NIR spectroscopy for predicting the nutritional, anthelmintic and environmental effects of sainfoin

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The role of sheep in saltbush domestication – what can they tell us?

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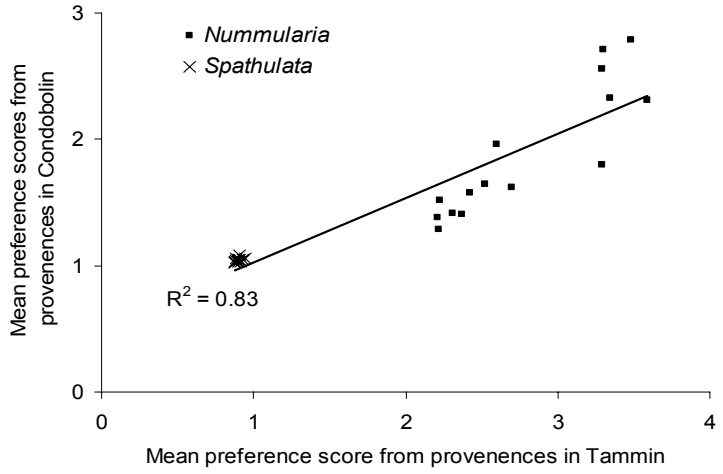
Introduction Climate change, leading to shorter and more variable seasons, represents a major challenge for livestock producers in southern Australia. Native perennial shrubs such as saltbushes (*Atriplex* spp.) are currently used in semi-arid and saline livestock systems to buffer seasonal feed shortages. A major limitation to saltbush systems is that the shrubs tend to accumulate high levels of salt (up to 30% of DM), have low organic matter digestibility (OMD) and accumulate a range of plant secondary compounds (Norman *et al.* 2010). The majority of commercial plantations of saltbush are based on 'wild' material and farmers report significant differences in the relative preference between shrubs within plantations. The palatability of forage concerns the interrelationship between flavour (odour, taste and texture) and postingestive feedback from nutrients and toxins (Burritt and Provenza 2000). What are herbivores telling us about saltbush when they selectively graze some shrubs before others? The aim of this work was to investigate differences in the relative palatability of provenances of old man saltbush (*Atriplex nummularia*). We tested the hypothesis that two flocks of sheep in different geographical locations will have similar preferences amongst 27 provenances of old man saltbush.

Materials and methods Provenances of old man saltbush were collected from 27 sites across Australia and grown in two plantations in Western Australia (Tammin) and New South Wales (Condobolin). The provenances included two subspecies; *Nummularia* (n = 16) and *Spathulata* (n = 11). Each plantation contained approximately 20 000 individuals (over 700 of each provenance), which were planted in randomly allocated groups of 4 plants within 8 replicate blocks. The plantations were fenced into 4 plots for grazing. Two years after establishment the plots were grazed with unrelated flocks of 1 year old Merino sheep at stocking densities of 25 and 50 sheep/ha (2 blocks per site for each). Defoliation of the shrubs was assessed when about half of the biomass had been eaten using a visual ranking with scores ranging from 0 (untouched) to 5 (all leaves removed). Laboratory analyses of biomass from the provenances were conducted according to the methods described in Norman *et al.* 2010. Linear regression was used to assess the relationship between relative preferences at the two sites.



F**igure 1** Relative preferences of sheep amongst 27 provenances of old man saltbush grazed at two sites. The higher the score, the greater the defoliation of shrubs within the provenance.

Results There was a significant relationship in the relative preferences of sheep amongst provenances at the two sites (R^2 = 0.83, P < 0.001). The sheep at Tammin and Condobolin demonstrated similar likes and dislikes across the range of provenances. The most striking result is that subspecies *Nummularia* was consistently preferred to subspecies *Spathulata*. Analysis of biomass samples from the provenances suggests that the animals could have been using nutritional wisdom – subspecies *Spathulata* had significantly lower OMD (P < 0.001), crude protein (P < 0.001) and higher salt (P < 0.001). Within subspecies *Nummularia*, differences in relative preference were significant (R^2 = 0.79, P < 0.01), indicating that shrub selection within the subspecies was not random; however it is unclear what was driving these differences. There were no significant linear relationships between preference score and OMD, OM, crude protein or the other minerals measured. It is possible that the sheep are selecting for or against a mineral or secondary plant compound that we have not measured. There was no significant linear relationship between relative preferences of provenances within subspecies *Spathulata.*

Conclusions The inclusion of herbivores in the early stages of plant domestication offers an opportunity to select lines with higher relative palatability thus leading to higher voluntary feed intake (VFI). Variation in VFI accounts for at least 50% of the variation that is observed in feeding value of forages (Ulyatt 1973). This approach also offers an opportunity to screen for nutritional or anti-nutritional traits that can be detected by the animals. We believe that this approach is valuable when dealing with perennial plant species that are new to agriculture.

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Intensification alternative for pasture-based systems in Australia: a complementary forage system whole farm study

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Introduction Australian dairy farmers are facing decreasing availability of land and water and declining terms of trade. In this context, systems that are able to increase milk production per ha from home-grown feed, beyond the potential of pasture only, are sought. The complementary forage system (CFS) was developed for this purpose, based on the integration into a pasture based dairy farm of a forage crop rotation that reported a utilised yield of more than 40 t DM ha^{-1} $year^{-1}$ (Garcia *et al*., 2008). The objective of the present study was to evaluate the feasibility of implementing a CFS to achieve 25 t dry matter (DM) of home-grown feed $ha^{-1}year^{-1}$ over the whole farm area and converting this into 35,000 litres of milk ha^{-1} $year^{-1}$.

Materials and methods A two-year whole farm study with 100 milking cows on 21.5 ha evaluated the implementation of a CFS that combined an area with a rotational sequence of two or three forage crops per year with an area of pasture in 35 and 65% of the farm area, respectively. Cows were fed 1 t DM of concentrates $lactation^{-1}$ as the only brought-in feed in the diet. Utilisation and nutritive value of all forages and milk yields of individual cows were measured daily; body condition and body weight weekly, and milk composition fortnightly. Individual cows and paddocks were considered as experimental units for animal related and forage related variables, respectively.

Results Over 26 t DM ha^{-1} $year^{-1}$ was utilised over the whole CFS farm for the two years of the study (Table 1). This utilised forage had a mean metabolisable energy value of 10.2 MJ kg DM^{-1} and crude protein content of 20.5% DM. The mean ± standard deviation daily DMI over the two years of the study was 20.4 ± 2.6 kg^{-1} cow^{-1} day^{-1} and the proportion of each feed fed was: 42.6% pasture, 9.8% legumes plus forage rape, 30% maize silage and 17.6% concentrates.

Table 1 Mean ± standard error of the mean forage yield (t DM $ha^{-1}year^{-1}$) for each forage section of the farmlet and total for the whole farm [complementary forage system (CFS)] for years 1 and 2.

		Pasture		Double crop		Triple crop		Total (CFS)	
Year		1	2	1	2	1	2	1	2
Pasture	(t DM ha^{-1} $year^{-1}$)	23.9 ±1.4	20.8 ±1.3	-	-	-	-	-	-
Forage rape	(t DM ha^{-1} $year^{-1}$)	-	-	-	-	6.6 ±0.6	7.5 ±1.2	-	-
Legumes	(t DM ha^{-1} $year^{-1}$)	-	-	6.4 ±0.2	9.6 ±0.2	4. ±1	5.2 ±1.2	-	-
Maize	(t DM ha^{-1} $year^{-1}$)	-	-	19.9	21.6	21.9	28.6	-	-
Total		23.9	20.8	26.3	31.2	32.5	41.3	26.0	26.6

Note: values in Table 1 are the arithmetic mean of individual paddocks yields and not a weighted average in relation to their size.

Table 2 Mean ± standard error of the mean milk yield (L cow^{-1} $lactation^{-1}$ corrected to 305 days and L ha^{-1} $year^{-1}$) and milk composition (kg) for the whole farm [complementary forage system (CFS)] and from home-grown feed meaned over the two years.

	Milk (L)	Fat (kg)	Protein (kg)
Yield cow^{-1} (lactation 305 days)	7,653 ± 105	321 ± 4	273 ± 10
Yield ha^{-1} $year^{-1}$ (from home-grown feed)	27,831	1,166	993
Yield ha^{-1} $year^{-1}$ (total)	34,499	1,445	1,231

Conclusions This study has shown that a high production forage crop rotation can be successfully integrated into a pasture-based farm system to increase the production of milk from home-grown feed well beyond the limits of a pasture only system. The total milk yield from home-grown feed of 27,831 litres of milk ha^{-1} $year^{-1}$, although below the initial target, was higher than any other whole farm study reported in the literature. This study warrants further investigation to determine the environmental and economic sustainability of the implementation of the CFS.

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Effects of two protein sources on the periparturient relaxation of immunity to gastrointestinal nematode parasites

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Introduction Studies in periparturient parasitized ewes indicate that increased supply of metabolizable protein (MP) at times of MP scarcity reduces the degree of the periparturient relaxation of immunity (PPRI), as shown by reduced worm burdens and worm egg excretion (Kyriazakis and Houdijk, 2006). However the extent to which increased metabolizable protein (MP) supply reduces PPRI differs between studies and it may be influenced by the digestible undegradable protein (DUP) proportion of the additional MP (Sakkas *et al*., 2010). This is because DUP is closer to the specific AA requirements that parasitism imposes to the host. Here we hypothesize that additional MP supply from xylose-treated soybean meal would be more effective than from field beans in reducing the degree of PPRI, as the former has a higher DUP proportion compared to the latter.

Materials and methods Sixteen twin- and eight triple- bearing Mule ewes were trickle infected with *Teladorsagia circumcincta* every Mon-Wed-Fri from day_{-49} until day_{31} (day_0 is parturition). On day_{-24}, ewes were allocated to one of three feeding treatments (n=8), balanced for FEC, number of lambs carried and body weight (BW). Ewes were fed at 0.9 times their metabolizable energy requirement and at either 0.8 (LP) or at 1.2 times their respective MP requirements (AFRC, 1993) using either xylose-treated soybean meal (HPS) or field beans (HPB) and feed intake was measured daily. Litter size was standardized to two at parturition. Daily food intake was measured from day_{-24} until day_{31}. Foods were analysed for DM and crude protein (CP) while metabolizable energy (ME), ERDP, DUP and MP and their average daily intake was calculated. Ewe and litter BW were measured weekly in order to calculate average daily weight gain. Ewe condition score (CS) was measured weekly. Regularly collected blood samples were analysed for plasma urea, albumin and pepsinogen concentrations. Ewe faecal egg counts were assessed twice weekly. Faeces were collected from day+19 to day+21 to measure dry matter (DM), DM digestibility, and daily nematode egg excretion during the lactation period. Averages of the parameters over the lactation period were analysed using ANOVA, with diet as a factor.

Results Parameter	Feeding treatments HPS	HPB	LP	SED	P
DM (g/day)	2667	2607	2596	73.7	0.582
ME (MJ/d)	27.91	26.59	26.22	0.753	0.083
CP (g/day)	416.0	455.3	297.2	11.96	<0.001
ERDP (g/day)	263.3	311.0	238.0	8.26	<0.001
DUP (g/day)	167.5	146.8	64.0	3.86	<0.001
DUP/MP ratio	0.50	0.43	0.30	0.00	<0.001
MP (g/day)	334.0	343.6	214.6	8.98	<0.001
DM digestibility	0.709	0.641	0.610	0.032	0.017
Ewe weight gain (g/d)	0.025	-0.051	-0.161	0.034	<0.001
Lamb weight gain (g/d)	0.606	0.550	0.503	0.031	0.013
CS	2.038	2.056	1.871	0.074	0.037
Albumin (g/l)	25.96	24.07	25.0	0.946	0.147
Urea (mmol/l)	11.14	10.05	7.0	0.817	0<.001
Pepsinogen (iu/l)	1.14	1.23	1.98	0.254	0.006
Nematode egg excretion (eggs/d) (x1000)	721.9	1366.4	1896.8	371.3	0.016

Results DM (g/d) and ME (MJ/d) intake was similar among our feeding treatments (P>0.05) while CP (g/d) and ERDP (g/d) intakes were significantly higher for HPB than HPS and higher for HPS than LP ewes (P<0.001). DUP (g/d) intake (P<0.001), DUP/MP ratio (P<0.001) and DM digestibility (P<0.05) was higher for HPS than HPB ewes, the latter being higher than LP ewes. MP (g/d) intake was higher for HPS and HPB than LP ewes (P<0.001). Ewe weight gain (g/d) was higher for HPS than HPB ewes and higher for HPB than LP ewes (P<0.001). Lamb weight gain (g/d) was higher for HPS than LP ewes with HPB ewes being intermediate (P<0.05). CS was higher for HPB and HPS ewes than LP ewes (P<0.05). Albumin concentration (g/l) was not affected by feeding treatments (P>0.01), while urea (mmo/l) (P<0.001) and pepsinogen concetration (iu/l) (P<0.01) was higher for HPS and HPB ewes than for LP ewes. Finally nematode egg excretion (eggs/d) was higher for LP than HPB ewes, and higher for HPB ewes than HPS ewes (p<0.016).

Conclusion The achieved DUP intake and the DUP/MP ratio were, as intended, higher for HPS ewes than HPB ewes. The lower DM digestibility of the HPB feeding treatment compared to HPS ewes imposed a penalty on ewe weight gain but not on lamb weight gain or to condition score. Urea concentration was similar for HPS and HPB ewes reflecting the similar MP supply of the two feeding treatments. Both protein sources resulted in reduced pepsinogen concentration which is indicative of reduced abomasal damage in response to abomasal parasitism. However nematode egg excretion was higher for HPB than HPS ewes suggesting extra MP supply from field beans is less effective in reducing the degree of PPRI than MP supplementation from xylose-treated soybean meal. Thus, dietary protein quality needs to be considered when formulating high MP diets for worm control in sheep.

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Crude glycerin as an alternative energy source for dairy cows in mid lactation

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Introduction The crude glycerin is the main subproduct generated from biodiesel production: for every liter of biodiesel produced, around of 100 ml of crude glycerin are generated (Thompson & He, 2006). In 2009, biodiesel production in Brazil was about 1.5 billions liters (ANP, 2011), resulting in a large glycerin surplus in the country. New uses of this residue must be tested, and animal nutrition is a good form to use this subproduct. The aim of this study was to evaluate the influence of different levels of crude glycerin (containing 83 % glycerol) in the diets of dairy cows on fat-corrected milk, milk composition, digestibility and efficiency.

Materials and methods An experiment was carried out at EMBRAPA Clima Temperado, located at Capão do Leão (latitude 31° 52' 20" south, longitude 52° 21' 24" west), Rio Grande do Sul, Brazil. Eight Jersey cows, in 2nd to 8th lactation, with 60 to 100 days in milk at the beginning of the trial, and kept in free-stall, were used. Treatments studied included: without crude glycerin in the diet; 4 % of crude glycerin in the diet (DM basis); 8 % of crude glycerin in the diet; 12 % of crude glycerin in the diet. The 4 % fat-corrected milk was calculated according (Gaines & Davidson, 1925). Milk composition was analyzed by infrared spectroscopy, according the AOAC (1996). The digestibility was determined using chromium oxide as an external marker for the fecal excretion; the dry matter intake (DMI) was measured directly. Feed efficiency was obtained by the ratio between the DMI and the milk production. Energy efficiency was obtained by the ratio between net energy of lactation (NEl) intake and the NEl produced, both estimated according the NRC (2001). The experimental design was a double latin square. The dates were analyzed by ANOVA and mean test (Tukey) and/or regression analysis using the procedure GLM of statistics program SAS.

Results The level of inclusion of crude glycerin did not influenced the organic matter (OM) digestibility, 4 % fat-corrected milk production, milk fat content, feed efficiency or energy efficiency (Table 1; $P>0.05$). Milk protein was greater with 12 % crude glycerin than without its inclusion (Table 1; $P<0.05$). NDF digestibility increased in a linear manner with the crude glycerin inclusion in the diet (Figure 1; $P<0.05$).

Table 1 4 % fat-corrected milk production, milk fat, protein milk, OM digestibility, feed efficiency [milk produced (kg)/DMI (kg)], energy efficiency (NEl from the milk produced/NEl intake)

	Glycerin inclusion (%)					
	0	4	8	12	Mean ± s.d.	p-value
4% fat-corrected milk	19.02	18.84	19.48	17.84	18.79 ± 3.03	0.2704
Milk fat (%)	3.77	3.67	3.55	3.4	3.60 ± 0.60	0.2658
Milk Protein (%)	3.61[b]	3.62[ab]	3.69[ab]	3.72[a]	3.66 ± 0.19	0.0230
OM Digestibility (%)	62.76	61.38	62.8	63.21	62.54 ± 4.05	0.4158
Feed effic. (milk prod/DMI)	1.01	1.01	1.05	0.96	1.01 ± 0.11	0.4153
Energy effic. (NE1 milk/NE1 intake)	0.24	0.22	0.24	0.22	0.23 ± 0.03	0.4266

[a,b]Different letters in the same line indicated a significant difference (Tukey 5 %)
OM = organic matter; DMI = dry matter intake; NEl = net energy of lactation

Conclusions Crude glycerin from biodiesel production, in Brazil, utilized with a level of inclusion up to 12 % in the diet, is a good alternative energy feed to milk cows, replacing corn grain without any modification in the milk production or efficiency. The supply of a quickly highly available energy source improved NDF utilization and increased milk protein.

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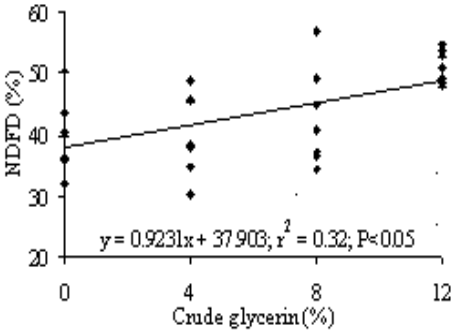

Figure 1 Effect of the crude glycerin on NDF digestibility (NDFD)

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Effects of irrigation and rates and timing of nitrogen fertiliser on proportions of maize plant fractions and nutritive value of whole crop maize silage for dairy cows

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Introduction Water and nitrogen (N) are the two most important nutrients that limit production of pasture and crops. Maize crop, which is one of the major feeds for dairy cows, can produce over 25 t dry matter (DM)/ha but requires up to 800 mm of water/ha (Garcia *et al*. 2008). Phillip *et al*. (2005) reported that increasing irrigation increases proportion of stem, fibre content and reduces metabolisable energy (ME) content of forages. However, there is less information on these aspects on maize crop grown for silage. This study therefore investigated the effects of different levels of irrigation water and rates of pre and post-sown N fertiliser on different plant fractions of maize before ensiling and on the nutritive value of maize silage for dairy cows.

Materials and methods The experiment was carried out at Camden, NSW, Australia using a 2 x 3 x 4 split-plot design with four replicates (blocks). Treatments were two pre-sowing N fertiliser (0 and 135 kg/ha) applied a day before sowing, three post-sowing N fertiliser (0, 79, 158 kg/ha) applied at the six leaf stage (6 leaves with visible collar) and four levels of irrigation water (0, 33, 66 and 100% water; i.e., 0, 153, 305, 480 mm/ha; plus rainfall, 235 mm). Pre-sowing N treatments were assigned to the main plots, and irrigation water and post-sowing N fertiliser were randomly allocated to the sub-plots within each main plot. Hybrid maize seed (Pioneer 31H50) was sown in 3.5 m x 3.5 m plots (96 plots) on 20 October and harvested at physiological maturity. Plants were cut at 15 cm above ground, weighed and chopped to a particle length of approximately 2.5 cm. The chopped material was ensiled in micro-silos (3.3 L capacity buckets). All buckets were opened 45 days after ensiling and pH was measured immediately. Samples were oven-dried and analysed for chemical composition. *In vitro* digestibility was determined using the rumen liquor from three fistulated lactating Holstein dairy cows.. Metabolisable energy (ME) content was estimated from *in vitro* DM digestibility, which was corrected for oven DM. Data were analysed using REML procedure, with treatments and blocks as fixed and random effects, respectively.

Results Increase in irrigation water (from 0 to 100%, respectively) increased ($P<0.001$) the proportion of grain from 9.3 to 31.5% with a concomitant reduction of the stover fraction. However, increased irrigation water also increased ($P<0.001$) plant height and weight (from 165cm and 98g DM/plant to 264cm and 186g DM/plant, respectively). This resulted in increased ($P<0.05$) neutral detergent fibre (NDF) content of maize silage (Figure 1). Increasing irrigation also decreased ($P<0.001$) crude protein (CP) and water soluble carbohydrate (WSC) contents by 33% and 64%, respectively (Figure 2), in association with a greater partition of nutrients to the cob. All together, these changes resulted in a total 10% decrease in ME content of maize silage as water irrigation increased. In contrast, application of post-sown N fertiliser increased CP (18%, $P<0.01$) and ME (21%, $P<0.05$) content of silage. There was also an irrigation x pre-sown N interaction for ME ($P<0.05$) content, but this effect was small compared to the main effect of treatments.

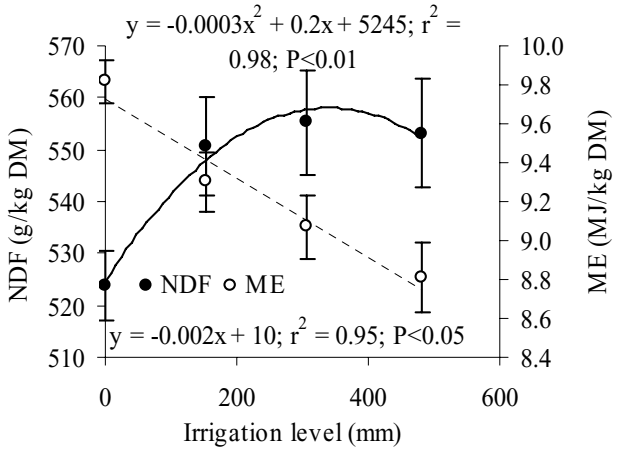

Figure 1 Relationship between irrigation level and (●) NDF and (○) ME contents of maize silage

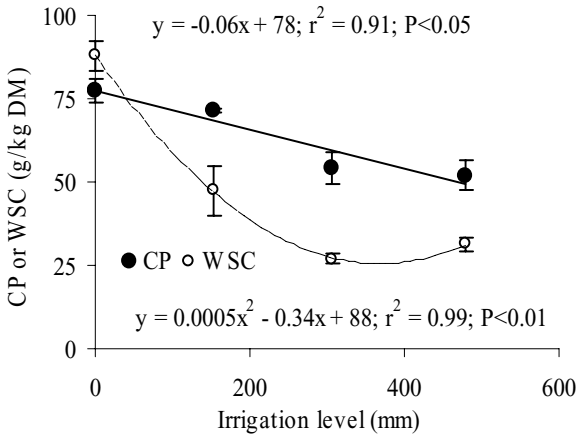

Figure 2 Relationship between irrigation level and (●) CP and (○) WSC contents of maize silage

Conclusions Results indicate that increasing irrigation water can adversely affect the nutritive value of maize silage for dairy cows by increasing NDF and decreasing CP and WSC contents and, therefore, ME content. This was despite a substantial increase in the proportion of grain in irrigated treatments. In contrast, application of N fertiliser, in general, increased ME content of maize silage, due mainly to an increase in CP content.

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Effects of dietary molybdenum, sulphur and zinc on the excretion and hepatic accumulation of copper in sheep fed palm kernel cake based diets

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Introduction Palm kernel cake (PKC) is a by-product of the palm oil industry; approximately 3 million tons is produced in Malaysia annually and exported mostly to Europe for use in diets of cattle. It is a good feed for ruminants, but because of its high content of Cu (11 to 55 mg/kg DM) its use in the sheep and goat diets is limited (maximum 30% of DM) due to the relative sensitivity of sheep and goats to the chronic Cu toxicity. However the sheep/goat is the main and expanding Malaysian ruminant production industry and potential user of PKC if the bioavailability of the Cu in PKC could be reduced to prevent the problematic chronic Cu toxicity. The already known dietary Cu-binding supplements of Mo, Mo+S and Zn (Suttle 2010) were used in the present experiment with lambs and their Cu binding efficacy was compared by measurements of the relative Cu body retention, and of blood plasma and liver concentrations.

Materials and methods Twelve male 8-month old lambs were used in a 6-month feeding experiment to determine the effects of dietary Mo, Mo+S and Zn supplements on the body retention and liver accumulation of dietary Cu. The lambs were divided into four groups of three and each group was fed *ad libitum* one of four diets. A control diet was based (DM basis) on PKC (86.1%) and grass hay (10%)*;* it contained 15.1% crude protein, 44.2% acid detergent fibre and 22.9 µ/g Cu. Three additional diets were the control supplemented either with Mo (20 mg/kg DM), Mo (20 mg/kg DM) + S (1g/kg DM) or Zn (500 mg/kg DM). The lambs had a free access to deionised drinking water. At three months of the experiment, faeces and urine were collected and sampled for six days. At the end of the experiment (6 months) blood was sampled and then the sheep were slaughtered. The liver was removed and sampled for chemical analysis.

Results In comparison with the control, each dietary supplement decreased ($P<0.05$) the Cu concentration in the liver, but only the Mo+S supplement decreased it to below 350 µg/g DM (Table 1). This was accompanied with the body retention of dietary Cu of 24.6%, 6.7%, 2.5% and 6.5% for the control, Mo, Mo+S and Zn treatments, respectively. The blood plasma concentration of Cu was decreased ($P<0.05$) by the Zn supplement, but was not affected ($P>0.05$) by the other supplements.

Table 1 Daily Cu intake, excretion and retention, and concentration of Cu in blood plasma and liver of lambs

	Diet							
	Control		+ Mo		+ Mo+S		+ Zn	
Item	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Intake (mg)	13.40	1.18	15.72	1.74	14.39	2.15	13.44	2.08
Fecal excretion (mg)	9.88^{a}	1.02	14.39^{b}	1.60	13.39^{b}	2.17	12.36^{ab}	1.74
Urinary excretion (mg)	0.22^{a}	0.04	0.27^{a}	0.12	0.64^{b}	0.06	0.21^{a}	0.05
Retention (mg)	3.30^{a}	0.23	1.06^{b}	0.11	0.36^{a}	0.08	0.87^{b}	0.31
Retention (%)	24.6^{c}	1.0	6.7^{b}	0.9	2.5^{a}	0.7	6.5^{b}	1.2
Blood plasma (µg/ml)	1.86^{b}	0.07	1.81^{b}	0.15	1.59^{ab}	0.31	1.29^{a}	0.23
Liver (µg/g DM)	1196^{c}	27	571^{b}	188	199^{a}	83	673^{b}	18

$^{a\text{-}c}$Means within the same row followed by the same superscript letter are not statistically different ($P>0.05$).

Conclusions It was concluded that from the supplements tested only Mo+S appeared to be effective in reducing the retention and liver accumulation of the dietary Cu to safe level; the liver Cu concentration range of 350 to 1000 µg/g DM is generally indicative of the liver Cu overload and marginal toxicity (Underwood and Suttle, 1999). Therefore dietary Mo+S supplement appears to be effective in preventing chronic Cu toxicity in sheep fed PKC-based diets. Indeed a follow-up experiment shoved that the supplement in the amount of 8 mg Mo and 1 g S per kg of the PKC DM used in the diet of sheep is optimal for reducing the hepatic Cu concentration to a safe level, without increase in the hepatic concentration of Mo (Alimon *et al*., 2011). Therefore, it can be concluded that PKC can be included in the diets of sheep and goats in any proportion if the appropriate amounts of Mo and S are mixed in the PKC portion before it is used for the dietary inclusion.

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Mineral supplementation of lambing merino ewes grazing dual-purpose wheat under Australian conditions increases twin-lamb growth rate

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Introduction Dual-purpose wheat forage is a valuable feed source to fill the winter feed gap in mixed farming systems of southern Australia. Wheat forage is highly digestible and has protein and calcium (Ca) levels sufficient for growing sheep, however it also has a high potassium (K), low sodium (Na) and marginal magnesium (Mg) content which may have implications for Mg availability and absorption (Dove and McMullen 2009). The growth rate of weaned lambs was significantly increased in Australian experiments by provision of loose-lick supplements containing Mg and/or Na (Dove and McMullen 2009). The higher demands for minerals of late-pregnant and of lactating ewes may exacerbate the deficiencies in wheat forage and result in metabolic diseases such as hypocalcaemia and hypomagnesaemia. The aim of this study was to assess the suitability of dual-purpose wheat for grazing by lambing ewes and the effectiveness of a loose-lick mineral supplement in preventing metabolic diseases in ewes grazing wheat forage in southern Australia.

Materials and methods In 2010, 292 merino ewes (mean weight non-pregnant 65.1 ± 0.61 kg; mean body condition score 2.70 ± 0.03) joined to lamb in June-July were allocated to one of 6 plots in a paddock of dual-purpose wheat (cv. EGA Wedgetail) on June 17 at Balldale, Australia. Ewes were previously identified as single-bearing or twin-bearing by ultrasound at mid-pregnancy, and were allocated so that stocking rate was equal (11.4 ewes/ha) across all plots and there was an equal number of scanned twin-bearing ewes per plot. Ewes in 3 plots were provided access to a loose-lick mineral supplement *ad libitum* consisting of magnesium oxide (Causmag® AL7), finely ground limestone and coarse salt in a 2:2:1 ratio. All lambs born were weighed within 24 hours of birth and recorded as being twins or singles. Lambs were weighed weekly throughout the trial. Grazing finished on August 10, on which date lambs were weighed for the final time. Individual plant samples of wheat forage were collected at the start and mid-point of the trial (10 samples pooled/plot/sample date) and were then dried, ground and analysed for mineral composition by near-infrared spectroscopy. Genstat for Teaching was used for statistical analysis, with lamb growth rates to final weighing analysed by repeated measures analysis of variance.

Results Results of analysis of forage samples are shown in Table 1.

Table 1 Comparison of mineral requirements for late-pregnant and lactating ewes with the mineral content of wheat forage.

	Ca g/kg DM	Mg g/kg DM	P g/kg DM	K g/kg DM	Na g/kg DM	K:Na	Tetany Index[2]
Requirement Pregnancy[1]	4.16	0.9	2.56	5.0	0.9	5.6	<2.2[2]
Requirement Lactation[1]	3.76	1.2	2.89	5.0	0.9	5.6	<2.2[2]
Wedgetail Wheat	2.71	0.8	2.61	50.6	0.24	211	6.4

[1] Estimated requirements based on CSIRO (2007) for a 65kg ewe with single foetus or single lamb. Requirements for twin-bearing ewes may be higher. [2] Kemp and t'Hart (1957)

Ewes consumed 24g/head/day of supplement on average. There was no significant difference in lamb growth rates for single-born lambs between treatment groups. However there was a small but significant increase ($p<0.05$) of 18 g/ day in the mean growth rate of twin-born lambs in supplemented groups. Whilst the survival rate of both twin- and single-born lambs was numerically higher in the supplemented plots, compared to lambs with the same birth status (twin or single) in control groups, this difference was not significant. No clinical cases of metabolic disease were observed in ewes during the wheat-grazing period in control or supplemented groups.

Conclusions The study showed that dual-purpose wheat can be a valuable source of feed for winter-lambing merino ewes. Whilst no clinical cases of hypomagnesaemia or hypocalcaemia were observed, the mineral profile of wheat forage shown in Table 1 indicates that the potential exists for metabolic issues based on the low to marginal mineral content (Ca, Mg, P). In addition, the very high tetany index, which exceeded the threshold of 2.2 in all samples, suggests magnesium supplementation may be necessary to prevent tetany, and the high K:Na ratio indicates Mg absorption from the rumen may be inhibited in ruminants grazing this pasture. While no response was seen in terms of animal health in this study, the higher growth rates of twin-born lambs demonstrates that provision of a loose-lick mineral supplement can improve production outcomes, particularly for ewes with higher net demands such as multiple lambs, and may be a cheap form of insurance against metabolic disease.

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Effects of pasture type and animal breed on bone metabolism of lambs grazing high altitude grasslands

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Introduction High alpine grasslands serve as feed resource for ruminant livestock species during summer. Low feed density and the steep slopes in alpine regions require large movement activities of the grazing animal (Liesegang *et al*., 2010). Due to their body size, sheep are well adapted to graze on those topographically and climatically harsh environments. Depending on magnitude and rate of strain, movement activities may influence bone metabolism in different ways (Bass *et al*., 2005). The present study aimed at investigating the effects of grazing on different alpine pasture types, characterised by vegetation type, slope and Ca content, and animal breed on bone metabolism of lambs.

Material and methods Lambs of two Swiss mountain breeds, Engadin Sheep (ES, 27; castrated males) and Valasian Black Nose Sheep (VS; 28; castrated males and females, 4:3), grazed for 9 weeks in paddocks allowing *ad libitum* forage intake on the following four pasture types: lowland control red/white clover-ryegrass ley, 0 % slope at 400 m a.s.l. (C), alpine *Crepido aurea-Festucetum* pasture, 0-5 % slope at 1950 m a.s.l. (F), alpine *Geo montani-Nardetum* pasture, >40 % slope at 2250 m a.s.l. (G) and alpine *Seslerio-Caricetum sempervirentis* pasture, >40 % slope at 2150 m a.s.l. (S). The initial live weights were 32.7±3.5 kg and 30.8±3.0 kg and ages were 26±2 and 18±7 weeks for ES and VS, respectively. Animals were balanced for gender, weight and age. Before and after the experimental period, markers of bone formation (bAP, bone-specific alkaline phosphatase) and bone resorption (ICTP, cross-linked carboxyterminal telopeptide of type I collagen) were analysed in blood samples and bone mineral density (BMD), bone mineral content (BMC) and cortical thickness (CT) measurements were conducted with peripheral quantitative computer tomography (pQCT) in the middle of the diaphysis on the left metatarsus. After slaughtering, maximum force (MF) to break the left metatarsus in the middle of the diaphysis was analyzed. Concentrations of Calcium (Ca) were analysed with COBAS MIRA® Autoanalyser in one pooled forage samples per pasture type. Data was subjected to analysis of variance considering pasture type, breed and the interaction of both. Furthermore, Pearson correlation coefficients were computed.

Results Concentrations (% DM) of Ca in forage samples were 0.54, 0.97, 0.50 and 1.62 for the pasture types C, F, G and S respectively, indicating a large variation in Ca supply of the lambs on the different pasture types. A trend (P=0.054) was found for a breed × pasture type interaction of BMD development. BMD increased during the experiment for ES by 101.6, 15.5, 24.8 and 48.1 mg/cm³ and for VS by 40.9, 97.5, 168.6 and 50.9 mg/cm³ on the pasture types C, F, G and S, respectively. The BMC increased during the experimental period for ES by 32.3 and 6.5 mg/cm and for VS by 5.0 and 33.8 mg/cm on C and F and even decreased for ES by 2.9 and 10.8 mg/cm and for VS by 22.8 and 10.6 on G and S, respectively. This results in pasture type differences (P<0.001), as well as a breed × pasture type interaction (P<0.001) for BMC development. The CT increased across all pasture types for both breeds during the experiment, but again (like for BMD) the increase in CT was higher for ES on C and for VS on the three alpine pastures (F, G and S), resulting in a significant breed × pasture type interaction (P=0.02). Further, a significant breed × pasture type interaction (P=0.004) as well as a significant breed difference (P=0.001) were found for MF to break the metatarsi in its middle. Metatarsi of VS were less stable than those of ES on the pasture types C, G and S while this was opposite for F. The qQCT measurements at the end of the experiment showed interestingly similar pattern for BMC, MF, BMD and CT although the development of those parameters during the experiment followed different ways. Close correlations were found between MF and BMC (R^2 =0.93; P<0.001) and between CT and BMD (r=0.94; P<0.001). Between BMC and BMD a significant correlation only developed during the experiment, with r=0.80 (P<0.001) at the end compared to r= 0.25 (P=0.064) at the beginning of the experiment. Calculation of modelling/remodelling rate (RMR) by dividing bAP through ICTP on both sampling dates gave a punctual impression of the bone metabolism of the lambs at those two specific dates. Significant pasture type differences (P<0.001) were found for RMR, as values decreased slightly during the experiment for both breeds on C and more clearly on G, whereas the decrease was remarkably stronger on both pasture types for ES than VS. This indicates that bone resorption outweighed bone formation processes, which corresponds with low Ca concentrations on C and G. Additionally, ES appears to be more sensitive in bone marker responds than VS. The RMR increased for both breeds during the experiment clearly on F and moderately on S, pointing towards facilitated bone formation processes on those pastures that had higher Ca concentration in the forage (F and S) compared to low Ca input pastures.

Conclusion Breed × pasture type interactions were found for BMC and CT development and also a trend for interactions for BMD development, indicating a different adaption of the two breeds to mountain pastures. The VS profit more from high alpine grazing compared to lowland grazing in terms of CT and BMD development while this was opposite for ES. The differences in alpine pastures characteristics, especially in slope, nutritional supply and also the individual movement behaviour of the lambs appear to influence BMC and RMR development throughout alpine grazing. Pasture types, including all characteristics mentioned, do have an important effect on bone metabolism of lambs and are tolerated by breeds to a different extent.

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Evaluation of fresh lettuce wastes in comparison with fresh pawpaw leaves, whole lettuce and cabbage wastes as sole feed ingredient for growing *Archachatina marginata* snails

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Introduction Large scale snail farming is needed in order to meet the animal protein need in human diet. There is a dearth in the supply of conventional feed concentrates which has greatly affected animal production in the tropics. This low level of livestock production in the tropics cannot meet the needs of the rapidly growing human populations. There is therefore the need to source for cheaper alternative sources of animal protein. This current investigation was designed to determine the performance of growing snails fed lettuce wastes, whole lettuce, pawpaw leaves and cabbage wastes as sole feed ingredient.

Materials and methods One hundred and twenty growing *A. Marginata* snails of mean weight of 132.91±2.13g were randomly allotted into 4 dietary treatments of pawpaw leaves (PL), whole lettuce (WL), lettuce wastes (LW) and cabbage wastes (CW). Each treatment was replicated thrice with 10 snails per replicate in a completely randomized design. Feed intake and weight gain were measured on a daily and weekly basis respectively. Shell length and width were measured with vernier calliper while micrometer screw gauge was used to measure the shell thickness on monthly basis. Other parameters determined were mortality and feed conversion ratio. The feeding trial lasted 6 months. At the end of the feeding trial, 9 snails per treatment were used to determine the dry matter digestibility. They were fed with the same diet fed during the feeding trial. Daily feed intake and excreta voided were recorded for each treatment. The daily excreta were dried in the oven at 60^0C and dry matter determined. Nine snails per treatment were also used for carcass analysis. Parameters determined were dressing percentage, visceral to live weight percent, shell to live weight percent and haemolymph per live weight percent. Proximate composition of the experimental diets as well as that of the foot of the snails was carried out (A.O.A.C, 2005). Parameters analyzed were dry matter, crude protein, crude fibre, ash and ether extract. The organoleptic evaluation of the cooked meat was carried out according to the method of Larmond (1977). All data were subjected to analysis of variance while the treatment means were separated using Duncan multiple range test (SAS, 1999).

Results The highest mean weekly feed intake of 8.79g was recorded in snails placed on WL while the lowest value of 6.45g was recorded for those on PL. Snails on CW recorded the highest mean weekly weight gain, dry matter digestibility and feed conversion ratio which were statistically similar to those on LW while those on PL recorded the least (Table 1). Snails on LW recorded the highest shell weight, shell per live weight and dressing percentage. The nutrient compositions of the meat in all the treatments were also similar. Dietary treatments also had no significant effect on the colour, taste, flavour, texture and overall acceptability of the snail meat.

Table 1 Performance characteristic of growing snails fed the experimental diets

Parameters (mean values)	Pawpaw Leaf (PL)	Whole Lettuce(WL)	Lettuce Waste (LW)	Cabbage Waste (CW)	SEM
Weekly dry matter feed intake (g)	6.45^{d}	8.79^{a}	8.18^{b}	7.81^{c}	0.32
Initial weight (g)	135.08	132.15	131.91	132.50	0.38
Final weight (g)	191.48^{b}	213.99^{a}	215.97^{a}	217.60^{a}	1.95
Weekly weight gain (g)	2.35^{c}	3.41^{b}	3.50^{a}	3.55^{a}	0.57
Total weight gain (g)	56.40^{c}	81.84^{b}	84.06^{a}	85.10^{a}	2.05
Monthly shell length gain (mm)	3.21^{b}	3.60^{a}	3.85^{a}	3.65^{a}	0.20
Monthly shell width gain (mm)	2.56^{d}	3.26^{a}	3.13^{b}	2.86^{c}	0.09
Monthly shell thickness gain (mm)	0.21^{a}	0.23^{a}	0.21^{a}	0.24^{a}	0.01
Mortality	0.00	0.00	0.00	0.00	
Dry matter digestibility	0.78^{c}	0.81^{b}	0.83^{a}	0.83^{a}	0.01
Feed conversion ratio	2.74^{a}	2.58^{b}	2.34^{c}	2.20^{d}	0.10

a,b,c,d: means along the same row with different superscripts are significantly different ($P<0.05$)
SEM – Standard Error of Means

Conclusion Lettuce wastes contain high nutrients which favour snail growth and development as evidenced in the total weight gain, feed conversion ratio, shell weight and dressing percentage. It could be fed fresh as sole feed or dried and incorporated into compounded ration as replacement for maize to enhance better growth of snails and increase in the supply of animal protein in Nigeria and so prevent these animals from going into extinction.

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Bacterial protein breakdown by different rumen protozoal groups

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Introduction Up to 50% of the microbial protein synthesized in the rumen is degraded to ammonia as a result of both bacterial and protozoal lysis and degradation (Leng and Nolan, 1984). Presence of rumen protozoa and their engulfment and digestion of bacteria is the most important activity regulating the turnover of bacterial N in the rumen. *Entodinium* is by far the most abundant protozoal genus in the rumen, and it has been suggested as the most important protozoan in absolute terms of bacterial protein breakdown (Wallace and McPherson, 1987; Ivan *et al*., 2000). However, the rumen protozoal population comprises more than 50 different species with a range of sizes and behaviours, and their capacity of bacterial breakdown is still largely unknown. The aim of this *in vitro* experiment was to identify the key protozoal groups most intimately related with bacterial protein breakdown. A differential fractionation procedure and several expression forms were used to evaluate how the protozoal type and size modified their activity.

Material and methods Protozoal activity was measured by the breakdown of ^{14}C leucine-labelled rumen bacteria *in vitro* as described Wallace and McPherson (1987) in two consecutive batches. Rumen protozoa and bacteria were isolated from rumen fluid obtained from four rumen-cannulated cows. Rumen bacteria were labelled by overnight culture using medium no. 2 of Hobson with ^{14}C-leucine (1.44 μ Ci/tube) at 39ºC. Bacteria were subsequently harvested, washed and resuspended in simplex type salts solution (STS, Williams and Coleman, 1992) containing ^{12}C-leucine to prevent re-incorporation of released ^{14}C-leucine. Bacterial protein concentration was determined with Folin reagent (Lowry *et al.,* 1951). For protozoal isolation, rumen fluid was diluted with two volumes of STS and protozoa were sedimented in a funnel at 39ºC for 1h. Plant components were removed from the sediment using a 250μm pore mesh, and the filtrate was then fractionated through 6 nylon meshes (80, 60, 45, 35, 20 and 5μm pore sizes). Fractions were resuspended in STS, a subsample was taken from each fraction and pooled to reconstitute the initial protozoal population. Every protozoal fraction was incubated in quadruplicate in Hungate tubes at 39ºC after addition of labelled-bacteria. A negative control was incubated to assess the rate of bacterial auto-degradation. Every tube was sampled every hour, samples were fixed with trichoroacetic acid, centrifuged (11,000 × *g* for 5 min) and the supernatant was diluted with scintillation fluid to determine the radioactivity by liquid-scintillation spectrometry. The bacterial breakdown at each incubation time was calculated from the acid soluble radioactive label and expressed as a percentage of the total counts per minute (CPM) present in the labelled bacterial suspension. The degradation rate per hour was calculated as the difference from the linear portion of the degradation curve. Concentration and size of every protozoa species was determined in each tube by optical microscopy. ANOVA was carried out to compare between fractions (blocking by batch) while multiple linear regression was used to predict the activity of each protozoal group studied.

Results Clear differences between fractions in the distribution of the 50 protozoal species were identified. However, the similar sizes of *Epidinium* and *Isotricha*, and *Entodinium* and *Dasytricha*, made their separation unfeasible. Estimations of protozoal activity by multiple linear regression showed that big *Diplodiniinae* can degrade 3 times more bacterial protein per cell than small *Diplodiniinae*, conversely the later group had double the activity per volume than the former. *Entodinium* and *Dasytricha* seem to have the lowest activity per cell but a greater activity per volume than *Epidinium* and *Isotricha*.

Table1 Protozoal fractionation and activity

Fraction	Initial	F80	F60	F45	F35	F20	F5	SEM	*P*
Big *Diplodiniinae*, %	6	44	28	4	0	0	0	1.26	<0.001
Small *Diplodiniinae*, %	25	8	14	28	29	31	8	1.76	<0.001
Epidinium, %	13	24	27	37	28	3	0	2.03	<0.001
Entodinium, %	13	1	1	1	1	10	36	0.66	<0.001
Isotricha, %	10	19	27	24	22	3	1	1.63	<0.001
Dasytricha, %	34	4	4	5	18	53	55	1.53	<0.001
Bacterial breakdown									
pg CP/ciliate·h	25	121	57	55	54	23	6	3.38	<0.001
ng CP/ciliate mm^3·h	103	170	98	138	158	195	92	18.8	0.001

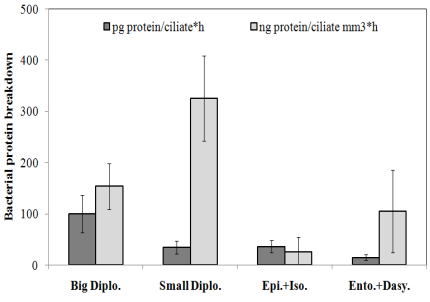

Figure 1 Bacterial breakdown by protozoal groups

Conclusions Our results show that the different protozoal groups have different capacities for bacterial protein breakdown. Protozoal cell size seems to determine the capacity of bacterial engulfment per protozoal cell while the protozoal genus determines their activity per protozoal volume unit.

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Microbial diversity of Archaeal community in the caecum of wild and domestic rabbits in Spain

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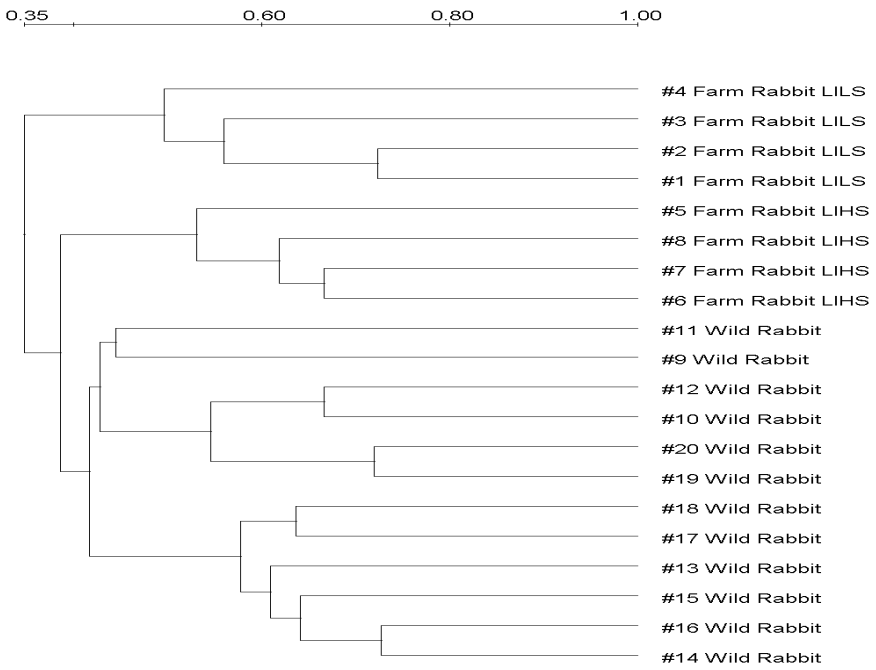
Introduction Rabbits are commonly used for intensive meat production in some European countries, mostly Italy, Spain and France. Although their microbial caecal population has been studied (Abecia *et al*. 2005; Monteils *et al*. 2008), scarce information is available on wild rabbits. Host genotype, feeding habits and environmental conditions may affect diversity of microbial digestive population of these animals. The importance of methanogenic community in the caecum of domestic rabbits has been recently studied (Micheland *et al*. 2010). Therefore, the aim of the present work was to characterise the biodiversity of the methanogenic Archaea community of the caecum of wild rabbits compared to that of domestic rabbits in intensive production conditions.

Materials and methods Sixteen wild rabbits were hunted in February 2009 in Magallón (Zaragoza, Spain). Besides, 8 commercial White New Zealand rabbits from the animal research facilities of the University of Zaragoza were weaned at 28 days and given diets (neutral detergent fibre, 370 g/kg) with two levels of neutral detergent soluble fibre (0.150, LS or 0.180, HS g/kg) until slaughtering at 49 days of age, according to the Spanish Policy for Animal Protection RD1201/05. Samples of caecal contents were taken and frozen in liquid N until analysis. DNA was isolated from thawed samples using the QIAamp® DNA Stool Mini Kit (QIAGEN Ltd., England) following the manufacturer's instructions. Quantitative and qualitative study of methanogenic *Archaea* was carried out by qPCR (Denman *et al*., 2007) and DGGE (Cheng *et al*. 2009), respectively. DGGE banding patterns were analysed by using Quantity One Software (BioRad) and the similarity among profiles was calculated with Dice coefficient and the clustering was done with the unweighted pair-group method using arithmetic averages (UPGMA). Banding profiles were quantified within each profile by determining the total number of bands, Shannon and Evenness indexes.

Results *Archaeal* DNA accounted for 0.948, 0.565 and 0.091 ng/ng total extracted DNA from caecum (RSD 0.6031; P=0.061) for LS, HS and wild rabbits, respectively. Averages of 22, 11 and 11 bands were detected by DGGE of samples, respectively, from domestic LS and HS rabbits, and wild rabbits. Shannon diversity index of Archaeal community in the caecum of domestic rabbits was affected by both the level of soluble fibre and the rabbit breed (3.07±0.104, 2.40±0.104 and 2.33±0.060 for LS, HS and wild rabbits; P<0.001). The Evenness index showed a similar trend, resulting 0.77±0.026, 0.60±0.0.026 and 0.58±0.015 for LS, HS and wild rabbits (P<0.001). Dendrogram from DGGE analysis of the *Archaeal* community (Figure 1) shows that both diet and breed clustered caecal communities separately. Similarity index between HS and wild rabbits was 0.40, whereas it was 0.35 between these and LS domestic rabbits.



Conclusions Results show a high biodiversity of Archaeal community in the rabbit caecum of both domestic and wild animals. It is not possible to link results in wild rabbits with their feeding habits, but in domestic meat producing rabbits it is largely dependent on the availability of fermentable input to the caecum in form of soluble fibre. Proximity of results between wild and HS rabbits might be explained by a potential feed selection. Further studies are required for identification of Archaea species inhabiting this ecosystem.

Figure 1 Dendogram derived from DGGE analysis of the Archaeal community in caecum of wild and farm rabbits.

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Evolution of bacterial diversity in the rumen in dairy calves from birth to weaning using a high throughput 454 GS FLX pyrosequencing

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Introduction The rumen in adult cow is an important site of fermentations, carried out by a microbial ecosystem that predominantly consists of bacteria. At birth, the calf rumen is sterile and milk is digested in the abomasum and the intestine. The rumen becomes functional later, with solid food intake. In lambs, Fonty *et al*. 1987 showed that the colonization of the rumen by bacteria begins during the first days of life and is sequential and Bryant *et al*. 1958 showed the same pattern from the first week of age in the calves. A lack of information persists on the establishment of the bacterial population during the very first days of life of calves, and most previous studies have focused on cultivable bacteria The aim of this study was to define, using molecular microbiology, the early implantation of the ruminal bacterial population and its evolution until weaning.

Materials and methods The experiment was carried out on 6 male Prim 'Holstein calves from birth (day 1) to 3 months of age (day 83), before weaning. The calves were separated from their mother at delivery and reared in individual pens. Body weight (BW) of the calves averaged 39.4 ± 1.7 kg and 120.5 ± 16.8 kg at birth and 83 days of age, respectively. A standard feeding program for unweaned calves, using a milk repacer, hay and a pelleted concentrate, was applied. The rumen samples were taken through a stomach tube every day until the 10th day after birth, then every 3 to 5 days to 83 days of age. Characterization of bacteria was achieved using V3 V4 hypervariable regions of the 16S RNA gene by pyrosequencing (454) on 132 samples. After cleaning and alignment, taxonomic assignment were performed using the open-source software Mothur V.1.12.3 (www.mothur.org) and a bacteria database based Silva 96 (1194263 reads). Number of and relative abundance of taxa according to phylum, class and order were analysed using a repeated-measures analysis of variance with a model that included the effects of age, calf and their interaction as fixed effects.

Results The compilation of all samples (*e.g.* 6 calves and. 22 sampling days) showed 430 different taxa. The number of taxa evolved with age ($P < 0.001$). Between 2 and 12 days after birth, the number of taxa increased from 185 to 229 (Figure 1). Between 15 and 43 days of age, the number of taxa decreased from 205 to 144 taxa. After one month of age, the number of taxa was stabilized at an average value of 155 taxa. On the 132 samples, there were 10 different phyla, the major being: Actinobacteria, Bacteroidetes, Firmicutes and Proteobacter (10, 15, 39 and 31%, respectively). Regarding classes, there were 17 different classes, the major being Actinobacteria, Bacteroidia, Bacilli, Clostridia, Betaproteobacteria and Gammaproteobacteria (11, 10, 9, 27, 9 and 15%, respectively). The importance of some classes changed between 2 and 83 days: Bacilli (from 16 to 8%) and Gammaproteobacteria decreased from 16 to 8% and from 25 to 12%, respectively, whereas Clostridia increased from 17 to 31%. Forty one orders were identified, the major being Actinobacteridae, Bacteroidales, Lactobacillales, Clostridiales, Burkholderiales, Enterobacteriales, Pasteurallales and Pseudomonadales (7, 11, 7, 28, 5, 3, 4 and 4%, respectively). Three orders were observed to change between 2 and 83 days: Lactobacillales and Enterobacteriales decreased from 13 to 5% and from 9 to 1%, respectively and Clostridiales increased from 16 to 31% (Figure 2) and showed a strong evolution during the first week.

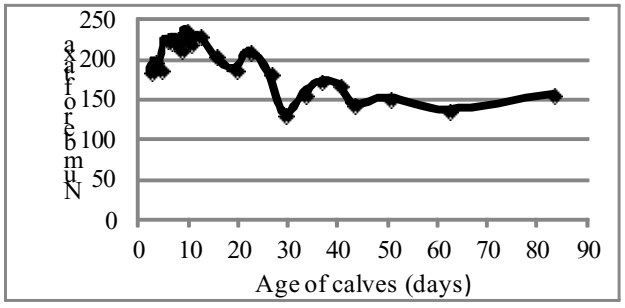

Figure 1 Evolution in the number of taxa according to the ageof calves from birth to 83 days

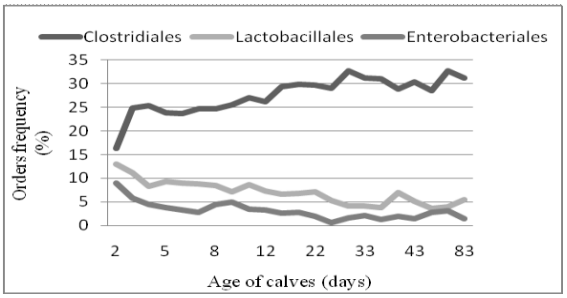

Figure 2 Evolution of 3 dominant orders freqency to the age of calves

Conclusions The use of 454 pyrosequencing showed an evolution of taxonomic diversity with the age of calves. This could be explained by successive steps of bacterial establishment in the rumen of calves from birth to weaning. The first one, between 2 and 30 to 40 days after birth would correspond to the establishment of pioneer species originating from the external environment. During the 2nd step the diversity reached stability, which could relate to an equilibrium between the microbiota and solid food arriving in the rumen.

