to be iso-energy and iso-protein to meet individual energy and protein requirements for production. Methane emissions were measured (days 23 to 27) when animals were in open-circuit respiration chambers. Daily intake, milk production and composition were also recorded. Intakes of gross energy and fiber were similar among diets, starch intakes decreased linearly ( $P < 0.0001$ ) and ether extract intake increased linearly ( $P < 0.001$ ) with increasing rapeseed oil amounts. Methane emissions (g/d), yield (g/kg dry matter intake) and intensity (g/kg milk) decreased linearly ( $P < 0.01$ ) when starch was replaced by rapeseed oil in the diets. Milk production and composition differ among diets ( $P < 0.001$ ). The linear decrease in milk fat yield with increasing amounts of oil suggests a milk fat depression with changes in milk fatty acids profile. Starch substitution by rapeseed oil decreased both enteric methane emission and performance in dairy cows fed grass-silage based diets.

Index terms: methanogenesis, mitigation feeding strategy, energy source, milk cow

<sup>(1)</sup>This study is part of the Joint Programming Initiative on Agriculture, Food Security and Climate Change (FACCE-JPI)'s "GLOBAL NETWORK" project and the "Feeding and Nutrition Network" (<http://animalscience.psu.edu/fnn>) of the Livestock Research Group within the Global Research Alliance Agricultural Greenhouse Gases ([www.globalresearchalliance.org](http://www.globalresearchalliance.org)).

## Effects of energy and tannin supplementation on N<sub>2</sub>O emission and emission factor from sheep's excreta

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**Abstract** - Livestock is one of the main activities responsible for greenhouse gases emission, such as nitrous oxide (N<sub>2</sub>O). The aim of the study was to evaluate the N<sub>2</sub>O flow and emission factor (EF) from feces and urine of sheep receiving corn ground and tannin extract supplementation. The animals were fed with ryegrass (*Lolium multiflorum* Lam.) haylage *ad libitum* and three different daily supplementation : 60 g of soybean meal (S; control); 140 g of cracked corn grain + 60 g soybean meal (SC); 140 g of cracked corn grain + 60 g soybean meal + 40 g kg<sup>-1</sup> of concentrated feed of *Acacia mearnsii* tannin extract (SCT). Feces (100 g) and urine (140 ml) collected from animals of the respective treatments were applied to the soil, besides a control treatment without excreta application. The feces of all treatments had similar flux and emission of N<sub>2</sub>O and did not differ from the control. However, the N<sub>2</sub>O flow of the urine samples from diets with corn grain were reduced ( $P < 0.05$ ) compared to that ones from the animals supplemented with soybean meal only. The EF for feces was similar among treatments with a mean of 0.18%. The average of EF from urine samples was 4%, moreover the EF from the SM treatment showed a tendency ( $P = 0.089$ ) of reduction in 60% compared to the S treatment. Corn grain supplementation may be a tool to mitigate N<sub>2</sub>O emissions in sheep, whereas the inclusion of *Acacia mearnsii* tannin extract has not been effective.

**Terms For Indexing:** *Acacia mearnsii*, *Lolium multiflorum* Lam., *Ovis aries*, ruminants, emission factor, mitigation.

## Enteric emission from beef cattle supplemented with Yerba mate extract<sup>1</sup>

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The objective of this study was to evaluate enteric methane emission from Nellore cattle with levels of inclusion of Yerba mate (*Ilex paraguariensis* St. Hilaire) extract in feed. Eight castrated male Nellore steers, rumen cannulated, were fed four experimental diet (0, 0.5, 1 and 2% of inclusion of Yerba mate extract); all the diets were composed with 10.4% crude protein and 65.9% total digestible nutrients. The experimental design was a double Latin square 4x4. Each period was composed by 14 days of adaptation and 5 days of methane collection by the tracer gas technique sulfur hexafluoride (SF<sub>6</sub>). Data were analyzed using the MIXED procedure of SAS software and a regression test was developed for P value < 0.05. For treatment 0, 0.5, 1 and 2% (respective values in the parenthesis), methane emitted daily (230, 238, 238 and 237 g/d), methane per dry matter intake (30.8, 31, 30.8 and 30.6 g/kg DM.d), methane per daily weight gain (215.4, 409.1, 239.8 and 219.3 g/kg gain.d), methane per body weight (497, 509, 514 and 505 g/kg BW.d), and methane conversion factor (9.6, 9.6, 9.5, and 9.5%) did not show difference (P > 0.05). This results may not have been significant due to the short adaptation time or low inclusion of the additive. The results show that Yerba mate extract on these levels of inclusion is not a good additive to modulate methanogenesis, however more researches are required.

Keywords: Nellore cattle, methane, feed additive.

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## Enteric methane emissions from beef cattle in N-fertilized grass or grass-legume pastures in North Florida

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Enteric methane emissions were assessed in grazing steers for three years (2016-2018). Treatments were allocated in a RCBD and consisted of: 1) N-fertilized ( $112 \text{ kg N ha}^{-1}$ ) bahiagrass (*Paspalum notatum* F.) pastures during the warm-season, overseeded with a mixture of cereal rye (*Secale cereale*, L.) and oat (*Avena sativa*, L.) +  $112 \text{ kg N ha}^{-1}$  during the cool-season (Grass+N); 2) Rhizoma peanut (*Arachis glabrata* B.) and bahiagrass pastures during the warm-season, overseeded with similar rye-oat mixture fertilized with  $34 \text{ kg N ha}^{-1}$  plus a mixture of clovers during the cool season (Grass+CL+RP); 3) unfertilized bahiagrass pastures during the warm-season, overseeded with similar rye-oat Grass+clover mixture +  $34 \text{ kg N ha}^{-1}$  during the cool-season (Grass+clover). The SF6 tracer technique was used to measure enteric CH<sub>4</sub> emissions and the marker dilution technique with Cr<sub>2</sub>O<sub>3</sub> and TiO<sub>2</sub> as indigestible external markers was used to determine dry matter intake (DMI). Enteric CH<sub>4</sub> emissions in  $\text{g steer}^{-1} \text{ d}^{-1}$ ,  $\text{g BW}^{(0.75)} \text{ d}^{-1}$ ,  $\text{g ha}^{-1} \text{ d}^{-1}$ , or  $\text{g kg of DMI}^{-1}$ , did not differ among treatments ( $P > 0.05$ ) in either warm or cool season. Emission intensity in  $\text{g of CH}_4 \text{ kg of ADG}^{-1}$  had a season  $\times$  treatment interaction ( $P < 0.01$ ), averaging 177, 140, and 123 in the cool season, and 397, 448, and 225 in the warm season for Grass+N, Grass+clover, and Grass+CL+RP treatments, respectively. In conclusion, increased quality of forages grazed in the cool season contributed to decrease CH<sub>4</sub> emissions intensity in grazing systems relative to that in the warm season.

Keywords: enteric methane, beef cattle, fertilized grass, legumes.



## Enteric Methane Production of Beef Steers Consuming Molasses, Cottonseed Meal, and Alkali Treated Bahiagrass Hay

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An experiment was conducted to evaluate the effects of bahiagrass (*Paspalum notatum*) hay treated with calcium oxide (CaO) on enteric methane production of beef steers supplemented with molasses and cottonseed meal. Twenty growing *Bos taurus* and *Bos indicus* steers ( $314 \pm 37$  kg of BW) were used in a generalized randomized block design. On day -1 and 0, steers were weighed and the average weight of both days was considered the initial BW. On day 0, steers were stratified by breed and BW, blocked by initial BW (heavy and light) and randomly assigned to one of two treatments: 1) bahiagrass hay as is (DH;  $n=10$ ) or 2) bahiagrass hay treated with 5% CaO (DM basis) + water (to 50% DM; CAO;  $n=10$ ). Steers were provided, individually, cottonseed meal and molasses at 0.3% of BW and 0.454 kg/steer/d, respectively. Steers were housed in four pens (108 m<sup>2</sup> each; 5 steers/pen) in a feed efficiency facility where individual intake of hay was measured via GrowSafe. Enteric CH<sub>4</sub> production was measured using the SF<sub>6</sub> tracer technique. Data were analyzed using steer as the experimental unit. The model included the fixed effects of treatment and the random effects of breed and block. No differences between treatments were observed on total CH<sub>4</sub> production (g/d;  $P = 0.535$ ) or grams of CH<sub>4</sub> produced per kilogram of OM intake ( $P = 0.694$ ), OM digested ( $P = 0.168$ ), and metabolic BW ( $P = 0.717$ ). Bahiagrass hay treated with CaO did not alter enteric CH<sub>4</sub> emissions in beef steers.

Index terms: *Bos taurus*, calcium oxide, green house gas, low quality forage

## Evaluation of Methane Emissions from Lambs Offered Fresh Grass Based Diets

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The present study aimed to evaluate the effect of feeding good quality grass on enteric methane (CH<sub>4</sub>) emissions of sheep. Forty-eight lowland lambs were used in a 2 breeds (Highlander vs. Texel) × 3 sexes (female vs. intact male vs. wether) × 2 diets (fresh grass vs. fresh grass plus 0.5 kg/d pelleted concentrate) factorial design study with a single period (23 days). Animals were 5 months old and weighing  $36 \pm 5.0$  kg at the commencement of the study. The CH<sub>4</sub> emissions were measured using respiration chambers. Lambs offered concentrate diets had greater DM intake and CH<sub>4</sub> production (g/d) than those given fresh grass only, but diets had no effect CH<sub>4</sub>/DM intake or CH<sub>4</sub> energy/GE intake (6.4% vs. 6.3%). Texels produced more CH<sub>4</sub>/DM intake than Highlanders, while sex had no effect on CH<sub>4</sub>/DM intake. The results indicate that high quality fresh grass may play a similar role to concentrates in mitigating CH<sub>4</sub> emissions of sheep.

Keywords: fresh grass, methane, respiration chamber, sheep

## Factors Affecting on Enteric Methane Emission from Dairy Cattle integrated with Paddy Rice Farming

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In 2018, in Sakon Nakhon province there were 4,524 dairy cows, 193 dairy farmers. They were integrated dairy cattle and paddy rice farming systems. The objective was to study the effect of farm size and feeding management on enteric methane emission from dairy cattle integrated with paddy rice farming in Sakon Nakhon Province. There were 2 parts of data, 1) primary data and 2) secondary data. Data were analyzed by least square analysis. There were 2 farm sizes, 1) small dairy farm (1-20 cows) and 2) medium dairy farm (21-100 cows). Milking cows from small and medium farms had an average enteric methane emission of 0.65 and 0.80 kg per head per day, respectively ( $P < 0.01$ ). The basic feeding were 21 %CP concentrate and rice straw. There were 3 levels of feed managements, 1) no supplement, 2) with 1 supplement source, and 3) with more than 1 supplement source. The milking cows of feed management level 1, 2 and 3 had average enteric methane emission 0.90, 0.74 and 0.56 kg per head per day, respectively ( $P < 0.01$ ). The cows with low and high TDN level had average enteric methane emission 0.81 and 0.65 kg per head per day, respectively ( $P < 0.01$ ). Farmers used manures as fertilizer, supported to reduce greenhouse gas emissions from chemical fertilizers and their transportation. It could reduce the GHG emission from rice straw burning. This dairy cattle and paddy rice integrated farming system supported food security, family income and friendly environment in Sakon Nakhon Province, Thailand.

Keywords: enteric methane, dairy cattle, paddy rice

## Factors Affecting on Enteric Methane Emission from Fattening Cattle Integrated with Paddy Rice Farming

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In 2018, in Sakon Nakhon province there were 3,378 fattening cattle, 964 fattening cattle farmers. They were integrated fattening cattle and paddy rice farming systems. The objective was to study the effect of age and feeding management on enteric methane emission from fattening cattle integrated with paddy rice farming in Sakon Nakhon Province. There were 2 parts of data, 1) primary data and 2) secondary data. Data were analyzed by least square analysis. Farmers raised Charolais crossbred. There were 3 levels of age, 1) 1-2 years, 2) 2.1-3 years, and 3) more than 3 years. Fattening cattle from 1, 2 and 3 level of age had an average enteric methane emission of 0.21, 0.31 and 0.55 kg per head per day, respectively ( $P < 0.01$ ). The basic feeding were 14 %CP concentrate and rice straw. There were 2 levels of feed managements, 1) with 1 supplement source, and 2) with more than 1 supplement source. The fattening cattle of feed management level 1 and 2 had average enteric methane emission 0.50 and 0.21 kg per head per day, respectively ( $P < 0.01$ ). Farmers used manures as fertilizer, supported to reduce greenhouse gas emissions from chemical fertilizers and their transportation. It could reduce the GHG emission from rice straw burning. This fattening cattle and paddy rice integrated farming system supported food security, family income and friendly environment in Sakon Nakhon Province, Thailand.

Keywords: enteric methane, fattening cattle, charolais, paddy rice

## Greenhouse gas emission from the composting process of broiler litter

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**Abstract**—The purpose of this study was to evaluate the greenhouse gas emission factors (EF) and the effect of aeration condition on the EFs of the composting process of broiler litter and propose strategies for reducing GHG emission. The broiler litter was piled in the composting plants with 3, 0.5 and 0 min/hr of aeration, respectively. The composting temperature was recorded daily. The GHG from the compost was measured triple a week. The composition of compost was analyzed once weekly. Total amounts of the greenhouse gases emitted during the composting processes were calculated by the GHG concentration of headspace gas of compost plants. The results showed the EFs of nitrous oxide were 0.62, 1.27 and 1.23% N<sub>2</sub>O-N/initial N and 1.59, 1.96 and 2.04% CH<sub>4</sub>-C/initial C, respectively. The more aeration time it was given, the less amount of methane emitted. Taking the power consumed into account, the total carbon dioxide equivalent of were 0.32, 0.43 and 0.42 kg/kg initial dry matter for the aeration condition of 3, 0.5 and 0 min/hr, respectively. The results of this study showed 3 min/hr of aeration could reduce 24% of the GHG from the broiler litter composting process.