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Functional genomic approach to assess dietary n-3 fatty acid effects on tissue function and lipogenesis in bovine muscle and adipose tissue

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Introduction Dietary intervention of farm animals with exogenous fatty acid sources has successfully been applied to improve meat quality traits (Nuernberg *et al.*, 2008; Scollan *et al.*, 2006; Woods & Fearon, 2009). As the underlying molecular mechanisms have not been completely elucidated as yet, the present study used a functional genomic approach to screen the expression of tissue function and lipogenesis related genes and gene products as affected by n-3 *versus* n-6 fatty acid based feeding regimes.

Materials and methods German Holstein bulls (n=27) were assigned to a maize-silage/n-6 fatty acid based control diet (control group (CG); n=14) or an isoenergetically formulated grass-silage/n-3 fatty acid based intervention diet (intervention group (IG); n=13) during a fattening period of 245±40 days until a live weight of 625±25 kg at an age of 477±40 days (CG) or 510±37 days (IG) was reached. Immediately after slaughter and exsanguination, *longissimus* muscle and subcutaneous adipose tissue samples were taken. Expression of lipogenesis and tissue function related genes (qRT-PCR) and proteins (Western Blot analysis), activities of lipogenic enzymes as well as fatty acid profiles (GC-FID) were analysed.

Results Gene expression analysis revealed that three major classes of genes were modulated by an n-3 fatty acid intervention: genes encoding (I.) lipogenesis related transcription factors (CEBPB, SREBP1c) and enzymes (ACC, FAS, SCD), (II.) intracellular lipid storage associated proteins (ADFP) and (III.) cell function and signalling associated proteins (SEMA3C, FDFT1, CHRNA1).
In *longissimus* muscle, reduced SREBP1c (P=0.02), ACC (P=0.00), FAS (P=0.10) and SCD (P=0.03) gene expression levels upon an n-3 fatty intervention corresponded to reduced protein expression levels of Δ6D (P=0.03) and SCD (P=0.03), diminished levels of SCD enzyme activity as well as reduced SFA (P=0.05), MUFA (P=0.02) and total FA (P=0.04) concentrations. Although lower degrees of functional genomic correlation between genes and gene products were obtained for subcutaneous adipose tissue, significantly reduced ACC (P=0.00) and FAS (P=0.01) gene expression corresponded to diminished SCD protein expression (P=0.02), SCD enzyme activity (P=0.03) as well as reduced MUFA (P=0.02), n-6 FA (P=0.00) and total FA (P=0.07) concentrations. Comparatively strongest functional genomic correlation between gene and gene products was detected in regard to the SCD axis (Figure 1).
Besides beneficial effects of dietary fatty acid intervention on lipogenesis, reduced expression of ADFP gene and enhanced expression of SEMA3C, FDFT1 and CHRNA1 gene in *longissimus* muscle upon an n-3 fatty acid intervention furthermore indicated improved tissue function *via* enhanced energy metabolism, vasculogenesis, innervation and mediator synthesis.

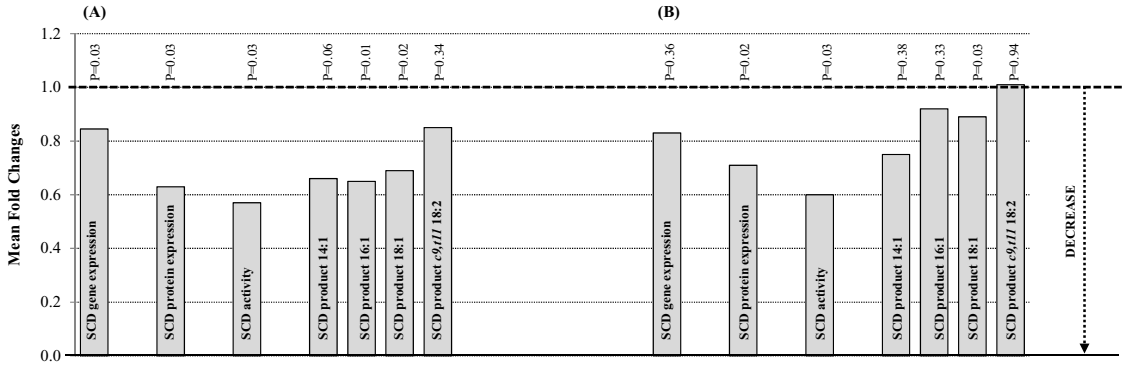

Figure 1 Mean fold changes of SCD gene expression, protein expression, enzyme activity and fatty acid products in longissimus muscle (**A**) and subcutaneous adipose tissue (**B**) upon an n-3 fatty acid intervention

Conclusions The study elucidated tissue-specific functional genomic responses to dietary fatty acid intervention and outlined the significance of n-3 *versus* n-6 fatty acid based feeding regimes on lipogenesis and tissue function in regard to quality tailoring of bovine tissues.

Acknowledgements The study was conducted and financially supported within the framework of EU Project ProSafeBeef (Project No. FOOD-CT-2006-36241). The authors kindly thank J.-F. Hocquette and co-workers (INRA, Clermont-Ferrand, France) for conducting microarray gene expression analyses, as well as B. Jentz and M. Dahm (FBN, Dummerstorf, Germany) for their gas chromatographic technical assistance.

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Integrating dual-purpose wheat to reduce supplement feeding in mixed crop and livestock farms in south-western Australia

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Introduction The use of dual-purpose crops (crops that are grown for both grain and grazing) has increased in southern Australia because of their potential to increase profitability in mixed farming systems (Kirkegaard, 2008). Using dual purpose crops effectively depends on the structure of various farm enterprises, as well as a range of factors including climate, soil types and commodity prices. The aim of this study was to investigate how the use of dual-purpose crops affects feed budgeting in different farm locations with different rotational sequences of crops and pastures.

Materials and methods A simulation experiment was conducted using the AusFarm biophysical model (Moore, 2007), based on a self-replacing Merino sheep and wheat growing enterprise with or without dual purpose cropping phases. The simulation experiment was located at 3 sites in Western Australia (East Binnu, Pingelly and Bakers Hill), based on meteorological data from 1908-2007, with an aim to represent the range of rainfall zones that exist in the main grain growing region of Western Australia. Two soil types ('Fertile' and 'Marginal') were selected at each site, with a different 4-year rotation sequence applied to each soil (a combination of either pasture (p), wheat (w) or dual-purpose wheat (d)). A single rotation sequence was used for Fertile soils (pwww), and one of four rotation sequences was used for Marginal soils (pwww, pppw, pddd or pppd). The proportion of pasture, supplementary feed and dual-purpose crop consumed by livestock in each farm model was determined. Differences in the level of supplementary feeding required across locations and rotation sequences were determined by 2-way analysis of variance.

Results When dual-purpose crops were made available for grazing, the combined amount of pasture and supplementary feed required by sheep decreased proportionally to the amount of dual–purpose crop that was consumed, as was expected. The amount of supplementary feed required was reduced most at East Binnu ($P<0.001$), which is the site with the lowest long-term annual rainfall. There was a significant interaction between location and rotation sequence ($P=0.004$), which may be related to a more consistent decrease in supplementary feeding across rotation sequences for East Binnu compared with the other 2 locations (Figure 1).

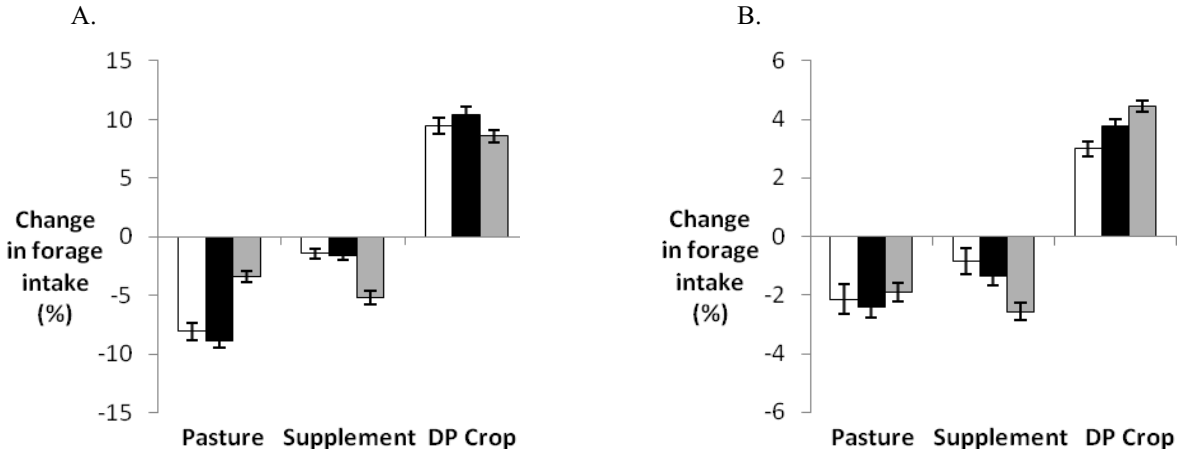

Figure 1 Change in simulated forage intake (%) from pasture, supplementary feed and dual-purpose crop when a dual-purpose crop is made available for grazing for A. pasture, pasture, pasture, wheat and B. pasture, wheat, wheat, wheat rotations on the 'Marginal soil' farm land. Three sites are used in the simulation study Bakers Hill (white bars), Pingelly (black bars) and East Binnu (grey bars).

Conclusions Dual purpose crops can be integrated in mixed farming systems to reduce supplementary feeding and lower pasture utilisation. The results of this study indicate that dual purpose crops may be most effective to reduce supplementary feeding costs in lower rainfall areas, whereas in higher rainfall regions a greater reduction in pasture utilisation is likely.

Acknowledgements This project was supported by the Australian Grains Research and Development Corporation and the CSIRO Sustainable Agriculture Flagship.

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Intake of various dry rangeland shrubs by fat-tailed Awassi sheep as affected by previous experience and offer in a multiple choice situation

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Introduction Increasing feed prices and competition for food between humans and animals demands for solutions to decrease the dependency on purchased feeds for animals. A possibility for sheep in Syria and elsewhere in the Mediterranean area could be to increase the contribution of feeds from rangelands in their diets. One option could be to establish fodder shrub plantations in the rangelands which at the same time may fulfil nature conservation purposes (Le Houérou, 2000). However, shrubs often contain unknown and unknown amounts of antinutritive factors thus limiting feed intake through low palatability or through the animal's lack of habituation with novel species. Providing the animals a choice between different shrubs might overcome such limitations (Provenza *et al*., 2007). Three hypotheses were tested in the present study: (i) animals fed a straw-based diet enrich their diets with fodder shrubs to a different degree depending on the shrub, and increase total nutrient intake when offered fodder shrubs, (ii) animals ingest more of the shrubs when they have experienced them earlier, and (iii) total feed intake increases when offered additionally to straw a choice among different fodder shrubs compared to only straw.

Materials and methods Dried leaves of five shrubs were tested in three subsequent choice-feeding experiments with 12 lactating fat-tailed Awassi sheep penned individually at the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria. Shrubs included *Artemisia herba-alba*, *Atriplex leucoclada*, *Haloxylon articulatum*, *Noaea mucronata* and *Salsola vermiculata*. In addition animals always received 1.1 kg/d concentrate supplemented with a mineral mix to cover requirements for maintenance and milk production in the afternoon. Barley straw was always available at *ad libitum* access. In the first experiment six sheep were used. These animals were offered all experimental shrubs in a random order together with barley straw in a binary choice situation for 4 hours every morning during 7 consecutive days. Between each testing phase straw was fed as forage for two days. This procedure was repeated until each animal had been exposed to every shrub once for 7 days. In the second experiment, the six 'adapted' sheep and six other sheep which had received only straw as forage in the meantime (non-adapted group) received for 14 days all experimental shrubs in separate troughs in a multiple-choice situation during 4 hours *ad libitum* every morning. During the rest of the day, straw was provided *ad libitum* together with concentrate. The third experiment consisted of another multiple-choice situation which included straw during the morning additionally to all shrubs for the test group (n=6, randomly consisting of 3 sheep of the adapted group and 3 sheep of the non-adapted group). The other half of the animals (control group, n=6) received only straw *ad libitum*. Again, after the 4 hour experimental feeding period straw *ad libitum* and concentrate was offered all sheep for the rest of the day. Intake was measured after each 4-hour morning feeding and after each 24-hour period. Feed samples were analysed for their proximate contents. Data was analysed with the Mixed Model procedure of SAS 9.2. and Tukey's procedure was used for multiple comparisons among means.

Results Crude protein was highest in *A. leucoclada* (180 g/kg DM) and lowest in the straw (49 g/kg DM). The digestible organic matter in DM (DOMD) was also highest in *A. leucoclada* (507 g/kg DM) but lowest in *N. mucronata* (202 g/kg DM). In the first experiment, *A. leucoclada* (238 g dry-matter per day (DM/d)) was the significantly most preferred shrub among the five experimental shrubs tested, followed by *S. vermiculata* (133 g DM/d). *N. mucronata* was almost entirely rejected by the animals (6 g DM/d) and the intake of *H. articulatum* and *A. herba-alba* was very low (28 g DM/d and 19 g DM/d, respectively; overall $P<0.001$ for species effect, overall SEM: 23.4). The total intake over 24 hours was higher ($P<0.05$) for the binary choice between *A. leucoclada* and straw compared to offering only straw (962 g *vs*. 756 g DM/d, SEM: 46.2). In experiment 2, adaptation had no significant effect on total daily and 4-hour shrub intake (total and individual). When given the choice of additional shrubs for 4 hours in the morning in experiment 3, intake was higher during 4 hours and daily (824 g DM and 1262 g DM) than when only offered straw as alternative (454 g DM; overall $P<0.01$ for group effect, overall SEM: 67.2, and 759 g DM; overall $P<0.001$ for group effect, overall SEM: 68.9).

Conclusions *A. leucoclada* and, to a lesser extent, *S. vermiculata*, were accepted by the sheep and showed potential to be used in supplementary feeding, while *N. mucronata* was almost entirely neglected by the experimental animals. As the most preferred shrubs had higher crude protein contents than the straw, they may improve rumen emptying due to improved fibre digestion. This may have also allowed total feed intake to increase. However, feeding a single shrub additionally to straw compared to only straw did only lead to a higher total intake in the case of *A. leucoclada*. Allowing animals to adapt to new fodder shrubs did not improve feed intake suggesting that the animals experiment with and adapt to new forages very quickly. Offering all five fodder shrubs in choice with straw, thus providing a more diverse diet, clearly increased feed intake compared to feeding only straw. The level of the effect was larger than in experiment 1 where only the most preferred shrub increased total intake (shrub plus straw). It could be concluded that a more diverse diet (here five shrubs plus straw) is more likely to improve total intake than adding a single component.

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Impact of concentrate supplementation on performance of grazing sheep in the steppe of Inner Mongolia

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Introduction An increasing human population and the growing demand for food of animal origin lead to an intensification of livestock production, overgrazing and desertification in the Inner Mongolian steppe, China. Strategies for a sustainable livestock production that protect the steppe vegetation and at the same time, satisfy farmers' economic interests are strongly needed. Hence, this study analysed the effects of a moderate concentrate supplementation on live weight gain (LWG) of sheep grazing at different intensities to evaluate its potential contribution to profitable livestock production at conservative stocking rates.

Materials and methods During the grazing season (July–September) in 2010, a grazing experiment with 337 non-pregnant and non-lactating female Mongolian fat-tailed sheep (30.2 ± 4.3 kg live weight) was conducted in the Xilin River Basin (E116°42' N43°38'). The study area is at about 1200 m above sea level and typical for the temperate steppe region, with high intra– and inter–annual variability in precipitation. Mean annual precipitation and temperatures are 335 mm and 0.8 °C (1982–2007). The study area was divided in grazed and ungrazed areas that were used for making hay. Two grazing systems were investigated: in the first system the same plots had been either used for grazing or hay-making in previous years; in the second system, grazing and hay-making had been alternated annually. In each system six grazing intensities (GI) from very light to very heavy grazing were realized. Plots had a size of 2 ha, except the lowest GI with a size of 4 ha. Every treatment was repeated in a flat and a sloped area. Stocking rates were adjusted every month to maintain similar herbage allowances (Schönbach *et al*. 2011). Per plot, four sheep were randomly chosen and received 250 g/d each of a corn–wheat–based concentrate feed (SUP), while four non-supplemented (NSUP) sheep were used as control group. The animals were weighed every month to determine their daily LWG. Data were analysed using ANOVA of R software 2.12.0.

Result Daily LWG of sheep was similar to LWG of animals in earlier studies in this area (Glindemann *et al*. 2009). It did not differ between systems (P=0.59) due to a similar herbage allowance and quality on the plots. LWG of SUP and NSUP sheep decreased with advancing grazing season despite similar herbage allowances (P<0.001; Table 1), probably due to a decline in herbage quality and/or sheep's feed intake (Glindemann *et al*. 2009). Increasing GI reduced daily LWG (P=0.044). The effect was only minor, maybe because the animals were able to compensate for a decreasing herbage availability by extending their grazing time (Lin *et al*. 2011). LWG was higher in SUP than in NSUP sheep (P<0.001). The effect of supplementation did not differ between GI's (P=0.73) and was higher in the beginning than at the end of the grazing season (P<0.001).

Conclusion A moderate concentrate supplementation of grazing sheep strongly increases LWG of individual animals and thus appears sufficient to maintain output per area even at lower GI's. It might compensate for farmers' economic losses due to the recent governmental de-stocking policies and could therefore offer a valuable contribution to a sustainable sheep production.

Table 1 Live weight gain (LWG) [g/d] of supplemented (SUP) and non-supplemented (NSUP) sheep grazing at different grazing intensities (GI) in July–September 2010 (mean ± standard deviation; n=4 observations per GI).

GI	1	2	3	4	5	6	Mean
SR	2.1 ± 0.3	4.3 ± 0.5	5.9 ± 0.9	7.3 ± 0.5	8.8 ± 0.5	10.3 ± 0.5	
HA	22.0 ± 7.1	9.2 ± 3.4	6.1 ± 1.6	3.3 ± 0.7	2.6 ± 0.4	1.8 ± 0.6	
SUP	126 ± 66	118 ± 54	129 ± 51	122 ± 52	126 ± 59	106 ± 77	121 ± 59^{Y}
July	211 ± 20	162 ± 56	188 ± 33	176 ± 28	186 ± 30	196 ± 44	186 ± 37^{E}
August	82 ± 19^{a}	127 ± 32^{ab}	114 ± 23^{ab}	124 ± 9b	135 ± 18^{b}	90 ± 12^{a}	112 ± 27^{D}
September	84 ± 25^{b}	67 ± 24^{ab}	86 ± 25^{b}	65 ± 25^{ab}	58 ± 17^{ab}	31 ± 30^{a}	65 ± 29^{B}
UNSUP	92 ± 36	87 ± 55	89 ± 50	94 ± 31	82 ± 35	71 ± 54	86 ± 44^{X}
July	132 ± 19^{b}	129 ± 61^{ab}	124 ± 51^{ab}	125 ± 8^{b}	107 ± 5a	112 ± 41^{ab}	122 ± 35^{D}
August	86 ± 14	98 ± 27	86 ± 42	99 ± 17	101 ± 10	88 ± 18	93 ± 22^{C}
September	58 ± 23^{ab}	33 ± 11^{a}	57 ± 42^{ab}	59 ± 16^{b}	38 ± 21^{ab}	13 ± 37^{ab}	43 ± 30^{A}

Different superscripts indicate significant differences (P≤0.05) between sheep at different GI's (a,b; within rows), between the mean LWG of sheep in different months (A,B,C,D,E), and between the overall mean LWG of SUP and NSUP sheep (X,Y); HA, herbage allowance [kg dry matter/kg live weight]; SR, stocking rate [sheep/ha].

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Impact of spatial arrangement of grass and legumes on performance and grazing behaviour of beef heifers

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Introduction Recent research examined the idea of allowing the grazing animal to express free dietary choice between legumes and grasses during grazing as a means towards achieving greater consistency of nutrient supply to the animal from pasture, and greater control of intake, and production. If ruminants have access to different forage species presented in a mixed pasture, they will always choose a mixed diet (usually showing partial preference for certain forages) even though one of those forages could meet the nutrient requirements of the animal (Chapman *et al.*, 2007). Evidence exists that, in low input grazing systems, more complex pasture mixtures can improve ecosystem function, primary production (plant), improve nutrient cycling, reduce weed invasion and also improve system resilience to climatic extremes such as drought. It is not clear, however, whether the benefits suggested by these limited-scale studies will apply more broadly to managed forage and grazing lands. The effects of greater pasture complexity at an individual animal level are more unclear and further research is needed in this area. There is also a need for more study of the interaction between pasture mixtures and sward type and animal management regimes. Preference has been defined as the ability of the animal to choose without physical restriction of access (spatially separated) to any of the components on offer (Parsons *et al.*, 1994). Preference is not the same as selection. Selection can be defined as the preference of the animal modified by physical constraints to access (mixed swards) to at least some of the components on offer. The searching cost involved in selection requires the animal to trade off total intake for dietary composition (Parsons *et al.*, 1994). Such trade-offs may have profound consequences for the performance of animals grazing conventional, intermingled grass-clover associations.

Materials and methods Twelve equal size paddocks (1.6 ha each) were no-till planted (treatments) with: 1) ryegrass (G) alone (*Lolium multiflorum*; 56 kg/ha); 2) berseem (*Trifolium alexandrium*; 22. 5 kg/ha), white (*Trifolium repens*; 5.5 kg/ha) and red (*Trifolium pratense*; 17 kg/ha) clovers alone (L); 3) ryegrass interseeded with berseem/white, and red clovers (M), and 4) adjacent paddocks of ryegrass and berseem/white and red clovers (ADJ) and fertilized according to soil test recommendations. There were 3 replications/treatment. Four heifers (initial BW = 211 ± 19 kg) were blocked by breed type and weaning weight to pastures and treatments and continuously stocked for 10 weeks. Heifers were weighed fortnightly and ADG and gains per hectare calculated. One heifer in each treatment replicate was fitted with a with an activity monitor. This monitor (IceTag, version 2.004; IceRobotics, Midlothian, Scotland, UK) was attached to a Velcro strap on the left rear leg just above the metatarsophalangeal joint. These units measure animal activity 8 times/s with an internal accelerometer. Percentage of time spent standing, active, and lying, and the number of steps taken by the cow were recorded. Data were downloaded from on board memory to a personal computer and analysed by IceTagAnalyser software (version 2.009; IceRobotics). Raw activity monitor data were transformed to partition out the amount of time the animals spend standing still, grazing, and walking without grazing as described by Boland and Scaglia (2011). Sward height was measured in all paddocks twice a week with 50 contacts in each using a plate meter. Forage from within 3 quadrats (0.25 x 0.25 m) in each replicate was clipped to ground level. Hand-plucked samples of forage (100 g fresh) were taken to simulate forage grazed by the animals. These samples were composited and analyzed for DM, ash, N, IVDMD. Data for testing treatment effect on animal performance and kg/ha produced was analysed using Proc MIXED (SAS). Behaviour variables were analysed with the same procedure using treatment, week of experiment and their interaction in the model. Treatment was the fixed effect. Paddock and heifer within rep were the experimental units for performance and behaviour characteristics, respectively. Orthogonal contrasts were used to evaluate the differences between G *vs* M/L/ADJ, M *vs* ADJ, G *vs* L. Level of significance was set at α=0.05. The REG procedure was also used to test the linear effect of week on grazing behaviour variables.

Results Average daily gains and beef produced per hectare for the entire experimental period were similar (P>0.05) between treatments with clovers (1.24, 1.34, 1.22 kg/d and 220, 239, 216 kg/ha for L, M, and ADJ, respectively) but different (P = 0.02) to G (0.99 kg/d and 175 kg/ha, respectively). The only differences in grazing behaviour between heifers on different treatments were in the time they spent walking. Heifers grazing M were more (P<0.05) active, as % time, than those grazing ADJ (6.2 and 5.5%, respectively, SED: 0.27), as well as in number of steps taken (4072 and 3657 steps, respectively, SED: 230), and minutes spent walking (24 and 10 min, respectively, SED: 4.6). Week of the experiment had a significant linear effect (P=0.0001) on all grazing behaviour variables. These coincide with the gradual decrease of forage mass and nutritive value of the grazed forage. No differences (P>0.05) between treatments were observed but, in time, forage mass declined from 2800 to 1410 kg/ha. As forage mass and height decreased heifers grazed for longer period of time (491 and 641 min in week 1 and 10, respectively) which is associated with more time remaining active (5.8 and 7.1% of the time, respectively) and number of steps (3820 and 4603 steps, respectively).

Conclusions The inclusion of legumes in a sward increased animal performance. Heifers on M were more active probably as a trade-off to be searching for legumes. Linear decreases in forage mass, height and nutritive value clearly explained the behaviour changes observed in heifers in all treatments. Search for appropriate bite imposed an increase in activity. Regardless, forage mass variables studied were not at a level that may have negatively impacted dry matter intake. Further research in this area is warranted.

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Forage proportion of diet affects efficiency of energy utilisation for milk production in lactating dairy cows

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Introduction Current energy evaluation systems for lactating dairy cows utilize key parameters such as the net and metabolizable energy (ME) at maintenance (NE_M, ME_M, respectively), efficiency of utilisation of ME intake for milk production (k_l), growth (k_g), and efficiency of utilisation of body stores for milk production (k_t). These parameters may be constant or a function of feed quality such as forage proportion in the diet. The aim of the present study was to investigate the effect of forage proportion in the diet on efficiency of utilisation of energy for milk production.

Materials and methods A database containing energy balance observations on 600 individual dairy cows was assembled from 35 calorimetry studies conducted in the UK. The data have been described previously by Kebreab *et al*. (2003). A meta-analytical approach based on Bayesian methods was used to analyse the data as the conclusion reached is valid across studies. The data contained information on cows fed a wide range of forage proportions from 0.31 to 1.00. The primary covariates considered in the analysis were ME intake, tissue energy gain, tissue energy loss and forage to concentrate ratio (F:C as a continuous variable). Student's *t*-distribution was used instead of the normal distribution because it has thicker tail probabilities and thus is more robust towards influential observations. The model was developed in the general purpose software for Bayesian modeling: OpenBugs (Thomas *et al*., 2006). Convergence was established using the Gelman-Rubin statistic as the main determinant and running three chains. Convergence was established after 10,000 samples, i.e. the burn-in period. Inference was based on an additional 100,000 samples from the posterior distribution with a thinning of 20.

Results The results show that there was a significant effect of forage proportion on parameters for NE_M (P = 0.015) and k_l (P = 0.004). However, k_g and k_t were not significantly different (P = 0.39 and 0.14, respectively) in cows fed various proportions of forage. Net energy for maintenance was estimated to be 0.25 MJ/(kg$^{0.75}$ d) (SD=0.024), where SD is the standard deviation. The overall equation that describes the effect of forage proportion in the diet on efficiency of energy utilisation for milk production was k_l = 0.52 (SD = 0.016) – 0.32 (SD = 0.096) × (F:C – 0.64) (when centred on the mean of the observations). The results agreed with Strathe *et al*. (2011) who reported that k_l was linearly related to metabolizability (i.e. ME/gross energy) of the diet, and predicted a 0.012 change in efficiency per 0.1 unit change in metabolizability. However, the magnitude of change in k_l was higher when the analysis was conducted based on forage proportion compared to metabolizability. This may be because the calculated metabolizability had a narrow range in the dataset (0.42 and 0.76) compared to a wider range in forage proportions because of the nature of diet formulation to meet energy requirements.

Table 1 Summary of the posterior distribution of selected parameters presented as posterior mean, standard deviation and 95% credible intervals

Parameter	Estimate (SD)	Lower	Upper
NE_M, MJ/(kg$^{0.75}$d)	0.25 (0.024)	0.20	0.29
ME_M, MJ/(kg$^{0.75}$d)	0.48 (0.034)	0.40	0.54
k_l	0.52 (0.016)	0.49	0.55
k_g	0.88 (0.041)	0.81	0.97
k_t	0.63 (0.047)	0.54	0.72

Conclusions The present analysis demonstrates that as the forage proportion in the diet increases by 0.01, the efficiency of utilisation of ME for milk production decreases by 0.32%. However, the efficiency of utilisation of ME for growth and efficiency of utilising body stored for milk production were not affected by forage to concentrate ratio.

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A new method for recording coordination between reticulo-rumen motility, methane eructations, and chewing activity in cattle

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Introduction In research work related to feed evaluation and ruminant health, monitoring rumen motility and the release of gas from the rumen is of interest. Primary contraction cycles (PCC) aid in mixing the rumen content, and secondary contraction cycles (SCC) are known to facilitate gas eructation by forcing the gas cap towards the cardia from where the gas is released through the oesophagus. Rumen motility has been studied by measuring changes in intra ruminal pressure using an inflated balloon or pressure transducers placed in the reticulum of rumen fistulated cattle. Tracheal cannulation or implantation of myoelectrodes in the reticulo-rumen and oesophagus are examples of invasive ways of studying the mechanisms of eructation in anaesthetized or fixated animals. This study presents a trial using a new method for simultaneous recording of methane (CH_4) eructation, reticulo-rumen motility and chewing activity in rumen fistulated cattle, introduced by Koch *et al.* (2009). This method enables investigation of factors affecting reticulo-rumen motility and eructation mechanisms, and variations caused by animal behaviour. The objectives of the study were to 1) evaluate the experimental method and 2) investigate short term effects of different types of concentrate supplementation on the coordination and frequency of CH_4 eructation, PCC, and SCC during eating, ruminating, and resting.

Materials and methods Two rumen fistulated non-lactating Jersey cows were given the treatments; three kg hay (H), and H supplemented with two kg ground barley, rolled linseed or beet pulp pellets, in an incomplete block design of 12 experimental days. Jaw movement oscillations (JMO) were recorded by a Hall sensor, reticulo-rumen motility by pressure transducers placed in the reticulum (PTre) and ventral ruminal sac (PTvs), and the CH_4 eructations by a CH_4 specific Taguchi gas sensor (TGS) mounted on a modified halter. The digitized signals (DV) from the sensors were sampled continuously at 20 Hz. Methane eructations were identified as peak TGS responses, and PCC and SCC were identified as bi- or triplephasic oscillations and single oscillations on PTre and PTvs response curves, respectively (Figure 1) as described by Backus *et al.* (1993). Frequencies of CH_4 eructations, PCC and SCC were summarized within 30 min intervals of standing eating, ruminating or resting behaviour, specified from JMO patterns, and ratios of SCC to PCC, and CH_4 eructations to SCC, were calculated. Effects of chewing behaviour and treatment on frequencies and ratios were analyzed as an incomplete block-design using the MIXED procedure in SAS.

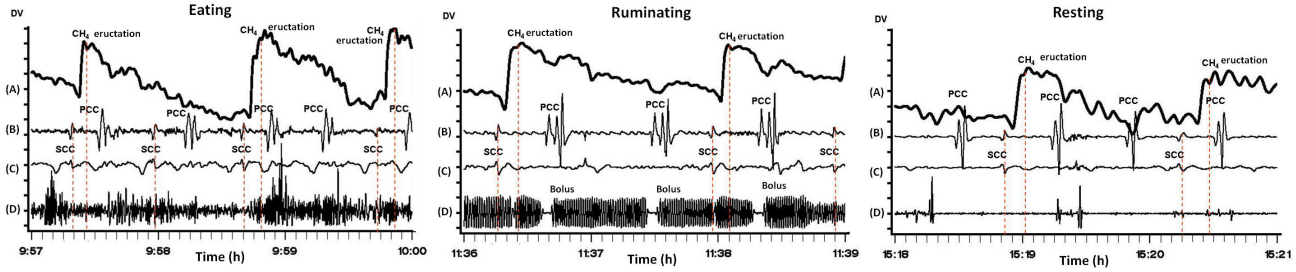

Figure 1 Plots of DV values from the sensors sampled from a standing cow while eating, ruminating, or resting. The upper curve (A) shows the peaks in CH_4 concentration observed as an eructation. The two curves beneath shows the pressure variation in the reticulum (B) and ventral ruminal sac (C) respectively, with occurrences of PCC and SCC marked. The bottom curve (D) shows JMO for instance clustered in regular cycles during rumination with regurgitation of boluses marked.

Results Identification of time occurrence of CH_4 eructations was possible. It was found that 90-94 % of the SCC lead to an eructation of CH_4, not significantly affected by behaviour or concentrate supplement. The frequencies of CH_4 eructations, PCC, and SCC were significantly higher during eating compared with resting ($P<0.0001$, $P=0.045$, $P<0.0001$) and ruminating ($P<0.0001$, $P<0.0001$, $P<0.0001$), the lowest frequencies were found during ruminating. The ratio of SCC to PCC was 0.5 ± 0.01during resting and 0.6 ± 0.01during eating and ruminating. No significant effect was found of concentrate supplement on frequencies CH_4 eructations, PCC, or SCC.

Conclusions The method enables recording of chewing behaviour and frequencies of CH_4 eructations as well as PCC and SCC simultaneously in tied up ruminants. Altered fermentation activity due to supplementation appears not to affect the rate of eructations. The results confirm the coordination between SCC and gas eructations, and in addition a consistent ratio between SCC and PCC dependent on chewing behaviour. Consequently, recording methane eructations alone appears as a simple indirect method for recor-ding the frequency of PCC as a biological indicator of rumen disorders like subclinical rumen acidosis, displaced abomasum or bloat.

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Effects of carbohydrate chemical structures on rumen microbial metabolism in *in vitro* culture system

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Introduction Methane emitted during rumen fermentation has been regarded as main green house gas in animal industry. Various efforts have been conducted to reduce methane emission from cattle by manipulating rumen fermentation. In this study, the effects of carbohydrates water solubility on rumen fermentation and microbial population were examined under *in vitro* culture conditions.

Materials and methods Total Mixed Rations for beef steer with isoenergy and isoprotein conditions (NRC, 2002) were prepared using pure compounds including glucose, starch, casein, xylan and filter paper (table 1). Five hundred mg of mixed feeds were added to 50 ml of *in vitro* culture solution and incubated at 39 □ under anaerobic condition. Samples were collected after 0, 3, 6, 12 and 24 hours of incubation and dry matter (DM) digestibilities, pH, methane production, ammonia concentrations and volatile fatty acid (VFA) concentrations were measured. Both DNAs and RNAs were extracted from samples and RNAs were used to synthesize cDNAs. Microbial populations were analyzed using quantitative PCR with 14 specific primers designed for *Fibrobacter succinogenes, Anaerovibrio lipolytica, Ruminococcus flavefaciens, Eubacterium ruminantium, Ruminobacter amylophilus, Selenomonas ruminantium, Ruminococcus albus, Treponema bryantii, Prevotella ruminicola, Prevotella albensis, Streptococcus bovis,* protozoa, methanogen and fungi. The PCR amplicones of microbes tested in this study were cloned and used for absolute quantification of individual microorganisms. Data were analyzed by ANOVA using SAS. Statistical significance declared at $p < 0.05$.

Table 1 Substrate composition for *in vitro* culture

%	A	B	C	D
glucose		30.2	15.1	
starch			15.1	30.2
casein	10.1	10.1	10.1	10.1
Xylan	17.0	17.0	17.0	17.0
Filter paper	57.4	17.0	17.0	17.0
Lignin	15.5	25.7	25.7	25.7

TDN:60%, CP:10.1%

Results After 3, 6 and 12 hr incubation, DM digestibilities were the highest in glucose group ($p<0.05$). Culture head space gas production in glucose group was significantly higher than the other groups after 3 and 6 hr incubation ($p<0.05$). However, methane production in glucose group was significantly lower ($P<0.05$) than those in starch groups. The change in chemical composition of substrates caused significant differences in microbial population (figure 2).

Table 2 Effects of carbohydrate solubility on DM digestibility (%), pH, head gas production (ml), methane production (ml), ammonia (mg/ml) and volatile fatty acid production after 12 hours incubation (mean ± SEM)

	DM digestibility	pH	Head gas production	CH_4	NH_3	Acetate: Propionate ratio
A	13.03±0.15^{c}	6.816±0.008^{a}	8.25 ±0.33^{b}	0.06±0.01^{c}	12.63±0.13^{a}	2.30±0.02^{b}
B	33.31±0.30^{a}	6.640±0.009^{b}	46.17 ±2.17^{a}	4.59±0.25^{b}	6.85±0.05^{b}	2.21±0.01^{d}
C	30.55±0.14^{b}	6.676±0.004^{b}	44.50±1.26^{a}	5.72±0.13^{ab}	5.59 ±0.09^{c}	2.41±0.01^{c}
D	28.53±0.35^{b}	6.616±0.011^{b}	43.00±0.42^{a}	6.30±0.15^{a}	5.10±0.07^{c}	2.55±0.01^{a}

$^{a-c}$ Means with different letter differ significantly between groups at the same time point ($P < 0.05$).

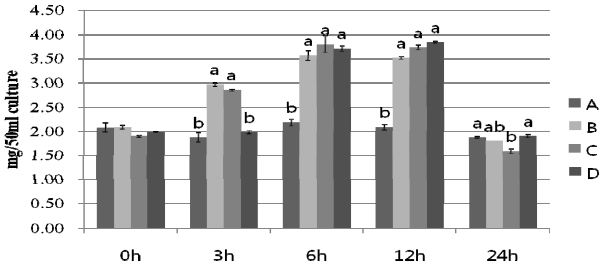

Figure 1 The concentrations of extracted total genomic DNA

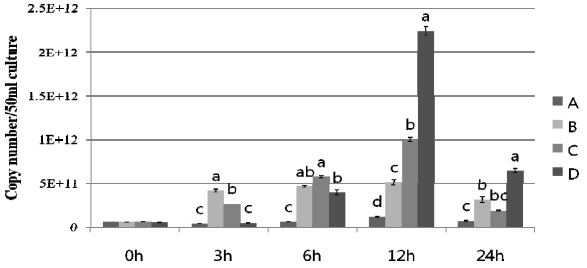

Figure 2 The population of *R. amylophilus* in the culture

Conclusions Increase in water soluble carbohydrate concentration in feed mixture caused increase in DM digestibility, ammonia production and decrease in methane production. However, these results were not exactly matched with cellulolytic, amylolytic, lipolytic and proteolytic bacterial population. In addition, there was no significant difference in methanogenic bacterial population among treatments even with differences in methane production.

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A new approach to measure the *in situ* degradation of small particles

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Introduction The *in situ* technique has been used widely worldwide to evaluate the rate and extent of nutrient degradation in the rumen (Lopez, 2005). A serious drawback of this technique is the particulate matter loss. Degradation characteristics of protein and starch in those particles cannot be measured *in situ* (Cerneau and Michalet-Doreau, 1991; de Jonge *et al*., 2009). This inhibits the evaluation of nutrient degradation of products containing a high content of small particles, such as wheat yeast concentrates. The aim of this study was to reduce this problem by modifying the rinsing method for incubated bags and the use of silica gel as a marker for the particulate matter loss. Firstly, since starch is the main component in small particles of various feedstuffs (Azarfar *et al*., 2007), the effect of modification of the rinsing method on the loss of starch from the bags in oats, peas and maize after 0 and 1 hour of incubation was examined. Secondly, the particulate matter loss as a function of the incubation time and particle size was determined by using silica gels. Finally, this new approach was used to measure the *in situ* degradation of protein in wheat yeast concentrate.

Materials and methods Maize, oats, and peas, but not wheat yeast concentrates and silica gels, were ground through a 3 mm sieve before incubation. *In situ* incubations were performed with nylon bags (inner size of 10 x 8 cm, pore size of 40 µm) containing approximately 5 gram DM of the feeds or silica gel. Incubations (1, 3, 6, 24, 48, 96, and 336 h) were performed using 3 lactating dairy cows according to the all-in all-out principle. For the new rinsing method, 4 bags were placed in a glass vessel containing 500 ml buffer solution pH 6.2 (de Jonge *et al*., 2009) and mechanically shaken during 30 minutes at a speed of 40 strokes per minute. In the extant method, the bags were rinsed in a washing machine. For both methods, the rinsed bags were dried at 70˚C, weighed and further analyzed. Nitrogen and starch was determined by using the Kjeldahl method and an enzymatic hydrolysis method, respectively. Incineration at 550 ˚C was used to determine ash content as indication of presence of silica gel. Analysis of variance was conducted using the GLM procedure of SAS (2002) to test the effect of treatment. The fractional disappearance rate of substrate or silica gel was estimated according to an exponential model with the NLIN procedure of SAS.

Results The loss of starch, especially for peas and oats, was lower for both incubation times in the new rinsing method compared with the extant method (Table 1). Only for oats, a decrease of the difference between both methods as function of the incubation time could be observed, which indicates the loss of small particles during the incubation itself. This effect, however, was numerically smaller than that of the rinsing method. This strong effect of the rinsing method compared to the incubation itself on particulate matter loss was also observations for silica gel (< 40 µm). The fractional loss for this silica gel after 1 hour of incubation was limited to 0.17 upon using the new method, but significantly increased to 0.93 by applying the extant method. The fractional disappearance rate of silica gel (< 40 µm) varied between 0.033/h and 0.072/h for the individual animals. The individual results, especially at short incubation times, showed a significant experimental variation. Silica particles larger than 40 µm were not removed during 336 hours of incubation. Upon applying the new rinsing method, an *in situ* degradation rate of 0.11/h was found for protein in wheat yeast concentrate after correction for particulate matter loss.

Table 1 Starch residue in bags (fraction of incubated amount of starch) in the new and extant method at 0 hour (n = 2) and 1 hour (n = 9) of incubation

	0 hour incubation			1 hour incubation		
Feed	New	Extant	Difference	New	Extant	Difference
Peas	0.94	0.52	0.42	0.98	0.54	0.44
Oats	0.99	0.36	0.63	0.74	0.21	0.53
Maize	1.01	0.91	0.10	0.99	0.89	0.10

Differences between the rinsing methods were significant ($P < 0.05$) for both incubation times within each feedstuff.

Conclusion This study showed that modification of the rinsing method can markedly reduce the loss of starch from nylon bags. This limited loss during incubation itself and the strong effect of the rinsing method was also observed for silica gel (< 40 µm), which indicated its potential use as a marker for particulate matter loss. The particulate matter loss was limited to particles smaller than 40 µm. Application of this new method enabled the estimation of *in situ* degradation behaviour of wheat yeast concentrates that could not be measured using the extant method.

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Digestibility parameters of a horse diet as observed under field conditions with acid insoluble ash as marker and response of young stallions to an improved dietary amino acid balance

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Introduction Currently the availability of digestibility data for feedstuffs or rations in horse nutrition is very scarce. Consequently, German recommendations for feed evaluation in terms of digestible energy are currently based on crude nutrient contents according to an equation as recommended by Zeyner and Kienzle (2002). Additionally, further development of the energy evaluation towards a metabolizable energy system takes into account the urine energy losses depending on dietary protein supply and methane losses depending on crude fibre supply (Kienzle and Zeyner, 2010), but due to the limited availability of digestibility data still based on crude nutrient contents. However, the variation of nutrient digestibility is expected to be very high, depending on quality of the main dietary components. Otherwise, currently no database is developed providing any verified information about availability of dietary protein resp. amino acids (AA) in horse nutrition. Several investigations were conducted in our lab to examine nutrient digestibility data of horses under field conditions in order to achieve more detailed information about energy and nutrient supply of horses. Based on this estimates, the current investigations aimed to measure the effects of improved amino acid balance in a diet for young stallions as applied under field conditions of a stud farm.

Material and methods The study utilized twelve stallions within the age period immediately before further selection for breeding (465 to 610 kg BW). Horses were individually kept in boxes under single feeding conditions and divided into two experimental groups (Control diet, fortified diet). The total experiment lasted 70d. On average, the individual daily feed supply consisted of 1kg straw, 8kg grass hey, 3kg oats, 0.65kg of a commercial pelletized concentrate including minerals and vitamins. Each ingredient was analysed for crude nutrients, hydrochloric acid (4n) insoluble ash (AIA) and amino acids (AA) according to German recommendations of VDLUFA (Naumann and Bassler, 1997). In summary, the average daily diet provided approximately 1106g CP, 553g DCP and 104.3 MJ DE, as calculated from feed tables. The fortified diet was added with L-lysine·HCl (20g/d resp. 15.6g/d pure lysine) and supplied in mixture with the concentrate. This added quantity was expected as the average of dietary lysine deficiency according to NRC (2007) and recommended AA profile (Graham *et al*., 2005). The daily training program was standardized for each of the stallions (25 min trot per day) and individual BW was weekly under control. Withers height was measured at start and at the end of the experiment in addition to further parameters (not reported here). Three individual venous blood samples (Start, middle, end of experiment) were analysed for 3-Methyl-Histidine (3-MH) and urea N (BUN). Statistical data analyses run within SPSS 15.0. Significant differences between mean values are identified by different superscript letters ($p<0.05$).

Results According to insignificant effect of collection period (ANOVA), summarized nutrient digestibility data are given in table 1.

Table 1 Crude nutrient digestibility (%) as derived from AIA based digestibility studies with stallions (n=35; Mean±SD)

	OM	CA	CP	CL	CF (n=28)	NFE
Digestibility (%)	57.0 ± *3.6*	52.6± *2,7*	68.3± *5.1*	52.3 ± *5.9*	35.4± *5.1*	66.8± *3.2*

Digestibility data reflect a crude nutrient digestibility as typical for young stallion diets under field conditions. As derived from digestibility data, real daily supply of digestible energy was 115.4 MJ (104.3 MJ/d calculated from feed tables) . The observed effects following improved dietary protein quality (Lys fortified diet AA-ratio**: Lys:Thr:Met = 1:0.61:0.26**) are summarized in table 2.

Table 2 Relative changes of blood parameters (%) and effect on withers height due to diet fortification with L-lysine

	BUN in blood as related to start of the experiment (%)	3-MH in blood as related to start of the experiment (%)	Gain in withers height during the experimental period (cm)
Control diet	-4.21	-4.00[a]	2.3 ±0.4[a]
Fortified diet	-14.94	-30.29[b]	3.2 ±0.7[b]

The results demonstrate significant effects of diet fortification on 3-MH, indicating more efficient protein deposition in the muscle. The observed trend of BUN changes ($p=0.058$) reflects a lower metabolic load according to improved dietary AA-balance.

Conclusions The applied procedure, making use of AIA as marker for digestibility studies provided acceptable results in horses and may contribute to improve information about real energy and nutrient supply of horses. In addition, enhanced dietary amino acid balance indicated improved metabolic efficiency in young stallions under field conditions. However, data about absorption and metabolic availability of AA as reported in farm animals are still lacking in horse nutrition. Ongoing studies with young stallions in earlier growth stages will increase the database to optimize AA-supply for growing horses in terms of "total AA".

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In vitro studies on the effects of grass silage containing true protein <50% in the total crude protein on protein metabolism in bovine ruminal fluid

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Introduction Feeding grass silages with true protein (TP) <50% in the total crude protein (CP) is associated with a higher incidence of peripartal diseases and digestive and metabolic dysfunctions as well as immunosuppression in Holstein Friesian dairy cows (Eicken, 2005). These silages provide higher concentrations of free amino acids that may be degraded in the rumen by a group of bacteria called hyperammonia producing (HAP) bacteria (Wallace, 1996). Because ruminally synthesized microbial protein is the main source of protein for ruminants an inefficient microbial protein production may cause marginal protein deficiencies (Nolan and Dobos, 2005). The aim of the present study was to investigate whether grass silages with low percentages of TP in the total CP affect protein metabolism in ruminal fluid via increased degradation of amino acids.

Materials and Methods Eight suspected grass silages (sGS) that had caused clinical diseases in dairy cows and showed TP <50% in the total CP were tested for their *in vitro* effects on protein metabolism in ruminal fluid using the long-term incubation system RUSITEC (RUmen SImulation TEChnique). Six grass silages with TP >50% that had not caused a high incidence of disorders were used as control silages (cGS). All silages were analyzed for their contents of CP, TP, and amino acids (AA). Each RUSITEC run started with a nine-day adaption phase (addition of 10.5 g DM hay/d). Afterwards, silages (10.5 g DM/d) were supplemented over a period of 10 days (experimental phase). The following recovering period (9 days, addition of 10.5 g DM hay/d) ended on day 28. Besides hay or silage 3.4 g of a concentrate (18.0% CP) where added daily to each fermenter. Samples of ruminal fluid were taken daily from days 11 to 19 always immediately before adding the silages and analyzed for their content of bacterial protein and ammonia concentration. Concentrations of branched-chain volatile fatty acids (BCVFA) were determined in the overflow of the system and used to calculate their daily productions. Bacterial protein and input of CP were used to calculate the efficiencies of fixation of N according to Czerkawski and Breckenridge (1985). Data were expressed as means and analyzed for differences between cGS and sGS using a two-tailed Student's t-test.

Results The sGS and cGS showed equal (P>0.05) mean contents of CP but total AA contents were lower (P<0.05) in the sGS. In contrast, percentages of free AA in the total AA were higher (P<0.05) in the sGS (Table 1). Addition of sGS increased (P<0.05) mean ruminal ammonia concentration (Figure 1a) and BCVFA productions (Figures 1b and 1c). Calculation of efficiencies of fixation of N in bacterial protein in fermenter fluid showed that the sGS lead to lower (P<0.05) fixation efficiencies compared to the cGS (Figure 1 d).

Table 1 Mean contents of crude protein (CP), amino acids (AA), and percentages of true protein (TP) in the total CP and of free AA in the total AA in control (cGS) and suspected (sGS) grass silages

	cGS (n=6)		sGS (n=8)		P
	Mean	SEM	Mean	SEM	
CP [g/kg DM]	173	8.49	187	8.07	>0.05
% TP in CP	56.0	2.29	39.5	1.35	<0.05
AA [g/kg DM]	77.2	4.64	55.5	3.80	<0.05
% free AA	33.5	3.09	68.5	4.87	<0.05

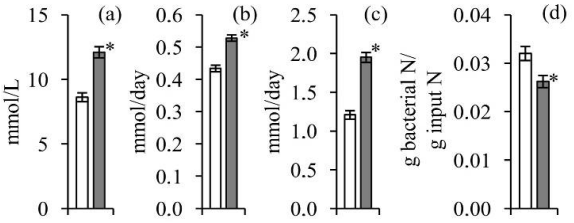

Figure 1 Mean concentrations of ammonia (a), i-butyric (b), and i-valeric acid (c) productions and efficiencies of fixation of N (d) in RUSITEC fermenter fluid during supplementation of control (▭) and suspected (▬) grass silages. Values are means ± SEM. *Means differ P<0.05

Conclusions Ammonia concentrations and BCVFA productions indicate that the sGS lead to an increased ruminal degradation of amino acids. A reason for this phenomenon may be an increasing population of deaminating and HAP species, respectively, that benefited from higher percentages of free amino acids in the sGS. In contrast, important proteolytic species were disadvantaged by the low percentages of TP. In the following, fixation of N in bacterial protein in fermenter fluid decreased as a result of the augmented N turnover and the changed bacterial population. The observed changes in ruminal protein metabolism might contribute to the aetiology of the above described disease but the changes are not severe enough to be judged as the only cause for its appearance.

Acknowledgement We thank Niedersächsische Tierseuchenkasse for financial support.

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Australian plants display anthelmintic activity towards equine cyathostomins *in vitro*

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Introduction Anthelmintic resistance in gastrointestinal parasites of horses is increasing in prevalence, particularly in cyathostomins (*Nematoda, Strongylida*), and is now one of the biggest problems for equine health (Kaplan & Nielsen 2010). For that reason there is a need for new sustainable approaches to parasite control to be investigated. One method that could be useful in lowering parasite burdens and reducing the reliance on anthelmintic drugs is the use of bioactive plants with anthelmintic properties. The aim of this experiment was to test the anthelmintic activity of a range of Australian plants on cyathostomins *in vitro.*

Materials and methods Dried material (100 mg) from 28 species of plants was added to 1 ml distilled water and shaken on a roller wheel for 48 hrs. The extracts were then centrifuged at 10000 rpm at 5ºC for 30 minutes. The supernatant was removed and filtered though a syringe driven filter unit and stored at -16 ºC until needed for the assays. Cyathostomin eggs were collected from the faeces of naturally infested horses that had not been exposed to an anthelmintic in the 8 weeks previous. The testing was done according to the protocol of Kotze *et al*. (2009). Briefly, the nematode eggs were recovered from the faeces by first passing through a series of fine sieves and then centrifuging on a sucrose gradient. The egg solution was placed into wells of 96-well plates on top of 200 µL plain agar, followed by 10 µL plant extract. Each plant extract was initially tested at 1400 µg/mL (equivalent to the water-extractable material in 1 mg of the original dried plant sample) and three 2-fold serial dilutions. The plates were incubated at 27ºC overnight and then fed with a broth culture of *E.coli*. After six days of further incubation the larvae were killed with iodine. The number of infective stage larvae (L3) present in each well was counted. Percentage larval development was calculated by expressing the numbers of L3 in treated wells as a percentage of the mean number of L3 in control wells (water only). IC_{50} values (concentration that resulted in a 50% inhibition of development) were calculated from percentage development data at a range of serial dilutions of the extracts using non-linear regression (GraphPad Prism).

Results Extracts from seventeen species inhibited larval development to less than 10% compared to control wells at the initial screening concentration of 1400 µg/mL (Figure 1). Of these species, seven completely inhibited development at 1400 µg/mL (*Acacia baileyana, A. melanoxylon, A. podalyriifolia, Alectryon oleifolius, Duboisia hopwoodii, Eucalyptus gomphocephala and Santalum spicatum). Dubosia hopwoodi* was the most potent plant, causing complete inhibition of larval development at as little as 175 µg/mL. Dose response relationships were demonstrated for the seven most active plants (Figure 2), the IC_{50} values of which ranged from 30.92-196 µg/mL.

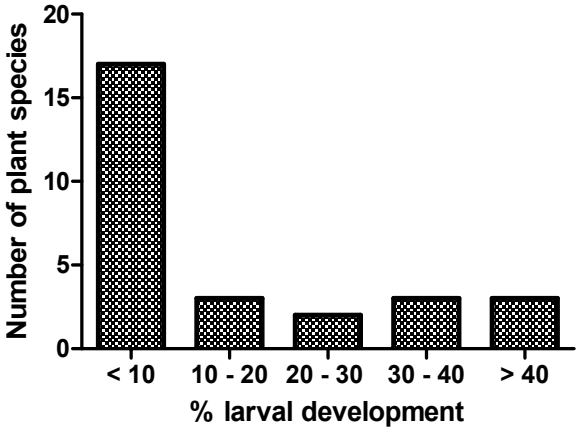

Figure 1 Frequency distribution of anthelmintic activity in extracts of various plant species (n=28) at a concentration of 1400 µg/mL.

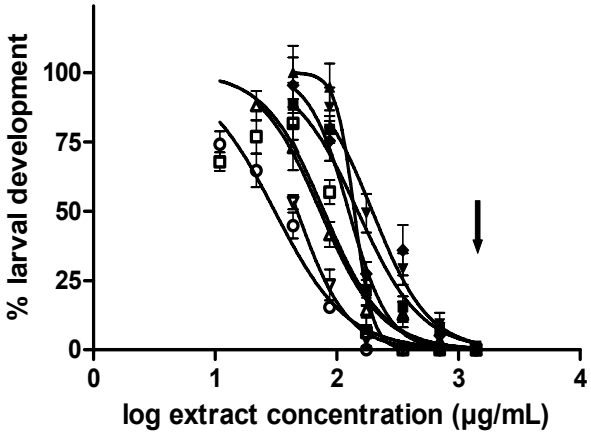

Figure 2 Anthelmintic activity of the seven most active extracts. The arrow indicates the approximate initial screening concentration for all extracts.

Conclusions Several species had significant activity against cyathostomins *in vitro*. *Duboisia hopwoodii* was the most active, completely inhibiting larval development at all concentrations. The IC_{50} values are similar to those in a previous study which found that several Australian plant species tested against the sheep parasite *Haemonchus contortus* had IC_{50} values ranging from 64-272 µg/mL (Kotze *et al*. 2009). Although further testing is clearly needed both *in vitro* and *in vivo*, these results suggest that it would be possible in the future to incorporate the use of anthelmintic plants in parasite control programs in horses.

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NIR spectroscopy for predicting the nutritional, anthelmintic and environmental effects of sainfoin

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Introduction Sainfoin (*Onobrychis viciifolia*) has been cultivated in Europe for over 450 years and used to be an important part of sustainable farming systems. It is a valuable feed for ruminants and an excellent nectar and pollen source for bees. Its tannins ensure that ruminants do not bloat when grazing sainfoin and are effective against parasitic worms. This EU project demonstrated that urinary nitrogen emissions and *in vitro* methane production were significantly reduced. Here we report the development of NIRS calibrations to support plant improvement of sainfoin in the future.