**Keywords:** emission factors, nitrous oxide, methane, aeration

## Greenhouse gas emission intensity from grazing beef steers with or without supplement in Argentina

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Beef production is a major industry in Argentina, but it is also responsible for the emissions of Greenhouse gases (GHG). The aim of the study was to measure the impact of supplementing grazing beef steers on their GHG emissions intensity. Twenty Angus steers ( $200 \pm 10$  kg) grazed tall fescue and alfalfa pasture without (P) or with maize grain as daily supplement (S, 1% body weight) during 90 days. Body weight was recorded every 21 days. Enteric methane (CH<sub>4</sub>) was measured on days 85 to 90 (SF<sub>6</sub> technique). Methane and nitrous oxide (N<sub>2</sub>O) emissions from dung patches were measured using close static chambers ( $n = 5$ ). Total CO<sub>2</sub>eq was equal to (enteric CH<sub>4</sub> + dung CH<sub>4</sub>) \* 25 + dung N<sub>2</sub>O \* 298. Average daily body weight gain (ADG) was 0.38 vs. 0.55 kg and enteric CH<sub>4</sub> was 4,045 and 4,300 g CO<sub>2</sub>eq·anim<sup>-1</sup>·d<sup>-1</sup> for P and S, respectively. Methane emission from dung patches decreased from 64.9 to 21.3, but N<sub>2</sub>O increased from 19.9 to 60.4 g CO<sub>2</sub>eq·anim<sup>-1</sup>·d<sup>-1</sup> for P and S, respectively. Although total emissions from P were numerically lower than S steers (4,130 and 4,382 g CO<sub>2</sub>eq·anim<sup>-1</sup>·d<sup>-1</sup>, respectively), total emission intensity were reduced by 27% (10.87 vs. 7.97 kg CO<sub>2</sub>eq·kg ADG<sup>-1</sup>, respectively) due to a 45% higher performance of supplemented steers. Although emissions from urine patches are still needed, this study demonstrated with local information that supplementing grazing steers have substantial impacts on improving productivity without altering enteric plus dung GHG emissions.

Keywords: cattle, methane, nitrous oxide, pasture, maize grain

## Greenhouse gas emissions from sheep fed with increasing levels of *Tithonia diversifolia*

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The increasing interest in livestock systems that are able to provide animal protein whilst reducing the impact on the environment has encouraged the use of shrub species in pasture systems as an alternative to high concentrate diets in conventional production systems. A novel approach for measuring *in vivo* gas emissions was used to evaluate the effects of the inclusion of *Tithonia diversifolia* (TD), a tropical shrub, in the diet of sheep. Four diets containing concentrate, Tifton hay and 0 (control), 0.09, 0.27 and 0.45 TD (as % of dry matter), were tested. Eight rams were individually allocated in ten respirometric chambers connected to a spectrometer (Picarro, Inc, USA) that allowed to continuously assess the concentration of CO<sub>2</sub>, CH<sub>4</sub>, NH<sub>3</sub> and N<sub>2</sub>O throughout a 48 h assay. The treatments were arranged in a Latin Square Design (4x4) and data was analyzed by ANOVA and Tukey test. The results showed a significant difference ( $P < 0.05$ ) between treatments (ppm/kgDMI) for CO<sub>2</sub> (697, 796, 816, 792), N<sub>2</sub>O (0.2617, 0.3198, 0.3361, 0.2959), NH<sub>3</sub> (4114, 4624, 4093, 4521) and CH<sub>4</sub> (35.8, 40.0, 39.6, 38.0) for T1, T2, T3 and T4, respectively. Overall, gas emissions were increased by the inclusion of TD when compared to control, however, higher inclusion (T4) accounted for lower concentrations when compared to T2. Further data processing still have to be made in order to assess the relationship of gas concentration, feed digestibility and volume of gas produced and also if higher inclusions of TD could reduce emissions.

Index Terms: Methane, Nitrous Oxide, Ammonia, Carbon Dioxide, Ruminants, Intensive Silvopastoral Systems

## Greenhouse gases and ammonia emissions from the breeding of black soldier fly (*Hermetia illucens* L.) larvae

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There is an increased interest in using insect larvae to recycle organic waste in high-quality protein products for livestock and aquaculture feeds. Different species can be used for this purpose and *Hermetia illucens* L., the black soldier fly (BSF), is one of the most promising. However, during rearing, due to the metabolism of these insects and their excreted material, greenhouse gases (GHG) and ammonia (NH<sub>3</sub>) can be produced and emitted. A study has been carried out to investigate carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), nitrous oxide (N<sub>2</sub>O) and NH<sub>3</sub> emissions during rearing of BSF larvae. The diet tested were based on agro-industrial by-products: corn stover (CS), residues from vegetable processing (VW) and brewery waste (BW). Three diets were formulated: a cereal-based diet (Gainesville) used as control (C), C+25%CS and BW+25%CS. Black soldier fly larvae (BSFL) were housed in containers (5 replications per treatment) placed in a climate-controlled room. Air flow rate and temperature were set according to rearing conditions used by commercial BSFL rearing farms. Gaseous emissions were determined with dynamic chamber method by Infrared Photoacoustic Detection. BSFL rearing was shown to produce sizeable NH<sub>3</sub> and GHG emissions. Estimated NH<sub>3</sub> losses ranged from 2.15 mg (C) and 8.52 mg (BW) per gram of produced insect crude protein (CP), whereas cumulative GHG emissions averaged 2.68 gCO<sub>2</sub>eq/gCP. A full evaluation of BSFL meal production system, including GHG related to energy used for BSFL rearing and processing as well as to the management of rearing residues, is already under study by our research group.



## Inclusion of calcium-ammonium nitrate in in vitro ruminal fermentation using a wheat forage based substrate

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A randomized complete block design was used to evaluate in vitro ruminal total gas and methane production, NH<sub>3</sub>-N concentration, and digestibility of wheat forage when incubated with calcium- ammonium nitrate (CAN). In vitro fermentations comprised 50 mL of a 4:1 buffer:ruminal fluid inoculum and 0.7 g of substrate [DM; wheat (*Triticum aestivum*; 100%) or wheat:corn (88:12)] incubated for 48 h. Batches were incubated on 3 days. Treatments included: 1) wheat (W); 2) wheat+corn (WC); 3) WC+2% CAN in the substrate DM (WCN); and 4) WC+0.67% UREA in the substrate DM (WCU). In vitro organic matter digestibility (IVOMD) was determined after incubation for 48 h with inoculum, followed by a 48 h incubation with a HCl-pepsin solution. The statistical model included the fixed effect of treatment and random effect of day (block). Total gas production was reduced by WCN compared with WCU and WC ( $P \leq 0.049$ ). The addition of corn without NPN increased ( $P=0.004$ ) total gas production compared to W. An increased ( $P=0.009$ ) IVOMD was observed for WC compared to W, while no differences ( $P=0.416$ ) were observed between WCN and WCU. Ammonia-N concentration was similar ( $P=0.463$ ) between WCN and WCU, and the inclusion of NPN increased ( $P \leq 0.026$ ) NH<sub>3</sub>-N concentration compared to W. Methane production (mmol/g OM fermented) was lesser ( $P < 0.001$ ) for WCN compared to WCU. Total VFA concentration and acetate:propionate were not affected by treatment ( $P > 0.05$ ). Therefore, CAN can potentially be used as a NPN source with the additional benefit of in vitro methane mitigation without negatively affecting IVOMD or total VFA concentration.

Index terms: *Bos taurus*, methane, digestibility

## Inclusion of yerba mate extract levels on lamb diets: methane emissions and animal performance <sup>(1)</sup>

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This study was carried out to evaluate yerba mate (*Ilex paraguariensis* St. Hilaire) extract (YME) as a natural additive and its effect on gas methane production and performance in lambs. Thirty-six male lambs (crossbreed Île de France x Dorper x Santa Inês), weighting  $23.77 \pm 3.70$  kg, were assigned into nine groups (following the initial weight), one of each animal inside of the group were assigned randomly to one of the four treatments (0, 1, 2 and 4% inclusion of YME), according to a randomized block design. The concentrate/roughage ratio was 60/40, body weight was measured every 14 days, dry matter intake was recorded daily and methane emissions were measured by SF6 technique during 5 consecutive days. The data were analyzed using PROC MIXED of SAS and the block like a random effect. The results show a tendency of YME to reduce linearly dry matter intake (DMI), final weight (FW) and daily methane production ( $P=0.0722$ ,  $0.0805$  and  $0.0709$ , respectively). However, daily weight gain (DWG;  $P=0.1526$ ), feed conversion rate (FCR;  $P=0.3848$ ), efficiency of conversion (EC;  $P=0.5015$ ), methane production per kg DMI ( $P=0.9909$ ) and per kg of live weight (LW) ( $P=0.5419$ ) show no differences. Although YME can decrease daily methane production, high levels of this natural additive can affect negatively the DMI. The performance index (FCR and EC) do not show differences because the proportion of total DMI in the experimental period I and body weight gain were the same between the treatments and the same behavior occurred with the proportion of methane produced per kg of DMI and kg of LW.

Terms for indexing: greenhouse gases, lamb production, methane, natural additive, yerba mate.

<sup>(1)</sup>Grant #2017/25319-0 and 2018/10308-6, São Paulo Research Foundation (FAPESP).

## Intensification of tropical pastures for beef cattle production in Brazil: intensity of greenhouse gases - GHG - emission and carbon balance (CO<sub>2eq</sub>)

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The objective of this study was to investigate the CO<sub>2eq</sub> balance in beef cattle production systems in the Atlantic Forest Biome as affected by different levels of pasture intensification. Twenty four steers (*Bos indicus*, Nellore breed) were distributed between four production systems (two replicate): DEG (degraded pasture), IHS (irrigated pasture - high stocking rate), DLS (dryland pasture - low stocking rate) and DHS (dryland pasture - high stocking rate). Intensities of GHG emission were obtained by measuring methane and nitrous oxide emissions in the pastures and enteric methane production by the experimental animals, for two years. The annual C accumulation rate was established by: C stock in the pasture soil minus- C concentration in the native forest soil divided by the number of years of land use as pasture (0-100 cm soil layers, Atlantic forest as reference). The CO<sub>2eq</sub> balance was calculated by: soil C sequestration in the pasture - GHG emissions in the system (the global warming potential by the IPCC, 1996). Data were analyzed by ANOVA and the Tukey test at 5% significance. Treatments DEG, IHS, DLS and DHS presented annual GHG emissions of 2.28, 10.43, 6.92 and 5.28 t CO<sub>2eq</sub>.ha<sup>-1</sup> (±0.65; P<0.05) and annual balance of -6.23, -13.40, 0.14, 1.3 t CO<sub>2eq</sub>ha<sup>-1</sup> (±1.90; P<0.05), respectively. As carcass yield per ha was higher in the irrigated pasture, the emission of CO<sub>2eq</sub>/kg carcass was lower that system than in the degraded pasture. Production systems with intensively managed dryland pastures have greater CO<sub>2eq</sub> sequestration capacity, mitigating emissions and generating C credits.

Keywords: carbon balance, carbon sequestration, beef cattle, emission, greenhouse gases, intensification

## Intensification of tropical pastures for beef cattle production in Brazil: the need for C sequestration by trees to mitigate greenhouse gas emissions.

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The objective of this study was to investigate the need for tree planting in pasture production systems for beef cattle for the mitigation of the emission of greenhouse gases, according to the application of intensification (different uses of correctives, fertilizers and irrigation in degraded pasture). With the results of the C (PAG - IPCC 1996) balance of production systems and with the annual C sequestration rate by the tree bark of *Eucalyptus urograndis* (trees produced under the same conditions) the trees were calculated for the mitigation of emissions of greenhouse gases. DEG (degraded pasture), IHS (irrigated high stock), DHS (dry high stocking) and DLS (dry low stocked) were evaluated for two years in the rearing of twenty-four steers (*Bos indicus* Nellore breed) per year. We performed a variance analysis of the results, with a comparison of means over time by the Tukey test at 5% of significance. For the degraded system (DEG) it would be necessary to plant 101.7 trees.ha<sup>-1</sup> for the mitigation of GHG emission and for the irrigated system (IHS) 218,75 trees.ha<sup>-1</sup>; (DHS) and low stocking rate (DLS), soil C sequestration was able to reduce emissions from the production systems and generate C credits equivalent to 2.34 and 21.27 trees.ha<sup>-1</sup> ( $\pm 37.19$ ,  $P < 0.05$ ). As carcass yield per ha was higher for irrigated pasture, the number of trees/kg carcass was lower than that of pasture degraded for the mitigation of GHG emission.

Keywords: beef cattle, carbon fixation, *Eucalyptus urograndis*, greenhouse gases.

## ***In-vitro* methane production of tropical forage diets under silvopastoral systems on a RUSITEC**

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Colombia has 24,4 million bovines grazing in 34,2 million hectares of pasture lands, emitting 1068Gg of enteric methane to the environment. Silvopastoralism is a technological alternative proven to be productive, efficient and generating associated environmental services incrementing sustainability in cattle production, especially through GHG mitigation at source. We evaluated the effect of different diets under silvopastoral arrangements in the Northern region of Colombia, on methane production and ruminal fermentation parameters under an *in-vitro* ruminal simulation system RUSITEC. Four different diet treatments(T) were considered: T0= 100% *Megathyrsus maximus forage-control*); T1= 70% *M. maximus* + 30% *Leucaena leucocephala*; T2= 70% *M. maximus* + 30% *Guazuma ulmifolia*; T3= 70% *M. maximus* + 30% *Crescentia cujete*). Methane production (mL/day) decreased ( $P<0.001$ ) by 26%, 19% and 29% for T1, T2 and T3, respectively, compared to T0. Neutral detergent fiber degradation (NDFD) was lower ( $P<0.05$ ) for T1, T2 and T3 vs T0. Organic matter degradation (OMD) increased ( $P<0.05$ ) for T1 in comparison to all other treatments. Raw protein degradation (RPD) increased ( $P<0.05$ ) for T1 vs T2 (15%) and T3 (18%). There was no effect on Volatile fatty acid production ( $P<0.05$ ). In conclusion, incorporation of tree forage resources into bovine diet reduces *in vitro* methane production and improves some ruminal fermentation parameters.

**Keywords:** Ruminal fermentation, enteric methane, silvopastoral diets

## ***In vitro* pure cultures of methanogenic archaea exhibit different characteristics between species and incubation media**

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In vitro incubations were conducted to characterize *Methanobrevibacter gottschalkii* Ho and *M. ruminantium* M1, incubated on growth media DSMZ 1523 (DSMZ GmbH, 2015) and DSMZ 119 (DSMZ GmbH, 2017). The experiment was conducted as a completely randomized design with a 2 × 2 factorial arrangement, plus 2 controls. Factors were methanogens species (*M. ruminantium* M1 or *M. gottschalkii* Ho), and growth media (DSMZ 1523 or DSMZ 119), and controls consisted of each growth media without inoculation with archaea culture. Media preparation, incubation, and growth conditions were based on recommendations by DSMZ, with modifications by Hackmann (2016). Incubations were conducted in triplicate. The model included the fixed effect of treatment and the random effect of incubation. Data were analyzed using the PROC GLIMMIX of SAS. *Methanobrevibacter ruminantium* M1 grew more slowly ( $P < 0.01$ ) than *Methanobrevibacter gottschalkii* Ho, particularly when incubated in DSMZ 1523 ( $P = 0.02$ ). *Methanobrevibacter gottschalkii* Ho had the greatest ( $P = 0.01$ ) CH<sub>4</sub> production when incubated in DSMZ 119 but was not different from M1 when incubated in DSMZ 1523. There was a species × media interaction ( $P < 0.05$ ) for the C isotopic signature of both the microbial pellet and the supernatant at the end of the incubation, as well as a tendency ( $P = 0.09$ ) for a species × media interaction for the C isotopic signature of CH<sub>4</sub>. Methanogens have differential growth responses between species and react differently to the same growing conditions in vitro.

Index terms: *Methanobrevibacter gottschalkii* Ho, *Methanobrevibacter ruminantium* M1, archaea, methanogens, methane, isotopic signature

## Long term effects of oilseed supplementation on methane emissions of grazing dairy cows<sup>(1)</sup>

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Oilseeds can decrease methane emissions of pasture and TMR fed dairy cows. The aim of this study was to evaluate the effects of supplementation of unprocessed oilseeds on methane emissions of grazing lactating dairy cows in a long term experiment (22 wk). In a randomized block design, 60 multiparous Holstein Friesian cows were allocated to 1 of 4 treatments: concentrate without oilseeds (6 kg/d, control), or concentrate with whole cottonseed (6 kg/d), rapeseed (5.3 kg/d) or linseed (5.5 kg/d), all on an as-fed basis. Concentrates were iso-caloric and iso-nitrogenous and the linseed, rapeseed and cottonseed concentrates contained 55, 53 and 56 g EE/kg of diet DM, respectively, compared to 34 g EE/kg diet DM of the control. All cows strip-grazed a perennial ryegrass-based sward. On weeks 9 (spring) and 20 (summer), methane emissions were estimated for 5 days using the SF<sub>6</sub> technique. Data were analysed by ANOVA separately for each season. There were no effects of the treatments on methane emissions expressed either as total methane production ( $P = 0.12$  and  $P = 0.61$  for spring and summer, respectively) or methane per unit of milk yield ( $P = 0.20$  and  $P = 0.09$ , in the same order). Lack of effects could be explained by the lack of processing of oilseeds, low level of lipid supplementation, or rumen adaptation to diets. In conclusion, the inclusion of whole oilseeds at the levels evaluated in this study was not an effective long term strategy for decreasing methane emissions of grazing dairy cows.

Keywords: oilseeds, methane, dairy cow, pasture, grazing, concentrate supplementation

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## ***Asparagopsis armata* added to lactating dairy cows' diet reduces enteric methane intensity by 60 percent**

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*In vitro* studies have shown considerable reductions in methane emissions using *Asparagopsis* spp. This is the first study investigating the effect of any species of *Asparagopsis* in dairy cattle *in vivo*. Our objective was to quantitatively evaluate the response of cows consuming *Asparagopsis armata* on methane production. Twelve post-peak lactating Holstein cows were randomly assigned to three treatments (control, 0.5% and 1% inclusion levels of *A. armata* on organic matter basis) in a 3×3 Latin square design with three 14-day periods. Enteric methane emissions were measured using the GreenFeed system. Methane production (g/d) decreased significantly by 26.4% at the low (0.5%) level of *A. armata* inclusion and 67.2% at the high (1%) level of inclusion. Hydrogen production increased 163 and 236% with low and high levels of *A. armata* inclusion, respectively. Feed intake was reduced by 10.8 and 38.0%, respectively, in low and high levels of *A. armata* inclusion. Methane yield (g/kg DMI) decreased significantly by 20.3 at low level and 42.7% at high level ( $P = <0.0001$ ). Hydrogen yield significantly increased with the addition of *A. armata* by 55.5 and 78.9% at low and high *A. armata* inclusion levels, respectively ( $P = <0.0001$ ). Methane intensity (g/kg milk yield) significantly decreased by 26.8% at low levels and 60% at high levels of *A. armata* inclusion. Bromoform concentrations in milk were not significantly different between treatments. Our *in vivo* results showed that *A. armata* has potential to be used as a feed additive to reduce enteric methane emissions.

Keywords: bos taurus, dairy cows, *Asparagopsis*, seaweed, enteric methane, greenhouse gas



## ***Plantago lanceolata* use as mitigation strategy for reducing nitrous oxide emissions from cattle urine patches in New Zealand pastureland**

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Urine deposited by grazing animals is the main source of nitrous oxide (N<sub>2</sub>O) emissions in New Zealand. Recent studies have suggested that certain pasture plants, for example plantain (*Plantago lanceolata*), can curb N<sub>2</sub>O emissions from livestock systems. This study aimed to i) evaluate the potential of plantain for reducing N<sub>2</sub>O emissions from cattle urine patches; ii) determine the effect of including plantain in animal diets on urine-N loading and its influence on N<sub>2</sub>O emissions; and, iii) evaluate whether any effects on N<sub>2</sub>O emissions reduction could be attributed to a 'urine' or a 'plant' effect. A static chamber method was used to measure N<sub>2</sub>O fluxes from urine that was collected from cows fed on a 0, 15, 30 or 45 % plantain diet. This urine was poured onto plots with the corresponding percentage of plantain in the sward. In addition, we measured N<sub>2</sub>O emissions from field plots with different proportions of plantain in the sward (0, 15, 30, 45, 60 and 100%), that received urine collected from cows fed on "standard" ryegrass/clover swards (*Lolium perenne*/*Trifolium repens*). Following applications of standard urine, nitrous oxide emissions reduced linearly as the proportion of plantain in the sward increased ( $r^2 = 0.969$ ). Urine-N loading rate was significantly lower when animals were fed on plantain than on ryegrass/clover ( $r^2 = 0.987$ ). There was a clear trend of lower N<sub>2</sub>O emissions with increasing plantain in the diet, although differences in emission factors were not statistically significant ( $P > 0.10$ ). The results suggest that the efficacy of plantain is due to both a reduction in urinary N excretion and a plant effect, the latter possibly due to biological nitrification inhibition (BNI) caused by the release of root exudates.

Keywords: Plantain, BNI, Greenhouse gases, Ammonium, Nitrate.

## Methane emission of young bulls grazing Marandu palisadegrass in different levels of pasture intensification

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We hypothesized that the improvement of grazing management by pasture fertilization will reduce enteric methane emission, contributing to its mitigation in Brazilian production systems. The experimental area consisted of 24 ha of Marandu palisadegrass pasture (*Brachiaria brizantha* 'Marandu') divided into 12 paddocks of approximately 2 ha each. The treatments consisted of three levels of intensification: zero (0N, no fertilization), moderate (75 kg N/ha/year; 75N) and high (150 kg N/ha/year; 150N), in a completely randomized design with four replicates (paddocks). The stocking rate over the experimental period was 1.7 (0N), 3.0 (75N) and 3.7 (150N) animal unit (animal unit = 450 kg)/ha. The source of N fertilizer was ammonium nitrate. Pasture were managed under continuous stocking to maintain grazing height fixed at 25 cm during the growing season, using the put-and-take methodology. Forty-eight tester and 31 put-and-take animals (initial body weight, BW of 295±16 kg) were used during rainy season that lasted from Nov 2018 until May 2019. The enteric methane emission was measured in 24 animals (8 per treatment) using the sulfur hexafluoride tracer-gas technique, in March 2019. The average daily gain (ADG) increased linearly ( $P < 0.0001$ ) from 0.73 (0N) to 1.00 (150N) kg/d with increasing level of intensification. However, methane production did not differ among treatments ( $P > 0.13$ ) and was in average 167.5 g CH<sub>4</sub>/d or 212.6 g CH<sub>4</sub>/kg ADG. Results indicate no effect of intensification achieved through N fertilization on individual enteric methane emission. São Paulo Research Foundation (FAPESP) (grant # 2017/18750-7; 2018/26492-0).

Keywords: ammonium nitrate, continuous stocking, nitrogen, pasture, sulfur hexafluoride

<sup>(1)</sup>Research funded by São Paulo Research Foundation (FAPESP) (grant # 2017/18750-7; 2018/26492-0).

## Methane emissions in response to supplementation in beef-cattle fed a tropical grass-based in Colombian Caribbean<sup>1</sup>

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In Colombian Caribbean, livestock base-diet is tropical-pastures. Finding the best strategy for feeding supplements is important to enhance the animal performance. The objective of this study was to evaluate the effects of energetic-protein supplementation (ENS) on growth, bodyweight (BW) and enteric methane emission of beef cattle grazing pastures. Twenty Brahman steers (474±34,05 kg) were distributed in four paddocks of six hectares each under a continuous stocking grazing system and assigned to a completely randomized design with four treatments: T1: grazing on *Cynodon nlemfluensis* (CN), T2: grazing on *Brachiaria hybrid* (BH); T3: CN+ENS and T4: BH+ENS. T3 and T4 received 1.3 kg.day<sup>-1</sup>.animal<sup>-1</sup> of supplement during two hundred seventy seven days. The methane was measured and collected in the last month before slaughter during three days at 6, 12 and 18 Hours using the laser methane detector (LMD). Daily weight gain (DWG), dry matter intake (DMI), emission factor (EF), grams of CH<sub>4</sub> per kilograms of BW (CH<sub>4</sub>BW), grams of CH<sub>4</sub> per kilograms of dry matter intake (CH<sub>4</sub>DMI), and net energy loss as CH<sub>4</sub> (YM), were evaluated. Statistical analysis was carried out using GLIMMIX-SAS. A significant effect by treatment diet (P< 0.05) was observed on performance animal (DMI, DWG) and methane emissions (CH<sub>4</sub>gd, CH<sub>4</sub>DMI, CH<sub>4</sub>LWG, and YM); obtained the best response with BH+ENS, increased above-average DMI 11.37%; and DWG by 3.05%. CH<sub>4</sub>DMI decreased by 49.1% and CH<sub>4</sub>BW by 44.8%, representing lower energy losses by 57.2% with YM = 5.01%. ENS animals improves beef production and reduces methane emissions compared to traditional grazing system.

Keywords: Beef cattle, greenhouses gases, supply, grazing

<sup>1</sup>Project from internal agenda, supported by Agrosavia.

## Methane emissions related to meat production by cattle grazing on pastures based in *megathyrsus maximus*<sup>1</sup>

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Agrosavia has developed a meat production system based on Romosinuano creole cattle crosses and grazing on improved pastures of *Megathyrsus maximus*, located in Sinú Valley, Colombia. The aim of this study was evaluating the enteric methane emissions related to this meat production system. Fourteen F1 Romo x Brahman (450.5 ± 37.18 Kg), were grazing on two *Megathyrsus maximus* pastures (Mombasa and Sabanera) under a rotational system with three occupation days and 28 resting days. Three methane (CH<sub>4</sub>) sampling during a 10-day period were taken in the last month before slaughter, measurements were collected 4 times during the day (5am, 9am, 1pm, 5pm), using the laser methane detector (LMD). Daily weight gain (DWG), dry matter intake (DMI), grams of methane per day (CH<sub>4</sub>gd), grams of CH<sub>4</sub> per kilograms of live weight (CH<sub>4</sub>LW), grams of CH<sub>4</sub> per kilograms of live weight gain (CH<sub>4</sub> LWG), grams of CH<sub>4</sub> per kilograms of dry matter intake (CH<sub>4</sub>DMI), and net energy loss as CH<sub>4</sub> (YM), were evaluated. Data were analysed by random design using the mixed procedure of SAS. The results showed that F1 animals grazing on both pastures were similar in DMI (8,8 Kg/d) and DWG (0,71 Kg). However, the animals, grazing on Sabanera reduced (p<0.0009) CH<sub>4</sub>LW by 4.98% and YM by 4.36% and consequently representing a methane emission reduction of 7.9% in this meat production system. These results confirm that grazing on improved pastures are essential to ecosystem functioning, contribute to lead to reductions in GHG emissions and drive to sustainable meat production.

Keywords: Sustainable, Green houses gases, Guinea, Romosinuano x Brahman.

<sup>1</sup>Project from internal agenda, supported by Agrosavia.

## Nitrous oxide and methane emissions from dung patches in tropical conditions

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In Colombia, cattle production is commonly based on monoculture pastures and farms with low productivity indicators, including nutrients use inefficiency. Poor nutritional balances generate loss of nutrients that are excreted in manure, contributing to emissions of greenhouse gases (GHG). This study aimed at evaluating methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) emitted by dung collected from cows that grazed two types of diet (*Brachiaria humidicola* and *B. humidicola* + *Arachis pintoi*). Dung patches were simulated in plots located in each grazing system. Soil GHG emissions from control (water added) and dung patches were monitored over a 43-day period through the static chamber technique. Milk production by cattle in the two pastures was quantified. The abundance of ammonia-oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA) was estimated in soil samples collected before grazing. The potential nitrification was estimated through a soil incubation experiment. The results showed that the associated *B. humidicola* + *A. pintoi* pasture generated less cumulative CH<sub>4</sub> and N<sub>2</sub>O fluxes from dung patches and better milk productivity compared to the *B. humidicola* pasture. Therefore, the monoculture presented higher emission factors for N<sub>2</sub>O and CH<sub>4</sub> (0.09% EF-CH<sub>4</sub><sup>+</sup>; 0.008% EF-N<sub>2</sub>O) than the legume-grass pasture (0.004% EF-CH<sub>4</sub>; 0.003% EF-N<sub>2</sub>O). There was no difference between the nitrification rate in both grazing systems and the abundance of AOA and AOB was low for the compared systems. Our findings suggest that the association of *B. humidicola* with *A. pintoi*, constitutes a mitigation strategy for greenhouse gases from deposited dung patches in soil.