Materials and methods Plant samples originated from a large EU '*HealthyHay'* sainfoin germplasm collection (> 300 accessions) at NIAB, Cambridge. A Foss NIRSystems 5000 instrument with ISI II software (Infrasoft Int. Inc) was used. NIR spectra were recorded between 730 and 2,500 nm. NIRS calibrations used modified partial least squares and a 1,4,4,1 mathematical treatment. Calibrations were established for several parameters. Pepsin-cellulase digestibility, tannins, prodelphinidins and *in vitro* methane production were determined according to Aufrère *et al.* (2007), Reed (1986), Gea *et al.* (2011) and Pellikaan *et al.* (2011).

Results NIR equations were developed for quantitative and qualitative tannin properties for the first time, i.e. content, polymer size and proportions of prodelphinidins and procyanidins. Good predictions were obtained for surprisingly different properties of sainfoin plants (*e.g.* botanical parameters, organic matter digestibility, *in vitro* methane production and anthelmintic effects).

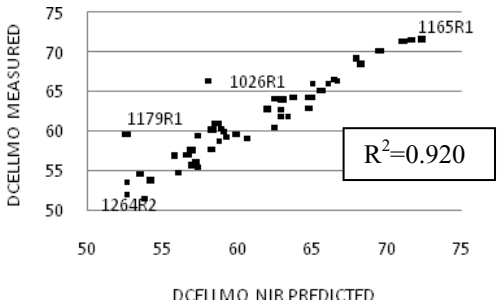

Figure 1a Pepsin-cellulase digestibility

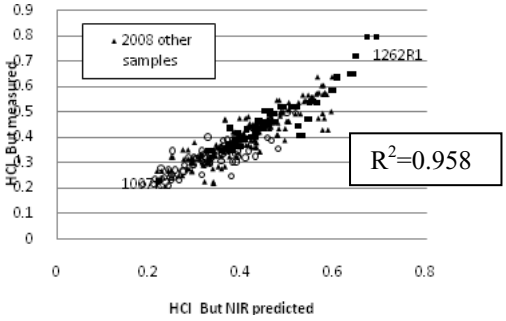

Figure 1b Tannins detected by HCl-butanol assay

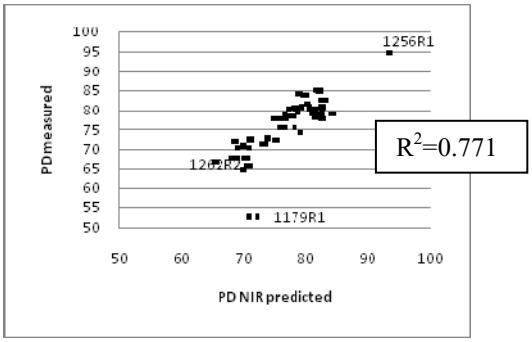

Figure 1c Proportion of prodelphinidin tannins

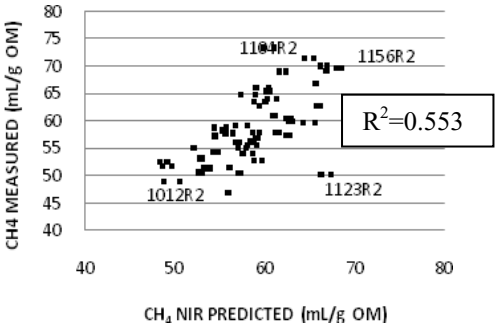

Figure 1d *In vitro* production of methane

Figure 1 Correlations between measured and NIR predicted sainfoin parameters

Conclusions This sainfoin germplasm collection exhibits a very wide range of properties in terms of plant composition and biological properties. This will be useful for a future plant improvement programme to develop a sustainable forage source for home-grown protein.

Acknowledgement Support from the EU (MRTN_CT-2006-035805, *HealthyHay* project).

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Validation of faecal near infrared spectroscopy to estimate digestibility and crude protein content of tropical forage diets consumed by cattle

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Introduction Near infrared spectroscopy (NIRS) can be used for analysis of constituents (e.g. N and fibre fractions) of forages and faeces. Because much of the NIR spectral information of consumed forages also appears in faeces despite digestion, NIRS of faeces (F.NIRS) can also be used to measure attributes of the diet including digestibility and crude protein (CP) concentration (Lyons and Stuth, 1992; Dixon, 2008; Dixon and Coates, 2009). F.NIRS allows frequent economical measures of the diet of grazing ruminants. The errors in prediction of diet digestibility and CP concentration were examined in a F.NIRS calibration data set (Coates 2004).

Materials and methods F.NIRS calibration equations require representative samples of both the diet and faeces, and measurement of diet attributes by conventional procedures. Samples of diets and faeces were obtained from cattle fed hays or freshly harvested forages in individual pens, or from oesophageally fistulated cattle grazing pastures. A F.NIRS calibration data set of 1223 faecal spectra representing over 300 diets, principally of tropical forages, was subdivided into 3 subgroups. Subset A (n = 1096) comprised diets of tropical C_4 pasture grasses fed alone or with *Stylosantheses* spp tropical legume, with other herbaceous dicotyledonous plants, or (for 2 diets) with *Clitoria ternatea* or *Dolichos lablab*. Subsets B and C (n = 62 and 65, respectively) comprised pen-fed diets consisting of (i) tropical pasture grasses with the tropical legumes *Clitoria ternatea*, *Centrosema pascuorum* or *Leucaena leucocephala*, (ii) lucerne *(Medicago sativa)* as a temperate legume, (iii) oats, wheat or ryegrass as C_3 temperate species forage crops, or (iv) sorghum as a C_4 tropical forage crop. These diets were allocated by stratified randomization to the B and C subsets. Calibration equations were developed using the chemometrics commonly used for NIRS analysis of forages (partial least squares models were developed from spectral data following derivative transformations). Calibrations based on the combined A+B or A+C data sets (Calibration P and Q respectively) were applied to data sets C and B, respectively, for validation.

Results The two validation data sets were predicted with a standard error of performance (SEP) = 22 and 26 g/kg for dry matter digestibility (DMD), and SEP = 12.8 and 16.4 g/kg for CP concentration. The R^2 of predicted *versus* measured relationships were ≥ 0.87 (Figure 1). The F.NIRS calibrations predicted both DMD and CP concentration with errors comparable with those for conventional laboratory (e.g. *in vitro*) analysis.

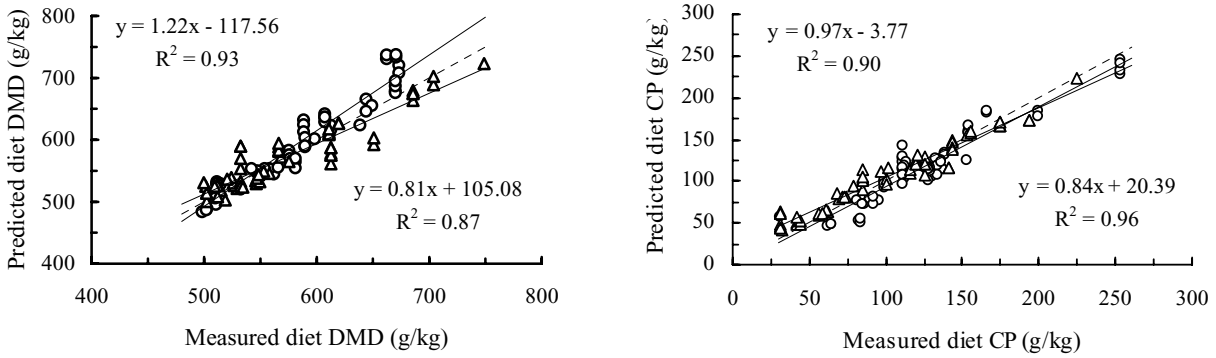

Figure 1 Regressions of DM digestibility (DMD) or crude protein (CP) concentration predicted from F.NIRS calibrations P (○) and Q (Δ) and the measured DMD or CP in independent validation data sets (C and B). The relationships Y = X are shown as (-------)

Conclusions A major constraint to the application of F.NIRS to estimate the diet selected by grazing ruminants is that calibrations need to be robust and reliable, and the pasture systems to which specific calibration equations can be reliably applied need to be delineated. The present study showed that F.NIRS calibration equations developed from cattle fed a variety of tropical forage diets in the seasonally dry tropics of northern Australia could be used to predict the DMD and the CP concentration of similar diets in the same region with errors which would be acceptable for many aspects of ruminant nutrition and management.

Acknowledgements The authors gratefully acknowledge financial support from Meat and Livestock Australia.

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Prediction of feed conversion efficiency in growing beef heifers based on N isotopic fractionation

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Introduction The conversion of feed protein into milk or meat protein is central to profitability and sustainability of ruminant production systems. The methods for estimating feed conversion efficiency (FCE) or Nitrogen-use efficiency (NUE) are laborious and so unsuited for evaluations with large numbers of animals. We have investigated an alternative approach to characterise NUE, based on the N isotopic fractionation. Some biochemical pathways discriminate between molecules containing ^{14}N and ^{15}N, leading to relative enrichment or depletion of products. Deamination and transamination pathways exhibit N isotopic fractionation (Macko *et al*. 1986), so that proteins (milk, plasma) are enriched and urine depleted in ^{15}N relative to the diet. Sick *et al*. (1997) showed significant relationships between protein intake and N isotopic fractionation in rats. However, we were not able to demonstrate relationships when comparing diets offered to dairy cows (Cheng *et al*. 2011), probably because of N isotopic fractionation associated with rumen processes. The objective of this work was to investigate the potential to use N isotopic fractionation to study differences in FCE between animals offered similar diets.

Materials and methods Eighty-six growing beef heifers (64 Simmental and 22 Simmental × Holstein-Friesian) were used. They were group housed in a shed with slatted floors, adapted to the diet for 3 weeks and then individually fed 2 kg/day concentrates and *ad libitum* grass silage. Daily feed intakes (through Calan gates) and growth rates (based on 3-weekly weighing) were recorded over a 12-week period. Blood samples were collected (vacutainers containing citrate) during week 11 and plasma harvested by centrifugation. Feed samples were analysed using standard techniques. Samples of the concentrates and grass silage (duplicates), as well as plasma (singly) were analysed for ^{15}N content by isotope-ration mass spectrometry (Iso-Analytical Ltd., Crewe, UK). Nitrogen-15 results are expressed in delta units relative to standard air ($\delta^{15}N$, ‰).

Results The concentrates and grass silage contained 139 and 133 g crude protein, and 215 and 510 g NDF/kg DM respectively. Silage DM intake averaged 4.1 (SD 0.73) kg/day, so that the forage proportion of total DM intake (g/g) averaged 0.70 (SD 0.038). Mean mid-test live weight was 334 (SD 47.4) kg, average daily gain was 0.51 (SD 0.191) kg and FCE (g/g) was 0.09 (SD 0.029). The $\delta^{15}N$ values of the concentrates, silage and total diet were 2.83, 4.91 and 4.26 respectively. The $\delta^{15}N$ value of plasma averaged 8.53 (SD 0.364), a 4.27 unit enrichment relative to the diet, and was unrelated to diet $\delta^{15}N$. Plasma $\delta^{15}N$ and plasma – diet $\delta^{15}N$ were related to FCE and animal weight ($W^{0.75}$) by equations [1] and [2], there being no significant relationship between FCE and $W^{0.75}$. The relationship between FCE and plasma – diet $\delta^{15}N$ is also shown in Figure 1.

Plasma $\delta^{15}N$ (‰) = 7.61 (SE 0.347)***
- 4.99 (SE 1.10) FCE***
+ 0.0174 (SE 0.00388) $W^{0.75}$***
N=86; r^2=40.6; SE (obs)=0.280; P<0.001 [1]

Plasma – diet $\delta^{15}N$ (‰) = 3.93 (SE 0.354)***
- 6.19 (SE 1.12) FCE***
+ 0.0112 (SE 0.00396) $W^{0.75}$***
N=86; r^2=38.0; SE (obs)=0.286; P<0.001 [2]

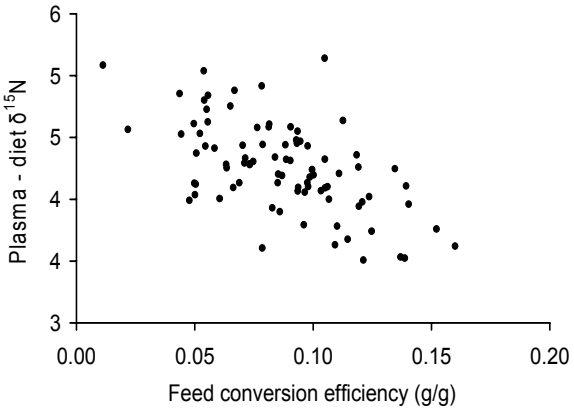

Figure 1 Relationship between feed conversion efficiency and plasma – diet $\delta^{15}N$

Conclusions The highly significant negative relationship between plasma $\delta^{15}N$ (and plasma – diet $\delta^{15}N$) and FCE is consistent with the anticipated effect of N isotopic fractionation in the deamination pathway. Whilst it was not possible to measure NUE directly, it is correlated with FCE and thus we anticipated a reduction in deamination, and so less isotopic fractionation, associated with increasing FCE. The effects of $W^{0.75}$ on plasma $\delta^{15}N$ can also be linked to N utilisation, since the N content of weight gain is higher in the earlier phase of growth. Plasma $\delta^{15}N$ explained more variation than single blood metabolites or hormones used to predict FCE in earlier studies (e.g. Kelly *et al*. 2010).

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Effect of the enrichment of ruminant rations with omega 3 fatty acids on the environmental impacts of beef production systems

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Introduction Mitigation of greenhouse gas emissions and other environmental impacts of livestock could be achieved by feeding strategies. The objective of this study was to investigate the environmental impacts of a standard beef production system in France in comparison with two systems enriched with omega 3 fatty acids (FA) and with a system including co-products rich in fibre, in order to find practical measures to improve the environmental performances of production systems.

Materials and methods Two grassland-based suckler cow-calf phases were St (Standard) and O3 (increasing omega 3 FA by using bale wrapping). Four bull-fattening phases were SM (using a diet rich in Starch based on Maize silage), SML (using a diet rich in Starch based on Maize silage supplemented with Linseeds rich in omega 3 FA), FC (using a Fibre based Concentrate diet), and SCL (using a Starch based Concentrate diet supplemented with Linseed). Weaned male calves from St were finished with either SM or FC; those from O3 were finished with either SML or SCL. Four studied beef production systems were thus St-SM, O3-SML, St-FC and O3-SCL. The life cycle assessment was delimited from cradle to farm gate for a one-year period, i.e. the studied system included the production and delivery of inputs used for grassland and cereals produced on-farm, of feed produced off-farm, herd management and associated upstream processes, emissions from the animals and manure storage. Enteric methane (CH_4) was estimated for each class of cattle according to the method developed by Vermorel *et al.* (2008). The effect of the supplementation of diets with lipids rich in omega 3 FA on enteric CH_4 emissions was taken into account based on Martin *et al.* (2010). Emissions from manure were estimated according to IPCC (2006). Carbon (C) sequestration from grassland were taken into account based on Arrouays *et al.* (2002). The impact categories considered were climate change (CC, kg CO_2 equivalent, eq) including gross CC and net CC (without and with C sequestration consideration, respectively), eutrophication potential (EP, kg PO_4^{3-} eq), acidification potential (AP, kg SO_2 eq), cumulative energy demand (CED, MJ) and land occupation (LO, m^2a). The environmental impacts were expressed per kg of carcass weight.

Results Gross CC and net CC per kg of beef carcass mass, in O3-SCL and in O3-SML were lower than those in St-FC (5%) and in St-SM (2%), respectively (Figure 1). These reductions originated from the decrease in enteric CH_4 and higher daily weight gain from animals fed with diets supplemented with omega 3 FA. CED value in O3-SCL was higher than that in O3-SML because yield per ha was lower for cereals than for maize silage and because more energy was required to produce starch based concentrate. The highest CED value in St-FC resulted from the energy required for lucerne and beet pulp dehydration to produce the fibre rich concentrate. Consideration of C sequestration induced a reduction of 9-10% of the gross CC impact. The contribution of the suckler cow-calf phase to the impacts ranged from 70 to 97% according to impact categories and systems. Enteric fermentation was the first contributor to gross CC, followed by grassland production, emissions from manure, and production of other feed (Figure 2). Grassland production was the major contributor for EP, AP and LO. Grassland production and other feed production contributed each a third of CED. The emissions from manure contributed 17-18% and 36-40% to EP and AP, respectively.

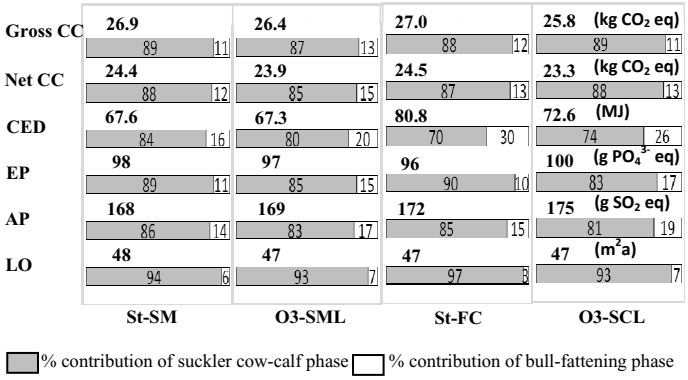

Figure 1 Environmental impacts per kg of carcass weight of beef meat produced in St-SM, O3-SML, St-FC and O3-SCL systems

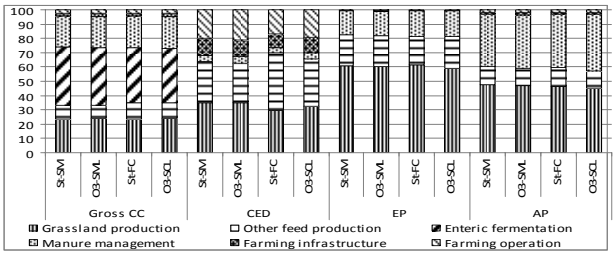

Figure 2 Contribution (in %) of main components in environmental impacts of St-MS, O3-MSL, St-FC and O3-SCL beef production systems

Conclusions Minor differences of the environmental impacts between four systems were observed. This study reveals that the research for mitigation of the environmental impacts of beef production needs to be focused on suckler cow-calf phase. Good practices for grassland and feed production, good management for pasture and manure along with mitigation enteric CH_4 strategy should be implemented to improve environmental performance of beef production system.

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Diurnal pattern of urinary and faecal nitrogen excretion by dairy cows fed ryegrass pasture twice daily indoors.

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Introduction The predominant component of the New Zealand dairy cow's diet is ryegrass pasture, which generally has a nitrogen (N) content in excess of cow requirement. Much of the surplus is excreted as urinary N which concentrates N in urine patches and contributes to groundwater and atmospheric (nitrous oxide) pollution. Urine patch soil N accumulation, and N loss to the environment, is influenced by urine volume and N concentration (Di and Cameron, 2002). However, the diurnal variation in dairy cow urine volume, and N concentration, is poorly understood. Grazing ruminants have a natural circadian grazing pattern, but strict pasture allocation may mean dairy cows consume up to 70% of their daily allowance in the first 3-4 h post-allocation (Gregorini *et al*., 2009). We tested the hypothesis that there is diurnal variation in the concentration and quantity of urine N excreted by dairy cows.

Materials and methods Six multiparous lactating Holstein-Friesian dairy cows (546±40 kg BW; 221±22 DIM) were housed in metabolism stalls and offered ryegrass (*Lolium perenne*, L.) pasture *ad libitum* for 10 days in March 2010 with measurements taken on d 9 and 10. Half the daily DM requirement of pasture was cut and offered to each cow at 0840 h. Feed was removed at 1300 h and cows milked at 1530 h. Fresh pasture was cut and offered to cows at 1600 h. Feed was removed at 2400 h and replaced at 0600 h prior to being removed again at 0730 h. Pasture intake was measured, and samples collected daily and stored for analysis by near infrared spectroscopy (NIRS systems 6500, FeedTech). Crude protein, NDF and ADF were 15, 59 and 32 g/100 g DM respectively. Milk production was recorded, and samples collected twice daily (0730 and 1530 h). Urine and faeces weight were determined every 2 hours for 48 hours on d 9 and 10 and sub-samples were taken on each occasion. Each 50mL urine sub-sample was immediately acidified with hydrochloric acid to a pH < 4 to prevent ammonia volatilisation and stored at -20^{0}C. Total Kjeldahl N and creatinine (Roche Diagnostics, kit number 11875418) were determined from these urine sub-samples. Each 50g faeces sub-sample was sealed in a zip-lock bag and stored at -20^{0}C for Kjeldahl N determination. Data were analysed as repeated measurements with a mixed model fitted using REML with Day and Time as fixed effects and Cow and Time within cow as random effects. An autoregressive of order 1 covariance structure was used to model the within cow measurements.

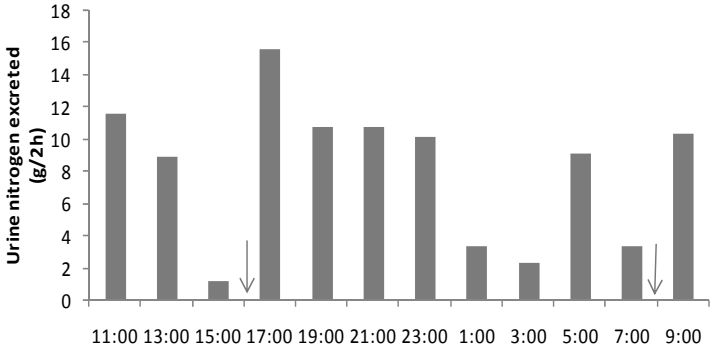

Figure 1 Urine nitrogen excretion (g/ 2h) over 24 h (mean = 8.11; SED=2.72). Arrows indicate when pasture was offered

Results There was no effect of day ($P > 0.05$) on the variables analysed so the data were analysed as a mean of each 2 h period for the two days. Urine N concentration (g/100g) varied with time ($P < 0.01$) with a peak at approximately 4 h after pasture allocation. Urine volume (L/2h) and N excreted (g/2h) (Figure 1) varied with time ($P < 0.01$) with a peak < 1h after feeding commenced. There was no effect of time on faecal N concentration (mean 2.3 g/100g), faecal N excreted (mean 10.9 g N/2h) and faecal weight (mean 480 g DM/2 h). Urine creatinine varied with time ($P < 0.01$) ranging from 2,653 to 1,287 µmol/L and creatinine also varied between cows from 7 to 12 mg/kg liveweight per d.

Conclusions The volume of urine and quantity of N excreted were more than doubled within an hour after feed was offered. Clark *et al*. (2010) reported dairy cow urination frequency increased during feeding compared with resting. In contrast, urine N concentration reached a peak four hours after pasture was offered. This delay in peak urine N concentration may be associated with proteolysis and microbial growth relative to feeding because Kolver *et al*. (1998) found rumen NH_3 concentration peaked three hours, and blood urea five hours after pasture feeding. There is good evidence that the quantity and concentration of excreted urine N is related to the timing of pasture ingestion. The diurnal variation has implications for mitigating N loss from grazed dairy farms because urinary N concentration changed from 0.3 to 0.5% (w/w) within a day, contrary to an assumed value of 100g N/m^2 used to describe the dairy cow urine patch soil N loading (Di and Cameron, 2002). If the high N loading events could be captured (see Clark *et al*. 2010) and spread evenly across a paddock, the amount of N loss from grazed dairy systems could be substantially reduced. Also, the timing of pasture allocation could be altered to prevent peaks and troughs of herbage intake. For instance, more frequent allocations of pasture may decrease peak urine N concentration, high N urine patch events and subsequent N loss to the environment.

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Effect of water-soluble carbohydrate in fresh forage on growth and methane production by growing lambs

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Introduction Previous studies in our institute have demonstrated benefits of feeding grasses high in water soluble carbohydrates (WSC) on animal performance (growth and milk production; Humphreys, 2005). It is hypothesised that elevated levels of WSC in forages may have an additional benefit on methane emissions from ruminants since recent observations demonstrate that any increase in WSC in perennial ryegrass leads to a reduction in methane production *in vitro* (Lovett *et al*., 2006). In this study, the impact on production and environment of a mixed sward consisting of three varieties with different heading dates of high WSC perennial ryegrass with or without white clover was assessed relative to a control perennial ryegrass, when offered to growing lambs.

Materials and methods Total 24 growing lambs (initial live weight approximately 30 kg, Texel-Mule cross-bred) were allocated to one of four forage treatments; 1) Control (*cv.* Premium), 2) Control with white clover (*cv.* AberDai), 3) Mixture of AberStar, AberMagic and AberAvon (high WSC varieties) heading dates are different) and 4) Mixture of AberStar, AberMagic and AberAvon with white clover namely Con, Con+WC, Mix and Mix+WC, respectively. The experiment lasted 8 weeks in total, 4 weeks diet adaptation followed by 4 weeks measurement period in zero-grazing study. Animals were individually fed the experimental forage on an *ad libitum* basis in two equal portions at 09:00 and 16:00. Fresh forage was cut in the morning using the Haldrup forage harvester, brought immediately into the research unit and stored at 4°C for the afternoon (16:00) and for the following morning (09:00) feeding. Following 4 weeks adaptation to the experimental diet, methane emission from an individual animal was determined using methane chambers developed for this study (see Figure 1) over a 3-day period. Ytterbium acetate was orally dosed to estimate a whole tract digestibility and faeces was collected manually twice a day while measuring methane emission using the chamber. At the end of methane measurement, all animals were weighed and slaughtered. An analysis of variance was conducted with diet as the main factor using GenStat (11th edition) statistical software.

Results Concentrations of WSC and total N of dietary forages during experimental period were distinct, averaging 27.4, 31.0, 28.3 and 33.6 for total-N (g/kg DM), and 163, 141, 205 and 153 for WSC (g/kg DM) for Con, Con+WC, Mix and Mix+WC, respectively.

Table 1 DM intake, live-weight gain (LWTG), methane production, digestibility and N-balance of growing lambs given experimental diets

	Con	Con+WC	Mix	Mix+WC	SED	P
DM intake (kg/d)	1.11	1.01	1.28	1.20	0.066	0.005
LWTG (g/d)	156	92	188	160	23.0	0.004
Methane production						
litre/d	35.7	30.2	34.8	31.7	2.91	NS
litre/kg DM intake	32.7	29.8	27.1	26.1	2.07	0.024
litre/kg LWTG	252	338	188	205	34.5	0.002
Whole tract DM digestibility (g/kg)	823	805	813	822	35.5	NS

Con=control, Con+WC=control with white clover, Mix=mixture of three high WSC cultivars, Mix+WC=mixture of three high WSC cultivars with white clover

Figure 1 Chambers to measure methane emission from small ruminant

DM intake and live-weight gain increased when animals were offered the mixture of high WSC forages compared to Con. Daily methane emission (litre/d) did not differ across all forage treatments (P>0.05), averaging 33 litre/d, which is broadly in agreement with figures from Intergovernmental Panel on Climate Change (IPCC) report (IPCC, 2006). However, when results were expressed as methane output per kg DM intake or per live-weight gain, ~ 15% (P=0.024) and 25% (P=0.002) of reductions in methane emission were observed when animals were offered Mix relative to Con, respectively.

Conclusions This *in vivo* study provides evidence that WSC content in grasses influences methane output. This effect could be due to the substrate specificity of the micro-organisms and may be associated with changes in the microbial population. Further studies are needed to elucidate the interrelationship between rumen microbes and plant components in the rumen especially, in terms of hydrogen transactions to help mitigate methane from ruminant pastoral livestock systems.

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Manipulation of the rumen microbial ecosystem to reduce methane emissions in ruminants through the intervention at early life stage of pre-ruminants and their mothers

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Introduction In ruminants, the development of the gastrointestinal tract involved the establishment of a fully functional and differentiated rumen, in which a diverse microbial population support fermentation and digestion of dietary fiber. The microbial colonization soon occurs in the rumen after birth, which causes extensive immune adaptation in the host. Previous observations point to an influence of the microbial colonization occurring during rumen development on the host-microbiome relationship in the adult animal (Yáñez-Ruiz *et al*., 2010). The aim of this work was to study whether interventions in early life of kids and their mothers, by treating them with an anti-methanogenic compound, have an impact on methane emissions and the rumen microbial ecosystem colonizing the rumen and whether the effects persist later in life.

Material and methods Eighteen goats giving birth to two kids were used. Nine goats were treated (G+) with 0,30 g/100 kg LW of bromochloromethane (BCM) after giving birth and over 8 weeks. The other 9 goats were not treated (G-). One kid per mother in both groups was treated with BCM (k+) while the other was untreated (k-), therefore resulting in four kids experimental groups: G+/k+, G+/k-, G-/k+ and G-/k-. K+ kids were treated over the 8 weeks period while with dam and for another month after weaning during which the four experimental groups were kept separately. After that all kids were grouped together and BCM treatment ceased. Kids´ weights were registered weekly. Methane emissions (average of 2 measurement days) from the mothers were recorded once at weaning and twice from kids, at weaning with all kids separated by treatment (week 8) and 8 weeks later when the kids were all grouped together and the BCM treatment had stopped (week 16). Rumen samples were collected three times from the mothers over the 8 weeks treatment (weeks 0, 4 and 8) and three times from the kids: at weeks 8, 12 and 16. Total DNA was extracted from rumen contents of mothers and kids and used for quantitative and qualitative study of methanogenic Archaea by real time PCR and DGGE, respectively (Denman *et al*., 2007; Cheng *et al*., 2009). Methane emissions and microbial numbers were analyzed by one-way and two-ways ANOVA for mothers and kids, respectively. DGGE banding patterns were analysed by using the Quantity One Software (BioRad) and the similarity tress constructed using the UPGMA method.

Results The kids in G+k+ group had a higher daily weight gain (141g/d) compared to the animals on the other three groups (127 g/d). Methane emissions by G+ goats were 38 % lower (P=0.008) than by G- goats (Table 1), which coincided with a tendency (P=0.088) towards higher milk yield in treated goats. In the mothers the numbers of methanogens in the rumen were not affected by the treatment. However a shift in the archaeal community composition was observed by DGGE (Figure 1). Within G+ mothers group, the DGGE study of the archaeal community revealed a gradual change along the 8 weeks treatment.

In the kids, one month after weaning (week 12) daily methane emissions by K+ kids were 48% and 59% lower (than from the K- ones in M+ and M- groups, respectively. At week 16 K+ kids remained lower (27%) emitters than K- kids, although this difference only persisted in those from G+ goats (table 1). Contrary to what was observed in the mothers, the number of methanogens in the rumen of kids at weaning was affected by the treatment received by the mother and by BCM treatment at weeks 8 and 12, but disappeared at week 16. With regards to the archaeal community structure, the DGGE analysis showed that was the mother the main source of inoculation, regardless the treatment received by the kid (not shown).

Table 1 Effect of BCM addition on CH4, milk yield and rumen archaeal numbers

Goats (week 8)	**G+**		**G-**		SEM	P value
CH_4 L/kg LW	0.349		0.564		0.059	0.008
Milk yield, g/day	1242		887		142	0.088
Archaea, log copies/g FM	7.88		7.96		0.161	0.753
Kids	**G+k+**	**G+k-**	**G-k+**	**G-k-**		
Methanogens (week 8)	7.63	8.19	7.28	7.02	0.328	0.027
CH_4 L/kg LW (week 12)	1.02	1.80	0.742	1.90	0.214	0.001
Methanogens (week 12)	7.31	7.96	6.72	7.81	0.289	0.222
CH_4 L/kg LW (week 16)	1.56	2.40	1.69	1.70	0.258	0.043
Methanogens (week 16)	7.74	7.64	7.52	7.51	0.125	0.232

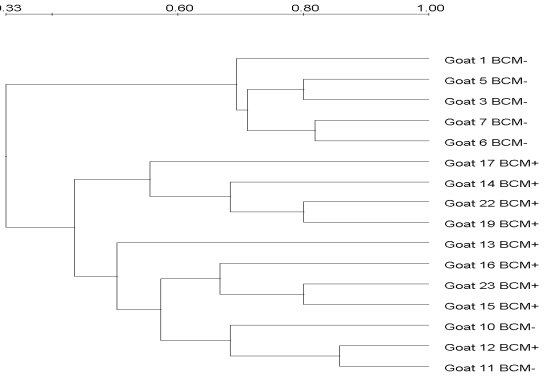

Figure 1 Dendrogram derived from DGGE analysis of the archaeal community in the rumen of goats at week 8.

Conclusions These results suggest that interventions at early life of ruminants cause differential microbial rumen colonization which result in modified rumen activity. The effect seems to be influenced by the mother and remains programmed in the adult animal.

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Determination of ruminal methane production using a fully automated *in vitro* gas system – a modelling approach

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Introduction Methane is one of the significant contributors to the 'greenhouse' effect, being second after carbon dioxide**.** Ruminants are a major source of methane production contributing about 20% of the total emissions. Methane production in ruminants is an energetically wasteful process, as approximately 6% of the gross energy intake is lost as eructated gas to the atmosphere. There are several strategies to reduce methane emissions such as dietary supplementation with oils or anti-bacterial agents. *In vivo* methods for measuring methane production are very laborious and expensive (respiration chambers) or possibly inaccurate (tracer methods) and difficult to standardize. *In vitro* methods have widely been described to measure methane production, but they don't take into account dynamics of digestion and passage kinetics in the rumen. The aim of the present study was to develop an *in vitro* method based on a fully automated *in vitro* gas production system that allows estimation of the kinetic parameters of methane production which then can be used in a mechanistic rumen model to predict methane production from different sample sizes.

Materials and methods Rumen fluid was collected 2 hr after the morning feeding from two cannulated cows (Swedish Red) at early lactation fed a silage based diet. Samples of 300, 600, 900 and 1200 mg of low quality timothy hay were weighed into serum bottles that were then filled with 60 ml buffered rumen fluid (1:5) and incubated in a water bath (39°C) while gently agitating. The gas production was recorded by a fully automated system described by Cone *et al*. (1996). Gas samples were drawn from each bottle by using a gas tight syringe at 2, 4, 8, 24, 32 and 48hr of incubation through the rubber suba seal. Methane was determined by injecting 0.2 ml of gas into a Star 3400 (CX series) gas chromatograph (Varian Chromatography, USA) equipped with a thermal conductivity detector. The second experiment was a 2 x 3 factorial design [two levels of concentrate (barley: grass silage 40:60 (LC) and 60:40 (HC)) and three levels of nutshell extract (0, 5 and 10 mg)]. Nutshell extract (NSE) was dissolved in 99.5% ethanol that was evaporated by leaving the substrate-containing serum bottles at room temperature overnight. The samples (1000 mg) were incubated as described above. Methane and total gas production data were fitted to a two-pool Gompertz model to estimate kinetic parameters. The kinetic parameters were subjected to a mechanistic rumen model described by Huhtanen *et al*. (2008) to estimate first-order production rate and total production of methane. The model simulations were made using 50 h rumen residence time that corresponds to the maintenance level of intake.

Results Methane production per gram of sample DM decreased as the sample size increased from 300 to 1200 mg. This reduction probably reflects the change in partitioning carbon between VFA and microbial cells. However, the first-order production rate was constant except for the smallest sample size (Table 1). The nut shell extract reduced linearly methane production, but the effect on total gas production was small. The decrease was approximately 50% at the10 mg level. The proportion of concentrate had no significant effect on methane or total gas production.

Table 1 The effects of sample size on methane production (ml/g DM)

	300mg	600mg	900mg	1200mg	SEM	L	Q
Asymptotic CH_4	45.6	40.9	39.7	36.3	0.98	<0.01	0.48
Rate (1/h)	0.052	0.046	0.046	0.045	0.001	0.001	0.10
Predicted CH_4	36.9	32.0	31.1	28.2	0.74	<0.01	0.20

Table 2 Effect of nut shell extract on methane and total gas production (mg/g DM)

Concentrate	L C			H C			SEM	Significant	
NSE	0	5mg	10mg	0	5mg	10mg		Concentrate	Lin
Asymptotic CH_4	43.7	33.5	21.9	43.0	34.7	24.0	2.36	0.45	<0.01
Rate (1/h)	0.057	0.062	0.062	0.062	0.062	0.066	0.0016	0.05	<0.01
Predicted CH_4	36.2	28.2	18.6	36.3	29.3	20.5	1.08	0.26	<0.01
Total gas									
Asymptotic	305	260	262	283	284	292	10.0	0.22	0.12
Rate (1/h)	0.10	0.10	0.10	0.13	0.13	0.10	0.014	0.10	0.31

Conclusion It is concluded that increasing sample size did not affect the kinetic rates of methane production and therefore, it is possible to use larger amounts of substrate (1000 mg) in the *in-vitro* system in order to decrease the possibility of substrate depletion in the system. In the second experiment the NSE showed a great potential to mitigate methane production without adversely affecting total gas production. Predicted methane productions (mean 7% of GE) for hay and mixed diets with additives are within the range of *in vivo* data.

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Development of Tier 3 enteric methane emission factors for Holstein and Norwegian dairy cows at two levels of concentrate supplementation

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Introduction The IPCC (2006) recommends three methods (Tier 1, 2 and 3) to estimate enteric methane (CH_4) emissions from livestock for development of national CH_4 inventories. Tier 1 default emission factors provide a fixed value for each species of animals in different regions of the world, irrespective of variations in animal physiological state and production level. The objective was to develop Tier 3 CH_4 emission factors for Holstein and Norwegian cows offered grass silages with two levels of concentrates.

Materials and methods Sixty-four first lactation dairy cows (32 Holstein and 32 Norwegian) were used in a 2 (breed) * 2 (level of concentrates) factorial design study. Each breed was offered two levels of concentrates (proportion of total diet) for days 1-100 (0.60 v. 0.30), 101-200 (0.50 v. 0.20) and 201-300 (0.40 v. 0.10) of lactation. Dietary ME concentration and CH_4 emissions were measured in indirect respiration calorimetric chambers using 4 cattle from each treatment at 80, 160, and 240 days of lactation. The data from production trials and chamber measurements were then used to calculate CH_4 emission for each cow during the 300 days of lactation. For the remaining 65 days in the dry period, feed intake and CH_4 emission for each cow were estimated using average feed intake in the last week of lactation, with subtraction of feed (ME) requirement for milk production and addition of feed (ME) requirement for pregnancy. Energy corrected milk yield was calculated from milk energy output divided by energy content (3.1 MJ) per kg of standard milk (4.0% fat, 3.2% CP and 4.8% lactose).

Results and discussion Live weight was similar between breeds but higher ($P < 0.001$) with cattle offered high than low concentrate diets (Table 1). Increasing dietary concentrate level enhanced DM intake and energy corrected milk yield, irrespective of breed. Holstein cows had a higher DM intake ($P < 0.01$) and milk yield ($P < 0.001$) within either concentrate level, although the difference in feed intake with low concentrate diets was not significant. The high feed intake with high concentrate diets resulted in a higher ($P < 0.001$) CH_4 output (i.e., Tier 3 emission factor) in terms of main effects or within each breed. Breed had no significant effect on CH_4 output, although with low concentrate diets Holstein cows produced more CH_4 than Norwegian cows ($P < 0.001$). Methane emissions as a proportion of energy corrected milk yield or DM intake were higher with Norwegian than with Holstein cows when offered high concentrate diets ($P < 0.001$). With low concentrate diets, Holstein cows had a higher CH_4/DM intake than Norwegian cows ($P < 0.001$), whereas the difference in CH_4/energy corrected milk yield between the two breads was no significant. The Tier 3 enteric CH_4 emission factors for Holstein and Norwegian cows offered low concentrate diets (119 and 110 kg/year, respectively) are close to the Tier 1 factor (117 kg/y) proposed by IPCC (2006) for use in Western Europe, whereas the Tire 3 factors for high concentrate diets (139 and 144 kg/year) are much higher than Tier 1 of IPCC (2006). However, when expressed as CH_4/milk yield, Holstein cows at high concentrate diets had a lower value than that of Tier 1 of IPCC (2006) (18.4 *vs*. 19.5 g/kg), while this variable in other 3 treatments was higher (22.4 to 23.2 g/kg). There was a significant relationship ($P < 0.001$) between CH_4 factor (y, kg/year) and energy corrected milk yield (x, t/year) for Holstein cows ($y = 8.749x + 72.6$, $R^2 = 0.59$) and Norwegian cows ($y = 22.250x + 4.5$, $R^2 = 0.80$).

Table 1. Effects of cow breed and plane of nutrition on enteric methane emissions

	High concentrates		Low concentrates			Significance		
	Holstein	Norwegian	Holstein	Norwegian	s.e.	Breed	Level	B*L
Live weight (kg)	537^{b}	528^{b}	489^{a}	481^{a}	10.9	NS	***	NS
DM intake (kg/year)	5581^{c}	5161^{b}	4381^{a}	4180^{a}	103.9	**	***	NS
E corrected milk yield (kg/year)	7559^{d}	6213^{c}	5338^{b}	4806^{a}	117.7	***	***	***
CH_4 output (kg/year)	139^{c}	144^{c}	119^{b}	110^{a}	2.8	NS	***	*
CH_4/EC milk yield (g/kg)	18.4^{a}	23.2^{b}	22.4^{b}	23.0^{b}	0.44	***	***	***
CH_4/DM intake (g/kg)	24.9^{a}	27.8^{d}	27.2^{c}	26.4^{b}	0.02	***	***	***

a - d superscript means with the same letter in the same row are not significantly different ($P > 0.05$)

Conclusions Tier 3 enteric CH_4 emission factors developed in the present study were influenced by dairy cow breeds and dietary concentrate levels, and can be predicted from milk yield of cows. This indicates that use of Tier 1 factor of IPCC (2006) to develop national CH_4 emission inventories can result in considerable errors.

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Feeding ground flaxseed to increase proportions of omega-3 fatty acids in beef from cull cows

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Introduction Increasing dietary intake of omega-3 fatty acids has been shown to play a role in brain development and function, and relief from inflammatory diseases (Ruxton *et al*., 2004). Research has also demonstrated reduced risk of coronary heart disease as a result of consuming diets rich in omega-3 fatty acids (Simopoulos, 1988). Beef from cattle fed cereal-based diets typically contains relatively low levels of omega-3 fatty acids due to the high levels of omega-6 fatty acids found in the grains they consume. Incorporating flaxseed into the diets of cattle affords the opportunity to alter fatty acid profiles of beef, resulting in increased concentrations of omega-3 fatty acids and improved omega-6:omega-3 ratios. Short-term feeding of diets containing flaxseed may be an effective strategy for improving the value of cull beef and dairy animals.

Materials and methods One hundred eighty two cull cows (BW 508 ± 54 kg) were utilized in a 68-d finishing trial to determine the optimum level of dietary flax in order to maximize performance and improve omega-3 content in beef. Twenty four hours after arrival cows were processed through the working facility receiving an internal/external parasiticide and uniquely numbered ear tags. Body weight was recorded and cows were blocked by weight and randomly assigned to 1 of 4 dietary treatments resulting in 20 pens (5 pens/treatment). Diets were based on steam-flaked maize and contained 0%, 5%, 10%, or 15% ground flax on a DM basis. Cows were transported to a commercial abattoir where carcass weight and incidence of liver abscess were collected at the time of slaughter. Samples of lean tissue from the triceps brachii, semitendinosus, and longissimus dorsi muscles, along with subcutaneous fat from the rib region were collected 24-h post-harvest from 4 carcasses per feedlot pen. Fatty acid profiles of tissues were analyzed as described by Sukhija and Palmquist (1988). Data was analyzed using the Mixed procedure of the Statistical Analysis System (SAS, 2004). Flaxseed concentration was the fixed effect, and pen was the random effect. Denominator degrees of freedom were estimated using the Satterwaith adjustment. Least-squares means for protected F-tests were separated using least significant differences.

Results Optimum dry matter intake, average daily gain, and gain efficiency were achieved when cows were fed 5% ground flaxseed, but no statistical differences were observed between treatments ($P \geq 0.32$). Similar to live performance, carcass weight was optimized when 5% flax was fed, but was not statistically different among treatments ($P = 0.89$). Omega-3 fatty acid content of beef increased in response to the proportion of flaxseed included in the diet ($P < 0.05$). Feeding flaxseed increased concentrations of alpha linolenic acid in triceps brachii ($P = 0.06$), longissimus dorsi ($P = 0.04$), semitendinosus ($P = 0.02$), and in subcutaneous rib fat ($P = 0.02$). Proportions of eicosapentaenoic and docosahexaenoic acids in muscle and adipose tissue were not affected by feeding flaxseed ($P > 0.7$). Omega-6 fatty acid content was unaltered by diet ($P > 0.35$), regardless of tissues evaluated. Feeding flax to cull cows decreased the omega-6:omega-3 ratio ($P \leq 0.06$) in muscle tissue due to unaltered omega-6 content and increases in omega-3 content.

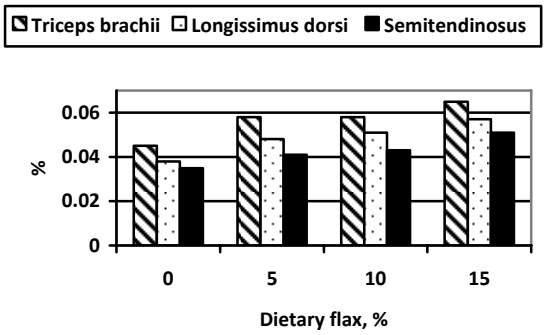

Figure 1 Effect of dietary flaxseed on concentrations of omega-3 fatty acids in tissues

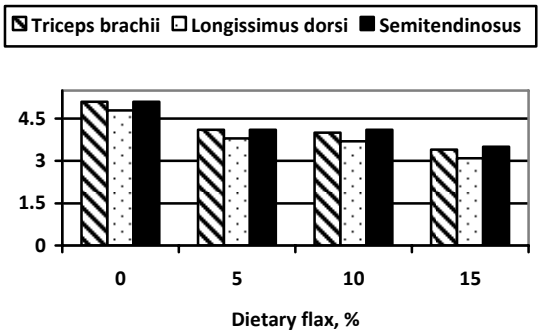

Figure 2 Effect of dietary flaxseed addition on omega-6:omega-3fatty acid ratio in tissues

Conclusion Feeding flaxseed to cull cows for 68 days prior to harvest increased concentrations of omega-3 fatty acids in the triceps brachii, semitendinosus, and longissimus dorsi muscles, and in subcutaneous rib fat. This increase in omega-3 fatty acid content resulted in a concomitant decrease in ratio of omega-6:omega-3 fatty acids. Producing beef that is enriched with omega-3 fatty acids may provide a marketing niche for value-added beef as a result of its appeal to health conscious consumers.

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Fatty acid composition of *longissimus dorsi* muscle of early and late maturing heifers offered supplements containing either safflower oil or ruminally-protected tuna oil while at pasture

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Introduction There is increasing interest in enhancing the nutritional value of ruminant-derived foods to improve long-term human health. Beef from cattle reared on grass contains more of the fatty acids that exhibit positive human health effects including the long chain n-3 fatty acids eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) and *cis*-9, *trans*-11 conjugated linoleic acid (CLA) than beef from concentrate–fed cattle (Noci *et al*. 2005). Supplementation with plant oils or marine lipids enhances tissue CLA concentrations but significant enrichment of EPA and DHA in beef requires the use of rumen protected fish oil (PFO) due to the extensive metabolism of long chain n-3 fatty acids in the rumen. In this experiment the potential of long-term supplementation with safflower oil (SAFF), a rich source of 18:2n-6 (Boles *et al*. 2005) or with PFO to increase muscle concentrations of *cis*-9, *trans*-11 CLA and long chain n-3 fatty acids, respectively, in grazing beef heifers was examined

Materials and methods Spring-born early-maturing Aberdeen Angus × Friesian heifers (AAF, n=24)) and late-maturing Belgian Blue × Friesian heifers (BBF, n=24)) were assigned, at four months old, to either a standard grass-based production system (Keane and Drennan, 2008) (CON), or within that system to continued supplementation with a SAFF or PFO-containing concentrate in a randomised block (bodyweight) design. Following slaughter from pasture at 21 months of age, *longissimus dorsi* (LD) muscle was collected and tissue lipids extracted, separated into neutral lipid (NL) and polar lipid (PL) fractions and converted to fatty acid methyl esters (FAME) as described by Noci *et al*. (2005). The composition of FAME was determined by GC-FID employing a CP-Sil 88 cyanopropyl fused capillary column (100m × 0.25mm i.d. × 0.2μm film thickness, Chrompack, Middelburg, The Netherlands) with H_2 as carrier gas (Shingfield *et al*. 2003). Data were subjected to Analysis of Variance for a split-plot design with block and breed (B) in the main plot and ration (R) and all interactions in the split-plot.

Results The fatty acid composition of LD muscle is presented in Table 1. Both the NL and PL from heifers supplemented with SAFF had a higher proportion of 18:1 *trans*-11 and *cis*-9, *trans*-11 CLA than NL from CON or PFO heifers which did not differ. The proportion of 18:2 in NL was higher for heifers supplemented with SAFF than with CON, with PFO than with SAFF, and for BBF than for AAF. The proportion of DHA in PL was higher for heifers supplemented with PFO than with CON or SAFF and for BBF than for AAF. There was an interaction between B and R for the proportion of 18:2 in PL such that for AAF, it was higher for PFO than for SAFF which was higher than CON whereas for BBF, the value was similar for SAFF and PFO (both higher than CON). There was an interaction between B and R for the proportion of EPA in PL such that it was higher for PFO in both breeds but it was lower in SAFF compared with CON for BBF only.

Table 1 Fatty acid composition (g/100g fatty acids) of bovine LD muscle

Fatty acid	AAF			BBF				P values		
	CON	SAFF	PFO	CON	SAFF	PFO	sed	B	R	B×R
Neutral lipids										
18:1 *trans*-11	2.33	11.17	2.49	2.48	10.89	2.49	0.522	NS	<0.05	NS
18:2	0.85	2.13	7.24	1.08	2.66	8.07	0.304	<0.05	<0.05	NS
cis-9,*trans*-11CLA	0.83	3.14	0.68	1.03	3.14	0.67	0.151	NS	<0.05	NS
Polar lipids										
18:1 *trans*-11	0.47	2.86	0.45	0.41	2.08	0.56	0.524	NS	<0.05	NS
18:2	6.70	26.81	33.53	9.55	30.19	28.17	2.172	NS	<0.05	<0.05
EPA, C20:5n-3	2.19	1.08	7.99	2.83	1.24	9.78	0.438	<0.05	<0.05	<0.05
DHA, C22:6n-3	0.35	0.40	2.60	0.54	0.40	2.98	0.140	<0.05	<0.05	NS
cis-9,*trans*-11CLA	0.27	0.93	0.05	0.26	0.71	0.10	0.143	NS	<0.05	NS

Conclusion Provision of a rumen-protected fish oil supplement to heifer calves for 17 months was an effective strategy for enhancing muscle EPA and DHA content, whereas provision of a concentrate containing safflower oil effectively increased muscle CLA.

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The impact of plant secondary compounds on animal product quality

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Introduction An increased attention to the production of safe animal products, together with the restrictions on the use of chemical growth promoters and anti-parasitic drugs form the basis for the ongoing research on the use of plants containing secondary compounds (PSC) in animal feeding. A number of studies have investigated the potential of PSC such as phenolic compounds (PhC), saponins and essential oils (EO) as dietary supplements for improving ruminants meat and milk quality. In this mini-review an overview of the effects of PSCs on products quality of ruminant livestock is reported.

Fatty acid profile Meat and milk fatty acid profiles are largely dependent on the ruminal microflora mediated biohydrogenation (BH) of polyunsaturated fatty acids (PUFA). Studies conducted *in vivo* have shown that tannins from various plants impair ruminal BH thus leading to the accumulation of PUFA at the expense of saturated fatty acids (SFA). Supplementation of quebracho tannins resulted in an accumulation of vaccenic acid (*trans*-11 C18:1, VA), rumenic acid (*cis*-9 *trans*-11 C18:2, RA) and total PUFA and in a reduction of C18:0 (stearic acid, SA) in lamb meat (Vasta *et al.*, 2009). Cabiddu *et al.* (2009) reported that the RA and VA were lower and linoleic acid (*cis*-9 *cis*-12 C18:2, LA) and linolenic acid (*cis*-9 *cis*-12 *cis*-15 C18:2, LNA) were higher in the milk from ewes fed *Hedisarum coronarium* foliage (condensed tannins: 2.6% in dry matter, DM) than that from ewes receiving this foliage along with polyethylene glycol (PEG). Supplementation of tannins from *Schinopsis balansae* (0.45% of DM intake) did not affect the overall fatty acid profile in cow milk (Benchaar and Chouinard, 2009). Feeding *Lotus corniculatus* foliage (condensed tannins: 2.6% in DM) resulted in increased RA, LA and LNA and reduced SA concentrations in cow milk compared with that in milk from cows fed this foliage along with PEG (Turner *et al.* 2005). The studies conducted so far suggest that saponin supplementation do not modify meat and milk fatty acid composition (Benchaar and Chouinard, 2009). *Quillaja saponaria* saponins at 30, 60 or 90 ppm in lamb diet reduced the concentration of only *cis*-9 C14:1 in meat (Brogna *et al*, 2011), while the overall fatty acid profile and meat cholesterol content did not change. The administration of 1 g of cinnamaldehyde/d (Benchaar and Chouinard, 2009) to dairy cows did not alter the milk fatty acid profile. Similar results were reported for goat milk by Malecky *et al.* (2009) on the infusion a monoterpenes blend (0.43 g/kg DMI) through the rumen.

Flavour The typical pastoral flavour of lamb meat has been attributed to the presence of skatole (3-methyl-indole), an indolic compound originated in the rumen by the action of ruminal microorganisms. Sometimes skatole confers a faecal flavour thus reducing meat acceptability by consumers. Some authors have succeeded to reduce the accumulation of skatole in lamb by impairing its production in the rumen by supplementing tannins from grape seed (Schreurs *et al.* 2007) or from quebracho (Priolo *et al.*, 2009) extracts. Roy *et al.* (2002) reported that grazing sulla for 14 days reduced skatole secretion in milk. The supplementation of some EO improved lamb meat flavour, for example the administration of EO of distilled rosemary (Nieto *et al.*, 2010a) or thyme leaves (Nieto 2010b) to pregnant ewes and growing lambs reduced the rancid odour note in meat.

Oxidative stability The oxidation of myoglobin and the consequent accumulation of metmyoglobin on the meat surface is the major cause of meat discolouration. The use of natural antioxidants could be promoted to prevent meat discolouration. Feeding lambs a concentrate-based diets with the inclusion of quebracho extract delayed myoglobin oxidation and extended the colour stability of meat (Luciano *et al.*, 2011). Similarly, the dietary administration (2% DMI) of seaweed (*Ascophillum nodosum*) extract, a rich source of phlorotannins, to goats (Gallipalli *et al*., 2008) or cattle (Braden *et al.*, 2007) improved the colour stability of chevon and beef. Recently, Nieto *et al.* (2010a; 2010b) found that the administration of distilled rosemary or thyme leaves (rich in EO) to ewes during pregnancy and lactation improved the shelf life of lamb meat, with a reduction of lipid oxidation and an extension of colour stability.

Conclusions Dietary PhC can modify ruminal biohydrogenation thus affecting meat and milk fatty acid composition. Supplementation of EO has considerable potential in extending meat shelf life and in preserving meat flavour from deterioration. The negative effects on animals of adding these compounds at high concentrations in diets must also be considered. The mechanisms responsible for the beneficial effect of PSC reported here are not yet clear. Several neglected and lesser known plants as well as agro-industrial by-products are rich in PSCs and their strategic incorporation in the animal diets could enhance quality and safety of animal products. This strategy would also contribute to converting the by-products associated disposal problems into opportunities for development.

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Effects of increasing the proportions of high quality grass silage after peak lactation on milk yield and milk composition in dairy cows

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Introduction Swedish dairy cows are among the highest producing in Europe with an average milk yield of >9300 kg energy corrected milk (ECM) year^{-1}. During the last decades, the feeding has been intensified and more based on concentrate feeds. The use of forage has decreased and is at present 400-500 g kg^{-1} dry matter (DM) of yearly consumed feed DM in conventional dairy production. Increased use of high quality forage may improve profitability in dairy farming. The high nutritional requirements of high producing cows make it necessary to find models allowing large amounts of high quality forage in dairy cow diets without jeopardizing health and production in early lactation. The aim of the present experiment was to evaluate the effect of high quality grass silage diets to dairy cows on milk production, using gradually increasing forage proportions after peak lactation and until drying off.