Keyword: Legume, Nitrification rate, Emission Factor, dung patches, milk production

## Pearl-millet grazing decreases daily methane emissions in dairy cows receiving total mixed ration

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Tropical pastures for dairy cows receiving total mixed ration (TMR) deserves further studies. This work aimed to evaluate the enteric methane emission from dairy cows receiving TMR exclusively (control), 75%TMR + pearl-millet (*Pennisetum glaucum* cv. Campeiro) pasture (TMR75) or 50%TMR + pearl-millet pasture (TMR50). Nine Holstein and F1 Jersey × Holstein multiparous cows averaging 136±40 days in milk and 520±41 kg body weight were distributed in a Latin square 3×3. The methane emission was measured using the SF6 and herbage intake by N-alkanes technique. Cows in TMR75 and TMR50 treatments strip grazed pearl-millet pastures between morning and afternoon milking (7 h/day). The crude protein and NDF content of TMR were 149 and 340 g/kg DM, respectively; for the pearl-millet pasture were 200 and 646 g/kg DM. The total DM intake was 19.4 kg/day in dairy cows receiving TMR exclusively and decreased linearly ( $P < 0.01$ ) to 18.4 and 18.2 in the TMR75 and TMR50 treatments, respectively. The daily methane emission decreased linearly ( $P < 0.01$ ) from 543 g/day (control treatment) to 495 and 451 g/day on TMR75 and TMR50, while the daily methane yield and methane intensity were similar between treatments, averaging 26.5 g/kg DM intake and 21.9 g/kg milk. The energy losses as methane in relation to the energy intake did not differ between treatments, averaging 7.91%. Dairy cows grazing pearl-millet pasture and receiving up to 50% of TMR “*ad libitum*” did not change daily methane yield and intensity, but decreased daily methane emissions.

Terms for indexing: *Pennisetum glaucum*, *Bos taurus*, dairy cows, grazing, methane emission, methane intensity and tropical pasture.

## Performance and methane emissions of lambs in two feeding systems in summer and winter pastures<sup>(1)</sup>

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The Brazilian sheep farming system is pasture-based, with different feeding and weaning strategies for lambs. We hypothesized that different feeding systems, commonly used in South of Brazil, can promote different animal performance and affect the methane emissions per kg of liveweight gain (LWG). We evaluated the lamb methane emissions in summer (2014) and winter (2016) pastures. The experimental design was a randomized complete block with two feeding systems and four replicates: (S1) lambs weaned at 60 days of age and supplemented with concentrate (2% LW.day<sup>-1</sup>) until slaughter at 40 kg LW; (S2) no weaned and no supplemented lambs until slaughter at 40 kg LW. 24 White Dorper x Suffolk lambs were studied for each feeding system in both years. The forage species were *Cynodon* spp (38.5%). and *Paspalum* spp. (28.5%) (summer); *L. multiflorum* Lam. (53%) and *A. strigosa* (21.5%) (winter). The CH<sub>4</sub> was collected by SF<sub>6</sub> tracer technique. Analysis of variance was performed by a GLM procedure. Weaned and supplemented lambs produced more methane (P=0.048) in summer (S1 - 288±74.8 x S2 - 106.3±17.1 g.kg LWG<sup>-1</sup>) with no difference (P=0.73) in winter (S1 - 90.1 ±15.7 x S2 - 78.1±14.2 g.kg LWG<sup>-1</sup>). In summer, live weight gain was 133 g/d for S1 and 45 g/d for S2 lambs (P=0,016) where as in winter, LWG were 195 and 140 g/d (P=0,04), respectively for S1 and S2 lambs. We concluded that methane emission can be modified by the feeding system, closely related to liveweight gain, in summer and winter pastures.

Keywords: Greenhouse gases, *Ovis aries*, production systems, sheep

## Plant extracts reduced enteric methane emission with increased growth performance and nutrient digestibility in Merino sheep<sup>(1)</sup>

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Plant extracts (PE) have the tendency to reduce methane emission from ruminants and are effective against pathogenic bacteria, disease and methanogens which impede growth and animal performance. This study tested the effectiveness of *Moringa oleifera* (MO), *Jatropha curcas* (JA) and *Aloe vera* (AV) PE on feed digestibility, methane emission, and growth performance of South African Mutton Merino sheep. Forty 15-week old Merino lambs were ranked according to their body weight and blocked into 4 treatment groups. Methanolic PE of MO, JA and AV were prepared and reconstituted by adding 96 g of each to 120L distilled water to produce plant extract solutions (PES). Lambs were housed and fed total mixed ration (42% roughage, 17% Soybean, 28% maize) and PES administered twice a day using drench gun at the recommended application rate of 50 mg/kg DM feed while the control group received distilled water. Methane emission was measured using open circuit respiratory chamber. Oral drenching of MO, JA and AV PES increased ( $P<0.05$ ) dry matter and crude protein digestibility of lamb when compared with the control. MO, JA and AV PES reduced ( $P<0.05$ ) methane emission from lambs by 21, 35 and 28%, respectively. Average daily weight gain of lambs on MO and JA PES were higher by 15.2 and 5 % respectively compared to the control. Application of 50 mg MO and JA PES/kg DM feed did not affect negatively rumen fermentation parameters and hence can be used as an alternative additive to reduce methane emission and improving animal performance.

Keywords: Nutrient digestibility, *Moringa oleifera*, *Jatropha curcas*, *Aloe vera*



## Potential anti-methanogenic effects of barley based finishing rations compared to maize using prediction pathway analysis

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Holstein Friesian bulls (n=120), blocked on the basis of age and liveweight, were offered one of four diets in a 2 (cereal type) x 2 (level of supplementary fat) factorial designed study. Cattle were offered either a (i) barley or (ii) maize based (70% of DM) dominant concentrate ration which was, in turn, supplemented with a partially rumen inert palmitate based at either zero (-SF) or 5%(+SF) of DM. Diets were offered on an ad libitum basis from 10 to 16 months of age at which time the animals were slaughtered. Feed intake was recorded for 70 days towards the end of the experimental period. Rumen fluid samples were collected from 15 animals across all four treatments, via stomach intubation, at the start and end of the feed intake recording period. Rumen microbial DNA was extracted with 16S rRNA amplicon libraries generated and sequenced on the Illumina MiSeq. Sequence analysis was carried out using DADA2 with predicted functional pathway analysis conducted using CowPI. A significant reduction in the abundance of archaea was observed in samples obtained from barley-SF compared to the maize-SF fed bulls (adj P <0.05). Treatment had a significant impact on pathways predicted to be associated with methane, with a higher methanogenic potential predicted in maize+SF compared to barley-SF fed bulls (adj P<0.05). In addition, regardless of SF, samples obtained from barley fed bulls had a reduced predicted methanogenic potential. This analysis suggests a greater potential methanogenic inhibitory capacity for barley compared to maize dominant finishing rations.

Index terms: bos taurus, beef production, rumen microbiome

## Potential of *Brachiaria humidicola* and dicyandiamide for reducing nitrous oxide emissions from cattle urine patches in the subtropical climate of Brazil

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Nitrous oxide (N<sub>2</sub>O) emissions from pasture-based livestock systems contribute about 34% of Brazil's agricultural greenhouse gas emissions. A field and a greenhouse experiment were conducted to (i) evaluate the potential of *Brachiaria humidicola*, a forage species known for its ability to inhibit soil nitrification, to reduce N<sub>2</sub>O emissions from urine patches, and (ii) determine the efficacy of the synthetic nitrification inhibitor dicyandiamide (DCD) to decrease N<sub>2</sub>O emissions. The field experiment was carried out in a split-plot experimental design, with forage species (*Brachiaria* and Guinea grass) as main plot treatments and cattle urine and DCD treatments (No urine, Urine and Urine+DCD) as subplots. Over the 67-day measurement period, N<sub>2</sub>O emissions were 20% lower from urine patches in the *Brachiaria* treatment compared to guinea grass ( $P < 0.10$ ). The results of the greenhouse experiment, using pots with either *Brachiaria* or guinea grass plants that received the same treatments as in the field experiment, suggested that this could have been due to lower soil nitrate (NO<sub>3</sub><sup>-</sup>) concentrations and intensities under *Brachiaria* forage than in guinea grass. This indicates that biological nitrification inhibition (BNI) could be a possible mechanism for lower N<sub>2</sub>O emissions from *Brachiaria*. The DCD application was effective in both forage species, decreasing N<sub>2</sub>O emissions by 40-50% ( $P < 0.10$ ) compared with the urine only treatment. Overall, the results demonstrated that *Brachiaria* and DCD use are strategies that can reduce N<sub>2</sub>O emissions from urine patches.

Keywords: BNI, Forage species, Livestock, Ammonium, Nitrate

## Potential of selected forages for reducing nitrous oxide emissions from New Zealand dairy systems

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To examine the use of selected forages to reduce the impact of nitrous oxide (N<sub>2</sub>O) emissions from New Zealand dairy farms, an integrated modelling assessment was conducted for the Waikato, Canterbury and Southland regions of New Zealand using the farm-scale models FARMAX<sup>®</sup> and OVERSEER<sup>®</sup>. Mid-intensification dairy farms were modelled, and six scenarios were examined; a Baseline scenario, two diverse pasture options (20 and 50% of the farm area; DP20 and DP50), two fodder beet options (10 and 20% of the farm area; FB10 and FB20) and a combined option (DP20+FB10). In addition to perennial ryegrass / white clover, diverse pastures also contained an extra grass and legume specie, and two forbs. Within each region, all scenarios were assumed to have similar pasture yields. The modelling assessment suggested that including diverse pastures and/or fodder beet in the diet of dairy cows could reduce total and paddock N<sub>2</sub>O emissions by up to 16 and 21%, respectively. However, the full N<sub>2</sub>O mitigation benefits from the inclusion of forbs in pastures and fodder beet were not assessed, since differential and seasonal plant N uptake, niche complementarity and root architecture remain to be fully captured by the modelling.

Keywords: Fodder beet (*Beta vulgaris*), prairie grass (*Bromus willdenowii*), chicory (*Cichorium intybus*), plantain (*Plantago lanceolata*), lucerne (*Medicago sativa*), red clover (*Trifolium pratense*).

## Promising Nutritional Strategies to Reduce Enteric Methane Emission from Ruminants – a Meta- Analysis<sup>1</sup>

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The present meta-analysis examined the effects of mitigation strategies on absolute methane emission (g/d) and yield [g methane/kg dry matter intake (DMI)], DMI (kg/d), average daily gain (ADG; kg), milk production (kg/d), and neutral detergent fiber digestibility (%). Only studies that reported measures of variance were included in the analysis [295 studies (66% cattle, 31% small ruminants, 3% other ruminants), 644 mean comparisons (treatment vs. control), and 4,087 observations]. A standard random-effects meta-analysis weighted by inverse variance was carried out. Mitigation effects based on the standardized mean difference (SMD; mean difference divided by standard deviation) were classified as small ( $-0.5 < \text{SMD} \leq -0.2$ ), medium ( $-0.8 < \text{SMD} \leq -0.5$ ), and large ( $\text{SMD} \leq -0.8$ ). Compared to control, inclusion of chemical inhibitors, alternative electron sinks, and lipids had large statistically significant effects (all  $P < 0.001$ ) on both absolute methane emission ( $\text{SMD} \pm \text{SE} = -2.1 \pm 0.5$ ,  $-1.6 \pm 0.2$ , and  $-1.3 \pm 0.2$ , respectively) and yield ( $\text{SMD} \pm \text{SE} = -1.8 \pm 0.3$ ,  $-1.2 \pm 0.2$ , and  $-1.0 \pm 0.1$ , respectively). The inclusion of chemical inhibitors did not impact animal productivity

(milk production and ADG;  $P > 0.15$ ), whereas electron sinks and lipids led to small decreases in DMI (SMD  $\pm$  SE =  $-0.2 \pm 0.1$ , and  $-0.4 \pm 0.1$ , respectively;  $P \leq 0.01$ ) without affecting animal productivity ( $P > 0.05$ ). Although these nutritional strategies effectively reduced methane emission without compromising animal productivity, their practical implementation will depend on proven long-term effects, economic feasibility, regulatory mechanisms, and consumer acceptance.

Terms for indexing: Cattle, sheep, goat, mitigation, CH<sub>4</sub>

<sup>1</sup>This study is part of the Joint Programming Initiative on Agriculture, Food Security and Climate Change (FACCE-JPI)'s "GLOBAL NETWORK" project and the "Feeding and Nutrition Network" (<http://anialsience.psu.edu/fnn>) of the Livestock Research Group within the Global Research Alliance for Agricultural Greenhouse Gases ([www.globalresearchalliance.org](http://www.globalresearchalliance.org)).

## Replacing urea with nitrate increase growth and reduce enteric methane production in sheep<sup>(1)</sup>

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Use of non-protein nitrogen (NPN) is a useful strategy to increase the nutritional value of protein-deficient diets. Urea is the most common NPN source but replacing it with nitrate may offers additional benefits in methane reduction, whereas the concurrent use of NPN and tannin extract offer potential for modulating rumen nitrogen metabolism. This study investigated the effect of urea or nitrate with or without the inclusion of Acacia tannin extract on dry matter intake, digestibility, growth and methane emission of sheep. Forty Merino lambs (95 days old) were randomly allocated to four groups fed diets containing: urea; nitrate; urea + tannin; nitrate+ tannin (tannin, 42 g/kg feed). The lambs were adapted to the diets for 21 days followed by sixty days of measures; methane was measured on open-circuit respiratory chambers. Dry matter and nutrient intake were not influenced by diets with the exception of the crude protein intake, which was higher ( $p < 0.05$ ) in lambs fed urea-based diets. A higher average daily gain (13 %) and lower methane emission (16 %) were recorded for lambs fed nitrate-based diets compared to those fed urea-based diets. In contrast, the inclusion of tannin reduced the final body weight and average daily gain of the lambs. Most haematology and serum variables were not affected by NPN source or tannin inclusion. This study demonstrated that nitrate could be safely used as a source of non-protein nitrogen with the additional benefit of reducing enteric methane emission and improving the average daily gain of sheep.