Materials and methods Twenty-one primiparous and 33 multiparous Swedish Red Breed (SRB) dairy cows were randomly assigned to three diets with different proportions of grass silage/concentrate on DM basis: low forage (L), medium forage (M) and high forage (H). The experiment ran through the whole lactation (305 days). The cows were fed according to a predetermined scheme during the first month of lactation, with a gradual increase in concentrate intake up to a maximum of 14 kg, and grass silage *ad libitum*. During the second and third lactation month, grass silage was fed *ad libitum* and the amount of concentrate was adjusted so that the forage proportion was 400 g kg^{-1} DM in diet L and 500 g kg^{-1} DM in diets M and H. From the fourth lactation month until drying off feed intake was restricted when intake of the cow exceeded 110% of her energy requirement. Starting from lactation month four, the proportion of grass silage was gradually increased from 400 to 500, 500 to 700 and 500 to 900 g kg^{-1} DM in diet L, M and H, respectively. The silage was made from an early first-cut ley of timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* L.) and red clover (*Trifolium pratense* L.) with 41% DM, 11.2 MJ metabolizable energy, 146 g crude protein and 443 g neutral detergent fiber (aNDFom) kg^{-1} DM. The ingredients in the concentrate mixture were oats, barley, peas, rapeseed cake, beet fibre, wheat bran, whole rapeseed (crushed), minerals and vitamins (234, 232, 200, 125, 90, 70, 25 and 24 g kg^{-1} feed, respectively). Feed intake was recorded automatically. The cows were housed indoors in an automatic milking system and had an average of 2.5 milkings day^{-1}. During the summer, the groups M and H were on pasture and fed concentrates indoors, while group L only had a paddock for exercise with all feeds offered indoors. Milk yield was recorded automatically at each milking and milk samples for analyses of milk composition were taken once every two weeks. Data were analysed using two-way ANOVA and means were separated using the PDIFF option of SAS®. Several independent variables and interactions were found non-significant and therefore the final model included only the fixed effects of diet and lactation number.

Results The average proportions of forage over the entire lactation were 490, 620 and 710 g kg^{-1} DM, with an average daily DM intake of 19.9, 19.4 and 19.2 kg^{-1} cow in diet L, M and H, respectively. Daily milk yield in kg milk and kg ECM from diet H were lower compared with the other two groups, while milk yield on diets L and M did not differ. Milk fat and milk protein did not differ among diets (Table 1).

Table 1 Milk yield and milk composition from Swedish Red Breed dairy cows fed low, medium and high proportion of high quality grass silage in the diet after peak lactation, least square means.

	Diet				
	Low forage	Medium forage	High forage	SEM	P-value
Milk kg day^{-1}	30.6^{a}	29.7^{a}	26.7^{b}	0.91	0.008
ECM kg day^{-1}	32.3^{a}	31.2^{a}	28.4^{b}	0.77	0.002
Milk fat g kg^{-1} milk	43.6	44.6	45.9	1.22	0.384
Milk protein g kg^{-1} milk	34.8	35.0	34.3	0.50	0.612
Milk lactose g kg^{-1} milk	48.5^{a}	47.6^{b}	47.4^{b}	0.24	0.007
ECM kg 305 d^{-1}	9846^{a}	9528^{a}	8667^{b}	235.1	0.002

ECM, energy corrected milk (4%); SEM, standard error of means, highest value chosen
$^{a-b}$ within a row, different superscripts differ ($P \leq 0.05$)

Conclusions The results suggest that when using high quality grass silage to dairy cows, the forage proportion can be gradually increased from 500 g kg^{-1} DM to 700 g kg^{-1} DM in the period following peak lactation until drying off, without significantly reducing milk yield, milk fat or milk protein content.

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Dietary levels of un-degradable dietary protein affects fibre diameter and nitrogen excretion in alpacas

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Introduction Alpacas can obtain most of their glucose from deamination of amino acids (AA) rather than from propionate (Van Saun, 2006). To reach their needs for glucose, alpacas, fed to meet their energy requirements for maintenance, may utilise most of the AAs absorbed from the small intestine. Consequently, the supply of AAs to meet their requirements for fibre growth might be compromised and it may be necessary to supply them with supplemental protein to optimise fibre production. Un-degradable dietary proteins (UDP) by-pass the rumen and are digested in the abomasum and small intestine where AAs are absorbed and used for synthetic processes including fibre growth or conversion to glucose. In sheep supplemented with UDP as expeller canola meal (about 50% UDP), wool growth increased by 11% compared to the wool growth of sheep fed lupins; a grain with a similar level of protein but only 25% UDP (Masters *et al*., 1999). In this study, we tested if the proportion of UDP in the diet could influence fibre growth of alpacas. We hypothesised that alpacas, fed at maintenance a diet containing canola meal protein high in UDP, will produce more quality fibre and excrete less nitrogen than alpacas fed a similar amount of canola meal protein with a low proportion as UDP.

Materials and methods Four groups of 8 alpacas were fed diets of similar metabolisable energy (ME) content at a level calculated to maintain body weight with varying proportions of UDP; 0%, 30%, 60% and 100%, in the form of heat-treated canola meal. The fibre growth of the animals was measured over two months by clipping mid-side patches at the start and end of the treatment period. The fibre diameter was measured using a representative sample from the mid-side patch. The behaviour of the animals in the two extreme groups (0% and 100%) was measured over five days using CCTV cameras and digital surveillance software.

Statistical analyses The mean fibre diameter and the dry fibre growth (in g/cm2) was analysed using ANOVA. The fibre diameter value obtained at the beginning of the treatment was used as a covariate. Pairwise comparisons between the four treatment groups were also tested using the Student-Newman-Keuls test (GenStat®, 11th edition, VSN International Ltd., 2008). Behavioural data from the two extreme treatment groups were normalised using an arcsine transformation and then analysed using ANOVA with repeated measures.

Results All alpacas consumed most of the food on offer and only a small amount of straw was not eaten by some animals. All animals maintained live weight (p = 0.662) and body condition (p = 0.278; Table 1). Fibre grown was similar between all treatment groups (p = 0.313). The fibre diameter was smaller in alpacas fed 0% UDP than that of alpacas fed the higher levels of UDP (p = 0.039). There were no differences between the 0% and 100% treatment groups for any of the observed behaviours, but alpacas fed 0% UDP spent a more time urinating than alpacas fed 100% UDP (p = 0.027).

Table 1 Mean change (±SE) in live weight, body condition, fibre growth and fibre diameter of alpacas fed diets containing different % of UDP over 14 weeks and time spent urinating. ^{ab} values within a row with different superscripts are different ($p < 0.05$).

	Proportion of UDP from canola meal in diet			
	0%	30%	60%	100%
Change in live weight (kg)	1.7 ± 0.28	1.5 ± 0.85	2.9 ± 1.11	1.5 ± 1.03
Change in condition score (1-5)	-0.6 ± 0.15	-0.2 ± 0.16	0.0 ± 0.19	-0.2 ± 0.16
Fibre growth (mg/cm^2)	33.8 ± 2.42	39.6 ± 3.29	42.2 ± 3.97	37.7 ± 3.10
Fibre diameter (μm)	18.1 ± 0.50^a	20.4 ± 0.93^b	21.4 ± 0.63^b	20.4 ± 0.82^b
Urinating	0.4 ± 0.13^a			0.1 ± 0.04^b

Conclusions The increase in time urinating in alpacas fed 0% UDP suggested that they produce more urine and excreted more nitrogen, and consequently less urea would have being recycled to the fermentative organs. Therefore, nitrogen may have limited microbial protein synthesis, so less amino acids were available for fibre production, as suggested by the decrease in fibre diameter.

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In vitro evaluation of four additives to reduce ruminal biohydrogenation

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Introduction Extensive lipolysis and biohydrogenation occur in the rumen, so that ruminant products are poor in polyunsaturated fatty acids (FA) which are considered as beneficial for human health. Numerous attempts have been made to limit ruminal FA metabolism, but only the chemical treatment with formaldehyde is efficient (Fievez *et al.*, 2007). The use of additives may act as a modifier of microbial activity. This experiment aims to study the effect on ruminal biohydrogenation process of four additives for which an effect on rumen FA metabolism is expected.

Material and methods Two sheep fed 600 g hay, 300 g barley and 100 g of a mixture of extruded linseed and chicory pulp (85:15) were used as ruminal liquid donors. Three series of *in vitro* incubations were carried out on 3 different days. Incubations were performed anaerobically at 39°C for 0, 5 and 24 h in 120-mL bottles containing 370 mg ground substrate (24.7, 42.3, 25.5, 6.4 and 1.1 % of straw, wheat, soybean meal, extruded linseed and chicory pulp, respectively), 25 mL Simplex buffer and either no additive (CON), 3 mg brown algae (*Ascophyllum nodosum*) (ALG), 13.1 mg cinnamon essential oil (CIN), 8 mg garlic essential oil (GAR) or 4 mg synthetic vitamin E (VitE). For each day, each time and each treatment, the total content of one bottle was freeze-dried before FA determination. Detailed procedures are described in Laverroux *et al.* (2011). Biohydrogenation was calculated as the % of disappearance of dietary unsaturated FA after 24 h of incubation. Data were submitted to the glm procedure of SAS. Means were compared using a Dunnett test with CON as the control level.

Results Concentrations of 18:2 *t*11*c*15 and 18:1 *t*11 after 5 h of incubation were higher for GAR than for CON but were not modified by the other additives (Table 1). This is consistent with the effect of allyl mercaptan, a compound of garlic essential oil on biohydrogenation, shown by Lourenço *et al.* (2010). Concentrations of the other main 18-carbon FA were not modified by additive supply. Biohydrogenation of oleic, linoleic and linolenic acids were decreased with garlic essential oil, especially the biohydrogenation of oleic acid which was twice lower after 24 h of incubation. The large increase in 18:2 *t*11*c*15 with GAR allows to venture the hypothesis of a decrease in biohydrogenation process after the first reduction of linolenic acid (from 3 to 2 double bonds). The increase in 18:1 *trans*11 and the decrease of oleic and linoleic biohydrogenation shows a more general effect on different steps of the biohydrogenation process.

Table 1 Concentration in major 18-carbon FA after 5 h of incubation and biohydrogenation after 24 h of incubation

	CON	ALG	CIN	GAR	VitE	SEM	P-value
Ruminal concentration after 5 h (g/100 g FA)							
18:3 c9c12c15 (linolenic acid)	3.81	5.36	5.06	4.84	4.73	0.518	0.345
18:2 t11c15	0.27	0.27	0.32	2.64**	0.27	0.185	<0.001
18:2 c9c12 (linoleic acid)	3.56	4.38	4.08	4.07	3.77	0.370	0.596
18:2 c9t11	0.11	0.11	0.11	0.12	0.11	0.009	0.876
18:1 t10	0.21	0.19	0.16	0.28	0.19	0.041	0.354
18:1 t11 (vaccenic acid)	3.75	3.83	3.72	5.64**	3.68	0.324	0.006
18:1 c9 (oleic acid)	3.12	3.83	3.47	4.13	3.42	0.351	0.355
18:0 (stearic acid)	64.93	61.33	62.80	58.19	63.72	1.984	0.224
Biohydrogenation after 24 h (%)							
18:3 c9c12c15 (linolenic acid)	87.8	85.5	84.7	79.6*	85.7	1.81	0.083
18:2 c9c12 (linoleic acid)	85.5	84.6	86.5	78.5**	82.5	1.25	0.008
18:1 c9 (oleic acid)	67.8	65.1	66.5	36.2**	77.1	2.40	<0.001

Differences between means: * $P < 0.05$; ** $P < 0.01$

Conclusions Addition of garlic essential oil in ruminal fluid decreases oleic, linoleic and linolenic biohydrogenations, with a general effect on different steps of the biohydrogenation process. Vitamin E, cinnamon extract and brown algae did not modify biohydrogenation.

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Auto-oxidation of *ortho*-diphenolic substrate and deactivation of polyphenol oxidases (catecholase) during wilting and post harvest damage in red clover

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Introduction Polyphenol oxidases (PPO) in red clover convert diphenolic substrate to highly reactive quinones which through their reaction with proteins increase the efficiency of N utilization and increase the proportion of beneficial polyunsaturated fatty acids in bovine products (meat and milk; Lee *et al*., 2009a,b). Auto-oxidation of phenolic substrate to quinone has been previously reported (Lim *et al*., 2005) but little information is available on the degree of auto-oxidation in forage crops during conservation, with all activity being attributed to enzymatic oxidation through PPO. This study investigated the degree of oxidation in wild type red clover (PPO+) and red clover with the PPO1 gene silenced by genetic manipulation (PPO-) to determine the degree of auto-oxidation under two damage regimes (heavy and light) and in addition the temporal deactivation of PPO.

Materials and methods PPO+ and PPO- red clover plants were grown under controlled conditions (temperature of 20/15 ºC, photoperiod of 16 h, and humidity of 0.6 kPa at both temps) and harvested at six weeks regrowth. The material was passed through a garden shredder as the light damage (LD) and half frozen at -20^{o}C as the heavy damage (HD). Material was left at room temperature and sampled at regular intervals for determination of PPO enzyme activity (active and total), PPO activation and formation of protein-bound phenol (PBP). For the PPO activity assay of LD and HD red clover material, plant tissue was extracted according to the method of Winters and Minchin (2005) and assayed according to the method of Robert *et al*. (1995). PBP analyses were carried out using a modified Lowry procedure described by Winters and Minchin (2005). All statistical operations were performed using Genstat 11.1 with PPO*Damage*Time regime as the treatment effect.

Results For PPO+ HD active PPO was higher (P<0.05) than PPO+ LD during the initial time points (0-0.5 h; Figure 1) whereas total PPO were comparable under both damage regimes (Figure 2). After 1 h both total and active PPO for HD dropped to below LD and trailed off to negligible activity at 24 h. Whereas with the LD active PPO increased (P<0.05) up to 1 h before declining and stabilizing after 4 h. PPO- had no detectible activity throughout the experiment. PBP formation as a measure of oxidation was highest initially on the PPO+ HD treatment up to 4 h where after PPO+ LD showed the highest concentration up to 24 h which was comparable with the PPO- LD treatment (Figure 3). Auto-oxidation was more prevalent at the earlier time points for PPO- HD than PPO- LD, but the inverse relationship was shown at 24 h.

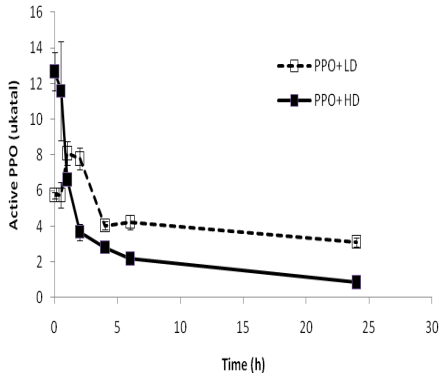

Figure 1 Deactivation of red clover active PPO under two damage regimes

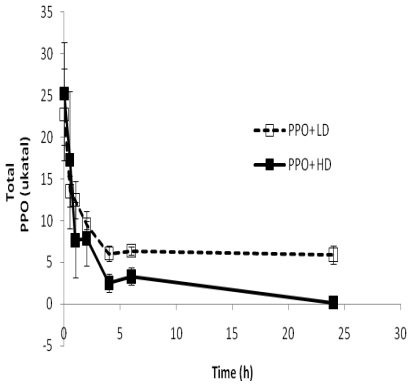

Figure 2 Deactivation of red clover total PPO under two damage regimes

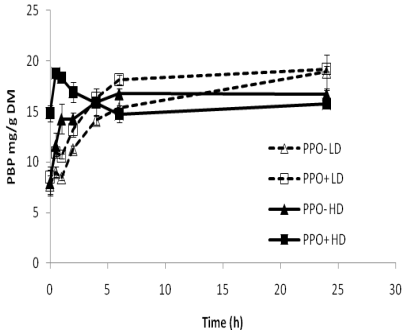

Figure 3 Protein bound phenol formation in PPO+ and PPO- red clover under two damage regimes

Conclusions The experiment highlights the temporal denaturation of PPO and the role of auto-oxidation in the formation of PBP in damaged red clover during wilting.

Acknowledgement This work was funded by the Biotechnology and Biological Scientific Research Council.

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Carcass quality of rabbits fed diets with increasing level of sulla (*Hedysarum flexuosum*) hay

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Introduction Sulla (*Hedysarum flexuosum*), short-lived perennial leguminous plant (Fabaceae) originating from the western Mediterranean region and North Africa, is a balanced source for growing rabbits. Kadi *et al.* (2011) estimated its nutritive value to 7.9 MJ/kg of DE and 62.9 g/kg of DP. In rabbit production, diet is known to affect carcass quality. This research aimed to evaluate the carcass traits of rabbits fed Sulla hay.

Materials and methods Ninety rabbits of a local Algerian population aged 5 weeks, were randomly allotted to three dietary treatments (thirty rabbits per group) in a completely randomized design. They were kept individually in cages under hutch housing conditions and fed *ad libitum* one of the three diets which contains 0 % (control, S0), 15 % (S15) or 30 % (S30) of sun-dried Sulla hay. After seven weeks of fattening (12 weeks old), ten rabbits per group were slaughtered (without fasting) in controlled conditions, according to World Rabbit Scientific Association (Blasco and Ouhayoun, 1996) recommendations. The parameters studied concern slaughter data (Slaughter live weight, Commercial Skin Weight (CSKW), Full Gastrointestinal Tract Weight (FGTW), Hot Carcass Weight (HCW), Chilled Carcass Weight (CCW), Dressing out Percentage (DoP) (% l.w.)), Chilled Carcass Composition (Liver weight (LvW), Kidney Weight (KiW), Reference Carcass Weight (RCW), Hind Part Weight (HPW)), Reference Carcass Characteristics (Perirenal fat weight (PFaW), Scapular fat weight (SFaW), Inguinal fat weight (IFW)) and Carcass linear measurements (Dorsal Length (DL) (cm), Lumbar circumference (LCL) (cm)). Data were analyzed as a completely randomized design with type of diet as the main source of variation by using the GLM procedure of SAS software (Online Doc®, SAS Inst., Cary, NC). Means comparison were done by using the test of Scheffe.

Results For all the traits, there was significant difference ($p<0.01$) only in two carcass characteristics (Table 1): Scapular fat weight (SFaW) which was lowest in rabbits fed diets with Sulla (S15 and S30) and Inguinal fat weight (IFW) which was lowest in rabbits fed diet with 30 % of Sulla.

Table 1 Effect of Sulla hay dietary level on slaughter traits

Traits	Diet S0	Diet S15	Diet S30	SEM	P
Slaughtering data					
Slaughter live weight (g)	2506.50	2433.70	2280.40	51.48	0.2064
Commercial skin weight (CSKW) (g)	287.85	281.10	254.37	8.34	0.2415
Full gastrointestinal tract weight (FGTW) (g)	423.76	403.97	395.53	9.36	0.4738
Hot carcass weight (HCW)(g)	1508.41	1473.99	1390.71	29.53	0.2871
Chilled Carcass Weight (CCW) (g)	1482.40	1448.30	1257.11	29.39	0.2388
Dressing out Percentage (DoP) (% l.w.)	59.23	59.31	58.90	0.27	0.8296
Chilled carcass composition					
Liver weight (LvW) (g)	112.56	97.73	86.21	5.36	0.1427
Kidney weight (KiW) (g)	19.43	17.02	16.68	0.52	0.0920
Reference carcass weight (RCW) (g)	1132.27	1084.00	1003.90	23.40	0.1251
Hind Part Weight (HPW) (g)	159.30	168.15	162.93	3.01	0.5892
Reference carcass characteristics					
Perirenal fat weight (PFaW) (g)	42.94	31.28	35.51	2.38	0.1958
Scapular fat weight (SFaW) (g)	14.90^{a}	9.87^{b}	9.33^{b}	0.46	0.004
Inguinal fat weight (IFW)(g)	12.68^{a}	12.95^{a}	8.23^{b}	0.53	0.0032
Linear measurements					
Dorsal Length (DL) (cm)	25.73	25.20	24.17	0.27	0.0763
Lumbar circumference (LCL) (cm)	18.34	18.23	17.84	0.14	0.3506

Means with the same letter are not significantly different ($P>0.05$)

Conclusions These results suggest a positive effect of Sulla on the adiposity of the carcass. However, no such a conclusion can be taken because Perirenal Fat Weight (PFaW), the better predictor of dissectible fat in the whole carcass, does not seem to be affected. Further investigations are necessary to determine the effect of this raw material on rabbit carcass quality, especially its adiposity.

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Effect of protein supplementation of lambs grazing natural pastures

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Introduction Uruguayan sheep production systems are mainly based on natural pastures. Lambs born in spring, and weaned at three months of age, start grazing in summer when forage presents low protein and energy content because most native species are in the reproductive stage. At this moment lamb protein requirement is high, and not satisfied by grazed pastures (Azzarini,, 1991) also there are significant effects of parasitic diseases. As a result, growth rates are slower than expected, and required for meat sheep production systems. This feeding period is a challenge to current feeding systems. The aim of this study was to examine the effect of protein supplementation on growth rate of lambs grazing natural pastures during the summer- autumn season.

Materials and methods The experiment was conducted at the Research Center "Dr. Alejandro Gallinal", belonging to the Uruguayan Wool Secretariat, (S 33º 52', W 55º 34') from 26 of January to 28 of April (12 days of adaptation period plus 87 days of measurements). Sixty 3 months old weaned Corriedale lambs were divided into ten homogenous groups according to sex, live weight, and body score, and were allocated into two paddocks (30 lambs/paddock, 10 lambs/ha, which differed in amount and composition of native species (2483 and 3660 kg of initial available DM ha^{-1}, 70 and 80 , and 700 and 690 g CP and NDF kg DM^{-1} for paddocks 1 and 2, respectively). In each paddock, animals were assigned (2 groups of lambs/treatment) to one of the following treatments: continuous grazing (Control), Control + proteic block (150 g $lamb^{-1}d^{-1}$; 940, 300 and 360 g kg DM^{-1} OM, CP, and NDF,respectively) (PB), Control + Soybean meal (100 g $lamb^{-1}d^{-1}$; 430 and 280 g kg DM^{-1} CP, and NDF, respectively) (SBM), Control + supplemental protein obtained from grazing 3 h day^{-1} of a natural pasture improved with *Lotus uliginosus* cv Maku (23 lambs ha^{-1}; 280 and 290, 560 and 600 g kg DM^{-1} CP, and NDF, paddocks 1 and 2 , respectively) (SP1), the last treatment was same SP1, except that grazing was allowed one every three days (SP2). Protein supplements were offered daily early in the morning in the grazing paddock. Lambs were weighed (beginning last day of adaptation period, and every ten days thereafter), body score (Jefferies, 1961) was determined at 30 days interval. Results of final body weight, average daily gain (ADG), and difference between initial and final body score (DIFBS), were analyzed (PROC GLM, SAS) in a completely randomized block (paddocks) with subsamplings (body weight and body score at dates of measurements) design (Tukey test), considering the group of lambs by replicate as the experimental unit.

Results Treatment effects ($P < 0.03$) were registered in final liveweight, ADG, and DIFBS (Table 1), appearing SP1 as the best ($P < 0.05$), treatment in reference to Control. Lambs supplemented with soybean meal presented similar ($p > 0.10$) final weight as those grazing the improved pastures 3 h day^{-1} ; while, lambs grazing the high protein pasture one every three days or supplemented with PB registered an intermediary ($P>0.10$) final body weight in reference to control. The ADG of lambs supplemented with soybean meal, or grazing the improved pastures one every three days, registered an intermediary response between those grazing the improved pastures 3 h day^{-1} and the control group. **L**ambs supplemented with soybean meal, or grazing high protein pastures (SP1 and SP2) registered the greater ($P<0.05$) DIFBS.

Table 1 Effect of protein supplementation on liveweight, average daily gain and, body score of lambs grazing natural pastures

	Control	PB	SBM	SP1	SP2	Pooled SEM	P treatment effect
Initial liveweigth, kg	19.03	18.58	18.89	18.90	19.41	-	-
Final liveweight, kg	25.33 ^{c}	24.03 ^{bc}	27.61 ^{a}	27.48 ^{a}	25.23 ^{abc}	0.49	0.02
Average daily gain, g $lamb^{-1}$ day^{-1}	46 ^{b}	51 ^{b}	71 ^{ab}	87 ^{a}	62 ^{ab}	5	0.02
Initial body score (IBS)	3.2	3.1	3.1	3.2	3	-	-
Final body score (FBS)	3.4	3.2	3.4	3.4	3.4	0.09	NS
Difference between IBS and FBS	0.06 ^{b}	0.09 ^{ab}	0.34 ^{a}	0.36 ^{a}	0.40 ^{a}	0.007	0.03

a,b,c : In the rows, $P<0.05$; NS: $P>0.10$
CG: continuous grazing; PB: CG + proteic block ; SBM: CG + Soybean meal; SP1: CG + grazing 3 h day^{-1} ; SP2: CG + grazing one every three days.

Conclusions Controlled access to grazing a high protein pasture appeared as a good protein supplementation for lambs grazing natural pasture. However, this "protein supplementation" is highly dependent on weather conditions. Soybean meal, appeared as an effective, and weather independent, protein supplement for lambs grazing pastures in seasons, and under pasture conditions similar to those tested in this study.

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Investigation of the relationship between sward organic matter digestibility and sward leaf proportion from 20 years data

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Introduction Animal output from grassland is dependent on the quantity and quality of the sward offered to the herd (Shalloo *et al*., 2007). Quantity parameters, such as herbage mass (HM), sward structure and density are interrelated with quality parameters such as sward organic matter digestibility (OMd) (Stakelum and Dillon, 2004). Highly digestible swards are perceived as having a low herbage mass and a high leaf proportion (LP) leading to high animal performance. Indeed leaf content was shown to be directly related to sward digestibility (Stakelum and O'Donovan 2000), but this study was conducted on a small number of samples back in the 1980s. Due to difficulties in laboratory determination of sward OMd it is not routinely analysed by farmers, but as mentioned above, it is a critical parameter in determining animal output. Thus the objective of this study was to investigate the relationship between sward OMd and the sward characteristic LP.

Materials and methods A database was constructed containing information from studies conducted from 1988 to 2009 on the research farms of Teagasc Moorepark (O'Neill *et al*., 2011). The database includes animal and grass variables from 522 grazing herds. Grass OMd was calculated *in vitro* using the neutral detergent cellulase method (Morgan *et al*. 1989). Leaf and leaf + stem/pseudostem (LS) proportions were calculated by manual separation. Datasets which contained OMd and LP and OMd and LS were selected from the database. Regression analysis (PROC REG) was used to ascertain the relationship between these sets of parameters.

Results and discussion From the database 150 data points were available for OMd and LP and OMd and LS. Leaf proportion explained 8.2% of the variation in OMd and LS explained 18.6%. For each 0.1 unit increase in LP OMd increased by 6.48 g/kg and for each 0.1 unit increase in LS OMd increased by 18.06 g/kg. Stakelum and O'Donovan (2000) reported that a 5.5 percentage unit change in leaf content equalled a 1 percentage unit change in digestibility. This equates to a 0.1 unit change in LP equalling an 18.2 g/kg change in OMd. The results of this study for LS were very similar to those of Stakelum and O'Donovan (2000), and were more comparable than the LP results. The grass plant is comprised of the leaf blade, leaf sheath (pseudostem), true stem and dead material. For most of the year the stem makes up a minor part of the grass plant and the pseudostem dominates. This pseudostem is highly digestible (Stakelum and O'Donovan 2000), and this likely accounts for the relationship between LS and OMd.

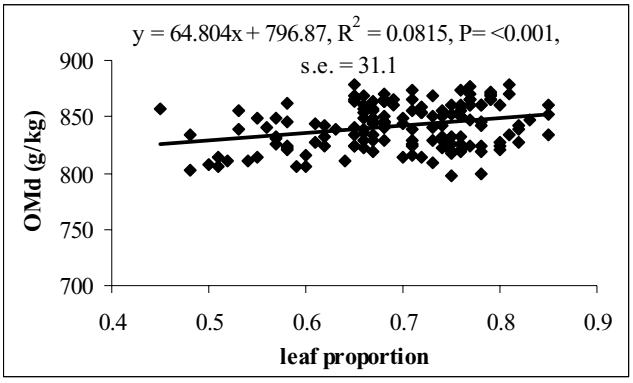

Figure 1 Relationship between leaf proportion and OMd for proportion for 150 sward samples

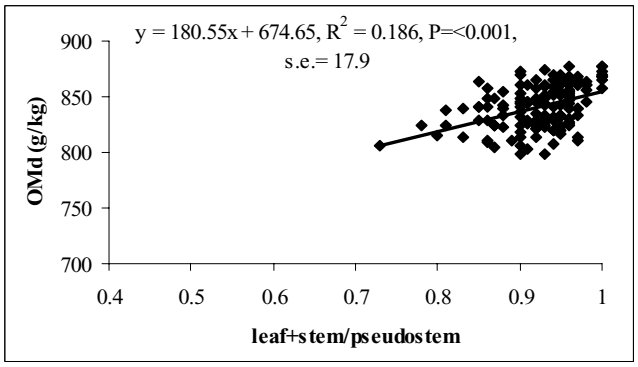

Figure 2 Relationship between leaf+stem/pseudostem and OMd for150 sward samples

Conclusion Of the parameters examined, LS explained the greatest variation in OMd. The relationships between LP and OMd and LS and OMd may be improved by increasing the numbers of data and especially by increasing the range of data available. Further work should also take into account season, previous post-grazing height and other management effects as Stakelum and O'Donovan (2000) demonstrated that previous management had a large effect on subsequent OMd.

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The effect of supplementing grazed grass with mixed ration on rumen pH and rumen ammonia, volatile fatty acid and lactic acid concentrations

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Introduction Ninety per cent of Irish dairy herds are spring calving, producing milk from grazed grass, with the mean calving date coinciding with the initiation of grass growth in early spring (Dillon *et al*., 1995). A 50% increase in milk production by 2020 is anticipated (DAFF, 2010) with the abolition of milk quota in 2015. This is likely to increase cow numbers and stocking rate, forcing some farmers to consider supplementary feeding e.g. partial mixed rations (PMR). This change in feeding strategy could have an impact on rumen parameters. For example, increasing the level of rapidly fermentable carbohydrate will increase propionic acid production, while fibrous forages with slow digestibility will produce more acetic acid (Monteny *et al*., 2006). Most of the information on rumen parameters derives from work done with diets other than grass. The objective of this study was to investigate the effects on rumen parameters of offering dairy cows diets composed of all grass compared to grass supplemented with a PMR.

Materials and methods Forty-eight spring-calving Holstein Friesian dairy cows (18 primiparous, 30 multiparous) were randomly assigned to one of 3 grass-based treatments for 8 weeks in mid to late lactation. The 3 treatments were i) low grass allowance (LGA), ii) high grass allowance (HGA) or iii) low grass allowance + partial mixed ration (PMR). The LGA group were allocated 14.4 kg DM grass/cow/day, the HGA group 19.3 kg DM grass/cow/day and the PMR group 13.9 kg DM grass/cow/day. The PMR group were also offered 4.1 kg DM PMR/cow/day, which was composed of maize silage (1.85 kg DM), straw (1.85 kg DM) and concentrate blend (0.40 kg DM). Six rumen-cannulated lactating dairy cows were arranged into 2 incomplete 3x3 latin squares. Two cows were allocated to each treatment for 4 weeks (period 1). Then the cows crossed over treatments and were allocated to a second treatment for a further 4 weeks (period 2). Measurements were made during the final week of each period. Rumen pH was measured using an indwelling rumen pH probe. The data were logged at 60-second intervals over the 48-hour period. Rumen fluid samples were taken on 2 consecutive days at 8am and 12 pm, strained through 3 layers of cheesecloth and frozen as-is for subsequent analysis of lactic acid and with 50% TCA for subsequent analysis of ammonia and volatile fatty acids (VFA). The data were analysed using the mixed procedure of SAS (SAS Institute, 2003) with treatment, period, square, cow and their interactions included in the model.

Results The proportion of acetic acid at 8 am was greatest on the PMR treatment ($P<0.001$). This agrees with the fact that structural fibre levels were greatest on this diet due to the inclusion of maize silage and straw. The branched chain VFAs iso-buyric acid, valeric acid and isovaleric acid are derived from the deamination of amino acids in the rumen. Thus an increase in their concentration is indicative of an increase in excess dietary rumen degradable protein. The proportion of valeric acid at 8 am was greatest on the LGA treatment ($P<0.05$) and the proportion of iso-butyric acid at 12 pm was least on the PMR treatment ($P<0.01$). This, along with the fact that ammonia concentration at 12 pm was greater on the LGA than the PMR treatment ($P<0.05$) indicates a greater excess of rumen degradable protein on the LGA than on the PMR diet. There was no effect of treatment on average rumen pH or on lactic acid concentration ($P>0.05$). Overall, average rumen pH levels were low (LGA=6.34, HGA=6.17, PMR=6.21, s.e. 0.053).

Table 1 The effect of supplementing grazed grass with mixed ration on rumen volatile fatty acid and ammonia concentrations

	8:00 AM					12:00 PM				
	LGA	HGA	PMR	s.e.	Sig	LGA	HGA	PMR	s.e.	Sig
Total VFA (mmol/l)	151	150	148	4.9	ns	186	193	192	6.3	ns
Acetic acid (%)	72.42^{a}	72.83^{b}	73.39^{c}	0.055	<0.001	68.23	70.34	68.51	0.713	ns
Propionic acid (%)	15.50	15.55	15.38	0.245	ns	18.38	17.04	18.71	0.586	ns
Butyric acid (%)	9.09	9.01	8.48	0.265	ns	10.09	9.33	9.84	0.168	0.06
Iso-butyric acid (%)	0.90	0.83	0.89	0.036	ns	0.97^{a}	0.98^{a}	0.81^{b}	0.027	<0.01
Valeric acid (%)	0.83^{a}	0.74^{b}	0.75^{b}	0.015	<0.05	0.94	0.94	0.99	0.033	ns
Iso-valeri acid (%)	1.26	1.03	1.11	0.059	0.09	1.39	1.37	1.14	0.062	0.07
Ammonia (mmol/l)	12.07	11.42	9.81	1.181	ns	27.89^{a}	24.04^{ab}	17.60^{b}	1.668	<0.05

Conclusions Rumen fermentation profiles are important in interpreting grazing studies effects on dairy cows. Rumen parameters suggest that supplementing a grass diet with PMR may increase fibre degradation and reduce excess rumen degradable protein.

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Effects of litter size and nutritional restriction over calcium and phosphorus deposition in fetus and gravid uterus of Santa Inês ewes

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Introduction The pregnancy is a fundamental step on animal production, since any kind of disturb on this phase can derail all the productive system. It has been demonstrated, although not well understood, that protein deficiency affects calcium metabolism in sheep (Sykes and Field, 1972; Braithwaite, 1978) due to an increased mobilization of bone because of problems generated in the intestinal absorption of calcium and deposition process at bone matrix. The effect of macronutrient restriction on the metabolism of phosphorus seems not be studied. This study aimed to evaluate the effects of litter size and the restriction of nutrients, energy and protein, on calcium and phosphorus deposition on gravid uterus of Santa Inês ewes with one or two fetuses.

Materials and methods A completely randomized design was used with a 2x2 factorial arrangement, composed by two litters size (one or two) and two nutritional planes (non and restricted group). Twenty multiparous Santa Inês ewes were allocated on the treatments, seven with one fetus and 13 with two fetuses. One fetus ewes were fed according to NRC (1985) for protein and energy requirements – not restricted group. The remaining ewes were fed with 15% of restriction on energy (TDN) and protein (CP) levels - restricted group. All animals received mineral supplement *ad libitum*. At 140 days of pregnancy, the ewes were slaughtered and the uterus was cut at the cervix. Full weight was registred. Fetal fluids, fetus and uterus (plus membranes and cotyledons) were separeted. The samples were dried at 55°C and pre-degreased by immersion in petroleum ether for 48 hours followed by a period of 12 hours of outdoor exposure for complete removal of ether by evaporation, with further drying for 24 hours in an oven at 55ºC. The samples were ground to 1mm and dried at 105ºC. The mineral solution was opened by wet-ashing using a nitro-perchloric solution composed by 75% nitric acid and 25% per-chloric acid (v/v) for the complete destruction of organic matter. The mineral solution was analyzed for calcium content by atomic absorption spectrometry and phosphorus content was analyzed by colorimetric methods. Values obtained for concentration and content of calcium and phosphorus were compared statistically using the Student Newman Keuss (SNK) test at 5% of probability.

Results None interaction was observed between the treatments. The content of calcium and phosphorus in the gravid uterus was affected ($P<0.05$) by the number of fetuses but was not affected by nutrient restriction applied as shown in Table 1. There was an increase of phosphorus deposition relative to the empty body weigh. This increment was about 1.47 times when the number of fetuses
was doubled. It can be observed that the fetus accounted for 90-98% of calcium and phosphorus content of the pregnant uterus.

Table 1 Total and relative content of calcium and phosphorus on gravid uterus of Santa Inês ewes

Treatments	Total content (g)		mg/Kg of empty body		% Fetus/gravid uterus	
	Calcium	Phosphorus	Calcium	Phosphorus	Calcium	Phosphorus
1 Fetus	48.50[b]	28.56[b]	1.057	0.064[b]	97.92	90.69
2 Fetuses	83.30[a]	44.99[a]	1.738	0.0953[a]	98.74	95.08
Restricted	63.91	36.58	1.409	0.0839	97.92	94.06
Not-restricted	67.90	36.97	1.386	0.0764	98.17	91.72
SD[1]	24.75	10.37	0.65	0.03	1.29	5.29

Means followed by distinct letters differ statistically ($P<0.05$) by SNK test. [1]Standard deviation.

The deposition of calcium and phosphorus on fetuses tissue of unrestricted ewes can be well predicted by the follow equations:
$Ca(g/g \text{ of fetus})=1.96635+0.0118323(W_f)$, $R^2=0.83$, $P(g/g \text{ of fetus})=0.442+0.00632(W_f)$, $R^2=0.88$, where. W_f is the weight of fetuses in grams. This means that, for an increase of 100 grams of fetus produced, there are an increase of 1.18g and 0.63g of calcium and phosphorus, respectively.

Conclusions The energy and protein restrictions did not influenced on the deposition of phosphorus and calcium. However the increase in fetal mass produced on gestation increased the deposition of these minerals. This occurred probably due to low restriction imposed and by the inadequacies of the requirements predicted by the NRC (1985) for native sheep from Brazil. Nevertheless, it may be noted that the increase in litter incurs in the increased demands of phosphorus, especially in late pregnancy, given the greater fetal growth.

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Application of neutral detergent soluble crude protein to estimate precaecal protein digestibility of concentrate feedstuffs for horses

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Introduction The horse depends on the supply of precaecally digestible crude protein (pcDCP) as its constituent amino acids (AA) can only be absorbed from the small intestine. Recently, a novel concept was presented (Zeyner *et al*. 2010), to evaluate CP of horse feed which included parts of the "Cornell Net Carbohydrate and Protein System" and concluded that there is a strong relation between pcDCP and neutral detergent soluble CP (NDSCP). This relation may lead to a more accurate and precise determination of protein supply than common systems which are based on CP and CP digested in the total tract. The aim of this study was to estimate pcDCP values of different concentrate feedstuffs for horses in order to evaluate the concept by Zeyner *et al*. (2010) and subsequently, to support future developments for recommendations in favour of a more precise and efficient protein supply of the horse.

Materials and methods This study included 43 commercial concentrate horse feedstuffs. Twenty-nine feedstuffs were especially mixed for sport horses, while 9 concentrates were declared as breeding feeds and 5 mixtures which were prepared for rearing foals and yearlings. Twenty-seven feedstuffs were available as pellets and 16 feedstuffs were declared as muesli. In addition to the standard chemical analyses, a modified method following the "Cornell Net Carbohydrate and Protein System" for ruminants, more precisely the procedure to determine the neutral detergent insoluble CP (NDICP) was used, in order to characterize and quantify fibre-bound CP in form of NDSCP as well as pcDCP. The following equations were used:
(1) NDSCP = CP - NDICP; (2) pcDCP = NDSCP x 0.9 (Zeyner *et al*. 2010).
PROC CORR of SAS 9.2 was used to test potential relations between fibre and CP fractions of the feedstuffs. Variables included NDICP and calculated pcDCP as well as PaNDF (neutral detergent fibre assayed with a heat stable amylase, determined by manual filtration on paper according to the recommendations of Licitra *et al.* (1996) and expressed inclusive residual ash) and aNDF (NDF assayed with a heat stable amylase and expressed inclusive residual ash; Van Soest *et al.* 1991). Pearson's correlation coefficient was reported from PROC CORR as an indicator of the strength and the direction of these relationships. Relations between these variables were considered significant at $P<0.05$, and a tendency toward significance was declared at $0.05<P<0.10$. P-values less than 0.001 are expressed as "<0.001" rather than the actual value.

Results Table 1 presents results of analyses and calculations. Overall CP in concentrates for sport horses ranged from 106 to 158 g/kg dry matter (DM) and estimated pcDCP ranged from 75 to 120 g/kg DM. Concentrates for breeding purposes contained CP from 163 to 189 g/kg DM and pcDCP from 125 to 142 g/kg DM. Concentrates for foals and yearlings included CP values between 169 and 193 g/kg DM and following 130 to 157 g/kg DM pcDCP. Correlation analysis revealed strong negative relations between PaNDF and CP ($r = -0.3397$, $P<0.001$), NDSCP ($r = -0.4010$, $P<0.001$) and pcDCP ($r = -0.4009$, $P<0.001$). Moreover, a trend for a positive correlation was observed between aNDF and NDICP ($r = 0.1903$, $P<0.10$). No relationship could be identified between CP, NDSCP, pcDCP and aNDF.

Table 1 Mean values (± standard deviation) for crude protein (CP), neutral detergent fibre (PaNDF, aNDF), neutral detergent soluble crude protein (NDSCP) and precaecally digestible crude protein (pcDCP) for horse concentrate feedstuffs, sorted after purpose (sport; breeding; rearing) and physical form (pellets; muesli). All values are expressed as g/kg DM

Purpose	Form	n	CP		PaNDF		aNDF		NDSCP		pcDCP	
Sport	Pellets	19	135	(±11.8)	370	(±47.2)	344	(±97.6)	108	(±12.3)	97	(±11.1)
	Muesli	10	127	(±16.0)	265	(±58.9)	261	(±9.7)	98	(±14.2)	88	(±7)
Breeding	Pellets	5	173	(±8.6)	299	(±40.5)	308	(±42.3)	147	(±6.2)	133	(±5.5)
	Muesli	4	174	(±7.0)	244	(±5.8)	261	(±90.4)	148	(±7.3)	134	(±6.2)
Rearing	Pellets	3	189	(±6.1)	195	(±15.0)	298	(±28.1)	170	(±5.7)	153	(±5)
	Muesli	2	184	(±14.5)	209	(±47.5)	262	(±12.5)	157	(±12.5)	141	(±12.5)

Conclusions The range of pcDCP values indicates that results are realistic and can be applied to various concentrate feedstuffs. The correlation between PaNDF and pcDCP confirms earlier findings, however, it has to be clarified in which way the type of analysis influences the outcome of results. Future research should include *in vivo* studies in order to establish and expand a database to specify and optimize protein supply recommendations for horse feedstuffs.

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First harvest forage yield and nutritive quality of four *Cratylia argentea* accessions in the humid tropics of México. I. Forage yield and chemical composition

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Introduction *Cratylia argentea* is a shrubby legume native to Brazil, Peru and Bolivia, that is well adapted from sea level to 900 m of altitude, in places with humid and sub-humid climates, dry season from 5 to 6 months, and to soils of medium fertility and good drainage (Lascano *et al*., 2002). The accession CIAT 18516 is the most evaluated and harvested every 12-14 weeks has yielded from 8 g of DM/plant in the Mexican dry tropics to 123 g DM/plant in the humid tropics of Costa Rica. It grows well in the dry season, producing about 30% to 40% of the annual forage yield. The basic agronomic and nutritional information of this species is scarce for the Mexican humid tropics, so the objective of this study was to assess the forage production and quality of edible plant components, of the first harvest after planting of four *C. argentea* accessions introduced from the International Centre for Tropical Agriculture.

Materials and methods The experimental site has a hot (23.5°C mean temperature) and humid (1991 mm of yearly rainfall) climate and Ultisol soils. Its coordinates are 20° 02' N latitude, 97° 06' W longitude and 114 masl of altitude, and is located in the State of Veracruz, México. The four accessions (18516, 18666, 18668 and 18676) were planted on 1 September 2006 and the first forage harvest was done on 25 August 2007, after ≈1 year of uninterrupted growth. Forage dry matter yield (DM, kg/ha) and chemical composition of plant parts leaf (LF), edible stem (ES) and non-edible stem (NS) were evaluated in a randomized complete block design with three replicates. The harvested material was hand separated into its components, which were dried at 62 °C/72 h, and then ground to pass a 2 mm sieve and then analyzed for crude protein (CP; AOAC, 1980); as well as neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (LIG) (Van Soest *et al*., 1991). The non-edible stem was not chemically analyzed. The analyses of variance included the effects of block, accession, plant component and accession x plant component interaction. Significance of model effects or differences between means by Tukey's procedure was declared if $P < 0.05$.

Results The four accessions were statistically similar in total and component dry matter yield. Nevertheless, the proportion of each component was different, with accession 18668 being significantly higher in leaf and lower in stem than accession 18666, which lead to a significant difference in the edible matter (LF+ ES)/non edible stem ratio (Table 1). The effect of accession was not significant upon chemical components (Table 1). However, the effect of plant part was significant, as LF showed a higher CP content than ES (22.0% *vs*. 16.2%), lower NDF (57.1% *vs*. 65.2%), lower ADF (36.8% *vs*. 47.5%), and higher LIG (15.1% *vs*. 13.3%).

Table 1 Dry matter yield, percent plant part and chemical composition of edible material (leaves and edible stems) from four *Cratylia argentea* accessions after 12 months of uninterrupted growth in the Mexican humid tropics.

Acc	DM yield (kg/ha)[1]				% of DM yield[2]			(LF+ES)/NS[2]	Chemical component (%)[1, 3]			
	LF	ES	NS	Total	LF	ES	NS		CP	NDF	ADF	LIG
18516	2500	43	2576	5120	48.96 ^{ab}	0.78 ^{a}	50.25 ^{ab}	0.99 ^{ab}	18.93	62.24	42.75	14.79
18666	2602	38	3002	5642	45.92 ^{b}	0.66 ^{a}	53.40 ^{a}	0.88 ^{b}	18.87	60.54	42.18	14.08
18668	2771	30	2113	4914	56.35 ^{a}	0.59 ^{a}	43.03 ^{b}	1.32 ^{a}	18.62	61.55	42.89	14.06
18676	2448	22	2083	4553	54.65 ^{ab}	0.45 ^{a}	44.89 ^{ab}	1.23 ^{ab}	19.97	60.21	40.94	13.80

[1] There was not significant differences between accessions. [2] Means with the same letter are statistically equal ($P<0.05$).
[3] Values are means averaged over leaves and edible stems.

Conclusions One year after planting, *C. argentea* accessions were capable to show around 50% of the nutritive leaf component, which is a remarkable trait as other tropical shrub legumes like *Gliricidia sepium* or *Erythrina poeppiggiana* shed their older leaves continuously, in particular during the critical winter and dry seasons. This would permit to offer the grazing ruminant good supplemental forage during the dry season. The present evaluation indicates that accessions 18668 and 18676 are the more recommendable due to their higher proportions of edible to non-edible material. Further studies aimed to find the best cutting management to maximize their edible DM yield, and also to explore how they respond to grazing are needed.

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First harvest forage yield and nutritive quality of four *Cratylia argentea* accessions in the humid tropics of México. II. Ruminal digestion

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Introduction The first part of this study showed that *Cratylia argentea* had at first harvest after planting, about 50% of its forage yield as edible matter with good chemical composition. To increase the nutritional information about this species the *in situ* DM degradability and *in vitro* gas production of edible plant components of four *C. argentea* accessions were assessed.

Materials and methods Dried and ground samples of edible leaves (LF) of four *C. argentea* CIAT accessions (18516, 18666, 18668 and 18676), were subjected to *in situ* dry matter disappearance (ISD, %) at 3, 6, 9, 12, 24, 48 and 72 h of ruminal incubation in triplicate on three rumen-fistulated cows, with the Ørskov and McDonald (1979) nylon-bag technique, without prior acid-pepsin treatment; zero time was DM disappearance after washing duplicates for 30 min in distilled water at 38 °C. Data were fitted to the model: $Y = a + b(1 - e^{(-c*X)})$, where: 'y', is the dry matter degraded at time 'X', 'a' is the highly soluble dry matter when X = 0 (%), 'b' is the slowly degradable dry matter (%), 'a + b' is the extent of degradation (%), 'c' is the fractional degradation rate of b (fraction/h) and 'X' is ruminal incubation time (h). Accumulated *in vitro* gas production (IVG) was assessed at 1-8, 12, 16, 20, 24, 28, 36, 44, 52, 60, 72 and 96 h of incubation on LF and edible stems (ES) according to Menke *et al*. (1979) and the data fitted to the Krishnamoorthy *et al*. (1991) model: $Y = b(1-e^{-c*(X-L)})$, where 'Y' (ml) is the accumulated gas production at time 'X' (h); 'b' is the potential accumulated gas production as 'x' $\rightarrow \infty$ (ml); 'c' is the fractional rate of gas accumulation with time 'X'; and 'L' is lag time (h). The ANOVA of each parameter could be made because one model per combination of accession x replicate x cow was fitted.

Results The mean R^2 of individual ISD curves was 0.90 with a standard error of ± 0.13. Accessions only differed on the a parameter: 29.97%, 30.06%, 33.15% and 31.32% for 18516, 18666, 18668 and 18676, respectively, being 18668 significantly higher than the other three that did not differ between them, but all had a similar fractional rate of degradation (c = 0.0488 ± 0.0192 per h) of the slowly degradable dry matter (b = 30.60% ± 4.52%) (Figure 1). The Krishnamoorty model parameters were not affected by accession and accession x plant component interaction. The fit of the individual IVG curves was very good, with a mean R^2 of 0.99 ± 0.01. Then, a single curve could be used to describe gas production dynamics of the four accessions: 164.15 ± 25.72 ml, 0.0332 ± 0.0015 per h, and 5.95 ± 0.46 h for parameters 'b', 'c' and 'L', respectively. The effect of plant component was significant on all parameters; respective means ± standard errors for LF and ES were: 146.67 ± 8.63 *vs*. 183.78 ± 7.96 for 'b'; 0.0263 ± 0.0005 *vs*. 0.0395 ± 0.0005 for 'c'; and 6.19 ± 0.16 *vs*. 5.73 ± 0.14 for 'L' (Figure 1).

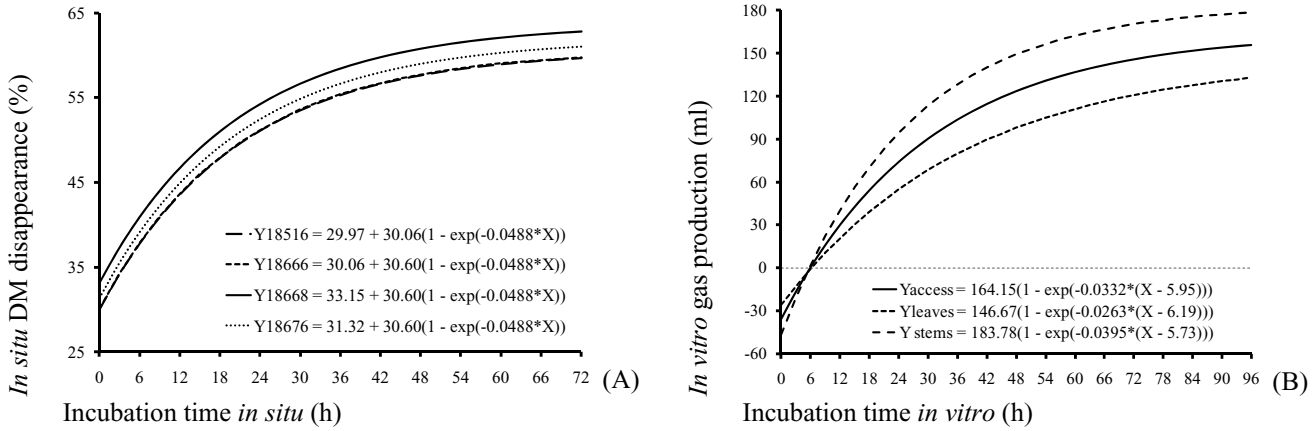

Figure 1 *In situ* DM (A) of first harvest leaves and *in vitro* gas production (B) of leaves, edible stems and averaged throughout all four *Cratylia argentea* accessions.

Conclusions The ruminal digestion of edible DM of the four accessions was similar between them, confirming previous chemical composition results. More research is required to show the possible application of these techniques to evaluate shrubby forages.

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Phosphorus concentration in protein supplements offer to cattle fed low quality hay

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Introduction The passage rate of diet is important information in ruminant nutrition because shows the flow of undigested residues through the digestive tract. This rate has direct influence on digestion and intake (Soares *et al*., 2001). The estimation of the ruminal kinetic parameters is necessary because they influence on the time available for rumen fermentation, microbial efficiency and passage rate of solids in the rumen. Factors as level of intake and the ratio between concentrate and roughage influence the passage rate in the rumen. This research was conducted to study the influence of phosphorus concentration in protein supplements for cattle consuming low quality hay on ruminal kinetics.

Material and methods Were used five Holstein steers implanted with ruminal cannulas and weighting 254 kg ± 22 kg of body weight. Steers were fed chopped hay with 6.17% CP (crude protein) (*Brachiaria humidicola* cv. Llanero) *ad libitum* and received protein supplements with five levels of phosphorus. In all treatments, supplements were fed at 1 g/kg body weight. Protein supplements contained: 2.5 g P/kg (2.5 P), 5 g P/kg (5P), 10 g P/kg (10P), 15 g P/kg (15P) and 20 g P/kg (20P). Experimental design was a 5 x 5 Latin square and the experimental periods lasted 31 days. The steers were weighted at the beginning of each experimental period, in order to adjust the daily amount of supplements to feed. The kinetic of liquid phase was determined after administration into the rumen of 30 g of Co-EDTA diluted in 500 mL of distilled water on day 24 of each experimental period. Co-EDTA was introduced into the rumen before offer the hay to determine the passage rate for liquids (Uden *et al*., 1980). The liquid phase kinetic parameters were calculated according Colucci *et al*. (1990). To determine the solid phase kinetics was used the technique of rumen evacuation on days 29 and 31, as described by Rinne *et al*. (1997) and Khalili and Huhtanen (2002). Data were analyzed by ANOVA and polynomial regression.

Results Supplementation with increasing amounts of P showed no influence (P>0.05) on following parameters: passage rate of liquid phase, retention time in the rumen, flow of liquids in the rumen, recycling rate, ruminal disappearance and digestion rate. The increasing concentration of P in the supplements produced a linear increase (P<0.05) in ruminal volume and a linear decrease (P<0.05) in the rate of transition from solid phase.

Table 1 Parameters of ruminal kinetics

	Means					Regression Equation	S	r^2
	2.5P	5P	10P	15P	20P			
Kpl	0.115	0.109	0.123	0.104	0.109	Y = 0.11194	NS	0.00
RV	59.38	68.85	69.23	77.38	81.49	Y = 59.64673+1.11814X	*	0.25
RT[1]	8.94	9.38	8.73	10.22	10.58	Y = 9.59866	NS	0.00
RF[2]	6.68	7.52	8.38	7.87	8.52	Y = 7.84252	NS	0.00
RR[3]	2.78	2.62	2.95	2.49	2.62	Y = 2.68657	NS	0.00
Kps	0.033	0.027	0.023	0.022	0.023	Y = 0.03032-0.00046457X	*	0.34
Kt	0.056	0.048	0.049	0.049	0.051	Y = 0.05046	NS	0.00
Kd	0.023	0.021	0.026	0.027	0.028	Y = 0.02517	NS	0.00

Kpl = passage rate of liquid phase (/h), RV = ruminal volume (L), RT = retention time (h), RF = rate of liquid flow (L/h), RR = recycling rate (times/d), Kps = passage rate of solid phase (/h), Kt = disappearance rate (/h), Kd = digestion rate (/h). S = level of significance, * = P<0.05; NS = P>0.05. [1]= 1/Kpl; [2]= Kpl * RV; [3]= 24/RT

Conclusions Phosphorus concentration in supplements increased the ruminal volume and decreased the passage rate of solid phase, in which may have contributed for increasing the digestibility of DM and NDF.