**Keywords:** Growth performance, Methane emission, Nitrate, Non-protein nitrogen, Serum biochemistry, Tannin

## Response to combination of yeast and oregano oil on feed degradability and methanogenesis<sup>1</sup>

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In recent years, a variety of plant bioactive compounds have been evaluated as rumen manipulators. Although the potential of essential oils to increase productivity and decrease methane emissions has been shown, adverse effects on fiber digestion and fermentation have been reported. In this study was tested a novel additive based on a Colombian native strain of yeast and oregano oil (rich in thymol, 70%) for its long-term effect on feed degradability and methanogenesis. The Rumen Simulation Technique was used to study the effect of a control diet and supplemented with yeast (*Meyerozyma guilliermondii*, 10<sup>9</sup> nonviable cells) plus 0.25% p/p of oregano oil (*Lippia origanoides*-Kunth) at 0.5 g.L<sup>-1</sup>. A single incubation period using 8 vessels inoculated with rumen fluid from four different cows (four replicates) was carried out. The experiment lasted for 16 days, using the first 12 days for adaptation and the last 4 for sampling. Fermentation gases were collected in gas-tight bags to measure total gas and methane production. Dry matter disappearance after 48 h incubation was calculated from the loss in weight. The residue was then analysed for organic matter (OM), nitrogen (N), Neutral-detergent (NDF) and Acid-detergent fibre (ADF) to determine nutrient disappearance. Data were analysed statistically by randomized block ANOVA, with individual cows as a blocking term. The novel additive decreased total gas (P=0.038) without affecting feed digestibility (P>0.05); a numerical decrease in methane was also observed. Our results showed the potential of the combination of yeast and oregano oil as feed additive to reduce gas emissions.

Keywords: Greenhouses gases, Yeast *Meyerozyma guilliermondii*, *Lippia origanoides*

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## Rumen dissolved gases saturation and enteric gas emission unaffected by forage particle sizes in goats

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This study investigated the effect of forage particle sizes on the saturation factor of rumen dissolved gases, microbial community and enteric gas emission in goats. Ten Xiangdong goats of average bodyweight of 37±5kg were randomly assigned to two treatments in a cross-over design. The animals were fed concentrate and forage with a theoretical cut length of 100 mm and 20 mm, respectively. The 100 mm forage showed a lower density (216.2vs237.3 g/L) and longer total chewing time, rumination count and bout/time (P<0.05) compared to the 20 mm, but the total tract nutrient digestibility was not significantly different. Although not significantly different from the 20 mm treatment, the 100 mm forage reduced the saturation factor of dissolved H<sub>2</sub> and CH<sub>4</sub> in the liquid phase (8.27, 4.81 vs 10.18, 5.29) and the H<sub>2</sub> and CH<sub>4</sub> concentrations increased in the rumen headspace. The CH<sub>4</sub> emission/kg of DM digested followed the same trend as observed in the rumen headspace. The rumen pH, total VFA and their specific molar percentages were not affected by the treatments (P>0.05). However, the 20 mm diet significantly increased ammonia concentration in the rumen liquids (+10.27%), the copy numbers of fungi (+2.67%), *Selenomonas ruminantium* (+1.44%), *Ruminococcus amylophilus* (+1.57%) and *Ruminococcus flavefaciens* (+4.36%). In conclusion, the two treatments stimulated the animals' rumen physiological functions in almost the same manner despite the vast difference in the diets' particle size. Nevertheless, from the evolution trend of dissolved gases in the present study, smaller particle sizes might significantly reduce the rate of enteric methane emission per time.

Index Terms: dissolved methane, hydrogen, feed physical structure, reticulo-ruminal contractions, effective factor.



## Rumen fermentation and microbial changes in grazing heifers treated with 3- nitrooxypropanol under tropical conditions<sup>(1)</sup>

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The aim of this trial was to study the effect of the methane inhibiting compound, 3-Nitrooxypropanol (3-NOP), an investigational product, on rumen metabolites and microbial community in cattle grazing tropical forage. Approximately 2 months prior to calving forty pregnant heifers were allocated to two groups and maintained in replicate paddocks at Lansdown Research Station (Australia). Each group received a treatment for 6 months: 3- NOP (2.5 g / animal/ day (DSM Nutritional Products, Switzerland; application form contains 10% 3-NOP mixed with silicon dioxide and propyleneglycol) or as a placebo control silicon dioxide/ propyleneglycol (50/50) (23 g placebo / animal/ day). The treatments were first mixed with steam flaked barley (88.8%) and molasses (7.4%) and offered daily at 8:00 and 14:00 h (675 g/head) to the respective groups. Rumen fluid samples were collected by stomach intubation at 3 h post feeding the morning treatment at 1.5, 4 and 6 months to determine the effect on rumen fermentation parameters and rumen microbial communities. No significant differences were observed on live weight between 3-NOP and control groups. Overall, increases in rumen formate, ammonia and all VFAs concentrations (except acetate) were observed in 3-NOP supplemented animals. Significant differences ( $P < 0.05$ ) in rumen bacterial composition between 3-NOP and control animals were observed with increases in *Prevotella* and *Succiniclaticum* and decreases in methanogenic species in the 3-NOP supplemented group. The response to 3-NOP feeding appears consistent with the methane inhibiting mode of action of the compound and elevated hydrogen levels observed in previous studies.

Index terms: methane inhibitor, microbiota, hydrogen sinks, volatile fatty acids.

## Small ruminants' methane emissions from biodiesel coproducts as alternative feed

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Reducing edible food used in livestock and enteric CH<sub>4</sub> emission are desirable as for food security and climate issues. The goal was to evaluate CH<sub>4</sub> emission (CH<sub>4</sub>e) for biodiesel coproducts (BC) replacing traditional feed (TF). Sheep were fed either with Control diet (CN: corn (28%), soybean meal (12%) and Tifton hay (TH, 60%)) or Coproduct diet (CP: sunflower meal (18%), corn (16%), cottonseed meal (6%) and TH (60%)) in two 21-day metabolism trial with 3 days' gas-collect chambers assay. Gases were sampled, CH<sub>4</sub> (%) was quantified using gas chromatography and outgoing air flow rate determined for measuring CH<sub>4</sub>e. Organic matter intake (OMI) and dry matter (DMI) intake were determined. ANOVA and Tukey test were performed. No differences ( $P < 0,05$ ) were observed for CH<sub>4</sub>e (CN = 15,7; CP = 17,0 L/day) as well CH<sub>4</sub>/DMI (CN = 11,8; CP = 14,0 L/kg) and CH<sub>4</sub>/OMI (CN = 15,3; CP = 19,4 L/kg). Replacing TF by BC at the proportion evaluated reduced CH<sub>4</sub>e per non edible food intake by 15% in metabolism trial. It is expected similar production efficiency, once there were no statistical differences on nutrients digestibilities.

Index terms: Ruminant sustainability, non-edible food, sheep.

## Soil Carbon Content of Canadian Native Grassland Improved by Grazing Management and Higher Species Diversity

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Study objective was to determine the effect of grazing management and native mixtures on soil carbon content (SOC) of a re-established native Canadian grassland. Native mixtures were seeded in 2001 on 16 paddocks of 2.1 ha that had cropland history of wheat and fallow. Study (2000-2017) was completely randomized (rep = 2) with Simple (7 species) and Diverse (12 species) native mixes and continuous (CG) and deferred-rotational grazing (DRG) systems. Paddock contained a ungrazed enclosure and were grazed (50% utilization) by steers ( $344 \pm 17$  kg) from 2001-2017. The CG and DRG stocking rates were 0.7 and 1.8 AU ha<sup>-1</sup>, respectively. Plant biomass, litter and dung were measured. Bulk density and SOC measurements occurred at three or five micro-sites per paddock at four depths (0-15, 15-30, 30-45 and 45-60 cm). For 2000 to 2015, most of the SOC change was in the upper 30 cm. Additional SOC between 30-45 cm and 45-60 cm was most pronounced in 2017. By 2017, most of the SOC change was below 30 cm. Average C sequestration rates from 2000 to 2017 were 0.24 and 0.34 Mg C ha<sup>-1</sup> yr<sup>-1</sup> to 45- and 60-cm, respectively. Ungrazed treatment gained more SOC from 2000 than the grazed, with effect more pronounced the diverse mix. Grazed had 0.3 to 0.8 Mg C ha<sup>-1</sup> more above ground C in form of plant biomass, litter and dung. The SOC at depths below 30 cm are important to account for in native grasslands and more long term research is needed.

**Keywords:** Soil organic carbon, Native grasslands, Grazing systems, Biodiversity

## Starch resulted in greater recovery of metabolic hydrogen than cellulose in methanogenesis inhibited rumen batch cultures<sup>(1)</sup>

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The objective of this  $2 \times 2 \times 2$  factorial arrangement experiment was to understand the effects of inhibiting methanogenesis in rumen batch cultures growing with different sources of energy (cellulose or starch) and nitrogen (ammonium or trypticase) on fermentation and microbial synthesis of protein and amino acids. 9, 10-anthraquinone was chosen as a model inhibitor based on previous results suggesting the greatest disruption of electron relocation among various methanogenesis inhibitors. Cultures were incubated for 96 (cellulose) or 72 (starch) h. 9, 10-anthraquinone decreased total gas production with cellulose but increased it with starch, and decreased methane production and increased dihydrogen accumulation with both substrates. 9, 10-anthraquinone increased final pH and acetate molar percentage with cellulose and decreased them with starch. With both substrates, 9, 10-anthraquinone decreased Eh and propionate molar percentage, and increased butyrate molar percentage. Apparent digestibility and total VFA concentration were decreased by 9, 10-anthraquinone with cellulose only. Total microbial amino acids-N was not affected by 9, 10-anthraquinone with either substrate. With both substrates, 9, 10-anthraquinone increased the percentages of glutamate and alanine in total amino acids and decreased tyrosine and phenylalanine. 9, 10-anthraquinone increased the percentages of serine and glycine in total amino acids with cellulose but not with starch, increased valine with starch only, and decreased leucine and isoleucine with cellulose only. The decline in the recovery of metabolic hydrogen with methanogenesis inhibition was lesser with starch than with cellulose and was not affected by amino acids biosynthesis.

Index terms: rumen, methane, fermentation, electron balance

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## Sulphur hexafluoride (SF<sub>6</sub>) tracer gas methodology modifications for highlands conditions<sup>(1)</sup>

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SF<sub>6</sub> as artificial tracer have been used to determine enteric methane emissions in ruminants, however, there is few technical information to set up the methodology under highland conditions. This study aimed to evaluated permeation rates of SF<sub>6</sub> tubes and capillary flow passage in Colombian highlands conditions. Two types of experiments were conducted to adjust the methodology. The first experiment examined the permeation rate from 15 permeation tubes saturated up to 2.2±0.1 g SF<sub>6</sub>, incubated at 39°C during 6 weeks and weighted weekly in Mariana (USA) at 51 MASL. After that, these tubes were relocated at 2547 MASL in Mosquera (Colombia), incubated at 39°C over 12 weeks and weighted weekly. The data set was compared between places by one-way design. The second experiment took place on Mosquera to determinate the final pressure inside the yokes by different capillary restrictions (0.3 to 1.2 sccm). A regression analysis was used to define the adequate restriction. For the first experiment, the SF<sub>6</sub> permeation rate was lesser ( $p < 0.05$ ) in Mariana respect Mosquera (1.49 y 2.03 mg.d<sup>-1</sup> respectively). In the second one, the restriction in the capillary increased ( $p < 0.05$ ) the vacuum inside the yoke, obtaining the regression equation  $Y = 7.75x - 15.74$   $R^2: 0.8$ , where  $x$  = capillary restriction. In highlands conditions, capillary flow of 0.48 sccm allowed to saturate up to 33% of total volume of yoke. The results suggested that is necessary to calibrate permeation tubes and capillary restriction according to environmental conditions to improve the estimate methane enteric emissions from ruminants.

**Keywords:** Enteric emission, greenhouse gases, methane production

<sup>1</sup>Proyecto FTG/RF-14653-RG, supported by Fontagro and Agrosavia.

## Supplementation of steers with polyclonal antibody preparations against methanogenic Archaea decreases methane production ex situ

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An experiment was conducted to evaluate the effect of supplementing fourteen ruminally cannulated Angus crossbred steers with different levels of polyclonal antibody preparations (PAP) from avian origin against the methanogenic archaea *Methanobrevibacter gottschalkii* Ho (Ho) and *Methanobrevibacter ruminantium* M1 (M1). The experiment was a randomized block design with a 3 × 2 + 1 factorial arrangement replicated in two periods. Factors were proportion of PAP against M1 and Ho in the mixture (100:0, 50:50, and 0:100 M1:Ho) and level of supplementation of each mixture (3 or 6 mL hd<sup>-1</sup> d<sup>-1</sup>). Steers were fed bermudagrass hay ad libitum and 2 kg hd<sup>-1</sup> d<sup>-1</sup> of corn gluten feed (CGF). Steers were adapted to PAP treatments for 14 days prior to the start of the experiment. Ruminal fluid from each steer (experimental unit) was collected and mixed individually with McDougal buffer (1:3 ratio) to inoculate serum bottles and polycarbonate tubes each containing 0.7 g of the same diet. Serum bottles and tubes were incubated for 48 h at 39°C for determination of methane production, fermentation profile, and in vitro organic matter digestibility. There was no effect of PAP supplementation level ( $P > 0.10$ ) on CH<sub>4</sub> production variables. Incubations containing PAP with 100:0 M1:0 decreased ( $P \leq 0.03$ ) total CH<sub>4</sub> production (mmol) and CH<sub>4</sub> production (mmol) per gram of OM fermented when compared with control. Incubations with 100:0 M1:Ho decreased ( $P = 0.02$ ) methane production when compared with 0:100 M1:Ho, while 50:50 M1:Ho were intermediate and did not differ from the other two proportions.

Index terms: *Methanobrevibacter gottschalkii* Ho, *Methanobrevibacter ruminantium* M1, Angus crossbred steers, ex situ incubations, passive immunization.

## Supplementing beef cattle diets with hydrolyzable tannins impacts manure emissions of ammonia and carbon dioxide

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This study examined the effects of feeding hydrolyzable tannins (**HT**) to beef cattle on ammonia and greenhouse gas emissions from manure. Eight beef heifers ( $480 \pm 29.2$  kg) were used in a replicated  $4 \times 4$  Latin square experiment that had four 28-d periods, with 7-d washout between periods. The heifers were fed a forage-based diet (19.7% crude protein), that was supplemented with the following treatments; control (no HT; **CON**), chestnut extract (**CHE**; 2% of diet dry matter [**DM**]), tannic acid (**TAN**; 1.5% of diet DM), and gallic acid (**GAL**; 1.5% of diet DM). Total tract digestibility of crude protein was greater for CON and GAL (average 69.0%) compared to CHE and TAN (average 62.4%;  $P \leq 0.001$ ). Samples of feces and urine were mixed according to excreted proportions to reconstitute 2.0 kg of manure (as-is basis). The manure was incubated in steady-state flux chambers for 96 hours to determine the effect of feed treatment on gas emissions from manure. After incubation, manure DM was greatest for CHE and TAN (16.2 and 16.8%, respectively), intermediate for GAL (15.5%), and least for CON (14.6%;  $P = 0.030$ ). While there was no effect of treatment on methane or nitrous oxide manure emissions ( $P \geq 0.35$ ), both CHE and TAN had greater ammonia and carbon dioxide emissions compared to CON and GAL ( $P \leq 0.018$ ). Dietary supplementation with GAL resulted in greater total tract digestibility of crude protein, and reduced manure emissions of ammonia and carbon dioxide compared to the other tannin treatments.

Keywords: chestnut extract, forage diet, gallic acid, greenhouse gas emissions, tannic acid

## Use of a novel nitrogen fertilizer to reduce nitrous oxide and ammonia emissions from pastures

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Nutrients losses to the atmosphere could be a limitation for livestock production worldwide. Novel forms of fertilizers are proposed to increase dry matter production and reduce nitrogen (N) gaseous emissions (e.g. NH<sub>3</sub> and N<sub>2</sub>O) in grassland systems. Thus, the objective of this study was to evaluate foliar N fertilization using a nanomaterial. For this, intact soil cores from a permanent pasture were collected in PVC lysimeters (0-15 cm) and transported to a controlled experiment (20°C) in laboratory. Three treatment were tested: a nanoformulation of Hidroxiapatite-urea (HA-urea), dissolved urea in water (uread, 1:10) and the traditional granular (ureag), at an equivalent rate of 25 kg N ha<sup>-1</sup>. A control treatment was also included (0 kg N ha<sup>-1</sup>). Cumulative emissions of N<sub>2</sub>O and NH<sub>3</sub> (kg N ha<sup>-1</sup>) were measured using static and dynamic chambers, for 58 and 21 days respectively. The experiment was set up on a complete randomized design with four replicates and results were analyzed by ANOVA. The results showed that the use of a nanoformulation decreased N<sub>2</sub>O emissions by 41% and 45% compared to granular and dissolved urea ( $p < 0.05$ ), respectively. However, for NH<sub>3</sub> emissions, N losses were higher for the two liquid formulations (HA-urea and uread) compared to ureag ( $p < 0.05$ ) given the presence of urease in plant leaves. Therefore, the use of HA-urea could reduce N<sub>2</sub>O emission, but further studies are required to understand its effect on NH<sub>3</sub> losses as well as on pasture yield and quality, including the testing of N- nanoformulations at different chemical forms and rates.

Index terms: Nanomaterial: material sized between 1-100 nanometers; Nanoformulation: synthesized enhanced material between the range of 1-100 nanometers. Pasture: land used for grazing; Nitrous oxide (N<sub>2</sub>O): greenhouse gas present in atmosphere due to soil ecosystem.

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## Use of Mootral™, a garlic and citrus based feed additive, to reduce enteric methane production in feedlot cattle

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Enteric methane production is the main source of greenhouse gas emissions from livestock globally, with ruminants particularly beef cattle contributing the most. Studies conducted *in vitro* have shown reduced methane production when using garlic and citrus extracts. However, limited research has been done investigating garlic and citrus extracts *in vivo*. The objective of this study was to quantitatively evaluate the response of Angus-Hereford cross steers consuming the feed additive Mootral™ on methane production. Twenty steers (419 ± 16 kg BW) were randomly assigned to two treatments: control (no additive) and Mootral™ applied at 15 g/d in a completely randomized block design with 3 week sampling intervals and 12 week data collection periods. Enteric methane emissions were measured using the GreenFeed system during the covariate period and experimental weeks 2, 6, 9, and 12. Methane yield (g/kg DMI) remained similar in both treatments for weeks 2 to 9. However, in week 12 there was a significant decrease in methane yield (23.2%) in treatment compared to control steers, this might be due to an increase in pellet consumption which contained the Mootral™ additive. Overall methane production during the entire experimental period was not significantly different. Carbon dioxide yield (g/kg DMI) was not significantly different between treatments. Dry matter intake, average daily gain and feed conversion efficiency also remained similar in control and supplemented steers. The *in vivo* results showed that Mootral™ has a potential to reduce enteric methane yield in beef cattle.

Index Terms: bos taurus, enteric methane, feedlot cattle, environmental sustainability, greenhouse gas

## Variations in Methane Emissions from Chinese Holstein Dairy Cows Offered Diets with Different Forage Concentrations

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This study was to investigate effects of dietary forage (maize silage and alfalfa hay) to concentrate proportions on enteric methane emissions from Chinese Holstein cows at different stages of lactation. Three different groups of 18 cows were selected from a commercial farm, and each group was offered 3 diets (6 cows/diet) of low, medium and high concentrate proportions (DM basis) during the early (44%, 53% and 59%), middle (46%, 53%, and 61%) and late (30%, 37% and 42%) lactation (average milk yield = 25.7, 20.2, and 17.9 kg/d, respectively). Each period consisted of 20 days for adaptation and 5 days for methane measurements using the sulphur hexafluoride technique. Methane emissions reduced linearly ( $P < 0.01$ ) with increasing dietary concentrate proportions during the early (392 vs. 347 vs. 325 g/d), middle (400 vs. 331 vs. 261 g/d) and late (396 vs. 329 vs. 242 g/d) lactation. A similar reduction in methane energy output as a proportion of gross energy intake ( $P < 0.01$ ) was also observed during the early (8.3% vs. 5.9% vs. 4.9%), middle (10.6% vs. 7.6% vs. 5.2%) and late (8.3% vs. 6.5% vs. 4.9%) lactation. The present data provide benchmark information for development of methane emission inventories for dairy production systems in China.

**Keywords:** Chinese Holstein cow, concentrate proportion, lactation stage, methane emission, sulphur hexafluoride technique

## 2.1 Adaptation (Oral)

### Teff diet with or without direct fed microbial and mixed enzymes affects the nutrient digestibility and methanogenesis

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**Abstract/** Teff (*E. tef*) is grown to produce gluten-free grains, this leads to production of sustainable abundance amounts of teff straw which is mostly neglected. Direct fed microbial and fibrolytic enzymes (DFM) can be used to upgrade the quality of teff straw and mitigate ruminal methanogenesis. Twelve Barki lambs were randomly allotted into three dietary treatments as: control (per kg DM, 500 g clover berseem hay (*T. alexandrinum*) and 500 concentrate), teff diet (the control diet proportionately replaced by 25 % of clover hay with teff straw), and DEM teff diet (0.5 DFM g /kg DM of teff diet). The tested DFM mainly contained *Saccharomyces cerevisiae*, species of *Bacillus* and *Propionibacteria*, cellulase, xylanase and phytase. The treatments were lasted for 21 days, while the last 7 days were assigned for the nutrient digestibility and nitrogen balance trial. Teff diet had higher neutral detergent fiber and lower crude protein than the control. The teff diet exhibited a reduction ( $P<0.05$ ) in the total volatile fatty acids concentrations and the digestibility of fibre fractions, while exhibited the highest ( $P<0.05$ ) methane production among the experimental diets. Similar nutrients digestibility, urine N excretions, and ruminal fermentation characteristics were found for DFM teff and the control diets except for ruminal pH. These results suggested that teff straw treated with DFM can replace partially the most traditional forage of ruminants in many developing countries.

**Keywords:** *Eragrostis tef*, yeast, *Bacillus* species, fibrolytic enzymes, fiber digestibility, methane emission

## 2.1 Adaptation (Poster)

### Blood Methaemoglobin in two sheep breeds during the process of adaptation to encapsulated nitrate supplementation<sup>(1)</sup>

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Sixteen ewe lambs (mean BW 32.7±3.86 kg) were used in a randomized complete block design with 2×4 factorial arrangement, two breeds (Santa Inês and Ile de France) and four levels encapsulated nitrate product (ENP) addition (0, 1.0, 2.0, and 3.0% DM), to evaluate adaptation to dietary ENP based on levels of blood methaemoglobin (MetHb). The ENP was increased by 1% every 4d from 0 to 1.0, 2.0, and 3.0% of dietary DM (12.2% CP and 0, 0.71, 1.4 and 2.14% NO<sub>3</sub><sup>-</sup> dietary DM). The MetHb (measured on day after ENP addition) and dry matter intake (DMI, measured on 3th and 4th day) were evaluated at 0, 4, and 8 h after feeding. There was an interaction ( $P<0.01$ ) between breed and ENP for MetHb measured 0 h before feeding when diet change from 2% to 3% ENP. The MetHb increased linearly ( $y=0.5619+1.0512*ENP$ ,  $r^2=0.44$ ) as ENP was increased from 2% to 3% ENP, whilst Santa Inês showed greater mean MetHb (15.12%) compared to Ile de France (3.9%). There was an interaction ( $P=0.047$ ) between breed and ENP for DMI at 8 h after feeding when level ENP increased to 0, 1, 2 and 3%, Ile de France showed a decrease in DMI (0.93, 0.91, 0.80 and 0.69 kg, respectively) when compared to Santa Inês (1.17, 0.99, 1.27, 1.26 kg, respectively). Ile de France breed was more sensitive to ENP supplementation than Santa Inês, showing a reduction in DMI according to ENP inclusion. Santa Inês showed greater ENP intake, which as associated to greater increase in blood MetHb.

Keywords: Methemoglobinemia, Nitrate poisoning, Slow-release.

## Ruminal fermentation and methane mitigation of salt tolerant Mediterranean shrubs in relation to their phenolics<sup>(1)</sup>

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Using salt tolerant polyphenol containing shrubs (STFS) may cover the feed limitation during drought in the Mediterranean. Chemical and phenolic composition of acacia (*A. saligna*), atriplex (*A. halimus*), leucaena (*L. leucocephala*) and moringa (*M. oleifera*) leaves were evaluated using the *in vitro* semi-automatic system for gas production. All comparisons were made using berseem clover hay (*T. alexandrinum*). The highest protein concentration was observed for leucaena compared to the other plants. Acacia had the highest total phenolics, while berseem had the lowest values among the experimental plants. Moringa and leucaena were similar in total phenolics, while they were different in their individuals. The most abundant phenolics found in moringa were rutin and caffeic, while in leucaena were pyrogallol and hisperdin. The major compounds of acacia were rutin and gallic, while pyrogallol existed in abundance in atriplex. Quercetin only existed in berseem. All STFS decreased methane ( $P<0.01$ ) compared to control. Among the STFS, moringa and atriplex had the highest ( $P<0.05$ ) values of nutrient degradability and total volatile fatty acids, while acacia resulted in the lowest ( $P<0.05$ ) values. Atriplex and leucaena had the lowest ( $P<0.01$ ) ammonia concentration and acacia reduced ( $P<0.05$ ) the protozoal count. These results indicated that STFS can substitute the traditional forages and reduce ruminal methane emission through different mechanisms.

Keywords: alternative forages, bioactive components, nutrient degradability, methanogenesis

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## **Session 3**

**Regional low carbon initiatives,  
Landscape, regional management and  
National Commitments**

### 3.1 Regional level (Oral)

#### Carbon neutralization potential of beef cattle system in cerrado biome of maranhao, Brazil

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This paper evaluated the municipal performance of beef cattle production systems in 2006 and 2017, in relation to the enteric methane emissions in CO<sub>2</sub> equivalent. The 109 municipalities evaluated belong to the Cerrado biome of the Maranhao state, Brazil. In this period the cattle population increased 20% and reached 3.3 million head; the pasture area remained at 2.6 million hectares. The total enteric methane emission, computed by the Agriculture and Land Use National Greenhouse Gas Inventory Software (ALU), was 3,867 Gg of CO<sub>2</sub>eq in 2006 and 4,654 Gg of CO<sub>2</sub>eq in 2017. The municipalities with higher cattle numbers were classified as “Regions of net CO<sub>2</sub> sink”. The performance score was calculated considering the pasture and forest area, herd size, enteric methane emissions and the stock of biomass. These scores were computed as a data envelopment analysis (DEA) measure for each year, under the variable return scale (VRS) hypothesis and output orientation. There is a statistically significant difference between years, with higher performance in 2017 (p-value 0.0193). We estimated the probability of a municipality being sink of CO<sub>2</sub> (p) considering the DEA-VRS score, the agricultural financing via ABC Program, the livestock financing and the agricultural GDP. The analysis, considering the two periods showed that there is a positive and significant effect of time, with higher p in 2017. It also showed that only the livestock financing has a positive and significant effect in p. These results show that the introduction of biomass stock technologies was beneficial to the performance of these municipalities.