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The effect of forage type on sheep NDF passage kinetics determined by slaughter

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Introduction Traditional digestibility coefficients measured in sheep fed at maintenance level combined with NDF kinetics are key parameters in modern dairy cow feed evaluation systems like the NorFor system (Volden, 2011). In NorFor fractional rate of degradation of digestible NDF is calculated based on sheep digestibilities, content of NDF and indigestible NDF (iNDF), an assumption regarding a rumen mean retention time (MRT) of 60 h, representing rumen MRT for sheep fed at maintenance, and a distribution of MRT between two rumen compartments (40%; 60%), as described by Weisbjerg *et al.* (2007). The aim of the present experiment was to determine the effect of forage type on iNDF MRT in different sections of the gastrointestinal tract of sheep.

Materials and methods Twenty castrated male Leicester sheep (101±11 kg) were allocated to one of four rations; maize silage (M), lucerne silage (L), high digestible grass-clover silage (HGC), or low digestible grass-clover silage (LGC). Forage was fed as the only feed, except for M where urea (12 g/kg DM) and sodium sulfite (1.9 g/kg DM) were added. Sheep were fed restrictively twice daily, 9.00 and 17.30, 950 g DM/d. The experiment consisted of a conventional digestion trial followed by subsequent slaughter trial and division of the gastrointestinal tract into eight different sections, rumen, reticulum, omasum, abomasum, small intestine, caecum, colon, and rectum. Feed, rumen content and small intestinal content were fractionated into small (<1.18 mm) and large (>1.18 mm) particles using wet sieving as described by Huhtanen *et al.* (2007). Content of iNDF was determined from 12 d rumen incubations. Pools of iNDF were determined for calculation of MRT (pool size/intake) and fractional rate of passage in the different sections, assuming first order kinetics (Paloheimo & Mäkelä, 1959). Data were statistically analysed for effects of forage type in SAS 9.2 using a general linear model including effects of feed (4), block (5) and time for slaughter (2), and weight at slaughter (20) as co-variate.

Results

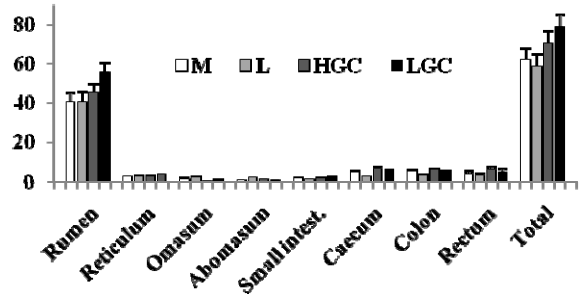

Figure 1 MRT of iNDF (h) in different sections of the gastrointestinal tract

Table 1 Rumen MRT of iNDF (h) in large particles, small particles, sum of large and small particles, and distribution of MRT between the two rumen fractions (%)

	Forage type					
	M	L	HCG	LCG	SEM	P
Large particles	20.4	19.4	28.2	35.5	3.4	0.02
Small particles	23.1	23.4	20.3	28.5	3.5	0.46
Sum	43.5	42.9	48.6	64.0	5.8	0.09
Proportion -Large	46.0	45.9	58.6	54.9	3.5	0.06
Proportion -Small	54.0	54.1	41.4	45.1	3.5	0.06

Intake of iNDF was 82, 227, 34, 72 g/d for M, L, HGC, LGC, respectively, and rumen iNDF pool size was significantly higher for L (386 g) than for M (138 g) and LGC (170 g), and the lowest rumen iNDF pool size was found for HGC (63 g). The highest mean retention time of iNDF was for all feeds observed in the rumen (Figure 1). Rumen retention time averaged 46 h and constituted on average 67% of the total tract retention time, followed by caecum (7%), colon (7%) and rectum (7%). There was no significant effect of forage type ($P>0.05$) on rumen MRT of iNDF and on rumen MRT of iNDF in small particles, whereas rumen MRT for INDF in large particles was higher for grasses compared to M and L (Table 1). Distribution of rumen MRT between the initial large particle compartment and the subsequent small particle compartment showed that the distribution of MRT between rumen compartments was close to 40%:60% for M and L and close to 60%:40% for grasses (Table 1). Total tract NDF digestibility was significantly affected by forage type ($P<0.001$) and was 78%, 71%, 60%, 39% for HGC, LGC, M and L, respectively.

Conclusions The rumen was the most important compartment with respect to MRT of iNDF. The observed rumen retention times were lower than expected, but the distribution of mean retention time between rumen compartments was close to 40%:60% or 60%:40%. The limiting factor for passage (e.g. highest retention time) was escape of small particles for M and L, and reduction in particle size for grasses.

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Effect of different levels of inclusion of weeds on nutritional quality and rumen fermentation kinetics of forage diets based on maize stover

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Introduction Milk production in Mexico's central highlands is dominated by *campesino* systems in which maize stover is the main forage in cows' diets. However, other forage resources are readily available during the rainy season, including weeds, which are widely used for feeding dairy cattle (Castelán *et al*., 2003). Thus, the objective of this work was to determine the effect of different levels of inclusion of weeds on nutritional quality and rumen fermentation kinetics of diets in which maize stover is the basal forage.

Materials and methods This study was conducted in two zones of the Toluca Valley during the months of August to October 2007; samples were collected in ten maize fields. The method proposed by Castelán *et al*. (2003) to sample weed species was used in the present work. After the weeds were collected, they were separated by species. A pool by species was obtained, including at least five individual plants from different plots. Ten pools were formed with the ten most abundant species. Then, six levels of inclusion of each of the ten species selected were combined with maize stover (basal diet), with the following proportions: 100/0, 80/20, 60/40, 40/60, 20/80 and 0/100, for weeds/maize stover, respectively. The samples were analysed for chemical proximal analysis, NDF, ADF, NDFdigestibility (NDFD), DMD and ruminal fermentation kinetics by the *in vitro* gas production technique as modified by the University of Reading (Mauricio *et al*., 1999). The experimental design was a split-plot design. The following model was used: $Y_{ijk}= \mu+P_i+A_j+\delta_{ij}+T_k+(ExT)_{ij}+e_{ijk}$, where Y is the response variable, μ is the general mean, P_i is the effect of period (i=1, 2, 3), A_i is the larger plot (i=1,..., 10), which are the weeds and δ_j is the error associated with the larger plot, T_k is the smaller plot (k=1,...6), which are the treatments, ExT is the interaction term between species and treatments, e_{ijk} is the effect of the residual error. Results were analysed by analysis of variance and were expressed in means with their respective standard errors. The difference between means ($p<0.05$) was calculated using the Tukey's test. Only the results for the treatments are presented.

Results Significant differences ($p<0.001$) were observed for CP content, in treatments 60/40 and 80/20 the CP content increased to 71 and 83.3 g/kg DM, respectively, in comparison with the 60.8 g/kg DM observed in treatment 0/100 (Table 1). In addition, in the 40/60 and 20/80 treatments the NDF content increased from 644.5 to 699.8 (g/kg DM), respectively. The inclusion of 20% of weeds increased the NDFD of the basal diet from 276 g/kg DM (0/100) to 528.7 g/kg DM. There were significant differences ($p<0.001$) for the gas production from the soluble carbohydrates fraction (**a** fraction); the 20/80 treatment decreased gas production to 16.6 ml gas/g DM compared with 45.8 ml gas/g DM produced from maize stover alone. This suggests that weeds do not provide a significant source of soluble carbohydrates to a basal diet based on maize stover. Gas production from the insoluble but potentially degradable carbohydrate fraction (**b** fraction) was significantly different ($p<0.001$) between treatments; the 20/80 treatment increased gas production compared with stover alone, however increasing levels of weeds depressed gas production from this fraction. On the other hand, the gas production rate of the **b** fraction (**cb**) improved with the 60/40 treatment ($P<0.001$), rising it to 0.03 in comparison with the 0.02 observed in the 20/80 and 0/100 treatments.

Table 1 Chemical composition and fermentation kinetics parameters of treatments

Treatments (weeds/maize stover)	CP	NDF	ADF	DMD	NDFD	a	ca	b	cb	lag
100/0	98.1^{a}	499.3^{e}	341.0^{e}	587.3	432.7^{c}	50.5^{a}	0.05	141.7^{c}	0.04^{a}	8.1
80/20	83.3^{b}	550.8^{d}	363.9^{d}	582.2	464.4^{c}	50.8^{a}	0.04	174.0^{c}	0.03^{c}	7.8
60/40	71.0^{c}	597.1^{c}	383.6^{c}	580.8	466.7^{c}	45.0^{a}	0.05	190.8^{c}	0.03^{b}	8.4
40/60	58.6^{d}	644.5^{b}	405.6^{b}	569.6	502.4^{bc}	30.5^{a}	0.07	230.9^{b}	0.02^{bc}	8.9
20/80	47.8^{e}	699.8^{a}	428.7^{a}	550.6	528.7^{ab}	16.6^{b}	0.08	256.6^{a}	0.02^{bc}	9.5
0/100	60.8^{d}	661.0^{a}	382.0^{c}	539.6	276.6^{a}	45.8^{a}	0.05	211.4^{c}	0.02^{bc}	14
S.E.	5.6	13.2	8.0	26.2	35.3	18.0	0.0	33.9	0.0	2.1
P	***	***	***	NS	***	***	NS	***	***	NS

Means between rows with different superscripts are significantly different (***=P<0.001), NS=non-significant (P>0.05)

Conclusions The inclusion of weeds in a diet in which maize stover is the basal forage improves the crude protein content of the diet. The inclusion of 20% of weeds in a maize stover diet improves the digestibility of the NDF, however high inclusion rates have no significant effect. Finally, the amount of gas produced from the soluble fraction of the diet's carbohydrates is reduced, on the contrary the volume of gas produced from the insoluble but potentially degradable fraction is improved but only at a low inclusion levels.

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Additive treatment and glycerol supplementation effects on *in vitro* digestibility and fermentation of a total mixed ration

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Introduction Glycerol is a by-product of biodiesel production and every 38l of biodiesel produced generates about 3.6 kg of crude glycerol. Glycerol production has increased with increasing biodiesel production in many countries hence the interest in using this relatively cheap ingredient to replace expensive energy supplements in livestock diets. However some studies have associated glycerol or the methanol in crude glycerin with adverse effects on rumen function (Roger *et al*., 1992; Paggi *et al*., 2004). The objective of this study was to examine the effect of glycerol addition on the *in vitro* digestibility and fermentation of a total mixed ration (TMR) for lactating dairy cows and to determine if rumen function modulating additives can mitigate potential negative effects.

Materials and methods Two isonitrogenous TMR containing corn silage 34.5% and alfalfa hay 10.8% and either 0 or 10% glycerol (DM basis) were treated without (Control, CON) or with four additives (0.35 g/L of Procreatin 7 yeast, Prince Agri,YE; 500 mg/L of Dry Apex essential oil, EO, BFI Liquid Feeds; 5 mg/kg of monensin, MO, Sigma; or 3.75 mg/L of sodium bicarbonate, SB, Sigma) and incubated for 48 h in buffered-rumen fluid in triplicate. The experimental design was completely randomized. Treatments had a 2 (glycerol) × 5 (additives) factorial structure. A statistical model containing both terms and the interaction was used for data analysis.

Results No glycerol x additive interaction was detected. Adding glycerol tended to increase molar proportion of propionate (P = 0.07, 20.9 *vs*. 19.5) to decrease pH (P = 0.06, 6.63 *vs*. 6.67), and decreased molar proportions of acetate (P = 0.04, 49.4 *vs*. 47.2). *In vitro* DMD (67.2 *vs*. 63.1%) was greatest (P < 0.05) for SB and least for MO. Compared to other treatments, EO and MO produced greater pH (P < 0.05, 6.83 *vs*. 6.62), lower total VFA concentrations (P < 0.001, 92.2 *vs*. 104.1 mM), and less methane (P < 0.001; 22.3 *vs*. 27.7 mol/100 mol). Butyrate and valerate molar proportions were lowest for MO-treated TMR (P < 0.05; 9.4 *vs*. 12.6 and 4.1 *vs*. 4.9). *Streptococcus bovis* counts were greater for EO than for other treatments (P < 0.05; 1.7 *vs*. 1.4 log cfu/ml).

	No glycerol					10% glycerol					SEM	P value[2]	
	CO[1]	YE	EO	MO	SB	CO	YE	EO	MO	SB		Trt	Gly
Digestibility													
DM, %	66.2^{abc}	63.6^{abcd}	59.2^{cd}	58.7^{d}	65.6^{abcd}	65.0^{abcd}	66.9^{ab}	64.1^{abcd}	61.4^{bcd}	68.8^{a}	2.45	0.04	0.11
Fermentation indices													
pH	6.57^{c}	6.70^{bc}	6.97^{a}	6.90^{ab}	6.63^{c}	6.67^{bc}	6.53^{c}	6.67^{bc}	6.77^{abc}	6.57^{c}	0.09	0.02	0.06
Total VFA, mM	103.9^{a}	107.8^{a}	97.5^{ab}	90.8^{b}	103.5^{a}	98.7^{ab}	106.6^{a}	90.6^{b}	89.9^{b}	104^{a}	3.82	0.001	0.27
Individual VFA, molar proportion													
Acetate	52.2^{ab}	54.4^{a}	46.5^{bc}	42.6^{cd}	51.6^{ab}	47.2^{bc}	52.2^{ab}	42.7^{cd}	39.7^{d}	50.6^{ab}	2.12	0.001	0.04
Propionate	20.2^{ab}	19.4^{ab}	20.4^{ab}	18.0^{b}	19.3^{ab}	19.5^{ab}	20.8^{a}	20.0^{ab}	20.9^{a}	22.0^{a}	0.97	0.78	0.07
Butyrate	13.1^{a}	14.2^{a}	10.6^{b}	9.6^{bc}	13.0^{a}	12.8^{a}	14.1^{a}	10.2^{bc}	9.1^{c}	13.0^{a}	0.51	0.001	0.41
Iso-Butyrate	5.3^{c}	5.9^{bc}	5.5^{bc}	6.7^{a}	5.5^{c}	5.5^{bc}	5.4^{c}	5.1^{c}	6.1^{ab}	5.2^{bc}	0.25	0.001	0.05
Iso-valerate	4.4^{ab}	4.9^{a}	4.2^{ab}	3.9^{bc}	4.4^{ab}	4.3^{ab}	4.3^{ab}	3.5^{c}	3.8^{bc}	4.0^{b}	0.24	0.02	0.02
Valerate	4.7^{ab}	5.0^{a}	4.9^{a}	3.9^{b}	4.8^{a}	5.0^{a}	4.9^{a}	4.7^{ab}	4.2^{ab}	4.8^{ab}	0.30	0.05	0.89

[1] CO, control; YE, yeast; EO, essential oil; MO, monensin; SB, sodium bicarbonate. [2] Trt, treatment effect; Gly, glycerol effect; Trt x Gly, interaction of treatment and glycerol. [a~e] Within a row, means without a common superscript letter differ (P<0.05).

Conclusions Glycerol addition had no adverse effects on the fermentation; rather it reduced the acetate to propionate ratio indicating improved efficiency of energy utilization. Adding of SB produced the greatest DMD. The essential oil and MO increased the pH and decreased DM digestibility, methane and total VFA concentration but YE only increased the molar proportion of butyrate.

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Utilization of nanotechnology for the development of a marker of fecal output in dairy cattle

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Introduction Marker is the term used to describe the material used in qualitative or quantitative estimation of physiologic or nutritional factors. The marker, therefore, is a reference compound used as a monitor of chemical aspects, like hydrolysis and synthesis, as well as physical aspects, like digesta flow, digestion process and/or of metabolites (Owens & Hanson, 1992). The validation of a marker is done based on alternative methods, such as the total collection of feces, or the comparison with another already validated marker. Saliba *et al*. (2003) developed an external marker based on the same principles of action cited above. This marker was obtained from the lignin found in the *Eucalyptus grandis*, which was isolated and enriched with phenolic groups. The marker is called LIPE®. The results obtained with this substance, coupled with the advent of nanotechnology led to the development of a nano marker called NANOLIPE. It was based on the same principle of LIPE® nevertheless possesses the attributes of the nano particles. The aim of this study was to evaluate the use of that nano marker in the estimation of fecal output in dairy cattle in comparison with the total collection of feces.

Materials and methods Thirteen crossbred heifers, with average weight of 560 kg, were used in this experiment, and were kept in tie-stall housing. The diet consisted of corn silage, concentrate and a mineral mixture, provided *ad libitum*. The animals spent an adaptation period of ten days to the facilities and diet. At the tenth and eleventh day the nano marker was then administrated in capsules containing 500 mg of marker each, via ruminal fistula. Feces for the analysis of the marker were collected once, directly from the rectum, at the twelfth day. Fecal output was also measured by total collection, during 24h, for a five day period (eleventh to the fifteenth day). The total dry matter (DM) content of the feces samples was then determined. Fecal output estimated by the nano marker was determined by Fourier Transform Infrared Spectroscopy (FT-IR). The statistical design was randomized blocks, with the animals being the blocks. For comparison of the average fecal output, determined by total collection and estimated by the nano marker. Tukey test was used at 5% probability.

Results No significant differences ($P > 0.05$) were found between the fecal output estimated by the NANO-LIPE and by the method of total collection of feces (Table 1).

Table 1 Fecal output (kg of DM) by the method of total collection and by the NANOLIPE

Method	Fecal output
Total collection	5,11 ± 1,62
NANOLIPE	5,14 ± 1,54
CV* (%)	10,68

*Coefficient of variation. Equal means by Tukey test ($P> 0.05$)

The nano marker in this study spent less time on adaptation period as well as on sampling period. It proves to be as efficient or even more than traditionally used markers. It is possible that the smaller particle size of the nano marker was able to increase its velocity of mixing with the digesta. Also the marker analysis by the FT-IR method represents a faster and accurate form of obtaining the fecal output data. All these associated factors account for less cost and execution time in studies of digestion.

Conclusions In this experiment the NANOLIPE was suitable to estimate the fecal output in dairy cattle with one single sampling.

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On-farm estimation of individual energy balance in dairy cows from body weight measurements and body condition scores

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Introduction Precise estimates of the dairy cow's energy balance are of great importance to health, reproduction and feed management. Energy balance is usually calculated as energy input minus output ($EBal_{inout}$), requiring measurements of feed intake and energy output sources (milk, maintenance, activity and growth). Except milk yield, direct measurements of these are difficult to obtain in practice, and estimates involve considerable error sources, which limit on-farm use. Alternatively, energy balance can be estimated from body reserve changes ($EBal_{body}$) using body weight (BW) and body condition score (BCS). Automated weighing systems exist and semi-automated body condition scoring techniques are emerging, thus enabling automated, frequent BW and BCS measurements. We present a method for deriving $EBal_{body}$ for individual cows using only frequently measured BW and BCS. Further, we evaluate the performance of the $EBal_{body}$ estimate against the traditional $EBal_{inout}$.

Materials and methods In the study 35 Holstein Friesian and 9 Jersey cows took part, 31 primiparous and 13 multiparous (lactations 2-4). They were fed a glycogenic (n=19) or ketogenic (n=25) diet. During 150 days from calving automated BW measurements were obtained at each milking, using a weigh platform in a voluntary milking system. From within-milking repeated BW measurements, a milk-free BW was derived, and from between-milking repeated BW measurements, a meal-related gutfill-free BW was derived by smoothing using a cubic spline function (Fig. 1). Changes in BW together with BCS were used to calculate changes in body protein, body lipid, and thus $EBal_{body}$. Because there is no accepted error free reference energy balance measure, $EBal_{body}$ was compared with the traditional $EBal_{inout}$ by isolating the term within $EBal_{inout}$ associated with most uncertainty, i.e. feed energy content (FEC). Thus, $EBal_{body}$ + (EMilk + EMaintenance + EActivity) = EIntake. Dividing EIntake by dry matter intake provided an FEC estimate.

Results We successfully modelled $EBal_{body}$ differences between breeds, parities and diets. $EBal_{body}$ relative to days from calving for the 2 breeds (Fig. 2) and parities compared well with others (Friggens *et al*., 2007). Also, estimated FEC agreed with FEC derived from feed tables, and FEC did not suggest any systematic bias in $EBal_{body}$ with stage of lactation.

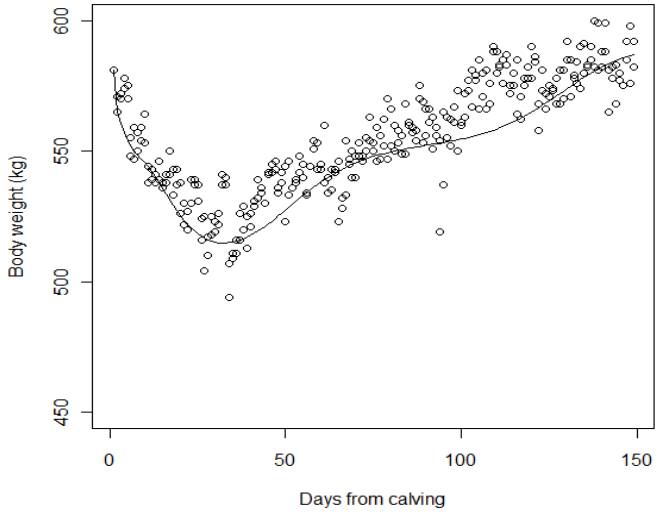

Figure 1 Example of milk-free (o) and smoothed, meal- related gutfill-free BW (-) relative to days from calving.

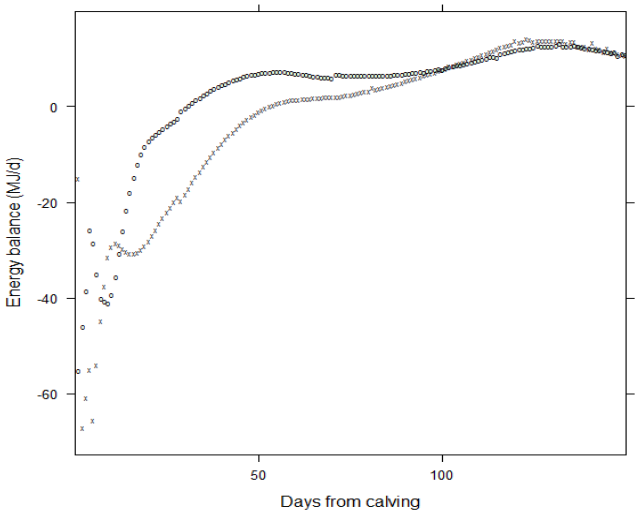

Figure 2 Average $EBal_{body}$ for cows grouped by breed: Danish Holstein (o) and Jersey (x) relative to days from calving.

Conclusions For the dairy farmer, an energy balance estimated for individual cows on-farm would be a highly desirable management tool. We have shown that estimating $EBal_{body}$ from daily, noise-reduced BW measurements combined with frequent BCS measurements can provide such a tool. Further, estimated FEC values agreed well with table values. The presented method would be a pragmatic solution to on-farm calculation of energy balance offering the perspective of improved estimation precision.

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A short-term test to assess horse feeding propensity towards pasture forages grazed as micro-swards

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Introduction The raising concern for stabled horses' welfare is encouraging an increasing use of grassland paddocks for both exercising and grazing, at least for short daily periods. Under Mediterranean conditions permanent pastures should be based on irrigated perennial forages adapted to grow under hot and dry summer and recover from trampling damages. A short-term test was run in order to evaluate stallions' propensity for grazing forages already tested for their resilience to horse trampling. The aim of the study was to rank these forages for their immediate palatability, gauged by measuring stallions' instantaneous intake rate which is particularly sensitive to factors acting at the level of sensory perceptions (Fleurance *et al*., 2009).

Materials and methods Eight perennial or short-lived forage monocultures were compared during the growing stage. Each treatment (T) was established by sowing in boxes the following forage species to establish micro-swards (Orr *et al*., 2005): *Bromus catharticus*, Vahl, cv. Samson (BC), *Dactylis glomerata*, L., cv. Amba (DG), *Festuca arundinacea*, Schreb., cv. Tima (FA1), *Festuca arundinacea*, Schreb., cv. Fawn (FA2), *Lolium perenne*, L., cv. Merlinda, (LP), *Phleum pratense*, L., cv. Climax (PH), *Trifolium repens*, L., cv. Regal (TR) and *Chichorium intybus* L., cv. Spadona (CI). The forages were offered to 8 stall-fed adult Anglo-Arab stallions (live weight - mean±SE - 549±7 kg) in a 8x8 Latin-Square design. Animals were first adapted to the grazing test routine using micro-swards sown with *Hordeum vulgaris* L. (training period, 5 days), and then submitted to the experimental treatments (experimental period, 8 days). During this period, between 10 and 12 a.m., the stallions were daily exposed in a random sequence to one of the treatments for about 4 min. (test period). The behaviour of the stallions was recorded during the test by video-cameras in order to calculate bite rate (BR, n/min). The micro-sward boxes were weighed before and after each test in order to determine the biomass removed, corrected for evapotranspiration losses (ET), measured using micro-swards of the same forage species. The sward surface height (SSH) of each micro-sward was measured by a sward stick before and after grazing; the same swards and the ones used for ET measurements were then cut at the root-shoot interface and oven dried at 85 °C for 18 hrs to measure DM and chemical composition (g/kg DM) of the swards before and after grazing. Bite mass and intake rate were calculated on both fresh (FMBM, g, FMIR g/min. grazing time) and DM (DMBM, g and DMIR, g/min. grazing time) basis. The behavioural variables were analysed by GLM with T, stallion (S) and test day (D) as fixed effects (df=7 for all). T means were separated by Tukey test ($P<0.05$). Regression analyses were performed to explore relationships between response and explanatory variables using mean treatment values.

Results Pre and post grazing SSH averaged 199±0.3 and 66±0.3 mm, respectively. T and S affected significantly all variables under study ($P<0.001$) while D did it ($P<0.001$) only for FMIR and DMIR. BR was higher in the grass treatments than CI, with TR acting as intermediate (Table 1). CI showed the highest FMBM and DMBM, although for the latter it did not differ from BC. FMIR was the highest in CI but the peak of DMIR was shown by FA1, which outperformed PH, TR and CI. Regression analyses showed that non fiber carbohydrate (NFC, Fig. 1) and water soluble carbohydrate (WSC, $R^2 = 0.58$) in the herbage on offer were among the best explanatory variables for FMIR ($P<0.01$). Pre-grazing SSH was the best explanatory variable for DMIR ($R^2 = 0.72$, $P<0.01$).

Table 1 Effects of forage treatments on horse behaviour

T	BR n/min	FMBM g	DMBM g	FMIR g/min	DMIR g/min
BC	24.8^{b}	4.03^{a}	0.69^{bc}	88.9^{a}	15.2^{bc}
DG	30.7^{cd}	3.22^{a}	0.46^{a}	96.4^{a}	13.8^{ac}
FA1	32.0^{cd}	3.14^{a}	0.53^{ab}	97.3^{a}	16.5^{c}
FA2	33.8^{d}	2.85^{a}	0.45^{a}	95.2^{a}	14.9^{ac}
LP	28.7^{bd}	3.28^{a}	0.50^{ab}	91.1^{a}	13.8^{ac}
PH	25.9^{bc}	3.45^{a}	0.50^{ab}	84.5^{a}	12.2^{a}
TR	22.9^{ab}	4.65^{a}	0.57^{ab}	103.3^{a}	12.6^{ab}
CI	16.6^{a}	8.45^{b}	0.83^{c}	132.0^{b}	13.0^{ab}

Values in columns with different superscripts differ at $P<0.05$.

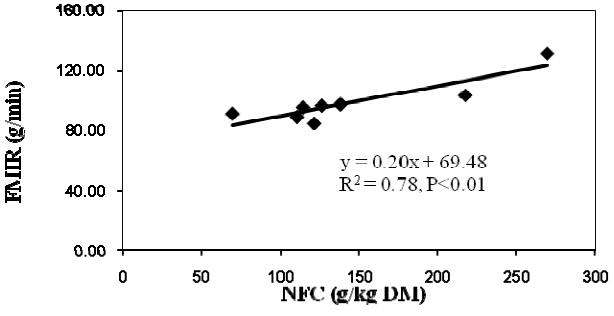

Figure 1 Regression of FMIR on forage NFC (N=8)

Conclusions This short-term test shows that stallion propensity, measured as FMIR, is by far the highest for chicory, in line with its high FMBM. However this does not result in higher DMIR, which shows tall fescue FA1 on top of the propensity rank. SSH, NFC and WSC contents in the herbage on offer are among the best single explanatory variables of these responses.

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The effect of direct-fed microbials (DFM) in diets for weaned Brahman calves in the south of Lake Maracaibo

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Introduction Direct-fed microbials (DFM) have been shown to increase daily weight gain and improve the health and performance of young calves (Krehbiel *et al*. 2003). Microorganisms used as DFM for ruminants include viable cultures of fungi and bacteria (Yoon and Stern, 1995). The aim of the present experiment was to determine the effect of a DFM bolus treatment containing microorganisms on ruminal fermentation and daily body weight gain in weaned Brahman calves.

Materials and methods Twenty weaned Brahman calves were allocated for two experimental treatments (Control, n =10 and with probiotic (DFM), n=10). All calves had the same diet, consuming Guinea grass (*Panicum maximum*) and mineral salts *ad libitum*. They were wormed with Ivermectin at 4% and given 10cc of Organic Modifier. The life zone where the research took place is characterized as tropical rainforest. Treatment group 1, the control group, consisted of 10 calves between 9 and 12 months old that did not receive the probiotic or DFM. Treatment group 2 consisted of 10 Brahman calves of the same age as those in treatment group 1 that received a bolus on day 1 of the study administered using an oral applicator. Each bolus contained a Total Live Cell Count 2.0 $X10^{9}$ CFU/gr of Colony-Forming Units (CFU), including Saccaromyces cerevisiae $5.5X10^{4}$ CFU/gr, Lactobacillus acidophilus$1.5X10^{8}$ CFU/gr, Lactobacillus lactis $1.5X10^{8}$ CFU/gr, Lactobacillus casei $1.5X10^{8}$ CFU/gr, Streptococcus diacetylactis $1.5X10^{8}$ CFU/ gr, Streptococcus faecium $1.5X10^{8}$ CFU/gr, Bifidobacterium bifidum $1.5X10^{4}$ CFU/gr, Bacillus subtilis $5.0X10^{2}$ CFU/gr. The 20 calves were weighed at the beginning, then at day 30 and every 15 days afterwards until the 2 more weighings contemplated had been completed (45d and day 60). Sixty days after inoculation and the final weighing, the rumen liquor was extracted for analysis; pH was determined immediately using a portable pH meter. For the sedimentation test, the sample was homogenized and placed in a graduated cylinder up to the 10 cc mark; three readings were taken at 10, 20 and 30 minutes, respectively. For the methylene blue test, the sample was homogenized, mixed with methylene blue at 0.03% and then observed; noting the time the rumen liquor took to return to its original color. Data obtained during the experiment was evaluated with a completely randomized design, using variance analysis through a Student´s *t*-test in the SAS (Statistical Analysis System, 2002).

Results Table I shows the effect of intraruminal application of DFM (probiotic) on weight gain obtained by weighings at 30, 45 and 60 days after inoculation with the probiotic via oral, observing no significant differences in weight gain was evident when comparing non-inoculated (Control Group) *vs*. Treatment 2 calves (P=0.7467 for the weighing at 30days; P=0.3526 for weight at 45d and P=0.0631 for weight at 60 days). Table II indicates that DFM had no significant effect on the pH (P=0.7083); rumen liquor pH did not vary between the control group and calves receiving treatment 2. Table II also presents results for the sedimentation rate and blue methylene tests, finding significant differences for both variables studied (P<0.0001 and P<0.0011, respectively); these values reflect the direct effect of DFM on liquor composition, specifically, on the sedimentation rate of particles suspended in the rumen liquor and on the time needed for the bacteria to reduce the blue methylene, shortened in this study due to an increase in the amount of bacteria in the rumen liquor.

Table 1 Effect of DFM in diets for weaned Brahman calves on weight at 30, 45 and 60 days post-inoculation with probiotic

Variables	Calves without DFM	Calves with DFM	Probability
Weight at 30d	148.71±1.87	1.47.78±1.87	P=0.7467
Weight at 45d	152.69±1.86	155.40±1.86	P=0.3526
Weight at 60d	157.26±2.3	164.23±2.3	P=0.0631

Table 2 Effect of DFM in diets for weaned Brahman calves on ruminal fermentation (pH, sedimentation and blue methylene tests)

Variables	Calves without DFM	Calves with DFM	Probability
pH	6.34±0.05	6.36±0.05	P=0.7083
Sedimentation Rate	$4.44±0.05^{b}$	$3.96±0.05^{a}$	P<0.0001
Blue Methylene Test	$7.05±0.34^{b}$	$5.55±0.34^{a}$	P<0.0011

Different letters(a,b) indicate statistically significant differences (P<0.05)

Conclusions The use of DFM or their incorporation in ruminant diets is an alternative nutritional plan design for weaned Brahman calves. These products provide benefits for the animals at a very difficult moment, since they undergo stress at weaning time and weight loss due to the change in their nutritional habits.

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Fecal shedding and persistence of *Escherichia coli* from cattle fed finishing diets containing corn or wheat distillers' dried grain with solubles

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Distillers' dried grain with solubles (DDGS) are byproducts of the bioethanol industry which are a valuable feed source for cattle, although feeding corn DDGS (CDDGS) to cattle in the USA has been linked to increased fecal shedding of *E. coli* O157:H7(Jacob *et al.* 2008). In Canada, DDGS is produced from wheat (WDDGS) which differs from corn in protein, fat and fiber content (Gibb *et al.* 2008). The aim of this study was to determine the relationship between incorporation of WDDGS or CDDGS and shedding or persistence *E. coli* O157 in inoculated and naturally-colonized feedlot cattle. To assess fecal shedding of *E. coli* O157:H7 in naturally colonized feedlot cattle, 9000 exotic cross bred yearling steers in 30 commercial feedlot pens were fed barley-based finishing diets containing 22.5% WDDGS or CDDGS compared to a control which had dry-rolled barley substituted for DDGS. Freshly voided pooled fecal pats were collected on a monthly basis, fecal pH measured and screened for *E. coli* O157:H7 by immunomagnetic separation (IMS) and enumerated by direct plating. Hide swabs were collected from randomly selected animals from each pen prior to slaughter. Performance data including slaughter weight, carcass weight and average daily gain (ADG) was calculated based on the number of animals sold for slaughter and average weight gain for each pen. To assess persistence of *E. coli* O157:H7 in feces, feces from cattle fed CDDGS, WDDGS or no DDGS for <14days or >14days were inoculated with a 10^9 CFU/g 5-strain naldixic resistant (Nal-R) *E. coli* O157:H7 mixture. Triplicate 300g samples of inoculated feces were incubated at 20ºC and weekly samples enumerated for Nal-R *E. coli* O157:H7 by direct plating and detected by IMS. To assess fecal shedding of *E. coli* O157:H7 in inoculated cattle, 32 Hereford X Angus steers housed in 8 outdoor pens were fed barley-based finishing diets containing 40% corn, 40% wheat, or 20%corn-20%wheat mixed DDGS or a control diet containing no DDGS. Steers were inoculated with a 10^{10} CFU/g 4-strain mixture of Nal-R *E. coli* O157:H7. Rectal grab samples were collected from each steer day 0, 1, 3, 5, 7 post inoculation, bi weekly for the next 4 weeks and weekly for the final 6 weeks of the experiment. Fecal pH was measured and Nal-R *E. coli* O157:H7 in feces was detected by IMS and enumerated by direct plating. In the commercial feedlot study, cattle fed WDDGS had decreased slaughter weight, carcass weight and average daily gain compared to CDDGS and control animals ($P<0.05$) but feeding DDGS had no effect on fecal shedding of *E. coli* O157:H7 ($P>0.05$). Fecal pH was highest for cattle fed WDDGS followed by cattle fed CDDGS and cattle fed control diet had the lowest fecal pH (6.94 *vs* 6.88 *vs* 6.77, $P<0.001$). Hide swabs collected from cattle prior to slaughter did not differ among dietary treatments in *E. coli* O157:H7 prevalence ($P>0.05$). There was no difference between dietary treatments in persistence of *E. coli* O157:H7 in feces or fecal pH. However, cattle adapted to finishing diets <14 days had greater ($P<0.05$) persistence of *E. coli* O157:H7 than did feces from cattle adapted for finishing diets for >14 days. In the inoculation study, fecal shedding, rate of disappearance of the organism or incidence of *E. coli* O157:H7 in feces did not differ among dietary treatments ($P>0.05$). Fecal pH had no effect on prevalence of *E. coli* O157:H7 and fecal pH did not differ between diets ($P>0.05$). Based on these studies, inclusion of CDDGS and WDDGS in the finishing diets of cattle had no effect on fecal shedding or persistence of *E. coli* O157:H7 and fecal pH was not associated with fecal shedding of *E. coli* O157:H7. However addition of WDDGS in finishing diets was associated with decreased feedlot performance and inclusion of DDGS in feedlot diets must be evaluated in conjunction with cost effectiveness.

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Effects of grass silages with low true protein on γ-aminobutyric acid in bovine ruminal fluid *in vitro*

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Introduction Grass silages with true protein (TP) content < 50% in the crude protein are considered to cause a severe non-infectious dairy herd disease ("factorial disease of dairy herds") in Holstein Friesian and consequently high economic losses in the northern part of Germany. Symptoms were described as follows: high incidences of cases with a high milk cell count, disturbances of fertility, digestive disorders, abomasal displacements, downer cow syndrome up to sudden death (Eicken 2005). As it was shown that a low TP content is accompanied by high γ-aminobutyric acid (GABA) levels (Table 1) and negative effects on health of animals could not be eliminated (Buchanan-Smith a. Phillip 1984), we tested in this study the hypothesis that high GABA concentrations in grass silages lead to a rise of GABA levels in ruminal fluid.

Methods In total 15 grass silages were investigated. Eight grass silages with TP < 50% (method: VDLUFA) caused clinical symptoms in cows and 7 grass silages with TP > 50% were from farms with healthy cows.

Furthermore the silages were tested for their effect on rumen fluid *in vitro*. A 28 day lasting test period started with an adaption period (9 days) fermenting hay (10.5 g DM/day), followed by silage input (10.5 g DM/day) for ten days and ended with a recovering period (9 days) fermenting hay again. A constant amount of concentrate was given every day. Six RUSITEC runs per silage sample were performed. Fermenter fluid samples were taken at days 0 (system start), 7, 8, 11-13, 16-21 and 26-28. Samples were analyzed by high performance liquid chromatography (HPLC) after fluorenylmethyl-oxycarbonyl-(FMOC)-derivatisation. Differences between silage additions were determined by student´s t-test.

Table 1 GABA content (g/kg DM) in high-TP (C-1 to C-7) and low-TP (S-1 to S-8) original grass silages

TP > 50%	GABA [g/kg DM]	TP < 50%	GABA [g/kg DM]
C-1	4.77	S-1	5.24
C-2	3.07	S-2	6.23
C-3	3.51	S-3	8.81
C-4	4.54	S-4	8.23
C-5	3.50	S-5	6.86
C-6	3.04	S-6	6.48
C-7	3.53	S-7	6.34
		S-8	8.01

Results The investigation of RUSITEC-samples revealed that the concentration of GABA rose distinctly after changing from hay to silage fermentation at day 9, moreover decreased after using hay again (day 20). Values in fermenter fluid deviated between 0.00 and 5.86 µmoles/L (2.15 ± 0.98) while fermenting high-TP silages and between 0.00 and 9.83 µmol/L (5.98 ± 0.88) using the low-TP-silages. It was shown, that fermentation of low-TP silages in RUSITEC led to a significant (P<0.01) higher increase of GABA concentration in fermenter fluid compared to high-TP silage fermentation (Figure 1).

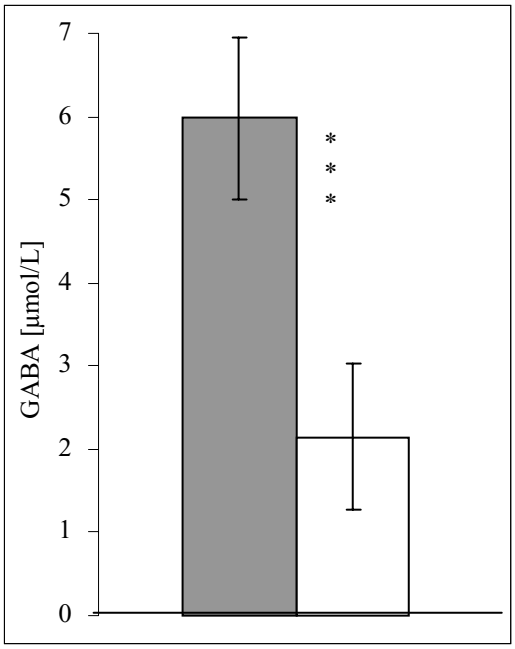

Figure 1 GABA concentration [µmol/L] in fermenter fluid during silage addition TP > 50% (▭) and <50% TP (▭); (***= P<0.01)

Conclusion Grass silages with a low true protein content show high GABA concentrations. The high GABA levels are also seen in ruminal fluid after addition of such grass silages to a RUSITEC system. It will have to be proven in further studies, whether GABA in silage could be a good indicator for silages containing low percentaged TP contingent with a high risk potential for the dairy herds health status.

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Effects of tannin extracts on *in vitro* growth of pathogenic and ruminal acidosis-causing bacteria

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Introduction Many biological activities and antibacterial-promoting effects have been reported for plant tannins, and their investigation is now increasingly relevant to replace widespread use of antibiotic feed additives by the North American feedlot cattle industry. Recently, Min *et al*. (2008) assessed antimicrobial activity of tannin extracts (TE) from perennial plants as well as commercial products against pathogenic bacteria *in vitro*. The authors observed that some plant TE were highly inhibitory to selected pathogens (Min *et al*., 2008). The objective of this study was to explore potential effects of TE on the *in vitro* growth inhibition against pathogenic and ruminal acidosis-causing bacteria (RACB).

Materials and methods Commercially available quebracho (QT), chestnut (CNT), and mimosa tannins (MT; Chemtan, Exter, NH, USA) were used as sources of TE. Exp. 1 was conducted to assess the growth inhibition of TE against *Escherichia coli* O157:H7 (EC), *Salmonella typhimurium* (ST), *Listeria monocytogenes* (LM), and *Staphylococcus aureus* (SA) in pure culture. An agar diffusion assay was used to evaluate the antimicrobial activity of TE at 1 mg TE/4 mL ethanol solution (1:5 dilution) and 1 mg TE/49 mL ethanol solution (1:50 dilution) against the bacteria. The *in vitro* experiment was performed in a 3 (source of TE) × 4 (pathogenic bacteria) × 2 (dilution rate) factorial design (n = 3). In exp. 2, two strains of RACB were used in a 2 (strain of RACB) × 4 (source of TE) factorial designed experiment (n = 3), and the two strains were *Selenomonas ruminantium* JY35 (SR) and *Streptococcus bovis* S81 A Xy2 (SB). The bacterial growth was measured by OD_{550} readings during 24-h incubation at 39°C in Hungate tubes under CO_2 with a typical beef steer finishing TMR extracted using an artificial saliva in the growth medium containing soluble protein and carbohydrate. All data in this study were analyzed with a model that included bacteria, TE, and the interaction between bacteria and TE as fixed effects using the proc mixed procedures of SAS.

Results In exp. 1 at 1:5 dilution, CNT depicted the most inhibitory response (2.05 mm) followed by MT (1.44 mm) ($P<0.05$; Table 1). The CNT elicited an inhibitory effect across all pathogenic bacteria (EC=ST>LM>SA, $P<0.05$). Mimosa tannins had less inhibitory effects compared with CNT, while QT did not affect bacterial growth. At 1:50 dilution, only the CNT inhibited bacterial growth (EC=ST>LM), but the overall response was lower than that in 1:5 dilution (1.10 *vs*. 2.05 mm). In exp 2, overall growth patterns of RACB differed in response to TE ($P<0.05$ for RACB×TE interactions). Adding TE decreased growth of SR starting at 2 h, and CNT was most effective to decrease growth of SR at 12 and 24 h followed by MT and QT ($P<0.05$; Table 2). At 24 h, the CNT decreased growth of SR at 48%. Growth of SB was inhibited by adding TE beginning at 4 h. At 12 and 24 h, the CNT elicited the least growth of SB followed by the MT and the QT ($P<0.05$). The CNT decreased growth of SB at 73% at 24 h.

Table 1 Inhibition zone (mm) of tannin extracts against selected food-borne pathogenic bacteria (n = 3)

Source of tannins	Pathogenic bacteria[1]				SEM	P
	EC	ST	LM	SA		
1:5 dilution						
Quebracho	0.01	0.01	0.01	0.01	0.028	<0.01
Chestnut	2.51[a]	2.42[a]	2.12[b]	1.14[c]		
Mimosa	1.94[a]	2.15[a]	1.65[b]	0.02[c]		
1:50 dilution						
Quebracho	0.01	0.01	0.02	0.01	0.018	<0.01
Chestnut	1.54[a]	1.54[a]	1.29[b]	0.02[c]		
Mimosa	0.02	0.02	0.02	0.02		

[1]EC = *Escherichia coli* O157:H7, ST = *Salmonella typhimurium*, LM = *Listeria monocytogenes,* and SA = *Staphylococcus aureus*.
[a-c]Means within a row that do not have a common superscript differ at $P<0.05$.

Table 2 Growth of *Selenomonas ruminantium* JY35 (SR) and *Streptococcus bovis* S81 A Xy2 (SB) at OD_{550} in response to supplementing tannin extracts in beef steer finishing diet during 24-h incubation in pure culture (n = 3)

	Incubation time, h			
	4	8	12	24
SR				
Control	0.22[a]	0.53[a]	0.62[a]	0.63[a]
Quebracho	0.15[b]	0.33[b]	0.40[bc]	0.55[ab]
Chestnut	0.10[b]	0.23[b]	0.32[c]	0.33[c]
Mimosa	0.12[b]	0.27[b]	0.43[b]	0.48[b]
SEM	0.021	0.034	0.027	0.032
SB				
Control	0.31[a]	0.86[a]	1.09[a]	1.08[a]
Quebracho	0.15[b]	0.40[b]	0.49[b]	0.48[b]
Chestnut	0.13[b]	0.23[c]	0.29[c]	0.29[d]
Mimosa	0.14[b]	0.29[c]	0.40[bc]	0.37[c]
SEM	0.028	0.026	0.037	0.017

[a–d]Means within a column at each incubation time for supplementation of tannin extracts that do not have a common superscript differ at $P<0.05$.

Conclusions Results from two *in vitro* experiments showed that the CNT exerted the greatest inhibition of pathogenic bacterial growth as well as RACB. The mechanisms for remarkable antimicrobial activity in the CNT on the tested bacteria are not clear. The potential value of plant TE needs to be further tested on the mode of action of tannins against microorganisms, particularly the structure-reactivity relationship of each tannin component.

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The influence of Iranian (Dezfoul) garlic (*Allium sativum*) on *in vitro g*as production parameters

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Introduction Currently, the use of plant herbs has resulted in improving rumen ecology (Kamra, 2005). Garlic (*Allium sativum*) has been used as spice and folk medicine since antiquity. It has a complex mixture of many secondary plant products such as allicin ($C_6H_{10}S_2O$). Garlic compounds also could manipulate rumen fermentation such as decreased in the proportion of acetate and increased in proportion of propionate and butyrate, inhibition of methanogenesis and decreased in the CH_4 production (Busquet *et al.,* 2006). Some studies have been conducted to determine the effects of plant herbs on rumen microbial fermentation (Hart *et al*., 2008), but, a wide range of different results have been obtained. Therefore the aim of this study was to determine the effect of different levels of *Iranian* garlic on *in vitro* gas production parameters of an experimental mixture diets (lucerne hay+wheat straw+sugarcane pith+ barley).

Material and methods Rumen fluid was supplied from two fistulated sheep were fed a 40:60 concentrate: forage (250 g concentrate, 550 g lucerne hay and 200 g wheat straw) in prior to the morning meal, homogenized in a laboratory blender, filtered through three layers of cheese-cloth and purged with CO_2, and was added to the anaerobic mineral buffer solution (1:2 v/v). The experimental samples were a mixture of lucerne hay (320 g/kg), wheat straw (200 g/kg), sugarcane pith (130 g/kg) and barley (350 g/kg) and 3 and 6 mg of DM Iranian garlic. Gas production were assessed by incubating approximately 300 mg experimental sample (1.0 mm screen, triplicate) with 30 ml of rumen buffer mixture in 100 ml glass syringes based on Menke and Steingass (1988) procedure. Gas production (ml) were recorded at 2, 4, 6, 8, 10, 12, 16, 24, 48, 72 and 96 h. Total gas values were corrected for blank with a known gas production. Cumulative gas production data were fitted to the exponential equation $Y=b\ (1-e^{-ct})$, where b is the gas production from the fermentable fraction (ml), c; the gas production rate constant (ml/h), t*;* the incubation time (h) and Y is the gas produced at time t. The values of organic matter digestibility (OMD) of experimental samples were calculated by the equation of Menke and Steingass (1988). Data of *in vitro* gas production, and OMD were subjected to analysis as a completely randomized design using the General Linear Model (GLM). Duncan's multiple range test was used to compare treatment means at $P<0.05$.

Results The effect of different levels of Iranian garlic (*Allium sativum*) on *g*as production parameters are shown in Table 1. Results of the present study indicated that garlic caused a significant ($P<0.05$) increase gas production from fermentable fraction and highest was for 6 mg DM garlic ($P< 0.05$). Under the conditions of the present study, organic matter digestibility (OMD) of diet treated with 6 mg DM garlic was the highest value (182.3 g/kg OM) ($P< 0.05$).The highest gas production after 24 hours incubation was for untreated diet ($P< 0.05$).

Table 1 Effect of different levels of Iranian garlic (*Allium sativum*) on *g*as production parameters *in vitro*

	Samples			s.e.m	P
	Garlic 0	Garlic 3	Garlic 6		
b (mL)	38.8^{c}	41.2^{b}	43.5^{a}	0.01	<0.05
c (mL/h)	0.02^{a}	0.02^{a}	0.03^{b}	0.001	<0.05
OMD (g/kg OM)	153.5^{a}	160.4^{b}	182.3^{c}	0.21	<0.05
GP 24h	31^{a}	26^{b}	23^{c}	0.13	<0.05

Conclusion Therefore, the results suggest that experimental diet treated with 6 mg DM Iranian garlic under the experimental conditions of this study has had the highest effect on alteration ruminal gas production parameters. It was previously demonstrated that some plant herbs may improve the cellulolytic activities of rumen microbiota (Khan and Chaudhry, 2008). These natural additives have the potential to alter the ruminal digestibility of ruminant feeds when used at appropriate concentrations, therefore this is essential to study these herbs by using different diets.

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The *in vitro* fermentation kinetics and gas production parameters of *prosopis cineraria* by rumen fluid

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Introduction The south-west of Iran are covered by *prosopis cineraria* tree that can be grazed by ruminants or harvested for use as livestock feed during feed shortages. Leaves of *P. cineraria* tree are highly palatable and nutritious, but contain 8–10% tannins (Bohra, 1980). Tannins are phenolic secondary compounds in plants that are able to complex with proteins and also carbohydrates although some authors have stated that tannins do not link directly to carbohydrates but to proteins which are part of the cell wall matrix (Pérez Maldonado and Norton, 1996). Among the beneficial effects, the protection of the protein against ruminal degradation has been described and, as a consequence, the supply of protein to the small intestine may increase (Waghorn *et al.* 1994). However, a reduced supply of protein to the small intestine has been also reported (Silanikove *et al.* 1996).The objective of this experiment was to determine fermentative parameters of *P. cineraria* (leave and pods) in ruminant nutrition by *in vitro* gas production.

Material and methods About 500±10 mg of oven dried and milled sample [1.0 mm screen, *P. cineraria* leave (LPC) and *P. cineraria* pods (PPC)] was incubated with 35 ml buffered rumen fluid (Rumen fluid was collected from two from two fistulated sheep (were fed diet containing 250 g concentrate, 550 g lucerne hay and 200 g wheat straw) in prior to the morning meal in 100 ml glass syringes, according to the method of Menke and Steingass (1988). All samples were incubated in triplicate (one run) with three syringes containing only incubation medium (blank) and gas production from the sample was corrected for the blank. Gas production was measured at 2, 4, 6, 8, 12, 24, 48, 72 and 96 h. Cumulative gas production data were fitted to the exponential equation $Y=B\ (1-e^{-Ct})$, where B is the gas production from the fermentable fraction (ml), C is the gas production rate constant for B, t is the incubation time (h) and Y is the gas produced at time t. Ammonia-N (NH3-N) concentration (mg/dl) was determined in supernatant samples at the end of the incubation time by macro Kjeltec System Tecator (Büchi 1030, Sweden). *In vitro* digestibility of organic matter (OMD, g/kg OM) and metabolisable energy (ME, MJ/kg DM) of samples were calculated by the equation of Menke and Steingass (1988). Data of gas production, ME, OMD, and NH3-N were subjected to analysis as a completely randomized design using the General Linear Model (GLM) procedure of SAS. Duncan's multiple range test was used to compare treatment means at $P< 0.01$.

Results Gas production and estimated parameters (ME, OMD and NH3-N) of *prosopis* during the fermentation period are given in Table 1. The potential (171.92 *vs* 34 ml) and rate of gas production for *prosopis* pods was more than leave ($P<0.05$). *Prosopis* pods had OMD and ME higher than *prosopis* leave (185 g/kg OM and 9.2 MJ/kg DM *vs* 166 g/kg OM and 5.4 MJ/kg DM, respectively) ($P<0.05$). *prosopis* leave caused to decrease NH3-N content in compared with *prosopis* pod (19.6 and 27.2 mg/dl, respectively).

Table 1 *In vitro* gas production parameters of *P. cineraria*

	Treatments		s.e.m	P
	LPC	PPC		
B (mL)	34^{b}	172^{a}	5.6	< 0.05
C (mL/h)	0.02^{b}	0.06^{a}	0.01	< 0.05
ME (MJ/kg DM)	5.4^{b}	9.2^{a}	0.12	< 0.05
OMD (g/kg OM)	166^{b}	185^{a}	2.2	< 0.05
NH3-N (mg/dl)	19.6^{b}	27.2^{a}	0.2	< 0.05

Conclusions Therefore, it appears that the *in vitro* fermentation, degradability and nutritive value of *prosopis* pods in ruminant nutrition are higher than *prosopis* leave that related to the lower tannins of *P. cineraria* pods. Tabacco *et al.* (2007) reported tannins significantly depressed gas production and rumen degradability, probably hampering rumen microorganisms.

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The effect of rumen protozoa on *in vitro* fermentation of sugarcane pith processed with high pressure steam

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Introduction The rumen protozoa cause 25-30% of total rumen microbial fiber digestion, therefore defaunation reduces fiber digestion, and that the ability of *Epidinium ecaudatum* to degrade microcrystalline cellulose is high (Lee *et al. 2*000). Sugarcane pith is a by-product of the final stage of the processing of sugar cane as it passes through rotary sieves to separate fine particle. Steam explosion has shown considerable potential as a method for the cost-effective pre-treatment of lignocellulosic material. By applying the steam explosion process to sugarcane bagasse about 60% of the hemicellulose fraction was hydrolysed and the susceptibility of cellulose to enzymatic hydrolysis was increased (Kling *et al*. 1987). Gas production technique is a useful procedure to assess digestible value of the ruminant feeds. The objective of this study was to evaluate the effect of high steam on the *in vitro* gas production parameters of sugarcane pith by rumen protozoa.

Material and methods About 200±10 mg of oven dried and milled sample (sugarcane pith as untreated (SP) or steam treated at 19 bar for 3 min, 210 °C (SSP), 1.0 mm screen) was incubated with 35 ml buffered rumen protozoa in 100 ml glass syringes, according to the method of Menke and Steingass (1988). Rumen fluid was collected from two fistulated sheep were fed a 40:60 concentrate: forage. To preparing rumen protozoa, rumen fluid was added to antibiotic solution (streptomycin sulphate, penicillin G and chloramphenicol, 0.1 mg/ml each) and fungicides (benomyle: 500 ppm/ml medium and metalaxyle: 10 mg/ml medium). All samples were incubated in triplicate (one run) with three syringes containing only incubation medium (blank) and gas production from the sample was corrected for the blank. Gas production was measured at 2, 4, 6, 8, 12, 24, 48, 72 and 96 h. Cumulative gas production data were fitted to the exponential equation $Y=B\ (1-e^{-ct})$, where B *is* the gas production from the fermentable fraction (ml), C is the gas production rate constant for B, t is the incubation time (h) and Y is the gas produced at time t. *In vitro* digestibility of organic matter (OMD, g/kg OM) of samples was calculated by the equation of Menke and Steingass (1988). Microbial biomass production (MB) was estimated by method of Blummel *et al*. (1997). Data of gas production, OMD and MB were analyzed as a completely randomized design using the General Linear Model (GLM) procedure of SAS. Duncan's multiple range tests was used to compare treatment means at $P< 0.05$.

Results *In vitro* gas production parameters, OMD and MB of the samples are shown in Table 1. All items were significantly influenced by the treatment. Gas production parameters of SSCP were significantly higher than SCP ($P< 0.01$). Steam resulted in increase OMD compared with the untreated samples (158 *vs* 151 ml per 200 mg DM). Value of MB was decreased when sugarcane pith was treated with steam.

Table 1 *In vitro* gas production parameters, OMD and MB of steam treated (STP) sugarcane pith by rumen protozoa

	Treatments			
	SP	SSP	s.e.m	p
B (ml)	18.5^{b}	21.6^{a}	1.2	<.0001
C (ml/h)	0.03^{a}	0.05^{b}	0.001	<.0001
OMD (g/kg OM)	151^{b}	158^{a}	0.3	<.0001
MB (mg/g)	52.3^{a}	38.1^{b}	0.2	<.0001

Conclusions It was concluded that *in vitro* gas production parameters, OMD and MB of sugarcane pith treated with high-pressure steam by anaerobic rumen protozoa were improved compared with the untreated samples. Steam solublised the hemicellulose fraction (Kling *et al*. 1987) which resulted in improve fermentation and gas production.

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The *in vitro* ruminal fermentative parameters of cotton seed meal treated with pistachio hull as a tannin source

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Introduction About 150,000 tons pistachio by-product is produced from dehulling process in Iran, annually. Pistachio hull is a natural source of phenolic contents, and the most important anti-nutritional factor in pistachio hull is tannin. Improvement of ruminant protein is a matter of practical concern that the amounts of protein and amino acids delivered to the intestine commonly limit productivity of these animals is shown by their responses to post ruminal supplementation (Ipharraguerre *et al.* 2005). Tannins are a diverse group of phenolic compounds with a high affinity for proteins, carbohydrates and other plant constituents. This property may reduce nutrient digestibility, especially in the rumen where tannins bind to dietary proteins and protect them from microbial degradation of protein sources (El-Waziry *et al.* 2007). At normal pH of the rumen, protein remains bound to the tannin, but at the acid pH in the abomasum the tannin–protein complexes may be cleaved resulting in an increase in the amount of dietary protein available for digestion in the intestine (Lascano *et al.* 2003). Therefore, the aim of this study was to determine the effect of different levels of pistachio hull tannin (25 and 45 g/kg DM) on gas production parameters and rumen fermentation of cotton seed meal (CM)**.**

Material and methods Rumen fluid was supplied from fistulated Holstein steers (400±12 Kg, body weight) fed twice daily a diet containing 5.72 kg lucerne hay and 3.08 kg concentrate mixture in prior to the morning meal, homogenized in a laboratory blender, filtered through three layers of cheese-cloth and purged with CO_2, and was added to the anaerobic mineral buffer solution (1:2 v/v). Gas production of samples (untreated cottonseed meal (UCM), cottonseed meal treated with 25 g/kg pistachio hull tannin (T1CM), cottonseed treated with 45 g/kg pistachio hull tannin (T2CM) were assessed by incubating approximately 200 mg experimental sample (1.0 mm screen, triplicate) with 30 ml of rumen buffer mixture in 100 ml glass syringes based on Menke and Steingass (1988) procedure. Gas production (ml) were recorded at 2, 4, 6, 8, 10, 12, 16, 24, 48, 72 and 96 h. Total gas values were corrected for blank with a known gas production. After 96 hours of incubation, the medium of each syringe used for determination ammonia-N (NH3-N) concentration using distillation method (Kjeltec 2300 Autoanalyzer, Foss Tecator AB, Hoganas, Sweden). Cumulative gas production data were fitted to the exponential equation $Y=b\ (1-e^{-ct})$, where b is the gas production from the fermentable fraction (ml), c; the gas production rate constant (ml/h), t; the incubation time (h) and Y is the gas produced at time t. The values of organic matter digestibility (OMD) and metabolisable energy (ME) of experimental samples were calculated by the equation of Menke and Steingass (1988). Data of *in vitro* gas production, ME, OMD, and NH3-N were subjected to analysis as a completely randomized design using the General Linear Model (GLM). Duncan's multiple range test was used to compare treatment means at $P<0.05$.

Results The results showed that pistachio hull tannin caused to reduce the fermentable fraction (b) and gas production rate constant (c) ($P<0.05$), and the lowest (b) and (c) was for CM treated by 45 g/kg pistachio hull tannin (87.5 ml, 0.01 ml/h, respectively). The value of ME and OMD were decreased by tannin, and cotton seed meal treated with 45 g/kg pistachio hull tannin had the lowest ME and OMD (8.5 MJ/kg DM, 153.5 g/kg OM, respectively). Concentration of NH3-N decreased ($P<0.05$) when CM treated with pistachio hull tannin and the highest was for untreated cotton seed meal.

Table 1. Gas production parameters of cotton seed meal treated with pistachio hull tannins

	Treatments			s.e.m	P
	UCM	T1CM	T2CM		
b (mL)	130.8^{a}	121.2^{b}	87.5^{c}	0.80	<0.05
c (mL/h)	0.07^{a}	0.04^{b}	0.01^{c}	0.01	<0.05
ME (MJ/kg DM)	11.4^{a}	10.3^{b}	8.5^{c}	0.23	<0.05
OMD (g/kg OM)	182.3^{a}	166.4^{b}	153.5^{c}	0.21	<0.05
NH3-N	25.2^{a}	18.3^{b}	14.6^{c}	0.30	<0.05

Conclusions Therefore it may be that ***in vitro*** degradation and fermentation of cotton seed meal are influenced by pistachio hull tannin content and the highest effect was observed for cottonseed meal treated by 45 g/kg tannin of pistachio hull ($P<0.05$). Tannin caused to altered microbial profiles in the fermentation, reduced microbial numbers and or enzyme production from the microbes available to ferment substrate (Apajalahti *et al.* 2004).

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The effect of Iranian anise *(Pimpinella anisum)* on *in vitro* ruminal *g*as production kinetic of Lucerne hay

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Introduction Ruminal fermentation of hexoses and amino acids is accompanied by losses energy and amino N, respectively. In fact, 8 to 12% of the digestible energy ingested by ruminants is lost in the rumen as methane (Tamminga, 1992). Modification of rumen microbial fermentation to decrease methane and ammonia N production using feed additives has proved to be a useful strategy to improve production efficiency in dairy cattle (McGuffey *et al.* 2001).The use of plant herbs that contain secondary metabolites, has resulted in improving rumen ecology (Wanapat *et al.* 2008). Anethol (1-methoxy-4-propenylbenzene; $C_{10}H_{12}O$) is the main active component of anise (*Pimpinella anisum*) and is responsible for its activities (Davidson and Naidu, 2000). *In vitro* studies with rumen fluid showed that anethol and anise oil decreased CH_4 production. The aim of this study was to determine the effect of different levels of anise on gas production parameters of lucerne hay *in vitro* condition.

Material and methods Gas production of experimental samples (lucerne hay or lucerne hay plus 3, 6 mg DM of anise (4 replicates per each sample) were assessed by incubating approximately 300 mg sample (1.0 mm screen) with 30 ml of rumen buffer mixture in 100 ml glass syringes based on Menke and Steingass (1988) procedures. Rumen fluid was supplied from two fistulated sheep were fed a 40:60 concentrate:forage (250 g concentrate, 550 g lucerne hay and 200 g wheat straw) in prior to the morning meal, homogenized in a laboratory blender, filtered through three layers of cheese-cloth and purged with CO_2, and was added to the anaerobic mineral buffer solution (1:2 v/v). Gas production (ml) were recorded at 2, 4, 6, 8, 10, 12, 16, 24, 48, 72 and 96 h. Total gas values were corrected for blank with a known gas production. Cumulative gas production data were fitted to the exponential equation $Y=b\ (1-e^{-ct})$, where b is the gas production from the fermentable fraction (ml), c; the gas production rate constant (ml/h), t; the incubation time (h) and Y is the gas produced at time t. The values of organic matter digestibility (OMD) of experimental samples were calculated by the equation of Menke and Steingass (1988). Data of *in vitro* gas production, and OMD were subjected to analysis as a completely randomized design using the General Linear Model (GLM). Duncan's multiple range test was used to compare treatment means at $P<0.05$.

Results The effect of different levels of anise (*Pimpinella anisum*) on *g*as production parameters are shown in Table 1. Results of the present study indicated that anise caused a significant ($P<0.05$) increase gas production from fermentable fraction of lucerne hay and highest was for 6 mg DM anise ($P< 0.05$). Under the conditions of this study, OMD of untreated lucerne hay was the lowest value ($P< 0.05$).The highest gas production after 24 hours incubation was for lucerne hay treated with 6 mg DM anise ($P< 0.05$).

Table 1 The effect of different levels of anise (*Pimpinella anisum*) on *g*as production parameters *in vitro*

	samples			s.e.m	P
	Anise 0	Anise 3	Anise 6		
b (mL)	39.8^{c}	$44.\ 2^{b}$	47.5^{a}	0.21	<0.05
c (mL/h)	0.02^{c}	0.04^{b}	0.05^{a}	0.01	<0.05
OMD (g/kg OM)	155.3^{c}	164.4^{b}	172.5^{a}	0.32	<0.05
GP 24h	23^{c}	26^{b}	31^{a}	0.13	<0.05

Conclusions Therefore, the results suggest that lucerne hay treated with anise under the experimental conditions of this study might alter ruminal gas production parameters and 6 mg DM has had the highest effect on these parameters. It was previously demonstrated that some plant herbs may have the antimicrobial activity (Cowan, 1999). Therefore, there is a need to test these herbs under conditions of using a wide range of different feedstuffs.

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The effect of sulphuric acid on *in vitro* gas production parameters of sugarcane top in Arabian sheep

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Introduction Approximately 1.4 million tons of sugar cane top (SCT) are obtained annually in the southern part of Iran (Khuzestan Province). It is a by-product obtained after the harvesting of the sugarcane plant, and is used as an important feed resource for ruminants. However, the low digestibility and high lignin are considered as the main reasons for unsatisfactory performance of animals fed these roughages (Osorio and Cruz, 1990). Ensiling is a conservation method for moist forage crops and the major goal in silage making is to preserve silage material with minimum nutrient loss. During the ensiling process, acids such as formic acid and sulphuric acid are used to immediately drop silage pH and stop the activity of enzymes and bacteria (Mahanna, 1997). The information on the effect of acid on *in vitro* gas production of sugarcane top is rare. The objective of this study was to investigate the effect of H_2SO_4 on *in vitro* gas production of sugarcane top (SCT) by Arabian sheep.

Material and methods Fresh sugar cane tops were cut into 3-6 cm pieces. Sugarcane top and acid were hand-mixed (DM basis) to obtain 3 kg mixtures on three levels of acid: 0 (control), and 0.9% and 1.8% (SCT, SCT1, SCT2, respectively). There were four replicates per treatment. Mixtures in polyethylene bags were completely sealed and tied with strings. Samples of ensiled chopped SCT were opened after 45 days. Ensiled mixtures were analysed for CP and NDF using standard methods. About 500±10 mg of oven dried and milled sample (1.0 mm screen) was incubated with 35 ml buffered rumen fluid (rumen fluid was collected from two fistulated Holstein steers (400±12 Kg, body weight) fed twice daily a diet containing 5.5 kg lucerne hay and 3 kg concentrate mixture) in 100 ml glass syringes, according to the method of Menke and Steingass (1988). All samples were incubated in triplicate (one run) with three syringes containing only incubation medium (blank) and gas production from the sample was corrected for the blank. Gas production was measured at 2, 4, 6, 8, 12, 24, 48, 72 and 96 h. Cumulative gas production data were fitted to the exponential equation $Y=B\ (1-e^{-Ct})$. Data of gas production and chemical composition of samples subjected to analysis as a completely randomized design using the General Linear Model (GLM) procedure of SAS. Duncan's multiple range test was used to compare treatment means at $P< 0.01$.

Results Silage chemical composition and *in vitro* gas production are presented in Table 1. The results indicated that ensiling SCT with 1.8 % sulphuric acid caused it to increase potential of gas production and rate constant in comparison with the other treatments (140.2 ml and 0.04 ml/h, respectively) ($P<0.05$). The content of CP of SCT ensiled with 1.8 % sulphuric acid was the highest (18.18 %) ($P<0.05$), Also, the content of NDF of this sample was the highest (79.8 %) ($P<0.05$).

Table 1 The parameters of *in vitro* gas production of sugarcane top ensiled with sulphuric acid

	Treatments			SEM	P
	SCT	SCT1	SCT2		
CP %	10.65^{c}	17.78^{b}	18.18^{a}	0.56	0.01
NDF %	75.4^{b}	76.3^{b}	79.80^{a}	1.2	0.01
B (ml)	89.3^{c}	114.1^{b}	140.2^{a}	0.1	0.20
C (ml/h)	0.01^{c}	0.02^{b}	0.04^{a}	1.2	0.10
GP 24 (g/kg OM)	18^{c}	22^{b}	34^{a}	0.2	0.31

Conclusions The ensiling SCT with 1.8 % sulphuric acid caused an increase in gas production in compared with the other treatments. This might be due to the effect of acid on the cell wall components, especially hemicellulose (Adesogan, 2006). The content of CP of SCT ensiled with 1.8 % sulphuric acid was the highest, which may be because on a reduced rate of deamination and decarboxylation of proteins (Rooke *et al.* 1998). Therefore, the results of the present study havedemonstrated that ensiling with sulphuric acid improved *in vitro* gas production parameters of SCT.

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Growth performance of growing goats fed on different concentrations of sodium bicarbonate under tropical field conditions

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Introduction High environmental temperature and rapid growth in growing small ruminants make excessive loss of HCO_3 in urine and thereby lowers blood buffering capacity and DMI in animals (Shahzad *et al*., 2007). Generally, high nutrients demand of growing small ruminants is usually satisfied by high concentrate diet which results in low acetate to propionate ratio and may lead to decreased feed consumption because of ruminal acidosis resulting reduced growth rate (Shahzad *et al*., 2010). Literature is available regarding favorable effects of SB supplementation in exotic large ruminants; however, the same is scanty for growing goats under tropical field situation. Moreover, physiological status, environmental condition and feeding strategies of goats vary from that of temperate cows which because the cause of this study with the objective to examine the influence of varying level of SB on performance of growing goats.

Materials and methods Sixty male goats of almost 10-12 months of age were divided into 5 groups of 12 animals each, in a randomized complete block design to examine the influence of varying dietary levels of sodium bicarbonate (SB) on feed consumption, nutrient utilization, nitrogen balance, acid base status and growth performance. Study lasted for 3 months. Five iso-caloric (2.32 Mcal/kg) and iso-nitrigenous [15.8% crude protein (CP)] total mixed rations (wheat straw: concentrate as 30:70) were formulated using different levels of SB supplementation .The C, LSB, MSB, HSB and VHSB diets contained 0, 0.4, 0.8, 1.2 and 1.6% SB. Nutrient intake, digestibilities and weight gain were taken into account. Feed and faecal samples were analyzed for dry matter (DM), CP, neutral detergent fiber (NDF), acid detergent fiber (ADF), serum bicarbonate and Na, K, Cl, Ca, Mg and S. Blood pH, urine pH, nitrogen balance and serum Na, K, Cl, Ca, P, Mg and S were also analyzed (AOAC, 2003). Analysis of variance technique was employed to analyze the data. Difference among the treatment means were compared using Duncan's New Multiple Range test.

Results Significant increase in nutrients (DM, CP, NDF & ADF) intake by growing goats was observed with increasing the dietary SB level while its reverse was true for nutrient digestibilities. Goats fed on HSB and VHSB diets retained higher nitrogen than those fed on C and MSB diets. Blood and Urine pH increased with increased level of SB. Serum Na increased with increased level of SB while serum K, Cl, S, Mg, P except Ca remained unaltered by SB supplementation. Goats fed VHSB diet gained maximum weight while minimum weight gain was recorded in goats fed C diet.

Effect of varying level of sodium bicarbonate[1] on feed consumption, utilization, blood profile and growth in growing goats

Intake (g/kg)	C	LSB	MSB	HSB	VHSB	SE	Dig. (%)	C	LSB	MSB	HSB	VHSB	SE
DM[2]	0.95^{c}	1.10^{bc}	1.53^{b}	1.8^{ab}	1.95^{a}	0.25	DM	68.9^{a}	68.9^{ab}	67.5^{b}	67.1^{bc}	66.7^{c}	0.51
CP[3]	150^{c}	173^{bc}	241^{b}	284^{ab}	308^{a}	14.71	CP	71.3^{a}	71.1^{ab}	70.2^{b}	69.8^{bc}	69.7^{c}	0.22
NDF[4]	336^{c}	389^{bc}	541^{b}	637^{ab}	690^{a}	25.41	NDF	64.5^{a}	64.1^{ab}	63.5^{b}	63.2^{bc}	62.9^{c}	0.61
ADF[5]	201^{c}	233^{bc}	324^{b}	382^{ab}	413^{a}	31.69	ADF	62.7^{a}	62.4^{ab}	61.7^{b}	61.2^{bc}	60.8^{c}	0.41
N balance	7.6^{c}	9.3^{bc}	12.8^{b}	17.2^{ab}	19.2^{a}	8.95	W. gain, g/d	85^{c}	95^{bc}	118^{b}	145^{ab}	158^{a}	5.93
Blood pH	7.12^{c}	7.21^{bc}	7.26^{b}	7.39^{ab}	7.42^{a}	0.02	Na mEq/lit.	126^{c}	127^{c}	129^{b}	132^{ab}	133^{a}	1.31
Urine pH	7.22^{c}	7.31^{bc}	7.41^{b}	7.46^{ab}	7.49^{a}	0.31	Ca mg/dl	9.81^{a}	9.65^{a}	9.31^{b}	8.75^{bc}	8.31^{c}	0.98
Serum HCO_3 mmol/l.	23.31^{c}	24.22^{b}	24.49^{b}	26.8^{ab}	27.5^{a}	1.66	Blood urea N mg/dl	17.7^{a}	16.0^{ab}	14.1^{b}	11.6^{bc}	10.5^{c}	2.97

Means within the same row having different subscripts differ significantly ($P<0.05$). [1]C, LSB, MSB, HSB and VHSB diets contained 0, 0.4, 0.8, 1.2 and 1.6% sodium bicarbonate, respectively. 2Dry matter, 3crude protein, 4neutral detergent fiber, 5acid detergent fiber

Conclusion In conclusion, growing male goats fed 1.2% SB ingested more nutrients and gained higher weight than those fed on diets without SB concentration in tropical field situation.

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Improving the vitamin E status of young sheep with native, salt tolerant shrubs (*Atriplex spp.)* used in the revegetation of saline landscapes in Australian farming systems

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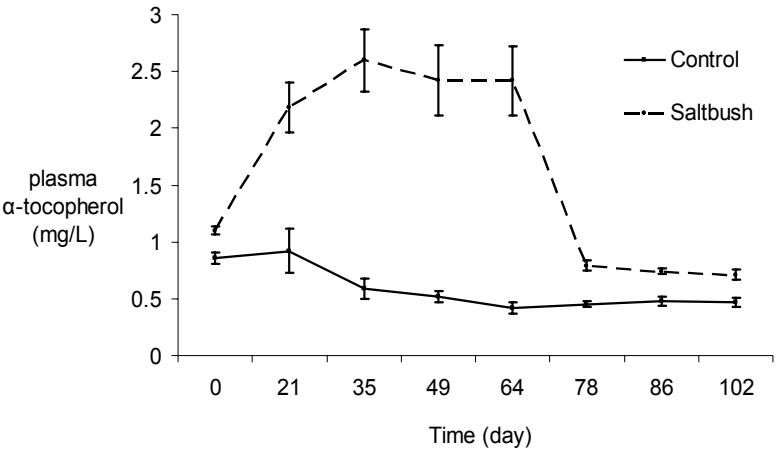
Introduction Vitamin E deficiency is widespread in young sheep across much of southern Australia during the long, dry summers because of the persistent lack of green feed. Deficiency in vitamin E can lead to nutritional myopathy, a disease that can cause heart and skeletal muscle damage, potentially leading to death. Deficiency is exacerbated in finishing systems where animals are fed grain-based rations that often contain low levels of vitamin E. To prevent vitamin E deficiency, supplementation with synthetic vitamin E is becoming increasingly common. But the use of synthetic supplements is costly, labour intensive and often requires frequent reapplication (Fry *et al.* 1993). One practical strategy that may prevent vitamin E deficiency is to utilise saltbush (*Atriplex spp.*), which are used widely in the revegetation of saline land in southern Australia, as green feed for livestock during summer and autumn. Saltbush has been shown to contain levels of vitamin E equivalent to good quality green pasture and can improve the vitamin E status of grazing sheep (Pearce *et al.* 2005). We tested the hypothesis that grazing saltbush during summer and autumn prior to a low-vitamin E finishing ration would improve the vitamin E status of young sheep and prevent vitamin E deficiency during finishing.



Materials and methods 10 month old crossbred wether lambs (n=48) grazed eight adjacent saline-land plots planted to either saltbush (*A. amnicola* and *A. nummularia*) or control pastures (dry, senesced annual pasture species) for 64 days during late summer, early autumn. Following this, animals were fed a low vitamin E, grain-based ration for a further 38 days. Plasma samples were collected approximately fortnightly throughout the experiment and analysed for α-tocopherol concentration using HPLC by the method of McMurray and Blanchflower (1979). The critical value for vitamin E deficiency was defined as 0.7 mg/L α-tocopherol in plasma (White and Rewell, 2007). Statistical analysis was done using a linear mixed model with fixed effects for diet and date and random effects for block and block.diet. Individual time points were compared using analysis of variance with Student Newman Kuels multiple comparisons in Genstat 12th edition (VSN International Ltd, Hemel Hampstead, UK).

Results Grazing saltbush improved the vitamin E status of young sheep up to 4-fold ($P<0.001$) and prevented plasma α-tocopherol concentration falling below 0.7 mg/L for 38 days after saltbush was removed from the diet. After grazing saltbush for 35 days, plasma α-tocopherol concentration peaked at 2.6 ± 0.1 mg/L and remained at this level until saltbush was removed from the diet. By contrast, at the same point in time, animals grazing control pastures had plasma α-tocopherol concentrations less than 0.7 mg/L and remained deficient until the end of the experiment. Plasma α-tocopherol fell rapidly once saltbush was removed from the diet but did not fall below 0.7 mg/L during the finishing phase and continued to remain above the levels in plasma from animals that grazed control pastures until the end of the experiment. ($P<0.05$).

Figure 1. Plasma α-tocopherol concentration (mean ± s.e.) of sheep whilst grazing saltbush or control pastures (day 0-64) and in a subsequent finishing phase (day 64-102).

Conclusions Saltbush provides an excellent source of vitamin E for grazing livestock during a time of year when deficiency is very common and the risk of developing nutritional myopathy, which can ultimately lead to death, is high. Further, grazing saltbush during summer can prevent plasma α-tocopherol concentration becoming deficient for at least 38 days after saltbush is removed from the diet. This indicates that strategic grazing of saltbush during summer and autumn could reduce the risk of vitamin E deficiency within sheep flocks and eliminate the need for synthetic supplementation. As a result of this work we are now investigating the potential for shorter term feeding of saltbush to boost vitamin E status and reduce the incidence of biochemical signs of muscle damage.

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Food habits of goats on rangelands with different cover of fourwing saltbush (*Atriplex canescens)*

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Introduction One species suitable for browse in areas of severe aridity is fourwing saltbush (*Atriplex canescens* (Pursh) Nutt.), an evergreen, highly palatable, drought tolerant browse for livestock (Pinos-Rodríguez *et al*. 2007). This shrub represents a neglected resource, which not only offers high quality winter forage, but also represents an opportunity to upgrade disturbed landscapes with a substantial increase in primary productivity in arid zones of the Chihuahuan desert (Goodin and Newton 1984). The purpose of this study was to create two treatments differing in the proportion of *A. canescens* to represent the variability that is present in the Chihuahuan desert. It was hypothesized that a high density of *A. canescens* increases its selection by goats and decreases the selection of other plants on offer.

Material and methods The study was established on an experimental rangeland in northern Mexico (24º 21' N, 101º 22' W; annual precipitation is 241 mm). Four sites (one ha in size) were established. Two of the sites were fenced and all shrub species, except *A. canescens*, were severed below ground level; this area was excluded from livestock grazing for 10 years. The two other sites were not fenced and were heavily grazed by livestock. In 2007, aerial cover was determined for each plant species and standing crop biomass in both the control and the *Atriplex*-dominated sites was determined. Samples of *Atriplex canescens* were collected and analyzed for nutrient content. Twelve esophageally fistulated adult criollo goats were used to collect ingesta samples in each treatment after vegetation collection, and diet botanical composition was estimated using the microhistological technique. Relative preference indices (RPI) for different plant species were also determined. Differences in forage production, and aerial cover between sites were analyzed using repeated measures analysis of variance. Scheffe's test was used to separate the means. Botanical constituents of diets were analyzed using analysis of variance.

Results The combination of livestock grazing exclusion over a ten-year-period and shrub removal led to a landscape with high cover (46.2 *vs* 14.6, across seasons; $P<0.01$) and high forage production of *A. canescens* (2281 *vs* 503 kg ha^{-1}, across seasons; $P<0.01$; Fig.1). Biomass production of *A. canescens* represented 90.0% of the vegetative composition in the *Atriplex*-dominated sites and 22% for the control pasture. Aboveground biomass production per unit area of this shrub was highest ($P<0.05$) in summer and lowest in spring). Shrub removal strongly affected goats feeding habits; goats grazing the *Atriplex*-dominated site consumed 4.5 times ($P<0.01$) the amount of *A. canescens* than goats on the untreated (control) pasture during all seasons. Shrubs were used heavily in all seasons by goats in both pastures, with higher ($P<0.01$) percentages (75.5 - 82.8) in the diets of goats grazing the control pasture, compared to goats grazing the *Atriplex*-dominated area (62.5 - 68.5%), with no differences between seasons. Goats in the *Atriplex*-dominated pasture were better grass users than goats grazing the control area, using more ($P<0.01$) perennial graminoids in summer (15.0%) and spring (18.3%) than goats on the control area (6.0% to 7.0%). Forbs were a variable but important component of diets, particularly in the *Atriplex*-dominated pasture, where goats utilized more ($P<0.01$) forbs than goats in the control pasture, with no differences between sampling seasons. In both pastures, preference indices were highest for *Sphaeralcea angustifolia* and lowest for *Stipa editorum*; *A. canescens* was used in proportion to its occurrence within the study area in both pastures.

Conclusions *A. canescens* recruitment in disturbed sites of *Larrea-Flourencia* plant communities would be enhanced by taking steps to protect during several years newly developing seedlings of this shrub along degraded areas previously cleared of shrubs. Successful revegetation of the treated pasture strongly affected goats feeding strategy, with a substantial increase in the use of *A. canescens*, forbs and grasses in all seasons. However, a disadvantage of using *A. canescens* as the main forages on offer to goats would be a suboptimum nutrient intake, due to the dilution effect of the abundant minerals in this halophytic shrub on energy density.

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Dietary cation-anion difference: effects on metabolism, health and lactation performance of periparturient cows in Karst area

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Introduction Low dietary cation-anion difference (DCAD) has been considered to be an efficient way to prevent milk fever (MF) or hypocalcaemia (Hy) in transition cows (Wu *et al*., 2008; Grünberg *et al*., 2011). However, there is also some suggestion that environment and climatic factors may influence the occurrence of MF or Hy on transition cows fed varying DCAD ration (Chan *et al*., 2005, Roche and Berry, 2006). To the best of the authors' knowledge, there is little information on the effect of DCAD on dairy cows in the Karst area, especially in Southwest China. The objective of this study is to evaluate the body metabolism, health status and subsequent lactation performance of periparturient cows fed varying DCAD diets in the Karst area.

Materials and methods Thirty Holstein multiparous cows were randomly allocated to 3 equal blocks based on their age (4yr), body weight (600kg) and expected calving date (21d); and were fed 1 of 3 diets with DCAD (DCAD=Na+ K- Cl- S, mmol/kg) level at +81 (control), +20 (Treatment 1) and -32 (Treatment 2), respectively. Anionic salt mixture ($MgSO_4$, $MgCl_2$, $CaSO_4$, $CaCl_2$) was used to reduce DCAD level. Cows were fed and milked 3 times daily. Animals were provided the same lactating diet after calving and had free access to water throughout the whole trial duration. Dry matter intake (DMI) was measured once a week. Urine pH was assessed on d -4 and -7 prepartum. Blood were taken from jugular vein and were centrifugated to harvest plasma for later determination of glucose (Glu), urea nitrogen (UN), Ca, P, Na, K and Cl. Individual milk yield were measured on d 14 and 28, while milk sample were collected for milk protein, fat and lactose analysis. Health status was declared from an experienced veterinarian. The GLM procedure of SAS software system (2000) was used to analyze urine pH, blood parameter, DMI and lactation performance data. The health status statistics were processed by Chi-square test. Duncan's multiple range tests was used to examine the significance of difference between means. A statistically significant difference was noted at $P < 0.05$.

Results Urine pH was reduced ($P < 0.05$) as DCAD decreased, reaching the lowest level at -32 (Figure 1). Compared with control treatment 2 resulted in higher ($P < 0.05$) plasma Ca (8.53: 8.67: 9.09 mg/dL, SEM 0.109) and Cl (109.23: 118.69: 124.38 mmol/L, SEM 4.71) concentrations, with no significant difference for plasma P, Na, K, Glu and UN observed by dietary treatments ($P > 0.05$). There were no statistical differences with respect to DMI, milk yield and composition among the three treatments ($P > 0.05$). There were no cases of MF in this study. However, lowering DCAD decreased the incidence of Hy (3/10: 1/10: 1/10) and retained placenta (4/10: 2/10: 1/10).

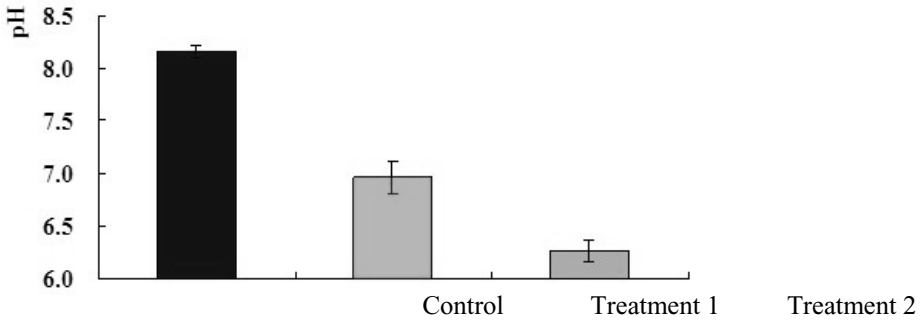

Figure 1 Urine pH of dry cows fed diets with varying dietary cation-anion difference

Conclusions In the Karst area, feeding periparturient cows with low DCAD diet can also induce a mild metabolic acidosis, improve plasma Ca homeostasis and health status. However, no significant improvement in lactation performance was observed in the study.

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The effect of addition of ensiled mulberry leaves in the diet of beef cattle on their growth and slaughter performance

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Introduction It has been established that mulberry leaf contains not only high protein as well as low fibre, but also certain natural active substances, making it a good livestock feed resource (Leterme *et al*. 2005). Through proper processing such as ensiling, mulberry leaves can be used as an energy and protein feed source for both ruminant and mono-gastric animals (Li *et al*. 2006). However, the information regarding the response of growth and slaughter performance of beef cattle to the dietary addition levels of ensiled mulberry leaves (EML) is lacking. The aim of the present study was to determine the effect of addition levels of EML in the diet of beef cattle on their growth and slaughter performance.

Materials and methods Eighty-eight beef cattle (44 Limousine crossbred cows and 44 local breed bulls) with 30 ± 3.2 months old and 468.0 ± 10.0 kg of initial body weight, were divided into 4 groups (n = 22) in a randomized complete block design with sex as a block. The basal diet contained (on DM basis) corn grains (48.0, 46.0, 44.0, 42.3%), cottonseed cake (1.0, 1.5, 2.0, 2.0%), brewer's grains (7.3, 6.3, 5.7, 5.5%), soybean curb residue (7.0, 6.0, 5.0, 4.0%), corn silage (30.0, 25.0, 20.0, 15.0%), dicalcium phosphate (0.25, 0.20, 0.10, 0.10%), limestone (0.56, 0.60, 0.30, 0.20%), salt (0.3%), sodium bicarbonate (0.50%), and premix (0.10%), respectively. The treatments were four addition levels of EML included in the basal diet (DM basis) as follows: 0 (control), 7.5, 15.0 and 22.5% of EML, respectively. All diets were isonitrogeous (11.7% CP, DM basis) and isonergetic (7.15 MJ/kg NE_m and 4.69 MJ/kg NE_g, DM basis). The trial lasted for a total of 100 days including 10 days for adaptation and 90 days for data recording. The body weight of all animals was measured before initiation of and after end of the trial, respectively. Average daily gain (ADG), dry matter feed intake (DMI) and feed conversion efficiency (Gain/Feed) were calculated. After the feeding trial completed, eight cattle from each group were randomly selected and slaughtered for measurement of slaughter performance including hot carcass weight, bone weight, net meat weight, backfat thickness and ribeye area. All the data were subjected to analysis of variance according to a randomized complete block design using a significant level of $P<0.05$.

Results The results (Table 1) showed that there were no significant ($P > 0.50$) differences in final body weight, ADG, DMI and feed conversion efficiency among 4 dietary EML addition levels. Addition of EML in the diet did not significantly ($P > 0.20$) change carcass parameters except hot dressing percentage and ribeye area (P□0.01). These results indicated that addition of EML at up to 22.5% of dietary DM had no considerable effects on growth and slaughter performance of beef cattle.

Table 1 Growth and slaughter performance of beef cattle fed the diet with different levels of EML

Item	Addition levels of EML (%)				SEM	P value
	0.0	7.5	15.0	22.5		
Growth performance						
Final body weight (kg)	475.0	470.8	464.6	464.8	10.8	0.885
ADG (kg)	0.77	0.82	0.86	0.83	0.05	0.628
DMI (kg/d)	7.53	7.34	7.41	7.33	0.12	0.602
DMI (%BW)	1.80	1.75	1.77	1.74	0.04	0.702
Feed conversion (G/F)	0.110	0.116	0.124	0.118	0.01	0.558
Slaughter performance						
Slaughter weight (kg)	444.3	454.3	466.8	442.0	19.5	0.800
Hot carcass weight (kg)	242.8	260.6	267.3	248.8	11.9	0.467
Hot dressing percentage (%)	54.0[a]	57.1[b]	56.8[b]	56.1[b]	0.70	0.018
Bone weight (kg)	29.3	30.3	30.8	29.9	1.36	0.873
Net meat weight (kg)	213.4	230.4	236.6	219.2	10.6	0.410
Meat to bone ratio	7.3	7.6	7.6	7.3	0.19	0.420
Backfat thickness (cm)	0.9	1.2	1.2	1.0	0.15	0.264
Ribeye area (cm^2)	62.2[a]	75.5[b]	75.4[b]	75.5[b]	2.81	0.004

Values with different superscripts are significantly different ($P < 0.05$).

Conclusions Addition of EML at up to 22.5% of dietary DM did not obviously influence growth performance and carcass characteristics of beef cattle, but it could significantly improve hot carcass percentage and ribeye area, indicating that ensiled mulberry leaves can be used as a feed resource for beef cattle feeding.

Acknowledgements This study was partially funded by the Earmarked Fund for Modern Agro - Industry Technology Research System (Beef Cattle and Yaks, CARS-38).

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Selection for nutrients by young and adult goats on a microphyll desert scrub

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Introduction Several studies in ruminants support the notion that ungulate herbivores select nutrients in amounts to meet their needs (Verheyden-Tixier *et al*. 2008; Villalba *et al*. 2008), and that this selection varies with the internal state (Kyriazakis *et al*. 1999; Mellado *et al*. 2011). As far as we know, no attempt has been made to assess the nutrient content of diets selected by goats in relation to increased nutrient requirement during active growth early in life. Although there is widespread recognition of the importance of adequate nutrition in preweaning kids (Fernandez *et al*. 2006), there is considerable uncertainty about its role in young grazing goats in landscapes with scarce and patchy vegetation, where severe malnutrition is common (Mellado *et al*. 1991). Therefore, this study was conducted in an attempt to elucidate the role of stages of body development on nutrition of goats in a microphyll desert scrub.

Material and methods The experiment was conducted during the dry and rainy seasons in a semi-arid rangeland of northern Mexico (25ºN; altitude 2150 m; 299 mm average annual precipitation). Ten pluriparous goats of undefined genotype and ten kids (<two months of age) were used. Goats were driven by a herdsman for daily 8-h grazing periods. The diet selected by goats was estimated by direct collection (four consecutive days) of plants from the goat's mouth after feeding bouts. Animals were restrained by a short light permanent rope tightened to their necks. Forages collected were pooled, oven-dried and ground for chemical analyses and DM digestibility (rumen fluid–pepsin *in vitro* technique) determination. Ash, neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose, crude protein and important macro and microelement content were determined in forages selected by goats. The effects of age, month of sampling and the age x month interaction on nutrient content of diets were analyzed by ANOVA using the MIXED procedure of SAS accounting for repeated measures.

Results Differences in ash content of diets selected by young (10.0 ± 1.6%; mean ± SD) and adult (7.9 ± 0.9%) goats were found, but ash values did not decline during the dry season. Across seasons, young goats selected diets higher ($P<0.01$) in crude protein (9.4 ± 0.5 *versus* 8.9 ± 0.5; mean ± SD) than adult goats; this nutrient did not meet the requirements of growing goats. Young goats made use of less ($P<0.01$) fibrous feeds than non-pregnant goats (across seasons, NDF content in forages selected by young and adult goats was 48.5 ± 3.9% and 53.1 ± 4.6%, respectively). However, young goats selected a diet less digestible (seven percent points lower; $P<0.05$) than adult goats**.** In order to cope with changing nutrient demands for growth, young goats adjusted their diet by increasing the selection of plants with on average 32% higher phosphorus content compared to forages selected by adult goats, with no differences between seasons. Calcium levels in forages selected by young and adult goats did not differ. The physiological state of goats did not alter the selection of forages with respect to their copper, manganese, iron and zinc content, but levels of all these microelements changed ($P<0.05$) with season.

Conclusions Active growth appeared to play an important role in determining forages selected by goats, as growing kids selected diets richer in CP and P and lower in structural carbohydrates during the dry and wet season, in order to sustain nutrient demands for their development. However, *in vitro* digestibility data indicated lower values for young goats compared with adult animals, apparently due to lower accessibility of tender leaves by young goats, because of their small size. This suggests that, despite their reduced stature and lack of grazing experience, very young goats foraged more selectively on the more profitable species than adult goats, and it seems that goats are capable of identifying potential food items according to their nutrient content.

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The influence of immune stress on the immune parameters and nitrogen metabolism in pre-ruminant calves

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Introduction The feed intake and growth performance of immunologically challenged animals is usually reduced (Kelley *et al.* 1994; Carroll *et al.* 2003) due to the proinflammatory cytokines, including tumour necrosis factor-a (TNF-α) and interleukin-1 (IL-1) released by activated macrophages (Webel *et al.*1997; Yi *et al*. 2005; Jiang *et al*. 2009). However, neither the synthesis or secretion nor the metabolic change of these cytokines following immune challenges has been investigated in calves. This study therefore aimed to examine the plasma cytokines and the changes of nitrogen metabolism following immunological challenge by bacterial lipopolysaccharide (LPS) in pre-ruminant calves.

Materials and methods Forty male Chinese Holstein calves of 24 days old were randomly divided into 2 groups and injected intraperitoneally with 2.5 μg *E. coli* lipopolysaccharides/kg BW at 24, 26 and 28 days of age, or an equivalent volume of sterile saline as control. Rectal temperature was measured 30, 90, 150 and 210 min after injection. All calves were on the same diet that was composed of soybean mill, milk powder, whey powder etc. A total collection of faeces and urine was conducted between 25 and 27 d for analysis of nitrogen metabolism. Plasma samples were collected 60, 120 and 180 min after injection for analysis of urea nitrogen, IL-1β and TNF-α. Data were analysed using the independent-samples t-test procedure of SPSS 15.0 (SPSS Inc., Chicago, IL).

Results Compared with the control, the body temperature of LPS-injected calves was elevated significantly 150 min (38.97 *vs* 39.36°C, SED: 0.08, P<0.05, for the Saline and LPS group calves, respectively) and 210 min (39.18 *vs* 39.82°C SED: 0.10, P<0.05) after the challenge of LPS (Figure 1); the plasma concentration of IL-1β in LPS-injected calves was increased significantly 120 min (196.7 *vs* 378.3 pg/ml, SED: 36.8, P<0.05) and 180 min (101.5 *vs* 230.7 pg/ml, SED: 27.6, P<0.05) after the challenge with LPS (Figure 2); the plasma concentration of TNF-α was increased significantly 120 min (41.8 *vs* 89.4 pg/ml, SED: 7.3 P<0.05) and 180 min (38.0 *vs* 67.0 pg/ml, SED: 7.7, P<0.05) after the challenge with LPS (Figure 2); and the plasma urea nitrogen in LPS-injected calves was increased significantly 120 min (1.4 *vs* 1.8 nmol/L, SED: 0.1, P<0.05) post-injection of LPS (Figure 1). The urinary concentration of nitrogen in LPS-injected calves was numerically higher than control (0.471 *vs* 0.508 g/kg $BW^{0.75}$/d, SED: 0.011, P>0.05), but the nitrogen retention (0.543 *vs* 0.486 g/kg $BW^{0.75}$/d, SED: 0.019, P>0.05) and apparent biological value of nitrogen (0.535 *vs* 0.487, SED: 0.014, P>0.05) were numerically lower in the LPS-challenged calves than control (Figure 3).

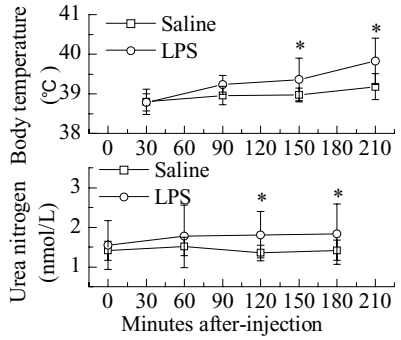

Figure 1 Body temperature and urea nitrogen in plasma of calves following a challenge of LPS. Asterisks indicate significant differences (P <0.05).

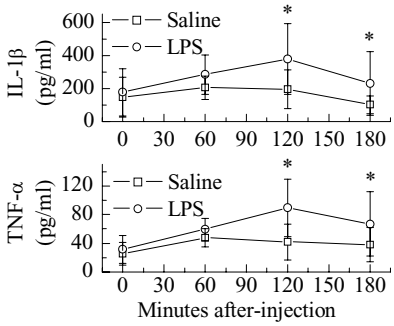

Figure 2 IL-1β and TNF-α in plasma of calves following a challenge of LPS. Asterisks indicate Asterisks indicate significant differences (P <0.05).

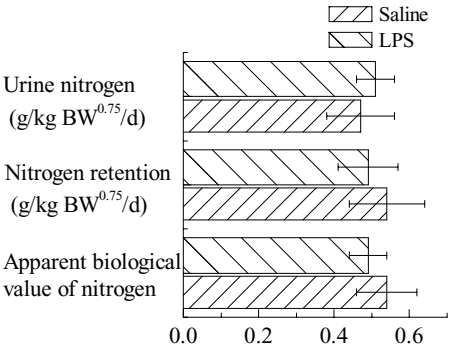

Figure 3 Nitrogen metabolism of calves following a challenge of LPS.

Conclusions The results suggest that the immunological stress activated immune responses and induced inflammatory responses, which consequently decreased the utilization efficiency of nitrogen in pre-ruminant calves.

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Preliminary comparative study on rumen fermentation in the grazing yak (*Bos grunniens*), indigenous cattle (*Bos taurus*) and cattle-yak (*Bos taurus* ♂ × *Bos grunniens* ♀) in the Qinghai-Tibetan plateau

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Introduction It has been shown that yaks developed special adaptive mechanisms to malnutrition in the harsh environment of the Qinghai-Tibetan plateau. For example, yaks relied on the recycling of nitrogen (N) to adapt to the harsh forage environment of the alpine Plateau (a lower average daily urinary N excretion and a greater N retention and urinary purine derivative N index than indigenous cattle, Wang *et al*. 2011); yaks have evolved morphological characteristics enabling them to consume a wide variety of plant species (Shao *et al*. 2010); Yaks have also developed special regulatory mechanisms in the kidney (Lower glomerular filtration rate, 3.9 $L/kg^{0.75}$ BW/d, and the ratio of net secretion in renal tubular load to total excretion of purine derivatives ranged from 26.2% to 66.3%. Wang *et al*. 2009).The aim of the present experiment was to determine if rumen parameters were affected by grazing seasons and animal's genotype living on the Qinghai-Tibetan plateau in China.

Materials and methods A grazing experiment was conducted at the Wushaoling Farm, situated in alpine meadow grassland of the Tibetan Autonomous County, Gansu province, altitude ranges from 2,700 to 3,300 m, the vegetation dominated by *Kobresia humilis*, *Elymus nutans*, *Polygonum viviparum*, *Dasiphora fruticosa*. It was divided into two periods (Period 1, from July 5 to 8 on the warm season, Period 2, from November 5 to 8 on the cold season), and nine castrated adult animals were allocated to one of three groups according to genotype (Yak, n=3, Indigenous cattle, n=3, and Cattle-yak, n=3) in each period. During the sampling period, animals came in from grazing and were in cages from 20.00 h to next 08.00 h without receiving any forage or supplement but fresh water was freely accessible at all times, then they grazed from 08.00 until 20.00. on day 1, 2, 3 and 4 of grazing season in each period, rumen fluid samples were taken at 20.00, 01.00 and 08.00h on the following day via the mouth using a rumen tube (Made in Japan).The pH values were determined immediately, and a 50-ml tube was used to collect rumen fluid, then centrifuged at 2,500 g for 10 min, sub-sample was stored – 80°C for later analysis of ammonia N (NH_3-N). Data obtained were analyzed using SPSS software procedure, and multiple comparisons were tested according to the Tukey method.

Results The pH values ranged from 6.82 SED 0.16 to 7.15 SED 0.21 for three genotypes, and there were no significant differences between genotypes or grazing season ($P > 0.05$); The NH_3-N concentration for yak (3.09 mmol/L, SED 1.77) was greater than for indigenous cattle (1.33 mmol/L, SED 0.68) on the warm season ($P < 0.05$). However, there were not significant differences between genotypes on the cold season (Yak, 2.28 mmol/L, SED 0.51, Indigenous cattle, 1.88 mmol/L, SED 0.97, Cattle-yak, 1.49 mmol/L, SED 0.51). The values for yak and cattle-yak were increased linearly from fasting 2h to 12h in July and November ($P < 0.05$), but the tendencies were not shown in cattle ($P>0.05$) (Figure 1 and 2).

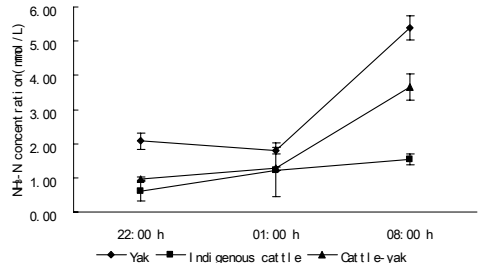

Figure 1 Response of ammonia nitrogen (NH_3-N) concentration to fasting time(2h, 5h, and 12h) on the warm season

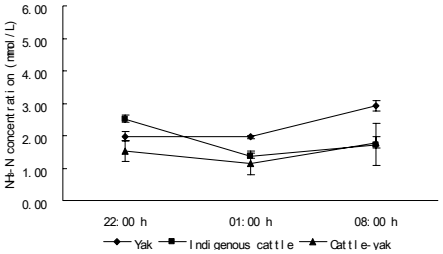

Figure 2 Response of ammonia nitrogen (NH_3-N) concentration to fasting time(2h, 5h, and 12h) on the cold season

Conclusions Grazing seasons had effect on the rumen fermentation for three animal's genotype living on the Qinghai-Tibetan plateau in China. Furthermore, the range of pH values may improve the digestibility of forage, and the greater NH_3-N concentration indicated that yaks can recycle more N than the indigenous cattle via saliva and rumen wall. This was identical to our experiment for yak's nitrogen balance, which indicated that yaks had a lower daily average urinary N excretion and greater N retention than indigenous cattle. In summary, the characteristics of rumen fermentation for yaks had advantage to adapt to the inadequate and poor forage environment on the Qinghai-Tibetan plateau. However, it was essential to highlight that, due to limited resources and local constraints; the number of animals used for this study was rather small.

Acknowledgments The work was supported by grants National Natural Science Foundation project 30730069 and National Universities special research fund lzujbky-2009-6 of China.

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A short-term test to assess sheep propensity towards Mediterranean forages offered as micro-swards

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Introduction The learning process of an animal to a particular food is the result of the association between pre-ingested stimuli (sensory perceptions) and consequences of post-swallowing (Iason & Villalba, 2006). The knowledge of the mechanisms that affect animal propensity towards a food has aroused new interest in recent years in management practice, especially when some constituent of the diet is rapidly changed. Animal propensity could be measured by intake rate (g DM/min), as this is strongly influenced by factors acting at the level of sensory perception. The aim of this experiment was to measure the instantaneous intake rate and the bite mass of dairy sheep fed on adjacent monocultures of equal or different forage species by using the 'micro-swards' method.

Materials and methods Six treatments (T), established by sowing in paired boxes the forage species to create micro-swards (Orr *et al*. 2005), were compared. Four treatments were featured by 2 adjacent monocultures of equal forage species: Italian ryegrass (*Lolium multiflorum* L., L) LL; alfalfa (*Medicago sativa* L., M) MM; sulla (*Hedysarum coronarium* L., H) HH; chicory (*Cichorium intybus* L., C) CC, while the other two treatments consisted of 2 adjacent monocultures of different forage species: HL and LH, varying only for laterality. Each treatment was offered to two homogeneous replicate groups of 6 animals each of Sarda lactating ewes in a 6x6 Latin-Square design. Animals were first trained to the micro-sward test with a species not included in the study, *Hordeum vulgaris* L. (training period, 4 days), and then submitted to the experimental treatments (experimental period, 6 days) that were offered to each subject in a rack for about 5 minutes (test). The behaviour of each experimental animal was recorded by a fixed camera (time of ingestion, number of bites) during the test in order to calculate bite rate (BR, n/min). The micro-sward boxes were weighed before and after each test in order to determine the biomass removed and calculate the intake rate (FMIR, g fresh matter/minute of feeding) corrected for evapotranspiration losses, measured in an ungrazed box of the same forage species as that being tested. On 3 occasions four ungrazed micro-swards containing the sown species were cut at the root-shoot interface and oven dried at 85 °C for 18 hours to measure chemical composition and dry matter on offer. Non fibre carbohydrates (NFC) were then calculated. Bite mass was calculated on both fresh (FMBM, g) and dry matter (DMBM, g) basis. Intake rate was also calculated on DM basis (DMIR, g/min). The behavioural responses (BR, FMIR, DMIR, FMBM, DMBM) were analysed by GLM with treatment (df=5), sheep (df=10), replicate (df=1) and test day (df=5) as fixed effects. T means were separated by the Tukey test ($P<0.05$). Regression analyses were performed to explore relationships between response and explanatory variables using mean treatment values.

Results All behavioural responses were significantly affected by T (Table 1). Although HH outperformed most other treatments for DMBM it did not differ from CC for FMBM and FMIR. DMIR was higher in HH than CC and LL with the other treatments as intermediates. LL tended to compensate its low average FMBM and DMBM, showing a higher BR than HH and CC ($P<0.05$). Positioning did not affect the responses of LH and HL, which were basically intermediate between those of corresponding monocultures (HH and LL). One of the most interesting relationships examined was that between FMIR and NFC (Figure 1).

Table 1 Effect of forage treatment on sheep behaviour

T	BR n/min	FMBM g	DMBM g	FMIR g/min	DMIR g/min
CC	33.3^{a}	2.31^{c}	0.18^{b}	75.1^{bc}	6.0^{a}
LL	52.0^{b}	0.78^{a}	0.10^{a}	42.9^{a}	5.6^{a}
MM	38.4^{ab}	1.43^{b}	0.20^{bc}	54.6^{ab}	7.7^{ab}
HH	36.5^{a}	2.75^{c}	0.26^{c}	98.2^{c}	9.5^{b}
HL	40.1^{ab}	1.57^{b}	0.16^{ab}	67.0^{ab}	7.1^{ab}
LH	41.0^{ab}	1.71^{b}	0.18^{b}	67.5^{ab}	7.2^{ab}

Values with different superscripts were significantly different ($P<0.05$)

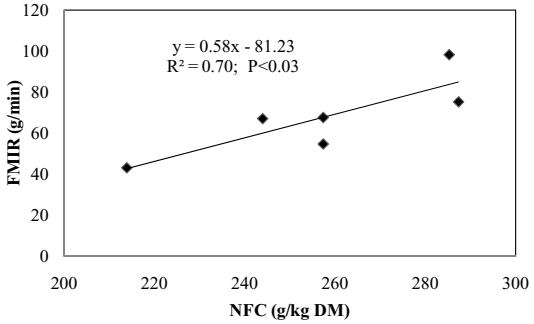

Figure 1 Regression of FMIR on forage NFC (n=6)

Conclusions This short-term test shows that sheep propensity, measured as FMIR or DMIR, is higher for sulla than Italian ryegrass with the other treatments as intermediate. NFC level in the offered forages was the best single explanatory variables for the observed responses.

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Effects of chicory / perennial ryegrass swards compared with perennial ryegrass swards on gastro-intestinal parasites in grazing beef steers

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Introduction The effective control of parasites is fundamental to ensuring both economically and environmentally sustainable livestock farming systems worldwide. Certain forages, including chicory (*Cichorium intybus*), can reduce gastro-intestinal parasitic infections in sheep (Marley *et al*. 2003). However, there has been little research to determine if chicory can reduce gastro-intestinal parasites in beef cattle. The aim of this study was to assess the effects of chicory / perennial ryegrass (*Lolium perenne*) compared with perennial ryegrass swards on the gastro-intestinal parasites of grazing beef steers.

Material and methods Triplicate field plots (2 ha) were established with either a chicory (cv. Puna II) / perennial ryegrass (cv. Premium) mix (7.4 kg ha^{-1} chicory / 22.2 kg ha^{-1} ryegrass) or a perennial ryegrass control (cv. Premium), sown at a rate of 29.6 kg ha^{-1}. Forty-eight Belgian Blue - cross steers (approx. 7 months of age at Day 0) were used for the experiment, with 8 animals grazing each replicate plot. All animals were naturally-infected with gastro-intestinal parasites. The experimental approach comprised of a standardisation and a measurement period. During the standardisation period of 28 days (Larsson *et al*. 2006), steers were placed on a standard ryegrass / white clover permanent pasture as one group. Animals were allocated to their respective treatment, on the basis of live weight, body condition score (BCS) and faecal egg counts (FEC) determined 7 days prior to the measurement period. The measurement period started on 25 May 2010 (day 0) and continued until the end of the grazing season on the 28 September 2010. Faecal samples were collected on Day 0, 28, 70, 84 and 126 for faecal egg count (FEC) and parasite culture determinations. Faecal samples for FEC were taken immediately prior to anthelmintic treatment (Eprinex Pour-on, 0.5% eprinomectin (Merial Animal Health, Harlow, Essex, UK) at a rate of 1 ml per 10 kg liveweight, given on Day 28, 84 and 126. FEC were determined using a modified McMaster technique (MAFF, 1997), with 1 egg representing 50 eggs g^{-1} of fresh faeces. Faecal cultures of *Trichostrongyle* type eggs to third stage larvae (L3) consisted of a 10 g faecal sample per individual steer, bulked per plot and incubated at 27°C ± 3°C for 7 days. Faecal dry matter (DM) was determined by placing a 15g sample of faeces at 95 □C for 48h. FEC data were adjusted for DM content prior to square root transformation to normalise the data. On day 126, a blood sample was taken from each animal to determine *O. ostertagi* antibody levels using an ELISA, with results expressed as an optical density ratio (ODR) (Charlier *et al*. 2005) and plasma pepsinogen levels using a colorimetric method, with enzyme activity expressed as units (U) of tyrosine. FEC were compared at each sampling point by analysis of variance with the previous sample value as the co-variate, and for all data the replicate plot of each forage was used as a block effect, using Genstat® version 11.1.