Keywords: Carbon sink, Carbon Source, GEE, Grassland Management, Livestock, Pasture Degradation

## Soil carbon in New Zealand hill country under contrasting phosphorus fertiliser and sheep stocking regimes<sup>1</sup>

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We examined soil carbon (C) stocks at the Ballantrae Hill Country Research Station, in southern Hawke's Bay. Soils were sampled to three depths in 2003 (0-75, 75-150, 150-300 mm) and to the two upper depths in 2014, in three farmlets under different annual phosphorus (P) fertiliser and stocked with sheep to maintain similar grazing pressure (i.e., stock units per unit pasture production) across farmlets since 1975. Farmlets: LFNF = no P applied since 1980, LFLF = 125 kg single superphosphate (SSP) ha<sup>-1</sup>, and HFHF = 375 kg SSP ha<sup>-1</sup> since 1980. Corresponding annual mean stocking rates were 6.0, 10.6, and 16.1 stock units ha<sup>-1</sup>. Soil C stocks in 2003 increased from 30.9 to 32.5 and 35.1 Mg C ha<sup>-1</sup> on the LFNF, LFLF and HFHF farmlets, but remained relatively unaffected at greater depths in 2003 and both depths in 2014. Adding these findings to earlier measures from the same farmlets provides a time series (1972-2014) that supports the view that soil C stocks are relatively stable under permanent pastures managed under the current conditions. In contrast, slope and aspect affected soil C stocks, evidenced by slope × farmlet and aspect × farmlet interactions ( $P < 0.001$ ). Data from this long-term study provide science, policy and industry with invaluable insights into the changes in soil C stocks in pastoral hill-country soils.

**Keywords:** Soil carbon stocks, pastures, phosphorus fertiliser, sheep grazing, long-term grazing experiment.



### 3.1 Regional level (Poster)

#### Carbon capture potential of different silvopastoral arrangements under stable stocking rate in the northern Coast of Colombia

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Carbon capture under silvopastoral systems with grazing cattle depends on the combinations of tree, pasture and shrub species. Evaluate carbon capture potential of 4 silvopastoral arrangements (SA), compared to traditional pasture (without trees), under stable stocking rate of 3,5UA/Ha (UA=Animal unit; 1UA=360kg live weight) and a paddock rotation system, in the Montes de Maria region located in the Northern Coast of Colombia. Treatments were: T0= *Megathyrsus maximus* (without trees); T1= *Eucalyptus tereticornis* (ET) + *Brachiaria* híbrido cv Mulato II (BhM) + *Cajanus cajan* (CC) - CIAT-18701; T2= ET + BhM + CC-CIAT-913; T3= ET + *Brachiaria brizantha* cv Toledo (BbT) + CC-CIAT-18701; T4= ET + BbT + CC- CIAT-913. For all SA trees were planted in stripes (6m) with 3 lines of trees each (2mx2m/tree), and 30m wide pasture stripes inbetween tree stripes, with 441 trees/Ha resulting density, and trees were 3.5 years old at first measurement. All treatments were managed under 6 paddocks (7 days occupation and 42 days rest) during 12 full cycles of paddock rotation. Full random block design was used with 6 Animals per treatment. Significant differences ( $P<0.05$ ) in Carbon Stock (CS) were found for groups T1 + T2 vs. T3 + T4 vs. T0 (T1= 82,47a; T2= 83,25a; T3= 75,58b; T4= 76,27b; T0= 70,28c TCO<sub>2</sub>/Ha). Pasture CS was higher ( $P<0.05$ ) for T0 vs. T1 + T2 vs. T3 + T4 (T0= 16,74a; T1= 16,25b; T2=16,19b; T3=12,94c; T4=12,90c TCO<sub>2</sub>/Ha). Tree CS(aerial+radicular portions) was higher ( $P<0.05$ ) for T1 and T2, and similar ( $P>0.05$ ) for T3 and T4 (T1= 94,13a; T2= 97,48a; T3= 82,56b; T4= 84,36b TCO<sub>2</sub>/Ha). Overall CS(including soil carbon until 80cm) was significantly higher ( $P<0.05$ ) for T1 + T2 vs. T3 + T4 vs. T0, which had the lowest carbon stock of all treatments (T1= 192,85a; T2= 196,92a; T3= 171,08b; T4= 173,84b; T0= 87,02c TCO<sub>2</sub>/Ha). Use of silvopastoral systems significantly improved the CS capture capacity (TCO<sub>2</sub>/Ha) in the region of Montes de Maria, Northern Coast of Colombia, with SA using BhM, showing the highest carbon immobilization capacity.

Keywords: Carbon immobilization, Trees+pasture, *Eucalyptus tereticornis*, *Megathyrsus maximus*, *Brachiaria* híbrido cv Mulato II, *Brachiaria brizantha* cv Toledo, *Cajanus cajan*

## Climate Ready Riverina - Adapting agriculture to reduce the impacts of climate change

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The Riverina is a productive agricultural region in south-eastern Australia around 57,000 km<sup>2</sup> in size and the major land use (78%) is agriculture. Grazing occupies over half of the agricultural land, with approximately 560,000 cattle and 5.5 million sheep in the region in 2017. The Riverina has extensive floodplains, tablelands, rangelands and irrigation districts with a variable long-term annual rainfall of around 240-620 mm. Recent studies have shown that the Riverina will be severely affected by climate change. Increased temperatures, variations in seasonal rainfall and changes to available irrigation water are expected to impact on livestock production and current land use. Broadacre and rangeland farm systems in particular will be highly impacted due to their dependence on dryland pastures. Livestock farmers have several options to negate the effects of climate change, including the use of best management practices, Climate Smart agriculture, greenhouse gas mitigation methods and climate adaptation options. Climate change has created an urgency to initiate discussions with farmers on preparing their farms to be climate-ready. This study provides a simple framework for farm advisors to identify and communicate relevant, industry-specific climate adaptation messages to livestock farmers. Livestock producers in the Riverina require management changes if current levels of productivity and profitability are to be maintained in the shorter-term. The framework considers the current farm practices and presents a hierarchy of adaptation priorities for livestock producers in the region, including building soil organic matter, proactively managing heat stress in animals and pastures, and developing animal and plant genetic adaptations.

## Enteric methane emissions in small-scale dairy systems: Five case scenarios

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The production of greenhouse gases by ruminants is influenced by factors such as diet composition, intake and digestibility, so that five scenarios (E) were evaluated using different supplements (E1: Without supplementation as a control diet, E2: 4.5 kgDM / cow /day of commercial concentrate (19% CP), E3: 200 gDM / kg of milk produced, commercial concentrate, E4: Corn grain and wet distillers grains and E5: E4 plus corn silage), using two pasture managements (cut vs grazing), and two varieties of legumes (Red clover vs. white clover). Six dairy cows per scenario in small scale systems, with average milk yield 13.9 kg / day in the second third of lactation, were used to estimate methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>) emissions per kg of milk FCM 3.5% using the equations proposed by the Intergovernmental Panel on Climate Change Tier 2. A completely randomized design with in a 5x2x2 a factorial arrangement (scenarios x pasture managements x legumes), with six replications (cows) was carried out for the evaluation of the supplementation strategies. Differences were found ( $P < 0.001$ ) in the scenarios and the pasture management with respect to the emissions of enteric fermentation, L CH<sub>4</sub> /day, g CH<sub>4</sub> /day and g CH<sub>4</sub> / kilogram of milk produced. E2 had the highest methane emission, 27.2 kg CH<sub>4</sub>/kg milk yield, and E3 and E4 produce the lower CH<sub>4</sub>/kg milk yield ( $22.6 \pm 0.2$ ). As a conclusion E3 and E4 reduce the environmental impact being more efficient in terms of methane / kg milk produced in small- scale dairy systems.

Keywords: IPCC, supplementation, grazing, milk, cow

## Insect Activity Affects N<sub>2</sub>O Emissions from Cattle Dung on Pastures

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Past studies have shown ivermectin reduces insect activity in dung pats. Using chambers, we monitored dung pats for two years (June 2016-June 2018) to assess the effects of insect activity (without vs. with mesh covers to deter insects), and the addition or exclusion of ivermectin, on N<sub>2</sub>O emissions from a semiarid native pasture. A bare surface soil was also included as a control resulting in a 2×3 factorial design with four replications. In both years, N<sub>2</sub>O emissions were higher from dung pats than bare soil. In Year 1, cumulative annual N<sub>2</sub>O emissions were affected by the presence/absence of dung, insect activity and their interaction; N<sub>2</sub>O emissions from dung decreased with insect activity and, in the absence of insect activity, N<sub>2</sub>O emissions were lower from dung without ivermectin. This equated to between 0.07 and 0.28% of the original N in the dung lost as N<sub>2</sub>O (emission factor = EF), but EFs were only affected by insect activity (0.09 with vs. 0.26% without insect activity). In Year 2, the presence of dung increased N<sub>2</sub>O emissions, while the other factors were not significant due to large variability between replications. The EF ranged from 0.24 to 0.85%, and did not differ between dung treatment or presence/absence of insect activities. Insect activity in dung may be important for mitigating N<sub>2</sub>O emissions on semiarid native pastures, but the conditions under which this mitigation occurs requires further study.

**Keywords:** greenhouse gas, dry mixedgrass prairies eco-region, native pasture, cattle grazing, nitrous oxide emission factor.

## Methane and nitrous oxide emissions from grazing steers' dung with or without supplement in Argentina

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Increasing the proportion of starch rich supplements in the diet of ruminants can decrease the emission of enteric methane (CH<sub>4</sub>) and can increase its emission from manure. However, the effect of diet composition on nitrous oxide (N<sub>2</sub>O) emission from manure is less known. The objective of this study was to quantify CH<sub>4</sub> and N<sub>2</sub>O emissions, and estimate their emission factors (EF) from fresh dung collected from 20 steers grazing pasture without (P) or with daily maize grain supplementation (S; equivalent to 1% steer's body weight). Emissions were measured, over 125 days, in static chambers (5 chambers/treatment plus 2 chambers as control) placed on a plot without grazing. Fresh dung was collected once from two groups of steers and homogenized to form a composite sample for each diet. One kilo of composite sample was placed on the ground inside the chambers. As dung dry matter in P was lower, S had a major carbon and nitrogen concentration. Even so, with greater amount of carbon, S cumulative CH<sub>4</sub> emissions (1738 mg CH<sub>4</sub>-C m<sup>-2</sup>) were lower than P (4033 mg CH<sub>4</sub>-C m<sup>-2</sup>; P<0.001). Instead, S produced significantly more N<sub>2</sub>O than P (392 and 123 mg N<sub>2</sub>O-N m<sup>-2</sup> respectively; P<0.01). P and S CH<sub>4</sub> and N<sub>2</sub>O EF were 0.94±0.10 and 0.30±0.06 kg CH<sub>4</sub> head<sup>-1</sup> year<sup>-1</sup> and 0.06 and 0.13% respectively. In conclusion, dung emissions responded differently to each diet. These preliminary results suggest that current EF (1 kg CH<sub>4</sub> head<sup>-1</sup> year<sup>-1</sup> and 2% of N<sub>2</sub>O-N) may overestimate dung contribution to the National Inventory.

Keywords: Greenhouse gasses, Livestock manure, Emission factors

## Milk and methane production in crossbreed tropical dairy cows grazing tropical pastures supplemented with concentrate

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The objective of this study was to evaluate the effects of different concentrate supplementation levels on milk production, estimate methane (CH<sub>4</sub>) and Nitrous oxide (N<sub>2</sub>O) emissions in dairy cows grazing native pastures. The study included 12 crossbred F1 dairy cows ( $\frac{1}{2}$  *Bos taurus* -  $\frac{1}{2}$  *Bos indicus*) over 60 days of lactation. Cattle grazed tropical grasses (*Paspalum spp.*, *Axonopus spp.*, *Brachiaria spp.* and *Cynodon neumfluensis*) and were supplemented with 0, 150, 300 and 450 g of concentrate per kg of milk yield, in three periods of 15 days each one in a crossover design. N<sub>2</sub>O emissions from excreta were calculated according of IPCC, 2006. The quantity of daily CH<sub>4</sub> production was calculated (kg • head<sup>-1</sup> • day<sup>-1</sup>). The data were analyzed with the SAS MIXED procedure and the means were subjected to a trend analysis using orthogonal polynomials. There were no differences (P >0.05) for live weight and Milk yield between treatments. The supplementation with concentrate in tropical dairy cows did not increase milk yield, but increased CH<sub>4</sub> and N<sub>2</sub>O (P <0.001) excretion per cow. The use of concentrates in crossbreed F1 dairy cows fed with local tropical pastures affects negatively the emission of greenhouse gases, producing more CH<sub>4</sub> and N<sub>2</sub>O per cow per day, however cow supplemented present a greater concentrate intake, which means more gross energy intake and higher dry matter ingested, but not showing more milk yield production.

Keywords: Greenhouse gases, Nitrous oxide, Tropical grasses, Crossbreed

## Nitrogen utilization and methane emission in lactating cows fed diets with different nitrogen contents

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Dietary nitrogen utilization, enteric methane emission, and milk production and composition in dairy cows fed diets with different crude protein (CP) contents were studied. Twelve Holstein multiparous cows averaging 70 days in milk, 645 kg of body weight, and 35 kg of milk yield, were randomly assigned to 1 of 3 diets, according to a replicated 3 × 3 Latin square design. Diets were composed, on dry matter basis, of 27% lucerne hay, 31% maize silage and 42% concentrate based on maize grain (MG) and soybean meal (SBM). Proportions of MG and SBM were such to result in diets with 13 (CP13), 16 (CP16) and 19% (CP19) CP content. Throughout the trial, cows were kept in individual pens. Each experimental period lasted 29 d (20 d for adaptation and 9 d of data and sample collection: 3 d for nitrogen balance, 1 d for blood sampling, and 5 d for enteric methane emission -SF6 technique-). Dry matter intake (23.8 kg/cow/d) and digestibility (74.7%) were similar among treatments. Increased CP intake resulted in an increased ( $P < 0.001$ ) milk (30.1, 32.4, 34.9 kg/cow/d) and milk protein production (0.93, 10.03, 1.13 g/cow/d), and urinary (120, 209, 318 g/cow/d) and faecal (131, 141, 157 g/cow/d) N excretion, for CP13, CP16, CP19, respectively. Daily methane emission (532 g/cow/d), intensity (16.6 g CH<sub>4</sub>/kg milk), and Y<sub>m</sub> (6.84) were similar among treatments. Milk and milk protein yields increased with increasing N intake at expenses of a threefold excretion of N to the environment without affecting CH<sub>4</sub> emission.

**Keywords:** dairy cattle, nitrogen balance, greenhouse gasses emission

## 3.1 National level (Oral)

### First Tier 2 enteric methane emissions national inventory for cattle in Mexico and analysis of spatially distributed emissions. A baseline for mitigation

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The aim of the present work was to calculate the inventory of enteric methane (CH<sub>4</sub>) emissions from cattle of the five geo-climatic regions of Mexico using the IPCC 2006 Tier 2 main method. We generated for the first time in Mexico country specific emissions factors through several experiments conducted in five open circuit respiration chambers (OCRC). Two chambers are located in the southern tropical region and three in the temperate climate region of Mexico. We then conducted a geo-spatial disaggregation of national cattle herd by dividing the country's territory into five geo-climatic regions: dry, very dry, temperate, tropical humid and tropical sub-humid and allocating the national cattle population to each region. The population of each region was divided into seven categories: calves, young steers, young heifers, steers, heifers, cows and bulls. The cow's category was further divided into dairy, beef and dual-purpose cows because cows represent the largest enteric methane producers and there is sufficient scientific evidence that CH<sub>4</sub> emission differ between them. We used ArcMap ver 10.2.2 in order to spatially allocate cattle to the corresponding geo-climatic region. A national survey was conducted in order to characterize the regional cattle production systems and feeding practices. Typical diets were defined for each region and then reproduced in the OCRC in order to generate regional specific emission factors for categories and subcategories. The total CH<sub>4</sub> emissions from enteric fermentation of cattle in Mexico calculated using the main method of the Tier 2 approach is 2,039.21 Gg of CH<sub>4</sub> year<sup>-1</sup>.

Keywords: Methane, inventory, Mexico, bovines, Tier 2.



## Partnerships for dairy and beef low carbon initiatives in France

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A voluntary initiative supported by French dairy and meat boards aims to reduce milk and beef carbon footprint at farm level by 15 to 20% from 2015 to 2025. To achieve this goal, a partnership has been built in order to reduce GHG emissions and increase carbon sequestration in French cattle farms. To implement the farm evaluation, a national Life Cycle Assessment tool CAP'2ER<sup>®</sup> has been developed to calculate milk and beef carbon footprint as other environmental burdens (eutrophication, acidification, energy consumption) and ecosystem services (carbon sequestration, biodiversity). Named Low Carbon Dairy Farm and Beef Carbon, the programs aim at improving farming practices efficiency through herd management and animal health, forage production, soil practices and manure management, energy consumption... A multi-stakeholder collaboration is in course with cooperatives, farmers' organizations, advice companies, milk and beef enterprises... By bringing together complementary skills in environment, technical and economic analysis of production systems, it represents an opportunity to reduce carbon footprint and to increase livestock sustainability. Thanks to training sessions, farm assessments, demonstrative farm network, farm advices in mitigation actions, 12,000 farmers (12 % of French cattle farmers) are now involved in. The national and regional coordination represents an opportunity to continue the dissemination to all farmers in France. The next step in course consist in improving methods for measuring, reporting and verifying the effects of mitigation actions, in order to communicate on the progress and to create collective carbon accounting projects for selling carbon avoided on the carbon market.

Terms for indexing: cattle, carbon, partnership, farming efficiency

## Pathways to a carbon neutral Australian red meat industry

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The Paris Climate Agreement of 2016-17 has seen 185 out of 197 countries ratify the Agreement. The targets will effectively require global greenhouse gas (GHG) emissions be net zero by 2050. This will require the major emitting industries to be carbon neutral by 2050. The Australian red meat industry is a large industry with a total value of \$18.4 billion including \$13.3 billion of export value. It is also a significant contributor to the national GHG emissions. We calculated baseline (2005) and current (2015) GHG emissions for the red meat sector (farm and processing sectors) based on the national inventory. Emissions from the Australian red meat industry have decreased from 124.1 Mt CO<sub>2</sub>e in 2005 (21% total national emissions) to 68.6 Mt CO<sub>2</sub>e in 2015 (13% of total national emissions), primarily through reductions in land clearing. This paper includes the rationale behind the feasibility of carbon neutrality at an industry level, including the likely costs and financial returns to producers and the community. The assumptions include changes in land management, adoption of new supplement technologies and new legumes to reduce enteric methane emissions from grazing livestock with clear links to established carbon market offsets. Our results show that it is possible for the Australian red meat industry to substantially reduce GHG emissions and feasibly become carbon neutral as determined by the Australian Greenhouse Gas Inventory. This will require substantial research and development investment along with policy support in carbon markets from private and government bodies.

Index terms: greenhouse gas, enteric methane, livestock, land management

### 3.1 National level (Poster)

#### Carbon Agri: A monitoring reporting verification process for capturing carbon reductions in French cattle systems

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Regarding the dairy and beef low carbon initiatives launched in France to reduce carbon intensity, there is a strong interest and need to improving methods for measuring, reporting and verifying (MRV) the effect of mitigation actions. Complying with the French Low Carbon Standard published in November 2018, a MRV process named CARBON AGRI has been developed for cattle farms. This methodology covers the whole farm considering dairy, beef and crops productions. To capture emissions reductions and carbon sequestration increases, CARBON AGRI measures progress from a range of mitigation practices as herd and feed management, crops fertilization, energy consumption, inputs purchased, grassland management, planting hedges, no tillage, cover crops,... Based on the national Life Cycle Assessment tool CAP'2ER®, the methodology consist in calculating the baseline carbon intensity of products for all farms involved corresponding to a collective or individual reference. On a five year crediting period, the carbon reduction represents the carbon intensity differences between the baseline and the project, multiplied by the milk and beef productions. By this way, CARBON AGRI permits to demonstrate additionality, track progress and communicate the efforts in a transparency way after a technical expert review. In order to implement new practices or make new investments to reduce carbon intensity the certified reduction permits to contract external financial supports for farmers. In that way, French representative's farmers created the France CARBON AGRI Association as an aggregator. The aim is to helping individual farmers for taking part in collective carbon accounting projects and selling carbon avoided on the carbon market.

Terms for indexing: MRV, cattle, mitigation, carbon

## Uncertainty of the Tier 2 enteric methane emissions inventory for cattle in Mexico: a Monte Carlo Approach

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The aims of this work are: 1) identification of the sources of uncertainty associated with the first Tier 2 enteric CH<sub>4</sub> emissions inventory for cattle; 2) estimation of the uncertainty of the Tier 2 method inputs using a bootstrap methodology, and 3) uncertainty propagation assessment of the input variables using Monte Carlo simulation (MCS). Uncertainty calculation comprises: calculation of the uncertainty associated to the input parameters and calculation of the uncertainty associated with the Tier 2 main method. The first was calculated by quantifying the inter-unit variability in activity and emission data. This step included choice of appropriated probability density function (PDF), estimation of its parameters and evaluation of the goodness of fit of selected PDF. We also used bootstrap simulations, means, standard deviations and 95 % confidence intervals to estimate the uncertainty of the inputs according with their PDF. For the Tier 2 method we used the uncertainty propagation approach. Mean and standard deviation of emissions and activity data were used as inputs to the Tier 2 method and MCS was carried out to propagate their uncertainty and to calculate the inventory's uncertainty. Finally, we conducted a sensitivity analysis to identify the variables that affect most the uncertainty of the inventory. Total CH<sub>4</sub> emission from enteric fermentation of cattle is 2,039.21 Gg CH<sub>4</sub> year<sup>-1</sup> with a level of uncertainty of -18.28 and +21.2 %. The variables that most affect uncertainty of emissions are gross energy intake, methane conversion factor, milk fat content, daily milk yield and dry matter intake.

**Keywords:** Methane, cattle, Mexico, uncertainty, Tier 2, Monte Carlo Simulation.

## Update of methodology for calculation of enteric methane emissions from dairy cows in Norway<sup>(1)</sup>

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In Norway, circa 50% of methane emissions derive from animal husbandry, with enteric methane production in about 200.000 dairy cows as major source. The objective was to update the methodology used in the national inventory for estimation of enteric methane emissions from dairy cows in Norway. The model development dataset from 2014 (n = 42) was extended with recent data to 65 treatment means in total. Number of individuals per treatment means was considered by using the WEIGHT statement in SAS. Updated equations for methane prediction were developed, which slightly differed from extant models used in Norway. The unchanged evaluation dataset from 2014 (n = 36) was used to compare prediction ability of the updated models (n = 2), the extant models (n = 2) and recently published models (n = 5). All tested models included dry matter intake as input variable. In addition, some models included one or several of the following parameters (g/kg dry matter): total fatty acids, neutral detergent fibre, crude fat. Model selection was based on low root mean squared prediction error, high concordance correlation coefficient and residuals versus predicted values. All models slightly overestimated methane emissions at low emission level and underestimated emissions at high levels. The updated model including dry matter and contents of fatty acids and neutral detergent fibre recommended for national inventory had the second lowest root mean squared prediction error (13.9%), greatest accuracy and precision of predicting methane emissions (concordance correlation coefficient = 0.69) and the smallest linear and mean bias.

Keywords: *Bos taurus*, inventory, model, methane production

<sup>(1)</sup>This project is funded by Miljødirektoratet, Oslo, Norway

# **Session 4**

## **International low carbon initiatives**

## 4.1 International Policies (Poster)

### Accounting for and reducing agricultural greenhouse gas emissions in Fiji

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The Paris Agreement aims to address climate change and keep global temperatures well below 2°C. At the heart of this agreement are Nationally Determined Contributions (NDCs), the voluntary commitments that countries make to reduce their greenhouse gas (GHG) emissions. Small Island Developing States contribute little to global GHG emissions but are disproportionately affected by the negative impacts of climate change. Many potential barriers exist when trying to reduce GHG emissions from developing countries due to the parallel issues of food security and the importance of maintaining livelihoods that are dependent on agriculture. This research analyses deemed emissions from the agriculture sector in Fiji and current commitments as defined by Fiji's NDC. Agriculture contributes around 40% of Fiji's GHG emissions with the majority (92%) of agricultural emissions coming from animal production as a result of enteric fermentation (51%) and manure (deposited on pastures 27%, effluent management 11%, applied to soils 3%). This project is working with the Fijian government to identify and prioritise livestock mitigation options in Fiji, based on which options have the greatest emissions reduction potential, the feasibility of various options and their associated cost. There is potential to apply the same process for analysing and prioritising livestock GHG mitigation options within other countries. The presentation will detail identified mitigation options, in the context of improving food security, consistent with agricultural policy, governance, land ownership, land tenure, legal and gender issues.

## Ensiling rice straw with different additive combinations impacts on *in vitro* gas and methane production

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This experiment was carried out to investigate the effects of rice straw silage with different additive combinations on *in vitro* rumen fermentation parameters, gas and methane production, and to select the most suitable additive combination for rice straw ensiling. Rice straw silage with different additive combinations (Treatment 1 was *Bacillus licheniformis*+*Aspergillus niger*+glucose, treatment 2 was *Bacillus licheniformis*+*Trichoderma viride*+bran, treatment 3 was *Bacillus licheniformis*+cellulase+starch, treatment 4 was *Lactobacillus plantarum*+*Aspergillus niger*+bran, treatment 5 was *Lactobacillus plantarum*+*Trichoderma viride*+starch, treatment 6 was *Lactobacillus plantarum*+cellulase+glucose, treatment 7 was *Enterococcus faecalis*+*Aspergillus niger*+starch, treatment 8 was *Enterococcus faecalis*+*Trichoderma viride*+glucose, treatment 9 was *Enterococcus faecalis*+cellulase+bran) were used as substrates, which were incubated for 72 h for an *in vitro* fermentation gas production test and a batch *in vitro* fermentation test. A pressure sensor was used to measure gas production through *in vitro* incubation at 39°C. The results showed that the concentration of ammonia nitrogen, total volatile fatty acid, propionic acid and butyrate acid in treatment 3 were significantly higher than those in other treatments ( $P<0.05$ ). Similarly, gas production in treatment 3 was significantly higher than that in other treatments ( $P<0.05$ ). However, the methane production in treatment 3 showed no differences with other treatment ( $P>0.05$ ), except for treatments 2 and 5. In conclusion, treatment 3 improved the *in vitro* fermentation properties and gas production without the increase of methane emission. Therefore, treatment 3 (*Bacillus licheniformis*+cellulose+starch) is the optimum additive combination for rice straw ensiling.

Index terms: rice straw silage, ensiling additives, *in vitro* fermentation, methane



## The ratio of 50:50 of *Urtica cannbinau* to *Leymus chinensis* improves rumen microbial fermentation and gas production *in vitro*

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This experiment was conducted to investigate the effect of the ratio of *Urtica cannbinau* and *Leymus chinensis* on rumen fermentation parameters *in vitro*. The experiment was divided into 5 groups according to the ratio of *Urtica cannbinau* and *Leymus chinensis*: 0:100 (A), 30:70 (B), 50:50 (C), 70:30 (D), 100:0 (E) respectively. The culture medium was collected to detect the fermentation parameters at 0, 1, 3, 6, 12 and 24 h after incubation. The results showed that: 1) Gas production and dynamic gas production parameters: Gas production in 0~1 h and 1~3 h were higher in groups A, B and C ( $P<0.05$ ), with group C recording the highest data ( $P<0.05$ ). The dynamic gas production parameters were all higher in both group A and group C ( $P<0.05$ ). What's more, there were no differences between group C and group A in theoretical maximum gas production, potential gas production and cumulative gas production. 2) Fermentation parameters: pH at 1, 3, 6 h, 24 h were higher in group A and group C, and was the highest in group C ( $P<0.05$ );  $\text{NH}_3\text{-N}$  concentration were higher in groups D and E at 3, 6, 12, 24 h ( $P<0.05$ ), and group C was lowest at 24h. 3) Bacterial protein and protozoal protein: The bacterial and protozoal proteins in group A and group C were higher at 1 h and 3 h ( $P<0.05$ ), and group C was the highest at 24 h. All in all, as for the ratio of *Urtica cannbinau* to *Leymus chinensis*, 50:50 is an optional level for rumen fermentation *in vitro*, by increasing the gas production and microbial protein, decreasing the ammonia concentration.

Keywords: *Urtica cannbinau*; *Leymus chinensis*; rumen microbe; gas production; *in vitro*

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methane, microbiome, rumen, 16s rRNA

sion potential during slurry storage.

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