Results The results of the gastro-intestinal parasite data are shown in Table 1. These data showed that the steers experienced a moderate challenge of parasitic nematodes over the grazing season. Faecal cultures indicated that *Ostertagia ostertagi* and *Cooperia* spp. were the main parasite species present in the steers. There were no differences in FEC, faecal DM or DM-adjusted FEC, *O. ostertagi* antibody or plasma pepsinogen levels of beef steers grazing either chicory/ryegrass swards or ryegrass only swards.

Table 1 Square root transformed FEC (gDM^{-1}), *O. ostertagi* antibody (ODR) or plasma pepsinogen (U) levels of grazing beef steers

	Chicory / Ryegrass	Ryegrass	SED	Prob
FEC				
Day 0	51.4	51.7	4.25	ns
Day 28	65.2	65.0	7.40	ns
Day70	31.2	25.9	2.70	ns
Day 84	45.6	37.2	3.94	ns
Day 128	23.1	20.8	7.64	ns
O. ostertagi antibody	0.70	0.72	0.06	ns
Plasma Pepsinogen	2.07	2.15	0.197	ns

Conclusions In this study, there was no difference in the FEC, *O. ostertagi* antibody or plasma pepsinogen levels of beef steers grazing chicory / ryegrass swards or ryegrass only swards. Further work is needed comparing animals either treated or not treated with anthelmintics to investigate the effects of chicory on gastro-intestinal parasites in beef finishing systems.

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In vitro gas production of ten pasture grasses grown in the humid tropics of México

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Introduction Several pasture grasses from the *Brachiaria* spp, *Panicum* spp and *Pennisetum* spp genera, are being used commercially in Mexico in the past 20 years, with some degree of success. There is information on nutritive value and ruminal digestion dynamics for some species, but for others, there is little, if no information at all. The objective was to study the *in vitro* gas production (IVGP) dynamics of ten tropical pasture grasses harvested over three climatic seasons at different ages of regrowth.

Materials and methods There were four *Brachiaria* spp grasses: *B. brizantha* (cv. Insurgente), *B. decumbens* (cv. Chontalpo), *B. humidicola* (cv. Chetumal) and *B. brizantha* x *B. ruziziensis* (cv. Mulato); three *Panicum maximum* cultivars: Guinea, Mombasa, and Tanzania; and three *Pennisetum purpureum* cultivars: Taiwan, Cuban and Purple. A randomized complete block design with three replicates was used. Harvests took place over five cycles (one for the 2008 dry season, two for the winter seasons of 2009 and 2010 and two for the rainy seasons of 2008 and 2009) each of which started with a standardization cut and continued with cuts at 3, 6, 9 and 12 weeks of regrowth. The IVGP (Menke and Steingass, 1988) was measured at 1-8 (hourly), 12, 24, 32, 48, 72 and 96 h, corrected for blank IVGP and readily fermentable NDF (Herrero and Jessop, 1996), and the data fitted to the model (Krishnamoorty *et al*. 1991): $Y = b(1\text{-}e^{-c*(X-L)})$, where 'Y' (ml) is IVGP at time 'X' (h); 'b' is potential IVGP (ml); 'c' is the fractional rate of gas accumulation; and 'L' is lag time (h). Parameters were analyzed by a two-way ANOVA. The chosen significance level was $P < 0.05$.

Results Table 1 presents parameter means for main effects and shows a satisfactory fit to the model, R^2 values being on average 0.98 (Table 1). Harvest cycle, regrowth age and their interaction were highly significant (P, 0.0001) for all parameters, while the other effects were not significant on any parameter ($P > 0.05$). The experiment did not detect differences in whole plant IVGP of tropical pasture grasses. Most variation in model parameters was due to environment *e. g.* harvest cycle and regrowth age. Both rainy seasons of 2008 and 2009 did not present statistical ($P > 0.05$) differences in b and c parameters, but the lag times were statistically different ($P < 0.05$). The b and c parameters for the 3-week regrowth were different ($P < 0.05$) from the other regrowth ages, that did not differ between them. The individual gas production curves of the grasses were practically the same (Table 1).

Table 1. Model parameter means for each main effect.

Variables	Levels	n	Means of model parameters				
			b	c	L	R^2	Sy.x
Harvest cycle	Dry 2008	40	296.15	0.0259	5.90	0.99	12.25
	Rainy 2008	36	348.85	0.0163	5.95	0.99	9.97
	Winter 2009	40	291.24	0.0205	5.90	0.99	10.62
	Rainy 2009	31	345.80	0.0152	6.71	0.97	14.60
	Winter 2010	39	374.27	0.0160	6.00	0.98	12.67
Regrowth age	3 weeks	50	276.98	0.0238	6.07	0.98	12.04
	6 weeks	47	351.36	0.0169	6.23	0.98	12.88
	9 weeks	43	346.76	0.0191	5.99	0.98	12.50
	12 weeks	46	349.94	0.0159	5.96	0.99	10.34
Grasses	Insurgente	18	353.81	0.0204	6.03	0.98	12.18
	Chetumal	19	360.83	0.0191	6.07	0.98	11.30
	Chontalpo	18	309.78	0.0195	6.18	0.98	12.82
	Mulato	20	319.83	0.0184	6.09	0.98	11.34
	Mombasa	18	313.82	0.0189	5.83	0.98	10.64
	Guinea	19	323.43	0.0171	5.95	0.98	10.57
	Tanzania	18	314.62	0.0196	5.99	0.98	11.61
	Cuban	18	334.19	0.0187	6.17	0.98	13.24
	Purple	19	333.33	0.0185	6.14	0.98	12.96
	Taiwan	19	333.08	0.0200	6.19	0.98	12.81

n: Number of curves used to calculate each mean.

Conclusions The potential nutritive value of the grasses, as assessed by the IVGP, was similar between them. Then, the selection of a best tropical grass for this particular environment should still be based on dry matter production.

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Dry matter intake and digestibility of rations with graded levels of ground pods of *Enterolobium cyclocarpum* as a replacement for concentrates in Pelibuey lambs

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Introduction In tropical Mexico, intensive sheep farming systems have been expanding during the last few years to meet the raising demand by the increasing population. *E. cyclocarpum*, a native leguminous tree present in the tropical regions of Mexico shed its pods during the dry season, and these can be easily collected for animal feeding. Pods of *E. cyclocarpum* contain 15.6% crude protein and 63% nitrogen free extractives suggesting the presence of substantial amounts of soluble carbohydrates. Moscoso *et al*. (1995) incorporated 36% of ration dry matter as ground pods of *E. cyclocarpum* reporting weight gains of 223 g/day in Katahdin x Blackbelly lambs. It has been shown that foliage of *E. cyclocarpum*, can decrease rumen protozoa by 25% and increase efficiency of microbial protein synthesis by 23% when fed (187 g DM/d) to sheep (Koenig *et al*. 2007). The aim of this trial was to assess dry matter intake and apparent dry matter digestibility of rations containing graded levels of ground pods of *E. cyclocarpum* in Pelibuey sheep.

Materials and methods Five entire male Pelibuey sheep with a live weight of 34±2 kg, were housed in metabolic pens. Before the start of the trial sheep were dewormed with Ivermectin® and an I.M. injection of vitamins ADE was also applied. Pods of *E. cyclocarpum* were collected manually from below trees. Mixtures of *E. cyclocarpum*:concentrates represented 90% of the DM offered while the other 10% was fresh chopped Taiwan grass (*Pennisetum purpureum*). Treatments were five levels of incorporation: 0, 20, 30, 40 and 50% (of ration DM), of complete pods of *E. cyclocarpum* mixed with concentrates; the rest of the concentrate mixtures being ground sorghum, soybean, cane molasses and a mineral mixture. The experimental design was a 5 x 5 Latin Square. Data was analyzed by ANOVA with SAS statistical software. The experiment lasted 60 days divided in five periods of 12 days; each experimental period had seven days of adaptation and five days for measurements. Sheep were fed *ad libitum* allowing a refusal of at least of 100 g of the feed offered the previous day. Voluntary DM intake was calculated as the difference between feed offered and the amount rejected the following day. Apparent digestibility of DM and OM was measured by total collection of feces, taking a 10% sample of feces produced daily during the last five days of each experimental period. At the end of each period, daily fecal samples (five days) were thoroughly mixed and aliquots were taken and frozen at -4 °C until analyzed.

Results No statistically significant differences (P>0.05) were found (Table 1) for DM intake and digestibility among treatments. The only significant difference (P<0.05) in OM digestibility was between the control treatment and the highest level of incorporation (50%) of ration DM as ground *E. cyclocarpum*. Nonetheless, DM and OM digestibilities showed a linear negative trend as the level of incorporation of *E. cyclocarpum* in the ration was increased. There were no differences (P>0.05) in intake of digestible dry and organic matter. No statistically significant differences (P>0.05) were found for ME intake among treatments.

Table 1 Voluntary intake and apparent digestibility of DM in Pelibuey lambs fed graded levels of *E. cyclocarpum* in the ration.

	Treatments						
	0	20	30	40	50	SEM	Linear effect
Intake (g^{-1} $kg^{0.75}$)							
DM	73[a]	87[a]	88[a]	94[a]	91[a]	6.0	*
OM	69[a]	81[a]	83[a]	88[a]	85[a]	5.7	*
ME	0.9[a]	1.0[a]	1.0[a]	1.0[a]	0.9[a]	0.07	**
Apparent digestibility (g^{-1} kg^{-1} DM)							
DM	780[a]	772[a]	758[a]	722[a]	710[a]	21.9	*
OM	795[a]	776[ab]	760[ab]	723[ab]	709[b]	19.9	*
Intake (g^{-1} $kg^{0.75}$)							
DDM	58.9[a]	67.3[a]	66.9[a]	67.6[a]	64.7[a]	5.0	**
DOM	55.8[a]	63.5[a]	62.7[a]	63.2[a]	60.2[a]	4.7	**

Rows with different superscripts differ significantly (P< 0.05).

Conclusions Ground pods of *E. cyclocarpum* are readily consumed by sheep when mixed with other feedstuffs**.** Incorporation of increasing levels of ground pods of *E. cyclocarpum* in the ration of Pelibuey sheep had no effect on intake and apparent digestibility of DM. At 50% incorporation of pods, OM digestibility was reduced. It is concluded that ground pods of *E. cyclocarpum* can be incorporated up to 40% of ration DM of Pelibuey sheep during the dry season when pods are widely available in tropical Mexico to reduce the use of imported usually expensive, concentrates.

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Faecal crude protein as a marker to estimate the digestibility of Italian Ryegrass for grazing sheep

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Introduction Italian Ryegrass (*Lolium multiflorum* Lam.) is relatively widespread as a forage source for ruminants in southern Brazil based on its nutritional quality and the regional climate adaptability. The assessment of pasture quality under grazing is one of the major problems for adequate pasture management studies. The evaluation of forage digestibility under grazing using the ratio between the protein concentration present in the faeces and the organic matter digestibility, as described by Lancaster (1949), has a great potential for estimating the pasture digestibility. However, very little information is available in relation to this estimation in subtropical areas, compared to temperate areas (Boval *et al*. 2003; Lukas *et al*. 2005; Fanchone *et al.* 2009). This study was conducted to assess the hypothesis that faecal crude protein can be used as a marker to estimate digestibility in sheep grazing Italian Ryegrass.

Materials and methods Seven experiments were carried out in metabolism cages over a period of 10 days of adaptation and five of sample collecting. Each trial represented different stages of maturity of Italian Ryegrass. Sixteen animals (Texel, entire males of 10 months of age and 35 kg live weight) were randomly divided into four treatments per period. The four dietary dry mater allowance levels were 1.5, 2.0, 2.5% of live weight and *ad libitum*. The forage composition, in each maturity stage (vegetative, pre-flowering and flowering were, respectively: DM = 14.2±1.9, 17.7±2.2 and 24.4±5.2%; CP = 24.5±1.2, 16.7±0.6 and 13.9±2.1%; NDF = 37.7±2.3, 54.8±4.2 and 61.5±3.4%. The intake was determined as the difference between the offered and refused feed. Total daily collection of faeces was used for the faecal nitrogen and ADF determination. Organic matter digestibility (OMD) was calculated using intake and faecal output data. Regression equations were derived between the OMD and faecal crude protein (fCP g/kg OM), using the hyperbolic model. The inclusion of faecal acid detergent fiber (fADF, g/kg OM) was also assessed in a multiple hyperbolic equation with the fCP. Based on the OMD estimated by the equations and OMD observed in the experiments, the relative prediction error (RPE) of each equation was calculated.

Results Based on findings in the literature on this theme (Boval *et al.* 2003; Fanchone *et al.* 2009), a hyperbolic equation was generated for the relationship between OMD and fCP (Table 1). A multiple hyperbolic equation was also generated including fADF together with fCP. The relative prediction errors in the estimate for the equations were around 5%, but the coefficient of determination (R^2) was higher for the multiple hyperbolic model (0.83) than the simple hyperbolic (0.77). Several authors consider that the hyperbolic model is best suited to explore the relationship between OMD and fCP (Boval *et al.* 2003; Fanchone *et al.* 2009). This is due to the biological relationship of the model, where there is a constant relationship between intake and metabolic faecal protein, consisting of cells of epithelial desquamation, digestive secretions and microorganisms, which does not occur with proteins from ingested food. The proteins of ingested forage can vary independently of ingestion, but are dependent on the nature of the diet (Boval *et al.* 2003). These authors also developed a number of leads showing the interrelations between the nitrogen content of faeces with the intake and digestibility. The hyperbolic model describes the rapid increase in organic matter digestibility per unit of faecal crude protein, followed by a relatively sharp bend before reaching maximum digestibility. The equation generated by the hyperbolic model using only the fCP, showed an acceptable coefficient of determination (0.77), but the inclusion of fADF increased the R^2 to 0.83, confirming the increased reliability of this equation for nutritional studies of sheep grazing Italian Ryegrass. The use of other components plus the protein content in the estimate of OMD, is reviewed in the literature (Lukas *et al.* 2005) and fADF has been the best performing component (Boval *et al.* 2003), a situation that was confirmed by data presented in this experiment.

Table 1 Relationship between the organic matter digestibility **(**OMD) and faecal components concentration

Model	Equation	R^2	RPE
Simple Hiperbolic	OMD = 1.01557 – 39.6067/fCP	0.77	5.87
Multiple Hiperbolic	OMD = 1.11581 – 23.4416/fCP – 0.000590151fADF	0.83	5.11

fCP = faecal crude protein; fADF = faecal acid detergent fiber; R^2 = coefficient of determination; RPE = relative prediction error.

Conclusions Faecal crude protein has good potential for use in estimating digestibility in sheep grazing Italian Ryegrass. The multiple hyperbolic model is recommended for estimating digestibility by using faecal crude protein and faecal acid detergent fiber for sheep fed on Italian Ryegrass.

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Effects of Crina and monensin on fermentation pattern and digestibility of high-concentrate diet in rumen simulation technique

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Introduction It has been shown that plants extracts such as essential oils (EO) are being evaluated by a number of research groups worldwide as possible alternatives to in-feed antibiotics (Benchaar *et al*., 2007). A commercially available mixture of EO (Crina Ruminant, DSM Nutritional Products, Parsippany, NJ) has been reported to increase the acetate to propionate ratio (A:P) and to lower protein degradation in the rumen but the effect may vary with diet composition. Wheat dried distillers' grain with solubles (DDGS) is becoming a common feed ingredient in western Canada; however, wheat DDGS contains high protein (40 – 44% of dry matter) which is highly degradable in the rumen. The objective of this study was to evaluate the effect of Crina supplementation *versus* monensin (MON) on ruminal fermentation of a high-grain beef cattle finishing diet using an *in vitro* rumen simulation technique (Rusitec).

Materials and methods The experiment was designed as a completely randomized block with a 2×2 factorial arrangement of treatment with 4 replications in each treatment. The Rusitec apparatus (Czerkawski and Breckenridge, 1977) was equipped with eight 920-mL volume anaerobic fermenters, and two groups of Rusitec apparatus were used. The finishing diet (10% barley silage, 55% barley grain, 30% wheat DDGS and 5% supplement) was supplemented with 0 or 90 mg Crina/kg DM combined with 0 and 28 mg MON/kg DM. Statistical analysis was conducted using the MIXED model of SAS accounting for the fixed effects of Crina, MON and their interaction.

Results Interactions of Crina with MON on fermentation and nutrient digestibility were not significant except for N (Table 1). Supplementation of Crina did not affect volatile fatty acid (VFA) concentration, but decreased (P<0.05) the molar proportion of acetate (40 *vs*. 39%) and reduced (P<0.02) ratio of acetate to propionate (A:P; 1.43 *vs*. 1.34). In contrast, MON did not affect the VFA concentration (57 mM) and molar proportions of individual VFA. Gas production (mL/d) was not affected, whereas production of CH_4 (mL/g digested OM) decreased (P<0.02) from 2.9 to 2.4 with Crina supplementation. Similarly, MON reduced CH_4 proportion in total gas (1.29 *vs*. 1.14%; P<0.01) or tended (P<0.09) to reduce CH_4 (2.9 *vs*. 2.5 mL/g digested OM). Crina improved (P<0.01) NDF digestibility from 28 to 31% but had no effects on digestibility of other nutrients. Whereas, MON reduced digestibility of DM (71.4 *vs*. 69.5%; P<0.04), and tended to reduce the digestibilities of N (71.4 *vs*. 68.8%; P<0.08) and of starch (88.4 *vs*. 85.5%; P<0.10).

Table1 Effect of Crina and monensin (MON) on fermentation nutrient digestibility, and gas production (GP) in Rusitec

	- Crina [1]		+ Crina [1]			P value		
Item	- MON	+ MON	- MON	+ MON	SEM	Crina	MON	Crina × MON
Total VFA, m*M*	59.5	57.6	55.8	55.7	2.33	0.25	0.67	0.71
VFA, mol/100 mol								
Acetate (A)	40.1	39.5	38.6	39.1	0.34	<0.01	0.88	0.13
Propionate (P)	28.1	28.2	29.1	28.9	0.50	0.11	0.90	0.77
A:P	1.44	1.41	1.33	1.36	0.033	0.02	0.95	0.44
Digestibility, %								
DM	70.5	69.5	72.2	69.5	0.78	0.30	0.04	0.29
N	69.4	69.6	73.4	68.0	1.33	0.37	0.08	0.05
NDF	29.2	27.6	30.7	31.3	0.77	<0.01	0.53	0.19
Starch	87.6	85.6	89.2	85.4	1.62	0.68	0.10	0.58
GP, mL/g digested OM	226.7	223.7	201.3	206.1	16.2	0.21	0.96	0.81
CH_4, % in gas	1.33	1.18	1.24	1.11	0.048	0.10	<0.01	0.84
CH_4, mL/g digested OM	3.1	2.7	2.6	2.3	0.19	0.02	0.09	0.56

[1]Diet consisted of barley silage, barley concentrate, and wheat DDGS in ratios of 10:60:30 (DM basis), supplemented with 0 (-Crina) and 90 mg Crina/kg DM (+Crina) combined with 0 (-MON) and 28 mg Mon/kg DM (+MON).

Conclusions Supplementation of Crina improved ruminal fermentation pattern with decreasing ratio of acetate to propionate, and reduced CH_4 production as well as increased NDF digestibility. The decrease of CH_4 with Crina is consistent with the effect of MON. The results suggest that addition of Crina in high-grain diets may potentially improve feed efficiency and alleviate methane emission although the effect seemed to be quantitatively limited.

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Improving the nutritive value of sugarcane bagasse for ruminants by culturing with a white-rot fungus *Ceriporiopsis subvermispora*

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Introduction Sugarcane is an important crop in the tropics and subtropics. In those countries, the vast amount of sugarcane bagasse (SB) is produced at sugar production plants. Around 80 to 90% of this SB is used as a heat source for sugar production and a part of the remainder has even tried to use for feed for livestock. However, the digestibility of SB is very low since the lignin content is higher than in grasses. In our previous study (Okano *et al.*, 2006), white-rot fungi as *Lentinus edodes* and *Ceriporiopsis subvermispora* were effective in improving digestibility of SB in *in vitro* digestion test. However, there is no report where nutritional evaluation of SB treated by white-rot fungi has been carried out by *in vivo* animal trial. The objective of this work was to determine the digestibility of SB cultured with a selective white-rot fungus *C. subvermispora* (Messner and Srebotnik 1994).

Materials and methods The sugarcane bagasse substrate (SBS) was prepared by mixing it with wheat bran at an air-dry weight ratio of 9:1. Four kg of SBS was placed on a polypropylene sheet and laid inside a plastic box. After sterilization at 118°C for 40 min, wheat grain spawn of *C. subvermispora* was inoculated with SBS. Twenty-four boxes were then cultured at 32°C for 8 weeks. To determine the change in chemical composition and *in vitro* digestibility, a part of the substrate was sampled from triplicate boxes randomly in 24 boxes before inoculation and 8 weeks after inoculation. Four Japanese Corriedale sheep (48.0 ± 5.0 kg) were used in a reversal design to determine the digestibility of SBS. The sheep were assigned to two treatments (*i.e.*, basal diet, and basal diet + SBS). The basal diet was composed of alfalfa hay cubes, wheat bran and soybean meal (70.3: 19.9: 9.8) and fed at 18.1 g dry matter (DM) per kg of body weight daily in two equal portions at 9:00 and 17:00 h. For the basal diet + SBS, 255 g/kg of the basal diet were replaced with SBS. During two consecutive 14 day periods, the sheep were adapted for 7 days followed by a 7-day sampling period to collect faeces. The chemical composition, *in vitro* organic matter (OM) digestibility (IVOMD), *in vitro* neutral detergent fibre excluded residual ash (NDFom) digestibility (IVNDFomD) and *in vitro* gas production (IVGP) data were subjected to a one-way analysis of variance.

Table 1. Change in chemical components, in vitro digestibility and in vitro gas production during 48 h of incubation (IVGP) of sugarcane bagasse substrates (SBS) cultured with Ceriporiopsis subvermispora for 8 weeks

	SBS before inoculation	SBS cultured for 8 weeks	SEM
Chemical composition			
OM (g/kg DM)	968	957 *	2.3
NDFom (g/kg DM)	862	692 **	4.0
ADFom (g/kg DM)	529	520	12.5
ADL (g/kg DM)	100	61 **	2.8
Coefficients of *in vitro* digestibility			
OM	0.430	0.719 **	0.0089
NDFom	0.360	0.611 **	0.0096
IVGP (ml/g OM)	122	207 **	4.0

Values within the same row followed by asterisks significant differ (* P<0.05; ** P<0.01).

Table2. Chemical composition and in vivo apparent digestibility of sugarcane bagasse cultured with Ceriporiopsis subvermispora for 8 weeks.

	Chemical composition (g/kg DM)	Coefficents of *in vivo* apparent digestibility
DM	1000	0.627
OM	955	0.647
Crude protein	44	0.138
Ether extract	4	0.277
NDFom	692	0.752
ADFom	557	0.799

Results OM, NDFom, and acid detergent lignin (ADL) content of the SBS before sterilization significantly decreased by culturing with *C. subvermispora*. However, there was no significant difference in acid detergent fibre excluded residual ash (ADFom) content of SBS between before sterilization and after culture for 8 weeks. The IVOMD, IVNDFomD and IVGP during 48 h of incubation significantly increased in the SBS by culturing with *C. subvermispora*. The coefficients of apparent OM, NDFom and ADFom digestibility of the SBS cultured with *C. subvermispora* for 8 weeks were 0.647, 0.752 and 0.799, respectively.

Conclusions Culturing with *C. subvermispora* for 8 weeks improved the digestibility of SBS to a level that was almost equal to middle quality of grasses. However, since the content of NDFom in SBS decreased by culturing with *C. subvermispora*, feeding SBS to ruminants would affect on their ruminating behaviour. In *in vitro* test, the digestibility of OM was higher than that of NDFom. In contrast, the apparent digestibility of OM was lower than that of NDFom as determined by *in vivo* test. This result indicates that the digestibility of ND-soluble fraction of SBS is not high as shown in *in vitro* test.

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Estimation of indigestible NDF of grasses and legumes from cell wall components and *in vitro* organic matter digestibility

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Introduction Indigestible NDF (iNDF) is the most important parameter for the estimation of available energy in the feed evaluation system for dairy cows, NorFor, which was introduced in 2006 in Denmark, Iceland, Norway and Sweden. Consequently, rapid and reliable methods for the estimation of this cell wall fraction are needed. The aim of the present study was to analyze the potential of cell wall components and organic matter digestibility (OMD) for the estimation of iNDF using correlation and regression analysis.

Materials and methods The data used is part of a larger dataset analyzed in our laboratory. Forages with information on OMD and the cell wall fractions NDF, ADL and iNDF were included. A total of 238 samples, consisting of 87 perennial ryegrasses, 25 pasture grasses, 19 lucernes, 15 fresh grass-clovers, 15 grass-clover silages, 15 red clovers, 18 festuloliums, 14 white clovers, 12 hybrid ryegrasses, six cocksfoots, four maize whole crops (WC), three pea WC, two green barley WC, two lupines and one barley WC silage harvested at varying maturity stages in different growths and years. *In vitro* OMD was determined based on the Tilley and Terry method, NDF analysis included amylase treatment, and NDF and ADL are expressed as ash-free. iNDF determination mainly followed the NorFor standard with 288 h incubation. Data from earlier iNDF determination with 504 h *in situ* incubation were also included using an earlier estimated correction factor. Forages were grouped into grasses and legumes.

Results The cell wall components and OMD displayed a large variation within and between forage types with the mean NDF concentration of 43.4 g/kg DM (SEM=0.59), ADL of 2.84 g/kg DM (SEM=0.10), iNDF of 7.70 g/kg DM (SEM=0.32) and OMD of 70.6 g/kg organic matter (OM) (SEM=0.52) over all forage types. Ratios were; ADL/NDF 0.07 (SEM=0.003), OMD/(iNDF in DM) 13.8 (SEM=0.60), OMD/(iNDF in NDF) 5.68 (SEM=0.22) and iNDF/ADL 2.68 (SEM=0.05). All ratios varied highly between the individual forage types. Across forage types, iNDF in DM correlated well to ADL in DM (r=0.87). ADL in DM and ADL in NDF showed high correlations to iNDF in NDF (r=0.87 and r=0.86). For legumes, OMD was highly negatively correlated to iNDF, both in DM (Fig.1) and NDF (r=-0.89 and r=-0.81). Supported by plots and Akaike`s Information Criterion the simple linear regression model on ADL in DM fitted well (with a better fit for iNDF in NDF than in DM) (Fig.2). Stepwise multiple regression analyses with NDF, OMD and ADL in DM (for iNDF in DM) or ADL in NDF (for iNDF in NDF) as variables in the model showed significant effects of all three parameters with ADL in DM as the most important predictor for iNDF in DM, whereas the significant effect of NDF disappeared for estimation of iNDF in NDF. When dividing samples into grasses and legumes, all parameters in the model had significant effects on iNDF for grasses, with ADL and NDF as the most important predictors. For legumes, the significant effect of OMD disappeared when estimating iNDF in DM and for iNDF in NDF, only ADL in NDF and OMD were shown to be significant.

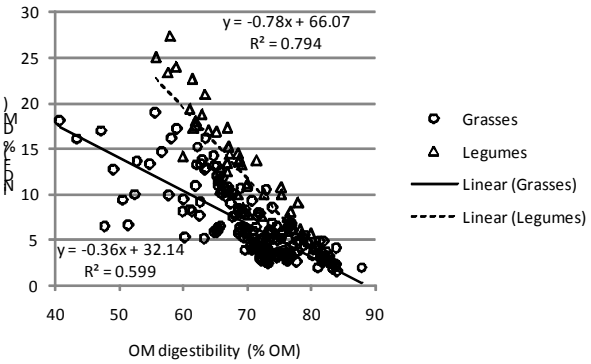

Figure 1 iNDF *vs*. OM digestibility

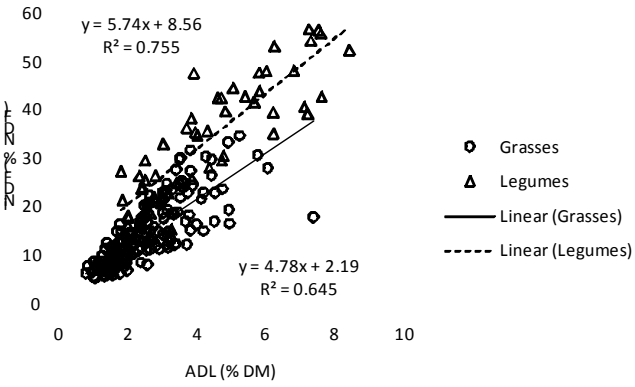

Figure 2 iNDF *vs*. ADL

Conclusions Estimation of iNDF based on cell wall components and OMD across forage types was inappropriate. For legumes ADL in DM seemed to be a potential predictor for iNDF in NDF. Multiple regressions were superior to simple regressions for estimation of iNDF and it remains to be analyzed if a different grouping of forage types according to the morphological structure of their cell walls could improve estimation of iNDF.

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Changes of membrane potential of abomasal smooth muscle cells and the influence of beta-hydroxybutyrate

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Introduction The left displaced abomasum (LDA) is a common disease in dairy herds and its incidence is associated with many risk factors including hypocalcemia and ketosis (LeBlanc *et al*., 2005). It is widely accepted that LDA is caused by a hypomotility of and a gas accumulation within the abomasum. The abomasal smooth muscle contractility is based on slow waves: a change in the membrane potential of smooth muscle cells initiated by the Interstitial Cells of Cajal. The aim of the present study was to characterise the membrane potential and the frequency of slow waves in abomasal smooth muscle cells of cattle and goats and to determine possible effects of beta-hydroxybutyrate (BHB) and of different calcium (Ca) concentrations.

Materials and methods Tissue samples of the abomasal corpus were obtained from 1) twelve bulls (beef bred) which had been slaughtered in a local abattoir, 2) five dairy cows under surgery for LDA in the Clinic for Cattle (University of Veterinary Medicine, Foundation, Hannover), 3) five goats fed a restrictive Ca diet and five goats fed a balanced diet (control group). All goats were slaughtered in the department of physiology (University of Veterinary Medicine, Foundation, Hannover).
All tissue samples were incubated in two different modified Krebs-Henseleit buffer solutions. A low Ca buffer solution (1mM ionized Ca) was used for the abomasal tissues of six of the bulls, the dairy cows and for the tissue of the goats previously fed the Ca restricted diet. A balanced Ca buffer solution (1.89mM ionized Ca) was used for the tissues of the remaining six bulls and those of the control goats. Mucosa and submucosa were dissected and removed from the underlying muscle layers. Muscle strips of 0.5x1cm were prepared and pinned into a small chamber coated with silicone, with the circular muscle layer facing upwards. The chamber was continously perfused with buffer solution. Circular smooth muscle cells were impaled with microelectrodes. All tissue samples were impaled twice: The 1st recording over 20 min served as time control and was analyzed in three five minute parts, during the 2nd recording 5mM BHB was added from minute five till twelve. Data between two conditions were compared with a two-tailed Student's t-test.

Results There was no significant time effect on basal membrane potential and the frequency of slow waves for all animal groups and buffer solutions (Tab. 1). Incubation in a low Ca buffer had no effect on slow waves' frequencies of abomasal muscles (Tab. 1). Treatment with BHB significantly decreased the frequency of slow waves in tissue samples from Ca restricted goats (Fig. 1). This effect was not reversible after withdrawal of BHB (Min 15-19). BHB showed no effect on basal membrane potential and the frequency of slow waves in all other tissue groups.

Table 1 Frequency of slow waves under control conditions (number of slow waves per minute, means±SEM)

	Min 1-5	Min 8-12	N
Bulls, balanced Ca buffer	4.5±0.6	3.5±1.1	4
Bulls, low Ca buffer	5.6±1.4	4.8±1.3	5
Dairy cows, low Ca buffer	10.4±1.8	10.5±2.0	4
Goats, low Ca buffer	6.7±1.9	6.8±1.6	4
Goats, balanced Ca buffer	6.4±3.7	6.8±3.8	3

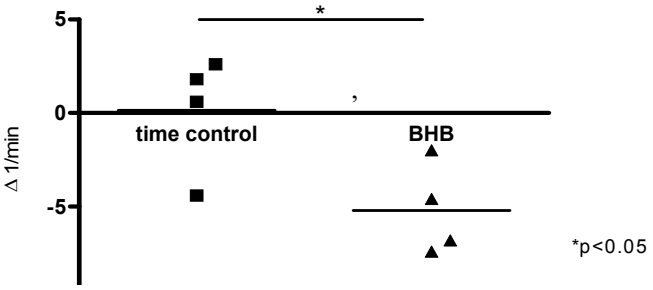

Figure 1 Change in slow wave frequency in the absence or presence of beta-hydroxybutyrate (BHB). Abomasal muscles from goats that had been fed a Ca restricted diet. Incubation in low Ca buffer.

Conclusion Low Ca levels together with high BHB levels have a negative influence on the frequency of slow waves in goats' abomasum and could contribute directly to the hypomotility of this organ in ketotic animals.

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Effect of pH on potassium transport in the digestive tract of ruminants

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Introduction Hypokalemia is often found associated with abomasal displacement (DA). The exact mechanisms connecting the two disturbances are, however, not yet fully examined. The low potassium levels may be a cause for impaired abomasal functions (Türck and Leonhard-Marek 2010), or the disrupted gastrointestinal functions may, in turn, prevent sufficient potassium absorption. Previous studies have shown that potassium can be absorbed from the rumen (e.g. Scott 1967). Thus, even cows with completely blocked gastrointestinal passage, as in abomasal volvulus, should be able to absorb potassium as long as the ruminal K concentration remains high, unless the ruminal transport processes are compromised. Electrolyte transport across the ruminal epithelium can be affected by pH (Gäbel *et al*. 1987) and K channels in different epithelia have been shown to alter their function with pH. Transition cows and cows with DA often face disturbances in their acid-base status. While the change to a high concentrate diet increases the risk of subclinical rumen acidosis, metabolic alkalosis can be a consequence of DA. Our study therefore aimed to examine the influence of pH variations on ruminal potassium absorption.

Material and methods Samples of ruminal tissue were obtained from sheep and cattle at a local slaughter house. Experiments were started within half an hour after slaughter.
The epithelia were separated from the muscular and serosal layers and incubated in Ussing chambers in a modified Krebs-Henseleit buffer solution under short circuit conditions. The mucosal buffer solutions were Na free, Na being replaced by N-methyl-D-glucamine (NMDG), and contained either 4 mmol/l K – low K buffer, or 100 mmol/l K – high K buffer. Then, the buffer pH was varied on the mucosal or serosal side, respectively, to simulate pathological circumstances. Current (I_{SC}) and tissue conductance were recorded and compared between treatments by analysis of variance and paired t-tests.
To simulate luminal acidosis, the pH was decreased on the mucosal side of ruminal tissue from sheep and cattle, from pH 7.4 to 5.5 in one experiment (N = 7 sheep / 7 cattle), and to pH 5.0 in another (N = 6 sheep / 6 cattle).
To simulate blood alkalosis, the pH was increased from 7.4 to 8.0 on the serosal side of ruminal tissue from sheep and cattle (N = 8 sheep / 10 cattle).

Results An increase in mucosal potassium concentration from 4 to 100 mmol/L led to an increase in current, as well as tissue conductance ($P < 0.05$ in all experiments), in line with previous results (Kronshage and Leonhard-Marek 2009).
A change from pH 7.4 to 5.0 on the mucosal side had no effects in a low K buffer. In a high K buffer, a reduction in mucosal pH from 7.4 to 5.0 decreased the short circuit current in both sheep and cattle from 2.2 ± 0.3 to 1.8 ± 0.3 µeq/cm²h, and from 2.2 ± 0.4 to 1.9 ± 0.4 µeq/cm²h, respectively ($P < 0.01$). This effect was fully reversible ($P < 0.01$), when mucosal pH was changed back to 7.4. Changing from pH 7.4 to 5.5 induced a similar effect, which did not reach statistical significance. Tissue conductance showed no clear trend of variation in either experiment.
Increasing the serosal pH from 7.4 to 8.0 had again no effect in a low K buffer, but led to a significant increase in short circuit current in a high K buffer in both sheep (from 1.8 ± 0.1 to 2.1 ± 0.2 µeq/cm²h, $P < 0.001$) and cattle (from 1.6 ± 0.3 to 1.8 ± 0.3 µeq/cm²h, $P < 0.05$). This effect was likewise reversible when serosal pH was changed back to 7.4. Tissue conductance showed no significant variations.

Conclusions Ruminal acidosis might impair potassium absorption, as shown by a reduction in transepithelial potassium current. If renal K excretion is not reduced in the same magnitude, this could promote potassium depletion and thus the development of hypokalemia in transition cows affected with DA. Metabolic alkalosis, in contrary, seems to lead to increased, rather than impaired, ruminal potassium absorption. Rumen epithelium from sheep and cattle seemed to react to the examined pH variations in a very similar manner, although most effects were slightly more pronounced in sheep.

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Effect of harvest time and physical form of alfalfa silage on chewing time and particle size distribution in boli, rumen content and faeces

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Introduction Intake of legumes is often higher than that of grasses at same digestibility (Wu *et al*., 2001) and with a lower NDF content. The NDF fibre in legumes are known to be more lignified, with a lower potential digestibility, but with higher rate of digestion compared with grass. There is limited information available about chewing activity and the breakdown characteristics of feed particles along the digestive tract when feeding alfalfa silage at different maturities and chopping lengths and especially alfalfa grown at Nordic latitudes. The objective of this study was to study the effect of harvest time and chopping on the structural value of alfalfa silage by comparing chewing activity and particle size distributions in forage, forage boli, rumen content and faeces from cows.

Materials and methods The alfalfa crop was harvested at two stages of growth (early: NDF 37%, late: NDF 44% in DM) and from each harvest a chopped (Theoretical cutting length: 19 mm) and an unchopped crop was ensiled in bales. The silages were fed restrictively to four rumen cannulated non lactating Jersey cows (391±26 kg) in a 4×4 Latin Square design. The cows were fed 80% of their *ad libitum* intake twice daily. Chewing activity was recorded continuously for 96 hours. Swallowed boli, rumen content, rumen fluid and faeces samples were collected, washed in nylon bags (0.01 mm pore size) and freeze-dried before dry sieving through 4.75, 2.36, 1.0, 0.5 and 0.212 mm pore sizes into 6 fractions. The length (PL) and width (PW) of particles within each fraction was measured by use of image analysis. The overall mean PL and PW values were estimated as weighted means of the different sieving fractions. The density distribution functions f(PL) and f(PW) were estimated as composite functions based on gamma distributions functions for γ_i(PL) and γ_i(PW) for the individual sieving fractions. The mode values were estimated by stepwise identification.

Results The mean eating time (min/kg DMI ($P<0.01$), min/kg NDF ($P<0.05$) and jaw movements (JM)/kg NDF ($P<0.05$)) were affected by harvest time. The mean ruminating time (min/kg DM) was affected by harvest time ($P<0.01$), physical form ($P<0.05$) and NDF intake per kg BW (min/day ($P<0.05$), min/kg DM ($P<0.01$), min/kg NDF ($P<0.01$), JM/day ($P<0.05$), JM/kg NDF ($P<0.001$)). The mean chewing time per kg NDF decreased due to chopping and early harvest time by approximately 10% each. The proportion of washed particle DM of total DM in boli, rumen content, rumen fluid and faeces was ($P<0.01$) highest by feeding late harvested alfalfa silage. Chopping of the silage decreased the mean PL and PW, the most frequent PL (mode) and 95 % percentile PL and PW values in boli. Chopping increased the mean PW ($P<0.05$) in rumen content. Early harvest caused longer and wider boli particles, thinner and higher length to width ratios of the particles in rumen fluid and faeces. The dimension size of faeces particles was not significantly affected by chopping. The stage of maturity at harvest and chopping affected the degradation of alfalfa silage particles along the digestive tract. The mode PW value was lower in rumen content and faeces compared with in boli ($P<0.001$) and the mode PL value was higher in boli and lower in faeces compared to rumen contents ($P<0.001$). The mean length and width values of fibrous particles decreased along the digestive tract by 95 and 90% relative to chopped silage, respectively, but without significant effect of harvest time or chopping. Two distinct local maxima on the density distribution of PL and PW value in boli, rumen content, rumen fluid and faeces were identified at each of the four treatments, and they were most likely related to the leaves and the stems, respectively. The lowest identified local maximum was considered as the mode value (Figure 1). These crop specific characteristics can only be seen because the particles are measured by use of image analysis.

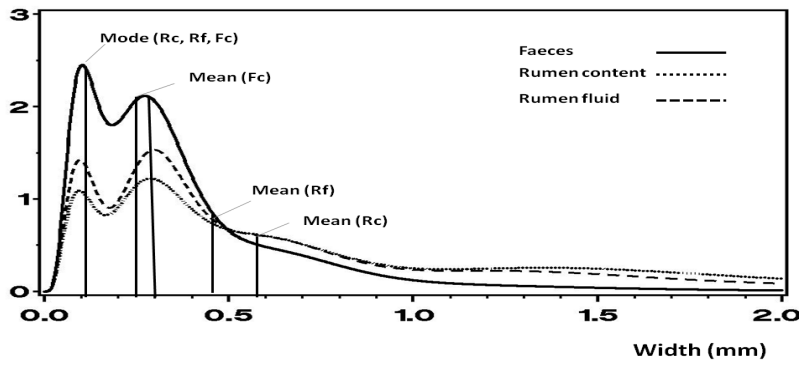

Figure 1 Density distribution of particle width in solid rumen content (Rc), fluid rumen content (Rf) and faeces (Fc) from Jersey cows fed alfalfa silage

C**onclusions** High NDF intake appears to cause a substitution of mastication activity during ruminating for mastication during eating and that was seen as a reflection in the particle size in boli with lower most frequent length of boli particles. When feeding chopped silage the mean total chewing time per kg NDF was reduced and that was reflected in a lower mode PL and mean PL, PW and PL:PW in boli even though the eating time per kg NDF was not significantly different between chopped and long silage. The density distribution of length and width values of boli and rumen content particles showed two maxima and they were most likely related to the leaves and the stems.

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Comparison of *in situ* and *in vitro* methods for assessment of *in vivo* rumen starch degradation

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Introduction Fractional rate of starch degradation (k_d) in the rumen is an important feed characteristic used in many modern feed evaluation systems. However, estimates of k_d for starch degradation have been highly variable within and among estimation methods. The *in situ* method is probably the most commonly used method for estimating k_d, but has been criticised due to the high loss of starch from the bags just by washing which is prevented using the *in vitro* method. The aim of the present study was to compare the *in situ* and *in vitro* methods for estimation of k_d for ruminal starch degradation.

Material and methods Eighteen starch rich feedstuffs comprising cereal, legume and maize seeds were used. Feeds were milled in 1.5 mm cutter mill for *in situ*, and 1 mm hammer mill for *in vitro* and chemical analysis. *In situ* study used 37 um Dacron bags, and incubation time 0, 2, 4, 8, 16, 24, 48 and 72 h, incubations were repeated in 3 dry cows fed standard maintenance ration. *In vitro* study used a modification of Sveinbörnsson *et al*. (2007) method with incubation times 0, 1, 2, 4, 8, 24, 48, 72 h repeated twice. Starch in feeds and residues were analysed from the difference in glucose before and after treatment with heat stable amylase and amyloglucosidase using a Hitachi 912 analyzer. Fractional rate of degradation k_d was estimated analysing profiles of starch residues. Residue profiles were fitted using PROC NLIN in SAS in the model $Y(t)$ = starch residue in mg per g feed DM weighed out at time $t = I+D*e^{-kd*t}$, where I is indigestible starch (g/g DM weigh out), D is degradable starch fraction (g/g DM weigh out), and t is incubation time.

Results A weak correlation (r=0.46, P=0.058) was observed between *in vitro* and *in situ* estimates of fractional rates of starch degradation (k_d). *In situ* k_d estimates generally were higher than *in vitro* estimates, except for four very low degradable samples. For some highly degradable samples *in situ* k_d estimates were up to 14 times higher (wheat sample) than *in vitro* estimates, and also far above the realistic range. *In vivo* degradabilities had been estimated for 13 of the 18 samples (Larsen *et al*., 2009). These 13 samples except one sample (sodium hydroxide treated wheat grain), were used for comparisons of *in vivo* rumen degradability with effective starch degradabilities (ESD). ESD was calculated based on the estimated *in situ* and *in vitro* k_d values, assuming a passage rate of 5%/h and a simple one pool rumen model assuming 1st order kinetics for both degradation and passage. *In situ* ESD and *in vitro* ESD correlated to *in vivo* rumen starch degradability with r=0.843 (P=0.0006) and r=0.764 (P=0.004), respectively (Figure 1). Sodium hydroxide treated wheat was excluded from the analysis as this was fed as whole grain *in vivo* and therefore it was problematic to make *in vivo* comparison with values from laboratory methods where samples have been milled.

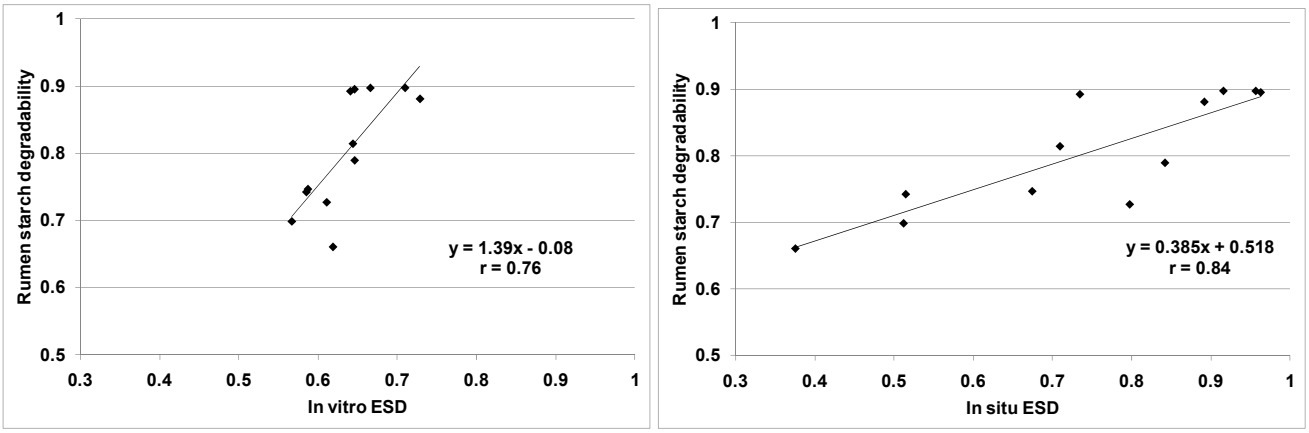

Figure 1 Relationship between *in vivo* rumen starch degradability and effective starch degradability (ESD) estimated from *in vitro* or *in vivo* estimated k_d.

Conclusion Weak correlations were found between rates of rumen starch degradation estimated with *in situ* or *in vitro* methods. However, when the estimated rates were used for calculating ESD, reasonable correlations were seen to *in vivo* rumen degradability for both methods, and although *in situ* k_d for some samples showed unrealistic k_d values, *in situ* ESD values correlated stronger with *in vivo* rumen degradability than *in vitro* based values.

Acknowledgements This work has been carried out with financial support from the Commission of the European Communities, FP7, KBB-2007-1.

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An evaluation of the herbage allowance and type of concentrate on rumen environment of grazing dairy cows during autumn

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Introduction In Southern Chile, milk production is predominantly based on perennial pastures as pasture-based milk production systems are more cost effective than indoor concentrate-based systems (Pulido *et al*., 2010). During autumn and winter, pasture availability is normally reduced due to low pasture growth rate, and supplementation is required to compensate the low availability at farm level (Pérez-Prieto *et al*., 2010). Synchronizing N and energy supply to the rumen can be achieved either by altering the energy source, or N source or both. Altering the diet forage/concentrate ratio can also be considered as a method of manipulating the synchronicity of diets (Cabrita *et al*., 2006). This study was undertaken to evaluate the influence of daily pasture allowance and concentrate type on ruminal pH, ammonia nitrogen (NH_3-N) and volatile fatty acid (VFA) concentrations in the rumen of autumn-calving dairy cows in early lactation.

Materials and methods Three Chilean Black Friesian cows fitted with rumen cannulas were used. The four dietary treatments were a combination of two pasture allowances above ground level (moderate, 25.5 *vs*. high, 36.0 kg of dry matter/cow/day) and two concentrate types offered at the level of 5 kg/cow/day, starchy (S) and fibrous (F). Cows were supplemented twice a day and managed under a strip grazing system on pasture consisting mainly of perennial rye grass. After an adaptation period of thirteen days, rumen liquor samples were taken at 0730, 0930, 1300, 1600, 1900, 2100, 0000 and 0300 h during days 14, 28, 42 and 56, and pH was measured immediately by glass electrode. The remaining samples were preserved for ammonia and VFA analyses. The samples were assayed with a gas chromatograph (model 5890 Series II; Hewlett-Packard, Avondale, PA) equipped with 1.8 m glass column packed with 10% SP 1200/1% H3pO4 on 80/100 chromosorb WAW (Supelco Inc., 1975) to determine the concentration of VFA. Nitrogen was the carrier gas and the temperature of the injection port and column was determined according to the procedures of Ipharraguerre *et al*. (2005). Statistical analysis was done using a linear mixed model with a factorial design of repeated measures.

Results The pH levels were not affected by pasture allowance ($P > 0.05$), whereas the effect of type of concentrate was ($P < 0.05$). The averages were 6.19 and 6.07 for starchy concentrate and fibrous concentrate respectively. Pasture allowance affected ($P < 0.05$) rumen ammonia concentration (9.7 and 18.2 mmol/L, for high and moderate pasture allowances, respectively) and total VFA (43.6 and 67.4 mmol/L, for high and moderate pasture allowances, respectively). The effect of type of concentrate did not affect NH_3-N concentration ($P > 0.05$).

Table 1 Least squares means for ruminal pH, ammonia-N (NH_3-N) and volatile fatty acid (VFA) by pasture allowance (HA: High = 36.0 kg pasture DM offered; Moderate = 25.5 kg pasture DM offered) and concentrate type TC (S = corn based concentrate; F = beet pulp based concentrate) on dairy cows grazing an autumn pasture.

	Pasture allowance			Concentrate type			$P \leq$		
	High	Moderate	SEM	S	F	SEM	HA	TC	HA x TC
Rumen pH	6.11	6.15	0.038	6.19	6.07	0.053	0.48	0.022	<0.001
NH_3-N, mmol/L	9.70	18.20	1.26	14.04	13.86	1.79	<0.001	0.92	0.92
Total VFA, mol/L[1]	62.6	48.5	2.74	43.6	67.4	3.87	<0.001	<0.001	<0.001
Acetate/total VFA	51.4	55.5	1.30	53.6	53.3	1.84	0.046	0.87	<0.001
Propionate/total VFA	22.0	22.3	1.01	21.5	22.8	1.44	0.81	0.38	0.64
Butyrate/total VFA	26.7	22.1	1.35	24.9	23.9	1.91	0.020	0.62	<0.001
Acetate /propionate ratio	2.53	2.75	0.17	2.68	2.60	0.24	0.36	0.75	0.65
Sutton´s ratio[2]	3.97	3.91	0.30	4.00	3.89	0.43	0.89	0.79	0.27

[1]Calculated as acetate + propionate and butyrate; [2] Sutton´s ratio = acetate + butyrate / propionate

Conclusions It can be concluded that offering a high pasture allowance to dairy cows would more efficiently synchronize ruminal fermentation. Supplementation with moderate amounts of concentrate maintains pH under physiological range, even though beet pulp-based concentrates produce a greater amount of ruminal VFA.

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Inclusion of ear elephant tree leaves (*Enterolobium cyclocarpum*) as a protein source for growing goats

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Introduction Mexico is a country, which because of its edaphically, topographic and climatic characteristics presents an important abundance of natural resources, mainly because of the diversity of plant species; nonetheless, the use of these species is limited. In tropical and sub-tropical regions, many of native forages exhibit low quality; nevertheless, several leaves, fruits and seeds represent an alternative as protein supplement to ruminants, which might be utilized as a supplementing source during the dry season. Smallholder livestock production systems, in which small ruminants predominate, are the main form of agriculture in central Mexico. Feeding is based on corn crop residues, which have little nutritive value, particularly low CP concentrations. However, such diet supplemented with other leguminous arboreal as elephant ear tree (*Enterolobium cyclocarpum*) can increase the CP and energy value in the diet. Therefore, the objectives of the present study were to determine the diet's effect on kid goat's voluntary intake and digestibility of the diet, when administered at different levels of elephant ear tree leaves (*Enterolobium cyclocarpum*) inclusion.

Materials and methods *E. cyclocarpum* leaves was obtained during the summer (March to May) from four municipalities in the state of Mexico, 1470 m above sea level and 800 mm average rainfall per year. It was collected 750 kg dry matter (DM) were collected and dried for 7 days in the field to reach 90% (DM). Nine kid goats, (LW 12.6 ± 3.2 kg; 3 months old), were fed with three experimental rations using a 3 x 3 Latin square design. The animals were located in metabolic cages. The control diet consisted of alfalfa hay (30%), corn grain (44%), SBM (20%), Megalac (3%) and mineral vitamin supplement (3%). *E. cyclocarpum* leaves replaced alfalfa hay at treatment levels of 15 and 30 % (DM basis). Rations were administered *ad libitum* twice a day at 08.00 h and 16.00 h. Each experimental period lasted 28 days, allowing 21 days for adaptation to the diet and 7 days for sample collection. Feed and refusals samples were collected on days 21 to 28, weighed and composited, both by each individual kid goat and across days. Fecal and urine output were weighed and sub-sampled (10% of wet weight). Diet, refusals and faecal samples were dried in a forced-air oven at 60°C for 48 h. Once dried they were ground (2.0 mm screen) with a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA), and analyzed for DM, organic matter (OM) and nitrogen (N) using the AOAC (1991) methods 930.15, 942.05 and 990.02, respectively. Urine samples were analysed for N content. Feed CP content was calculated by multiplying the N content by 6.25. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined by the method of Van Soest *et al*. (1991). The *in vivo* tests were submitted to an effect of the analysis according to a 3 x3 Latin square design, following the model: $Y_{ijk} = \mu + D_i + A_j + P_k + \varepsilon_{ijk.}$ where μ is the mean, D_i is the effect due to the diet, A_j is the animal effect, P_k is the effect due to the experimental period, and ε_{ijk} is the experimental error. The averages were compared by Tukey's *t*-test.

Results The total DM and OM Intake (g/day; $P \geq 0.05$; Table 1) was unaffected with increasing *E. cyclocarpum* leaves inclusion. NDF intake was higher (P<0.05) for 15 and 30 % *E. cyclocarpum* inclusion. The absence of differences in DM and OM intake levels related to *E. cyclocarpum* leaves inclusion would confirm the acceptable palatability of this product. The inclusion of the different levels of *E. cyclocarpum* leaves affect the DM, OM and NDF digestibility of the diets, being lower (P<0.05) with the 30% DM than 0%. Average daily gain (g/d) and feed conversion was unaffected (P>0.05) for the *E. cyclocarpum* leaves inclusion

Table 1 Intake (g/day**)** and digestibility (g/100g) balance in growing kid goats fed with different inclusion levels of elephant ear tree leaves (*E. cyclocarpum*) inclusion.

Variable	*E. cyclocarpum* inclusion						*E. cyclocarpum* inclusion				
Intake	0%	15%	30%	SEM†	P<	Digestibility	0%	15%	30%	SEM†	P <
DM	1034.03	1115.59	1148.91	197.69	NS	DM	79.18^{a}	76.18^{ab}	69.11^{b}	7.77	0.05
OM	1007.06	1114.42	1145.92	68.87	NS	OM	82.54^{a}	79.02^{ab}	71.78^{b}	6.88	0.05
NDF	309.52^{b}	419.78^{a}	429.77^{a}	195.51	0.05	NDF	63.10^{a}	58.61^{ab}	41.65^{b}	14.89	0.05

Values are expressed as mean. Different letters indicate significant differences ($P < 0.05$). NS = not significant at $P > 0.05$. †Standard error of mean.

Conclusions The chemical composition of *E. cyclocarpum* leaves, its digestibility indicates that it is medium quality forage. Furthermore, the intake levels related to the inclusion confirm the acceptable palatability of this product in kid goat's diets. The decision to utilize *E. cyclocarpum* in ruminant rations should be based on cost, availability and nutrient characteristics of the ration.

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In vitro and *in situ* determination of sweet sorghum bagasse ensiled with urea or urea plus molasses

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Introduction The objective of the current study was to determine the nutritive value of sweet sorghum bagasse silage (BS) with or without urea (U) and molasses (M) and comparing them with corn silage (CS).

Materials and methods Experimental silages with three replicates were prepared as: 1. Corn silage (CS), 2. Sweet sorghum bagasse silage (BS), 3. Sweet sorghum bagasse silage+1% urea (BSU), 4. Sweet sorghum bagasse silage+1% urea+5% molasses (BSUM; based on dry matter (DM)). Factors such as pH, concentrations of effluent, DM, ash, CP, EE, water soluble carbohydrates (WSC), non-fibre carbohydrates (NFC), VFA, ethanol, lactic acid, NH_3-N, NDF, ADF and ADL, in addition to *in vitro* true dry matter digestibility (IVTD) and protein fractions of Cornel Net Carbohydrate and Protein System (CNCPS) of silages after 45 and 90 days of ensiling were determined. Also *in situ* degradability (DM and CP) and gas production (GP) of silages in 90 days after ensiling was measured.

Results Showed that pH in silages containing urea (3.8) were lower than silage containing urea plus molasses (3.9; $P<0.01$). Ethanol, acetic acid, NDF, ADF, b_{DM} and a_{CP} (*in situ*) in silages containing urea were greater than silages containing urea plus molasses. Nonetheless, silage containing urea plus molasses in factors such as, DM, ash, NFC, a_{DM}, c_{DM}, b_{CP}, c_{CP}, ERD_{DM} and $(a+b)_{DM}$ *in situ* degradability were greater than silage containing urea. Silage containing urea plus molasses in DM, CP, NH_3-N, A (CNCPS), gas production rate (c), OMD, ME, a (DM and CP), ERD_{DM}, ERDP, (a+b) DM and CP *in situ* degradability were greater than silages with no additives. There were no differences in CNCPS fractions between 45 and 90 days silages, except proportion of B_3 fraction which increased with 90 compared to 45 days of ensiling. Totally, BSUM in many characters has no different with CS and it has as valuable as corn silage, and sometime it is better than it, such as ethanol, DM or ADF in Iran condition.

Conclusions It is suggested that it is better to use urea and molasses simultaneously as additives to increase the nutritional value of bagasse silage. Inclusion of urea increases part A of CNCPS so the addition molasses is necessary to improve the synchronised use of urea by rumen micro-organisms.

Table 1 Fermentation characteristics of corn and sorghum bagasse silages with or without additives (after 90 days ensiling; %DM).

Silage	Parameters						
	pH	NH_3-N	NH_3-N/N	ethanol	lactic acid	acetic acid	effluent
CS	3.8^{b}	0.85^{a}	12.9	1.95^{a}	7.2^{a}	2.14^{a}	3.6
BS	3.8^{b}	0.25^{c}	15.8	0.19^{b}	2.5^{b}	1.04^{bc}	2.6
BSU	3.8^{b}	0.55^{b}	17.9	0.21^{b}	2.7^{b}	1.27^{b}	1.7
BSUM	3.9^{a}	0.49^{b}	17.3	0.15^{c}	2.7^{b}	0.93^{c}	2.4
SE	0.01	0.034	1.72	0.077	0.088	0.097	0.66

Table 2 Chemical composition of corn and sorghum bagasse silages with or without additives (after 90 days ensiling; %DM).

Silage	Parameters										
	DM	CP	EE	WSC	NDF	ADF	ADL	Ash	NFC	IVTD	$IVD_{(ADF)}$
CS	20.3^{c}	8.3^{a}	3.3	4.1	50.2^{a}	27.2^{a}	7.9	7.6^{a}	32.0^{b}	70.6	33.6
BS	39.5^{b}	4.1^{c}	2.3	3.9	47.0^{ab}	26.0^{ab}	9.6	7.0^{ab}	40.7^{a}	66.2	30.2
BSU	38.2^{b}	7.4^{ab}	2.5	5.7	49.9^{a}	26.4^{a}	8.8	5.9^{b}	35.5^{b}	67.7	29.0
BSUM	42.2^{a}	7.4^{ab}	2.7	6.3	44.3^{b}	23.6^{b}	7.8	7.1^{a}	39.5^{a}	71.9	34.6
SE	0.51	0.22	0.28	0.55	0.83	0.54	0.88	0.31	0.83	1.61	3.41

Table 3 Protein characteristics (CNCPS) and gas production (GP) parameters of corn and sorghum bagasse silage with or without additives (after 90 days ensiling; % CP).

Silage	CNCPS					Gas production (GP) parameters						
	A	B_1	B_2	B_3	C	GP	GP_{MAX}	b	c	ME	NEl	OMD
CS	61.6^{b}	7.9	13.3	4.8	12.4^{b}	41.6^{a}	72.9^{a}	75.4^{a}	0.030	9.6^{a}	4.9^{a}	561^{a}
BS	46.0^{c}	11.4	14.0	5.1	23.6^{a}	32.0^{b}	59.3^{b}	61.5^{b}	0.031	7.5^{b}	3.8^{b}	456^{b}
BSU	70.2^{a}	6.5	8.2	1.7	13.4^{b}	36.6^{ab}	63.8^{b}	65.2^{b}	0.033	8.5^{ab}	4.4^{ab}	511^{ab}
BSUM	69.1^{ab}	6.4	9.6	2.3	12.5^{b}	38.7^{ab}	64.4^{b}	63.9^{b}	0.041	8.9^{ab}	4.6^{a}	531^{a}
SE	1.74	1.44	1.86	1.23	1.13	1.63	1.73	1.89	0.0035	0.30	0.16	14.6

Effects of different dietary supplementation levels of ensiled mulberry leaves on growth performance and serum parameters of Chinese yellow cattle

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Introduction Mulberry trees have been planted due to their special function in the protection of riverbanks from erosion in Yangtze River submerging zone near the Three Gorges Dam in China. A big problem must be solved because a huge amount of mulberry leaves are available each year. It has been shown that mulberry leaves is a good protein and forage feed source for ruminant animals because of its high protein content (> 21% in dry matter; Yao *et al*., 2000). The aim of this experiment was to determine the effect of different dietary supplementation levels of ensiled mulberry leaves (EML) on growth performance and serum parameters of Chinese yellow cattle.

Materials and methods Thirty-six healthy bulls with similar age (average 21.5 months) and average initial body weight (232.0±14.0 kg) were selected and randomly divided into 3 groups, with 12 bulls in each group. Each group of animals received one of three treatment diets: (A) control diet (no EML); (B) diet with 10% EML (DM basis); and (C) diet with 20% EML (DM basis). All mulberry leaves were ensiled over 60 days before feeding, and were used to replace part of mixed concentrate and roughage in diet B and C. The three diets had similar metabolic energy (ME) content (about 1.94 Mcal/kg, DM basis) and crude protein (CP) content (about 14%, DM basis). All bulls were tethered using neck straps in tie stalls and were individually fed the diet twice a day (06:30 and 18:00). Water was freely available to the bulls. The trial was carried out over a total of 88 days, including 7 days for adaptation and 81 days for data collection. Daily gain and feed intake were recorded during the trial and blood samples were collected at the end of the trial for measurement of some biochemical parameters. All the data were subjected to one way ANOVA on SAS and the differences among means were tested using Duncan's New Multiple Range Test.

Results Compared with the control, bulls receiving 10% EML and 20% EML diets had no significant changes in average daily gain, dietary dry matter intake and feed conversion efficiency (Table 1). However, both serum low-density lipoprotein (LDL) and high-density lipoprotein (HDL) tended ($0.05<P<0.1$) to be higher for bulls fed 20% EML diet than bulls fed control and 10% EML diet.

Table 1 Growth performance and serum biochemical indexes of Chinese yellow cattle fed the diet with different levels of EML

Item	Addition levels of EML (%)			SEM	P value
	0	10	20		
Growth performance					
Final body weight (kg)	314.8	316.5	320.3	17.8	0.975
ADG (kg)	1.01	1.05	1.09	0.07	0.747
DMI (kg/d)	8.55	8.87	9.33	0.40	0.395
DMI (%BW)	3.17	3.32	3.41	0.14	0.430
Feed conversion (G/F)	0.12	0.12	0.12	0.01	0.994
Serum parameter					
Urea (mg/dL)	6.59	6.09	6.03	0.23	0.211
Glucose (mmol/L)	4.24	4.27	4.21	0.14	0.949
Total protein (g/L)	79.1	78.7	79.0	1.37	0.975
Total cholesterol (mmol/L)	4.19	4.04	4.65	0.28	0.259
Triglyceride (mmol/L)	0.14	0.14	0.21	0.05	0.580
Low-density lipoprotein (mmol/L)	1.90	1.78	2.49	0.22	0.067
High-density lipoprotein (mmol/L)	3.27	3.26	3.84	0.21	0.088

Conclusions Feeding growing bulls with diets containing 10% and 20% of EML (DM basis) had no effect on their growth performance. Both serum LDL and HDL tended to be higher for bulls fed 20% EML diet than bulls fed control and 10% EML diet.

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Adoption of improved feeding and mating strategies and their impact on productivity of Bali cattle under small holder conditions in Lombok, Indonesia

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Introduction Lombok is part of the west Nusa Tenggara regency of eastern Indonesia, which is one of the country's main Bali cattle producers. Talib *et al*. (2003) reported that the most important constraints to improving productivity of Bali cattle in the region are low calving rate (52%), low calf birth weight (12.7 kg) and high calf mortality rate (15%). Consequently, the overall herd productivity is low. Panjaitan *et al*. (2008) showed that the calving rate can be improved to 85%, and calf mortality can be reduced to 5%. Another study (Dahlanuddin *et al*., 2008) showed that the growth rate of newly weaned Bali calves increased from 0.2 kg/d to 0.38 kg/d by feeding fresh *Sesbania grandiflora* (at 30% of diet). This paper summarizes results of an adaptive research project to scale out these improved management strategies to 36 farmer groups involving about 1200 farmers in Lombok from 2007 to 2010.

Materials and methods The feeding and mating strategies are part of an integrated village management systems (IVMS) consisting of a) controlled natural mating to enable calves to be born at times when feeds are in good supply and of high quality, b) mating cows from 40 days after calving, c) mating heifers when they reach 180 kg live weight, d) weaning calves at 6 months of age to reduce the nutritional load on the cow, and e) introduction of improved forages and better use of existing high quality forages, especially tree legumes. This IVMS was communicated to the farmers through a trained 'on ground team' (OGT) who worked closely with the farmers. Project activities included farmer training on feed quality and feed requirements based on the physiological status of animals, year round feed budgeting, bull feeding and mating management. The OGTs also facilitated farmers to improve pen sanitation and infrastructure to enable efficient feeding and to minimize the risk of disease and parasite infestation.

Results Controlled mating with a selected bull at 40-60 days after calving was the most commonly adopted management component (73.1% farmers), followed by better feed for cows during late pregnancy (66.4% farmers), weaning calves at 5-6 months old (60.3%), better feed for cows during lactation (41.9% farmers), better feed for newly weaned calves (38.4% farmers), planting and use of improved forages (34.5% farmers) and mating heifers at 180 kg (12.8% farmers). High adoption of mating cows from 40-60 days after calving was due to the availability a selected bull in the collective housing system and the better mating performance of the bull. There was lower adoption of improved forages and mating heifers at 180 kg because only 60% of the farmers have access to land and not all farmers have heifers or retain female calves for replacement. The most common introduced forages were *Brachiaria brizantha x ruziziensis* (cv. Mulato) and *Panicum maximum* (cv. Simuang). Mulato and Simuang are now widely distributed throughout the area. Compared to the introduced legumes (*Centrosema pascuorum, Stylosanthes guyanensis* and *Clitoria ternatea*) grasses are preferred because they are easy to establish, produce a large biomass and better regrowth. Adoption of the IVMS improved bull condition and bull mating performance (to more than 100 cows in 6 months) increased the calving rate to 86.6% (more than 30% increase), increased birth weight to 16 kg (more than 3 kg improvement), reduced calf mortality to 4.8 % (reduced by about 10%) and increased weaning weight from 70 kg to 90.2 kg. Cumulatively, these strategies increase beef production by 139% and significantly improved income of the small holder farmers.

Conclusions Productivity of Bali cattle under small holder conditions can be more than doubled by facilitating adoption of the IVMS that aims primarily to improve supply and quality of nutrients according to the physiological status of the animals. Successful adoption of the IVMS depends greatly on the ability of the field facilitators (OGTs) to communicate the management system to the farmers and provide on-going support. Adoption of improved forages by the small holders in Lombok is constrained by lack of access to land.

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Limitations associated with recording reticulo-rumen motility and methane eructations in cattle

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Introduction A trial was conducted using a method for simultaneous recording of methane (CH_4) eructation, reticulo-rumen motility and chewing activity in rumen fistulated cattle, introduced by Koch *et al.* (2009). The method enables recording of chewing behaviour and occurrence of CH_4 eructations as well as primary (PCC) and secondary contraction cycles (SCC) in the rumen of tied up ruminants. However, previous observations from similar trials indicate inconsistent sensor responses when animals were lying down. The objective of this study was to gain knowledge about eructation mechanism in ruminants and to evaluate the experimental method in terms of applicability to lying animals.

Materials and methods Four rumen fistulated non-lactating Jersey cows were fed four different cuts of grass silage diets in a 4x4 latin square design. Jaw movement oscillations (JMO) were recorded by a Hall sensor, reticulo-rumen motility by pressure transducers placed in the reticulum (PTre) and ventral ruminal sac (PTvs), and the CH_4 eructations by a CH_4 specific Taguchi gas sensor (TGS) mounted on a modified halter. The digitized signals (DV) from the sensors were sampled continuously at 20 Hz. The type of behaviour (eating, ruminating, or resting) and standing (n=114) or lying positions, left (n=50) *vs*. right (n=62), of the animals were registered at random time intervals between 7 to 16 h. An evaluation summary of sensor response interpretability within ± 5min of each registration time was made. Methane eructations were identified as peak TGS responses, and PCC and SCC were identified as bi- or triplephasic oscillations and single oscillations on PTre and PTvs response curves, respectively, as described by Backus *et al.* (1993) and Koch *et al.* (2009). A total of 226 registrations were included in the analysis. Effect of lying position, cow no, behaviour, and diet on odds ratio of occurrence of CH_4 eructation, PCC, and SCC was analysed by logistic regression using the GENMOD procedure in SAS 9.2.

Results Methane eructations were identified in 96 % of all registrations of standing cows which was significantly higher than for lying cows (P<0.001). Methane eructations were identified at all registrations of animals lying on left side, unlike cows lying on their right side (P<0.001) where only 50 % of the registrations showed occurrence of eructations. Significant difference of CH_4 eructation was found between cows (P=0.047). No significant differences were found between diets or behaviour types. SCC were identified from the PTvs response at 64 % of all registrations of lying cows and at 95 % of standing cows, which was significantly different (P<0.001). No significant difference was found between right and left side registrations, cow no, diet, or behaviour. PCC from the PTre response were easy to identify at all animal positions. Examples of plotted data from a standing cow and a cow lying on its right side are shown in Figure 1.

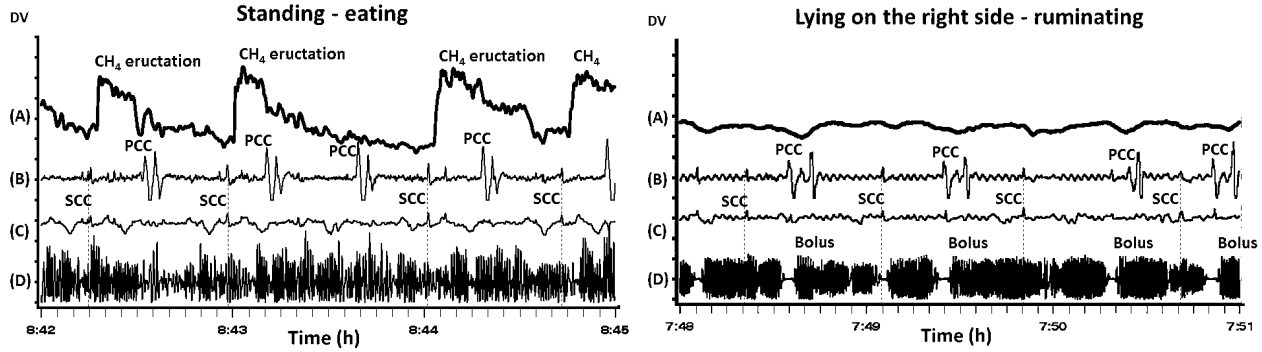

Figure 1 Plots of DV values from the sensors sampled from a cow standing while eating and lying on the right side while ruminating respectively. The upper curve (A) shows the CH_4 concentration observed with peaks marking an eructation. The two curves beneath shows the pressure variation in the reticulum (B) and ventral ruminal sac (C) respectively, with occurrences of PCC and SCC marked. The bottom curve (D) shows JMO for instance clustered in regular cycles during rumination with regurgitation of boluses marked.

Conclusions In general occurrences of SCC are difficult to indentify from the pressure variations in the ventral ruminal sac in lying cows. Analysis of logged data in similar experiments should consider position of the cows, since standing cows are preferred both regarding recording of eructation mechanisms and the coherent reticulo-rumen contractions. We consider leakage of rumen gas along the fistulas and rumen content or fluid covering the cardia area as the most likely cause for inconsistent CH_4 eructations from animals lying on their right side. This consideration may influence quantitative studies of eructated gas from rumen fistulated animals.

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Effect of essential oils of *Zataria multiflora* on *in vitro* rumen fermentation using fattening lamb diet

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Introduction Inclusion of antibiotics in animal rations as growth promoters has been limited due to the occurrence of multi-drug resistant bacteria that may cause a risk to human health. In the recent years many attempts have been devoted towards developing alternatives to antibiotics. It is documented that certain essential oils have the potential to modulate rumen fermentation by decreasing proteolysis and methanogenesis in rumen, and thus enhance the digestibility of diet (Benchaar *et al*. 2008).
Due to the presence of carvacrol and thymol, the essential oils of *Zataria mutiflora* (ZMEO) have strong inhibitory effects against some pathogenic bacteria (Amin *et al*. 2010). To our knowledge, no literature is available on the effects of ZMEO on rumen fermentation kinetics and feed digestibility. Therefore, the objective of this study was to assess the effects of ZMEO on the kinetics of fermentation, synthesis of microbial biomass, digestibility of feed and population of protozoa *in vitro*.

Material and methods The experiment was conducted in three runs in which the cumulative gas production technique of Menke and Steingass (1988) was used to determine the effects of different levels of ZMEO essential (0, 150, 300, 450 and 600 μg/ml) on fermentation characteristics of a commercial finishing ration of fattening lambs as a fermentation substrate. To estimate the fermentation kinetics 200 mg substrates were weighed into syringes that were subsequently filled with 30 ml buffered ruminal fluid. The gas volume recorded at 2, 4, 6, 8, 12, 48, 72, 96 and 120 h after incubation. Gas production profiles were fitted by iteration for individual incubation flasks to the mono-phasic model as described by Groot *et al*. (1996) and shown in the following equation:
$GP= A/(1+(B/t)^S)$ where GP is the cumulative gas production (ml g^{-1} incubated OM), *A* is the estimated asymptotic gas production (ml g^{-1} incubated OM), B is the time (h) after incubation at which half of the asymptotic gas production has been reached and *S* represents a constant that determines the sharpness of the switching characteristic of the profile. The *in vitro* cumulative gas production profiles were further characterised by estimating the maximum rate of gas production (R_{max}, ml h^{-1}) and the time at which this maximum rate was reached (T_{max}, h) according to Groot *et al*. (1996). The data were analyzed using generalized linear model ANOVA procedures (SAS, 8.1) and polynomial linear (L), quadratic (Q) and cubic (C) contrasts were used to test the effect of levels of ZMEO on parameters.

Results As the level of ZMEO was increased, the volume of produced gas decreased. At the level of 600 μg/ml, the profile of GP showed a multiphasic manner. The addition of ZMEO linearly decreased the A (Table 1). Increasing the level of ZMEO from 450 μg/ml to 600 μg/ml drastically increased the B. The parameter S was influenced in a linear manner by ZMEO (Table 1). The R_{max} was linearly decreased, whereas T_{max} was increased by the inclusion of ZMEO. The highest increase in T_{max} was observed when the ZMEO was increased from 450 μg/ml to 600 μg/ml.

Table 1 Effects of different doses of ZMEO on the estimated parameters of *in vitro* GP

	Doses of ZMEO (μg/ml)						Contrasts		
Parameters	0	150	300	450	600	SEM	L	Q	C
A	602.2	438.2	455.9	255.7	271.6	37.72	***	NS	NS
B	18.8	11.6	24.1	26.9	58.7	2.62	***	***	NS
S	1.2	1.5	1.4	2.8	4.2	0.89	**	NS	NS
R_{max}	22.7	23.3	11.7	7.5	4.9	1.29	***	NS	***
T_{max}	1.9	3.7	6.6	19.9	50.0	5.49	***	***	NS

A: asymptotic GP (ml); B: the time (h) when half of A is produced; S: the variable regulating the switching characteristics of the GP profiles; R_{max}: the maximum fractional gas production rate (h^{-1}); T_{max}: the time (h) of R_{max} occurrence; L: linear; Q: quadratic; C: cubic;** P<0.01; *** P<0.001; NS: non-significant; SEM: Standard error of the means.

Conclusion The inclusion of ZMEO decreased gas production which was an indication of decreased fermentation activity of microorganisms. The time (h) of incubation at which half of the asymptotic gas production has been formed was elongated with the higher level of ZMEO. The increase in parameter S supports the change of curve shapes from exponential to sigmoidal as the level of ZMEO was increased. The degree of sigmoidicity represents the possible lag process occurring at the early stages of incubation (Groot *et al*. 1996). The current study showed that ZMEO influenced rumen fermentation *in vitro* in a dose dependant manner. This illustrates that ZMEO has the potential to modulate ruminal fermentation.

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Effects of linseed supplementation on milk composition and on liver, adipose and mammary gland metabolism of periparturient dairy cattle

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Introduction Linseed supplementation in rations for dairy cattle has been used to increase the proportion of poly-unsaturated fatty acids (PUFA) in milk fat. Dietary PUFA are also known to reduce lipid accumulation, up-regulate fatty acid (FA) oxidation in liver and skeletal muscle, and increase total body glycogen storage in rodents (Jump and Clarke, 1999). Furthermore, human and animal studies have shown that PUFA are important modulators of immune reactions (Calder *et al*., 2002). The sudden changes in lipid metabolism in periparturient dairy cows influence hepatic functioning and immune reactions (Drackley *et al*., 2005). Possibly, linseed supplementation may not only influence milk FA composition, but may also affect liver, adipose and mammary gland metabolism when fed to periparturient dairy cattle. Therefore, the effect of dietary linseed supplementation on milk composition and on gene expression in liver, adipose and mammary gland metabolism was studied in periparturient dairy cows.

Material and methods Fourteen HF dairy cows were randomly assigned to control or linseed supplementation. Experimental treatments started 3 weeks before the expected calving date and lasted until 6 weeks after calving. All cows received *ad libitum* a forage mixture that consisted of maize silage, wilted grass silage, wheat straw (before calving) or grass hay (after calving) and soybean meal. Concentrate mixtures were fed individually through computerised feeders. Daily concentrate allowance was increased gradually from 0 kg on d-21 to up to 1.9 kg DM on d-1 and from 1.9 kg DM on day of calving up to 7.9 kg DM on d16. This level of concentrates was maintained until d42. The concentrate mixture for the linseed treatment contained 130 g of linseed/kg DM. Milk yield was registered daily and milk was sampled weekly for fat, protein and lactose analyses. Milk samples for FA analyses and biopsies of liver and adipose tissue were taken in wk-3, wk1, wk4 and wk6; mammary gland tissue was sampled in wk6. In the liver, changes in mRNA abundance of glucose transporter 2 (GLUT2), pyruvate carboxylase (PC), cytosolic phosphoenolpyruvate carboxykinase (PEPCK), carnitine palmitoyltransferase I (CPT1), glycerol-3-phosphate acyltransferase (GPAT1), solute carrier family 22 member 5 (SLC22A5), microsomal triglyceride transfer protein (MTTP), fatty acid transport protein 2 and 5 (FATP2 and FATP5, respectively), fatty acid binding protein 1 (FABP1), peroxisome proliferator-activated receptor (PPARα and PPARδ) were measured using real-time PCR assays. In adipose tissue, changes in mRNA abundance of fatty acid synthase (FASN), lipoprotein lipase (LPL) and PPARγ were analysed. Beta-actin (ACTB) was used as reference gene. The RNA of each mammary gland biopsy was amplified, biotin-labelled, and hybridised to single-dye Affymetrix GeneChip® Bovine Genome Array (#900493). Results of liver and adipose tissue were analysed using a mixed-effects linear model that accounted for the random effect of each cow nested within treatment, and the fixed categorical effects of dietary treatment, time, and the interaction between dietary treatment and time. Gene expression in the mammary gland was analysed as described above but without the time effect.

Results and discussion Linseed increased milk yield, lactose yield, and decreased milk fat concentration, which coincided with higher proportions of stearic acid and PUFA (mainly linoleic acid and conjugated linoleic acid). Linseed did not result in considerable transcriptional changes in adipose and liver tissue. However, the GLUT2 in liver tissue was greater in cows supplemented with linseed, suggesting that more glucose was released by the liver thereby increasing the proportion of available glucose for lactose synthesis compared with cows fed control diet. In the mammary gland, gene sets related to cell proliferation and remodelling, as well as immune system response were predominantly up-regulated by linseed supplementation, suggesting an effect on mammary gland tissue integrity and health. The expression of genes involved with lipid and carbohydrate metabolism was reduced. Furthermore, the concentration of short-chain and medium FA in milk was mainly positively correlated to most of the lipogenic genes, whereas that of long-chain FA was negatively correlated, suggesting a regulatory role for these components at the transcriptional level of mammary gland lipid metabolism.

Conclusion Including linseed in diets for periparturient dairy cattle not only affects FA composition in milk, but also reduces the expression of genes involved in lipid and carbohydrate metabolism in the mammary gland. Further studies are required to validate whether the up-regulation of genes related to immune system response by linseed supplementation has a direct effect on udder health.

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Ingestive behaviour of Hereford steers under different summer grazing managements of a mixed grass and legume pasture

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Introduction Performance of grazing beef cattle in Uruguay is affected by summer heat stress. Previous research has shown that removing cattle from pastures during the hottest hours of the day to a shaded area improves liveweight gain of steers compared to free grazing animals without access to shade in the grazing paddock (Beretta *et al.* 2005). It is probable that even when time of access to pasture is reduced cattle adapt foraging behaviour to maintain dry matter intake (Chilibroste *et al.* 2007). However, this response could be affected by pasture forage allowance. The aim of the present experiment was to characterize the ingestive behaviour of grazing Hereford steers under different summer grazing managements varying in forage allowance and total time of access to pasture.

Material and methods The study was conducted in Uruguay (32º S, 58º W) during summer 2005 (January 14 to March 18) on 40 hectares of a mixed grass and legume pasture based on *Festuca arundinacea, Trifolium repens* and *Lotus corniculatus*. Thirty-two Hereford steers (298±38,0 kg liveweight, LW) were randomly allocated to one of four treatments as result of a factorial arrangement of 2 levels of forage allowances (FA): 6 or 12 kg DM/ 100 kg LW; and 2 grazing managements (GM): free grazing (FG, cattle grazed all day long) or restricted grazing (RG, animals were removed from the pasture from 10:00 am to 05:00 pm and restrained in a shaded area with water *ad libitum*). Each treatment grazed independent paddocks in daily strips, being moved to a new strip at 10:00 am. FA was adjusted weekly varying strip area. Pre-grazing pasture biomass (kg DM/ha) and height were measured weekly to characterized grazing conditions. Random pasture samples were cut, oven dried and analyzed for crude protein, NDF and ADF content. On weeks (W) 2, 5, 6 and 8, during 2 consecutive days, grazing activity, rumination and idling were recorded by visual appraisal, every 15 min during day time (7:00 am to 9:00 pm) on four animals per treatment (no records were taken in RG treatments while out of pasture). Bite rate was recorded every 2 hours as number of bites in one minute. On same dates, pre-grazing and residual pasture biomass and height were measured. Behaviour data were submitted to LOGIT transformation assuming binomial distribution and analysed through a generalised linear model as follows: $Ln(p_{ijkl}/ (1-p_{ijkl})= \mu + FA_i + GM_j + (FA*GM)_{ij} + W_k + (FA*W)_{ik} + (GM*W)_{jk} + (FA*GM*W)_{ijk} + day_l(W_k)$; where Ln(p/1-p) is the probability to find an animal a particular behaviour activity. Bite rate and pasture data were analysed through a generalized model for repeated measurements including principal effects and interactions as previously described.

Results Average pre-grazing pasture biomass was 2797±40.8 kg DM/ha and 21±0.9 cm height, with 42.0% dead forage and 33% legumes, 12.0% CP and 57.2% NDF and 32.4% ADF. The effects of treatments on grazing activity, rumination and idling are presented in Table 1.

Table 1 Effect of forage allowance (6 *vs* 12% liveweight) and grazing managment on the probabilty of finding an animal grazing, ruminating or idling during day-time.

Treatments*	FG 6%	FG 12%	RG 6%	RG 12%	SE	FA	GM	FA*GM
Grazing	0.52	0.44	0.80	0.82	0.015	P>0.05	P<0.01	P<0.05
Ruminating	0.10	0.13	0.05	0.06	0.009	P>0.05	P<0.01	P>0.05
Idling	0.18	0.13	0.11	0.07	0.010	P<0.01	P<0.01	P>0.05

* FG: free grazing; RG: animals were removed from the pasture from 10:00 am to 05:00 pm and restrained in a shaded.

A significant interaction FA*GM was observed only for grazing activity. Reducing time of access to pasture increased the grazing activity independent of FA. Grazing activity of free grazing cattle, however, was increased as FA was reduced (P<0.05). Considering total time of access to pasture during the observation period and based on observed probabilities for grazing activity it was estimated a total grazing time of 437 and 370 minutes for FG at 6% and 12% FA, respectively, and 336 and 344 minutes for RG at 6% and 12% FA, respectively. Restricted grazing treatments also showed higher mean bite rate compared to FG and this effect was independent of FA (25.9 *vs*. 13.2 bites/min, SE: 0.82; P<0.001). Daily grazing pattern varied depending on GM: although no differences were observed during first grazing session, up to 3 hours after cattle entered a new strip (P>0.05), RG steers mantained a higher grazing activity 3 to 6 hours after (P<0.05) and early in the morning (P<0.05). Neither the GM nor the interaction FA*GM affected residual pasture biomass and height (P>0.05) indicating similar sward utilization for the different managments.

Conclusions Beef cattle modify foraging behaviour to compensate for changes in grazing conditions. Animals with reduced time of access to pastures change their grazing pattern, increasing grazing activity and bite rate to contribute maintain forage DM intake. Also, as forage allowance increases restricting time of access to pasture has a larger impact on grazing activity. Results show that during summer, animals removed from pasture to a restrained shaded area during the hottest hours of the day could benefit from better thermal conditions while adapting their ingestive behaviour to compensate for reduced time of access to pasture.

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Effects of Yerba Mate (*Ilex paraguariensis*) supplementation during the dry period on dairy cows' milk yield and oxidative status

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Introduction The transition period of dairy cows (3 weeks pre-prepartum to 3 weeks post-partum) is particularly important for dairy cow health and subsequent performance. During this period, dairy cows are subjected to dramatic changes in endocrine function and metabolism associated with calving and the initiation of lactation (Drackley, 1999). The transition period is also characterised by a depleted antioxidant status often resulting in oxidative stress. Oxidative stress results when the amount of reactive oxygen metabolites (ROS) exceeds the capacity of antioxidants to neutralise these which are associated with disruption of normal metabolism and physiology (Celi, 2011a). It seems that ROS and antioxidants are involved in some relevant physiological functions such as milk yield and therefore it might be beneficial to supplement cows with antioxidants (Celi, 2011b). Yerba Mate (YM; *Ilex paraguariensis*) exerts antioxidant activity due to its content of several compounds such as polyphenols (Heck and De Mejia 2007). In a previous study we were able to observe that YM supplementation during mid lactation sustains milk yield when cows are fed with maize silage (Celi and Raadsma, 2010). Therefore, the objective of this study was to evaluate the effects of supplementation of the diets of dairy cows YM during the dry period on their milk yield and oxidative status.

Materials and methods In February, a total of 37 pregnant Holstein-Friesian cows of mixed parity (1-7), homogenous for age (6.5 ±1.6 years), body condition score (BCS; 3.3 ±0.2 and liveweight (LW; 680 ±60kg) were enrolled in the study. Cows were fed a typical Australian pasture-based diet (control diet: pasture plus concentrates at milking). Cows were randomly allocated to the three following dietary treatments: Control diet (n = 9), YM 250 group (n = 14): control diet + 250 g/cow.day YM and YM 500 (n=14): control diet + 500 g/cow.day YM. Cows received the dietary treatments once daily for a total of 4 weeks before calving. Because of the limited pasture availability during the trial, maize (*Zea mays*) silage was supplemented to the cows (5 kg DM/cow.day) after the afternoon milking; the cows were fed the silage on a feed pad as a group. The cows received their concentrate (6 kg/cow.day) allocation twice daily at milking. Blood samples were taken from all cows, before the start of the administration of the dietary treatments and on monthly intervals from February till July; LW and BCS were also monitored on these occasions. Blood samples were centrifuged and plasma was analysed for, advanced oxidative protein products (AOPP; Witko-Sarsat *et al*. 1998), Glutathione peroxidase (GSH-Px; Cayman, Item No. 703102, Sapphire, NSW, Australia), reactive oxygen metabolites (ROMs) and biological antioxidant potential (BAP; Diacron International, Grosseto, Italy). The degree of oxidative stress was estimated by the ratio of (ROMs/BAP)*100 given that the relationship between the level of oxidative stress and pathology is higher when ROMs and BAP measurements are so combined (Celi, 2011a). Cows were milked 2 times daily and milk yields were recorded daily for individual cows.

Statistical analyses The effects of time, treatment and their interaction were analysed for all measured variables by a repeated measures ANOVA in GenStat v12 (VSN International Ltd, Hertfordshire, UK).

Results Overall, daily milk yield averaged 28 ± 0.4, 29 ± 0.5 and 30 ± 0.5 L/cow/day in the control, YM 250g and YM 500 g groups respectively. A significant effect of the interaction time of sampling × diet ($P<0.01$) was noted on milk yield with the YM 500g cows having higher yields that the Control ones in August and September. Plasma concentrations of ROMs, BAP, AOPP and GSH-Px were not affected by Yerba Mate supplementation, however a significant effect of time ($P<0.001$) was noted. Both ROMs and AOPP concentrations significantly increased over time. A significant effect of the interaction time of sampling × diet ($P<0.05$) was noted on oxidative stress index, with both YM groups presenting significantly lower levels that the Control ones in April and June.

Conclusion. This study indicates that supplementation of dairy cows' diet with YM during dry period seems to improve milk yield; however, as the oxidative status of the cows was mildly affected by YM supplementation, further studies are required to investigate the mechanisms by which YM affects milk production. The observation that AOPP concentration significantly increased after the cows were fed maize silage is highly relevant as high level of AOPP could indicate the presence of inflammatory process which can compromise the correct embryonic development in dairy cows (Celi *et al*., 2011).

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A meta-analysis of the responses to non-protein nitrogen supplementation by cattle grazing native pastures in the seasonally dry tropics

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Introduction In the seasonally dry tropics the nutritive value of senesced dry season pastures is usually low. The diet selected by grazing cattle is often < 0.50 dry matter digestibility and < 50 g crude protein/kg. Cattle grazing such pastures often lose liveweight (LW), but LW is often improved substantially by non-protein nitrogen (NPN) or protein meal supplements. Both the magnitude of the responses of cattle to NPN supplements, and the carryover effects, are clearly important for optimal herd management.

Materials and methods Information was collated on the LW responses of tropically adapted grazing *Bos indicus* cross cattle to NPN supplements given during the dry season in the seasonally dry tropics of northern Australia. Experiments had been conducted in the Speargrass and Mitchell native grass pasture systems and with moderate stocking rates so that pasture availability was not expected to constrain voluntary intake (McLennan *et al*. 1981; Foster and Bligh 1984; Winks 1984; Dixon 2008). Treatments were replicated in all experiments and included unsupplemented (control) and NPN supplemented groups of cattle. Responses to the NPN supplement were measured in 37 cohorts, each representing an independent group of cattle except that where 2 age groups of cattle grazed together they were considered separate cohorts. Measurements were made at 5 sites between 1968 and 2004, but 20 of the cohorts were measured at one site 100 km SSE of Townsville. NPN supplements were fed for on average for 5.3 (s.d. 1.8) months and were provided as molasses-urea mixes fed via roller drums, or as urea-based loose mixes also containing salt and ammonium sulphate. In some experiments (29 cohorts) the cattle continued to graze without supplements through the subsequent wet season and LW was measured to determine the carryover effects of the NPN supplementation following compensatory growth.

Results Mean LW of the various cohorts of cattle at the commencement of supplementation averaged 248 (s.d. 74) kg. LW change of unsupplemented control cattle during the dry season was on average -0.08 (s.d. 0.20) kg/day, equivalent to a LW change over the entire dry season of -9 (s.d. 38) kg. On average the NPN supplements improved LW change by 0.13 (s.d. 0.068) kg/day, equivalent to 20 kg LW over the entire dry season. The improvement in LW ranged up to about 0.25 kg/day and was generally related to the severity of the dry season. On average 68% of the improved LW due to NPN supplements was retained at the end of the subsequent wet season due to compensatory growth effects in some cohorts (Figure 1). Compensatory growth ranged between cohorts from nil to complete compensation. There was high variation among cohorts and between years in both the response of cattle to NPN supplements and the proportion of the improved LW which was retained through the following wet season.

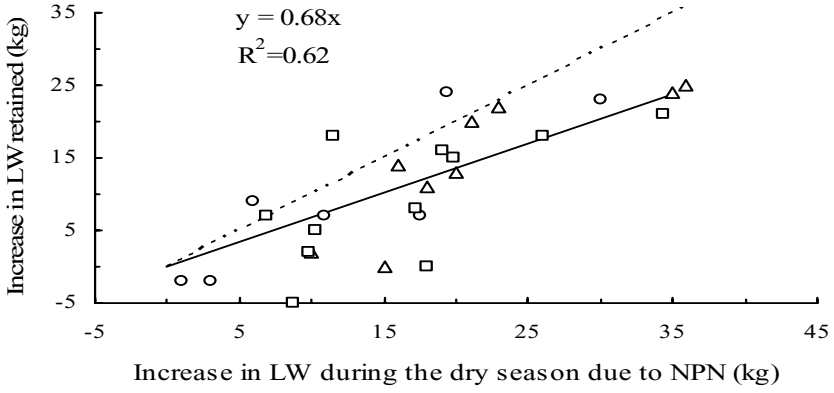

Figure 1 The relationship between the increased LW due to supplementary feeding of NPN supplements during the dry season retained at the end of the subsequent wet season, and the increased LW due to the supplements at the end of the dry season. The cohorts of animals were initially weaners (o), one year old (Δ) or two year old (□). The relationship Y = X is shown as (-------)

Conclusions NPN supplements can improve dry season cattle performance in the seasonally dry tropics in some circumstances, but compensatory growth during the subsequent wet season may reduce the LW benefit. The greatest benefits of NPN supplementation are likely to be to reduce late dry season mortality and to achieve earlier reconception of breeder cows in the wet season. There may be benefits for young cattle where higher LW in the late dry season increases the value of the animal for a specific market or through improved meat quality.

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The relationship between the concentration of phosphorus in the diet and in faeces of cattle grazing tropical grass and grass-legume pastures

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Introduction Phosphorus (P) is a limiting nutrient for cattle production in many grazing systems. P deficiency is common in the tropical rangelands of Africa, South America and northern Australia. Evaluation of the dietary P status of grazing cattle depends on estimation of the P concentration in selected forages, but this is difficult to measure directly. A procedure to estimate diet P concentration from faecal samples would provide opportunity to improve management of P nutrition in grazing cattle.

Materials and methods Data from 5 studies with *Bos indicus* cross cattle in northern Australia were collated. At site 1 (Springmount, Mareeba; Hendricksen *et al.,* 1994; Miller *et al.,* 1998) growing and reproducing cattle grazed native grass or native grass - *Stylosantheses* spp pastures. At site 2 (Lansdown, Townsville; Ternouth *et al.,* 1996, Ternouth and Coates, 1997) growing and reproducing cattle grazed native grass - *Stylosantheses* spp or *Urochloa* - *Stylosantheses* spp pastures. At site 3 (Narayen, Mundubbera; McLean and Ternouth, 1994) growing cattle grazed *Cenchrus ciliarus* - *Macroptilium atropurpureum* pastures. In each study the concentration of P in the diet and diet dry matter (DM) digestibility were measured using oesophageally fistulated animals and P tracers to correct for salivary P contamination of dietary extrusa. Faecal P concentration and faecal DM output, and hence intake of DM and P, were measured in resident cattle using slow-release rumen chromic oxide capsules.

Results There was a linear relationship (Figure 1) between the concentration of P in the faeces and in the diet. Thus the concentration of P (g/kg) in the diet could be estimated as: [Diet P] = 0.514 [Faecal P] - 0.12 (n = 63, RSD = 0.205). This close relationship indicates that the concentration of P in faeces can be used to estimate the concentration of P in the diet selected by cattle grazing tropical pastures. However, an important limitation was that only a limited range of sites and pastures were examined. It is not known how closely these results represent the variety of pasture systems across tropical rangelands, or of rangelands in other environments. Furthermore the results presented were for grazing cattle not fed P supplements; limited evidence from other treatments in the same experiments where P supplements were fed suggested that the same relationship would not apply in cattle ingesting P supplements.

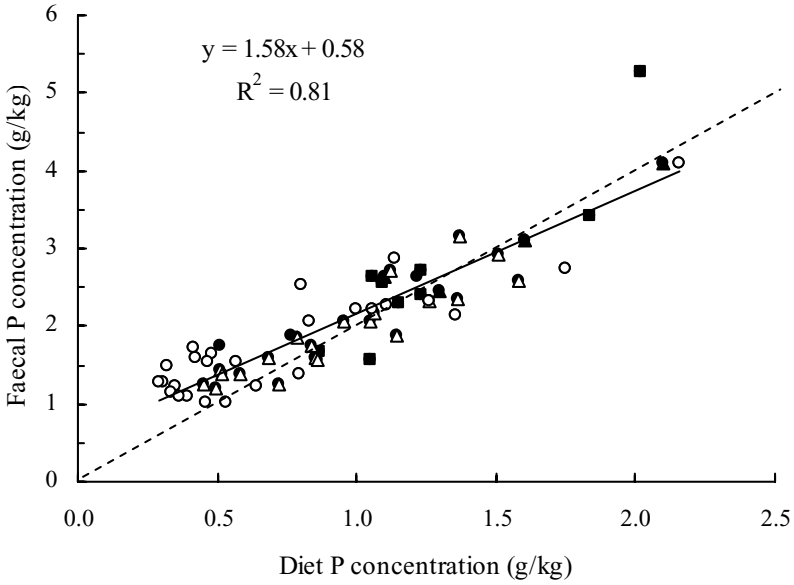

Figure 1 The relationship between phosphorus (P) concentration in faeces and P concentration in the diet of grazing cattle ingesting tropical forage diets (n = 63). Results are from growing animals at site 1 (●), site 2 (▲) and site 3 (■), or of reproducing cows at site 1 (○) and at site 2 (Δ). The relationship Y = 2X is shown as (-------)

Conclusions The concentration of P in the faeces of cattle grazing tropical pastures can be used to estimate the concentration of P in the diet selected providing P supplements are not being fed. These observations are comparable with previous reports of correlations between the concentration of P in the diet and in the faeces of cattle ingesting forage diets. Estimation of diet P concentration from faecal P concentration, in conjunction with estimation of diet digestibility and nitrogen concentration by faecal near infrared spectroscopy, is being used to improve management of P nutrition of cattle grazing in extensive rangelands in northern Australia.

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Effects of fat to protein ratio in early lactation on subsequent milk yield, milk lactose, urea nitrogen, citric acid and somatic cell count of dairy cows

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Introduction Dairy cows are in a state of negative energy balance (NEB) during the early lactation. Negative energy balance may supress immune system and increase risk of disease such as ketosis and mastitis. The degree of NEB is critical for productivity and health status. Monitoring of NEB at individual cow level is diffucult under field conditions. On the otherhand, milk composition analysis has been regular done in most dairy herds. It has been shown that the fat to protein ratio (FPratio) in milk may be used as a indicator of energy balance (Heuer *et al.* 2004). The FPratio higher than 1.5 can be accepted as a good predictor of ketosis (Buttchereit *et al.* 2010). There are few studies about effect of ketosis in early lactation on subsequent milk production and composition (van Straten, *et al.* 2009). Therefore, the objective of the present study was to investigate the effect of early lactation FPratio on subsequent months milk yield, milk lactose, urea nitrogen, citric acid and somatic cell count (SCC).

Materials and methods Forty-nine Holstein-Friesian cows (parity 2, days in milk 31±7.5, 25.6±5.92 kg/d) were observed for a total period of 10 months between June 2009 and March 2010. The animals were housed in a free-stall barn and fed diets as total mixed ration (TMR) ad libutum throughout lactation. The components of the TMR were maize silage, alfalfa hay, and home blend concentrate. Phase feeding was applied by keeping cows in three lactation stages (calving to month 1, month 2 to month 5, and month 6 to dry). The proportion of concentrate in the TMRs, averaged over all, was 570 g/kg DM ranging from 540 to 600 g/kg DM. The crude protein and net energy lactation (NE_L) contents of the TMRs varied from 160 to 173 g crude protein/kg DM and 6.86 to 8.0 MJ/kg of DM. The cows were milked three times daily at 0400, 1500 and 2300 h, and individual milk yields were recorded. Individual milk samples were collected at afternoon milkings monthly. Somatic cell count was measured (CellCount, DeLaval, Sweden) in fresh milk. Milk samples were preserved by bronopol (2-bromo-2-nitropopane-1,3-diol) at 4°C pending analysis. Milk fat, protein, lactose, urea nitrogen (MUN), citric acid contents were determined by infrared analysis (Milkoscan FT 120, Foss Electric, Hillerød, Denmark). The FPratio in first 2 months after calving were averaged indivually, and 2 FPratio groups (FPR< 1.5 and FPR≥ 1.5) were performed. Because of non-normality of the distribution of SCC, normality was approximated by using the natural logarithm to the SCC value in statistical analyses. Data were analysed using the PROC MIXED of SAS (2004). The model included the fixed effect of the FPratio group, month (M), the FPratio group by month interaction (FPratio x M) and random effects of cow nested within month. The effect of FPratio x M was removed from the statistical model, because it was not significant. Values were reported as least square means.

Results Daily milk yield and cumulative milk yield (sum of 8 months) were not different (P> 0.05) for the FPratio groups (Table 1). Milk composition and SCC were also similar (P> 0.05) for the FPratio groups.

Table 1 Effect of fat to protein ratio in early lactation on subsequent milk production, composition and quality

	FPratio			
Item	<1.5	≥1.5	SED	*P-value*
Milk yield, kg/d	29.17	28.88	1.254	0.814
Cumulative milk yield, kg/cow	7637.17	7202.25	646.05	0.504
Lactose, %	4.63	4.61	0.034	0.687
Milk urea nitrogen, mmol/l	6.50	6.62	0.122	0.350
Citric acid, mmol/l	8.22	8.23	0.190	0.969
SCC, x 1000/ml	308□75	287.08	101.76	0.123

Conclusions The results of the present study demonstrate that FPratio in early lactation did not effect subsuquent milk production, composition and quality. Further investigation of the relationship between ketosis and milk meaures is needed on a larger data set.

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The effects of essential oils of thyme oleaster and orange peel on milk fatty acid composition in dairy cows

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Introduction Essential oils (EO) have been suggested as a potential means to manipulate bacterial populations involved in ruminal biohydrogenation to modify the fatty acid composition of ruminant-derived food products such as milk and meat (Jenkins *et al*.,2008). The fatty acids have been shown in several animal studies to contribute to cancer prevention, decreased atherosclerosis, improved immune response, and altered energy metabolism (Banchar., 2009). This study aimed at investigating the effects of thyme, oleaster and orange peel oils on milk fatty acid composition.

Materials and Methods Twenty eighth lactating Holstein cows having 501±9.12 kg BW, 25.19±0.73 kg milk yield, 68.1±5.8 DIM, 2.99±0.06 BCS and 2.03±0.19 lactation number were used. Cows were allocated into four experiment groups and housed in individual stall barn. During 28 days period cows fed twice daily (08:00 and 16:00 h) for *ad libitum* intake a TMR (%90.8 DM,15.5% CP, 31.54%, NDF, %22.68 ADF, %2.3 EE) consisting of alfalfa hay (40%) and concentrated (%60). TMR was formulated as isocaloric and isonitrogenic. Treatments were as follow: control (no additive), or supplemented with thyme (108 ppm; THY), oleaster (108 ppm; OLE), or orange peel oil (108 ppm; ORG). Individual milk samples were collected twice weekly for fatty acid analyses and milk yield was recorded each milking. Fatty acid profiles of fat extracted from milk samples were determined by gas chromatography (GC) of methyl esters (AOAC, 1990). Data were analyzed using the GLM procedure of SAS.

Results The results showed that dietary supplemental essential oils of thyme ,orange peel or oleaster , had no significant effects on milk fatty acid profile except total omega-3. Essential oils decreased total of omega-3 compared to control (Tables I).

Table1 Effects of essential oils of thyme oleaster or orange peel oil on feed intake, milk yield and milk fatty acid composition (g/100 g of total fatty acids) in lactating dairy cows.

	CON	ORG	THY	OLE	SEM	P
DM intake kg/d	21.66	23.47	23.56	22.03	0.69	0.14
Milk yield , kg/d	23.05	22.97	23.31	23.98	0.99	0.88
Fatty acid composition						
Total	94.80	94.87	94.52	94.55	0.15	0.28
Unknown (g/100 g of total fatty acids)	5.57	5.73	7.41	5.83	0.59	0.13
Omega-3 (g/100 g of total fatty acids)	0.61	0.52	0.53	0.47	0.02	0.01
Omega-6 (g/100 g of total fatty acids)	4.24	4.29	4.24	4.09	0.24	0.94
Omega-9 (g/100 g of total fatty acids)	28.77	28.15	29.62	28.61	1.18	0.85
PUFA (g/100 g of total fatty acids)	36.61	35.92	37.40	36.19	1.28	0.86
MONO (g/100 g of total fatty acids)	32.07	31.28	32.81	31.76	1.22	0.84

Conclusions The results revealed that thyme, oleaster and orange peel essential oils did not have the potential to alter ruminal biohydrogenation process for improving product quality and modify fatty acid profile of milk when dairy cows fed with TMR containing 108 ppm thyme, 108 ppm oleaster or 108 ppm orange peel essential oils. Further investigations are needed to determine the factors (dose, active components, concentrate roughage ratio etc.) that limiting or encouraging the effects of these essential oils on ruminal microbial populations involved in the biohydrogenation processes of unsaturated fatty acids.

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Anionic salt supplementation and intra-rumen delivery of 25OHD increase urinary calcium excretion in steers fed a forage diet

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Introduction Hypocalcemia in clinical (milk fever) or subclinical form is a common malady after calving in dairy cows. An increase in urinary Ca excretion occurs when excess ionised Ca enters the extracellular pool (Kurosaki *et al*., 2007). Increased urinary Ca prior to calving is a direct indication that Ca homeostatic mechanisms have been initiated, permitting greater resistance to a sudden fall in plasma Ca at calving. Diets that are anionic (dietary cation-anion difference (DCAD) is negative) increase intestinal absorption of Ca (Horst, 1986). Negative DCAD results in a mild metabolic acidosis, which increases the sensitivity of small intestinal Vitamin D receptors (Goff *et al*., 1991). The combination of anionic salts and elevated levels of available 25-hydroxy vitamin D (25OHD) in plasma may activate Ca mobilisation from the small intestine more effectively than anionic salts alone. This study was designed to determine if intra-rumen supplementation via slow release boluses containing 25OHD would increase urinary Ca excretion in steers fed positive or negative DCAD diets**.**

Materials and methods Crossbred beef steers (n=18; 395kg ±SD 29, with rumen fistulae) were allocated to two dietary treatments, DCAD +150 and DCAD -120 mEq/kg. DCAD was manipulated in a forage based diet by addition of $MgCl_2$. Steers were allocated standard commercial monensin slow-release rumen boluses that contained either monensin (DCAD+150-D) or monensin and 45.6mg of 25OHD (DCAD+150+D). Boluses were allocated in a randomised double blind design. Daily Ca excretion rates were determined from the ratio of Ca to creatinine in 'spot' urine samples combined with a prediction of daily creatinine excretion rate based on individual live weight and stage of maturity. Blood samples were taken at two separate times to correspond with bolus degradation speed. A regression analysis was performed on urine Ca and plasma 25OHD. Urine Ca excretion rates of steers in DCAD-120-D and DCAD+150-D treatments were compared by AOV.

Results Urine Ca excretion was higher ($P<0.05$) in steers in treatment DCAD-120-D than in DCAD+150-D (4.6 ±SE 1.5, *versus* 0.3 ±SE 0.1 g/d), but there was no change in Ca excretion with increasing levels of plasma 25OHD. In the presence of negative DCAD, however, urine Ca excretion rate was positively associated with 25OHD in plasma (Figure 1).

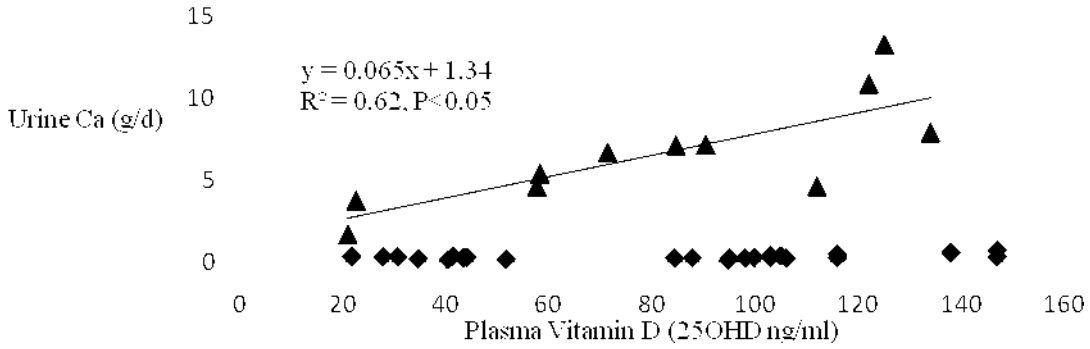

Figure 1 Urinary Ca excretion in steers was increased by a combination of supplementary anionic salts and 25OHD. DCAD -120 (▲), DCAD +150 (♦).

Conclusion Intra-rumen supplementation of 25OHD and negative DCAD resulted in higher Ca excretion than negative DCAD alone. 25OHD in plasma, at about double endogenous levels, was only associated with increased urinary Ca excretion when DCAD was negative. The interaction between 25OHD and negative DCAD may be a consequence of an increased Vitamin D receptor sensitivity in the small intestine during mild metabolic acidosis and increased activity as a result of increased 25OHD availability, with a consequent improvement in dietary Ca digestibility. Further research using preparturient dairy cows may confirm the usefulness of both 25OHD and anionic salts **i**n preventing clinical and subclinical hypocalcemia.

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Responses of pregnant dairy goats to feed restriction

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Introduction Maternal nutrient deficiencies during critical times of pregnancy can permanently affect development of fetal tissues especially in the last weeks before delivery when occurs about 70% of fetal growth. Therefore, significant adjustments in maternal body are necessary and partition of nutrients may favor fetal growth rather than the requirements of pregnant females, which use their body reserves in order to maintain adequate supply of nutrients (Charismiadou *et al*. 1999). Goats have some peculiarities such as a thin deposition of subcutaneous fat and their main adipose depot is the abdominal, consequently it is the first to be mobilized. Moreover, in the context of goat production there are little information regarding the growth dynamics of pregnant uterus, and thus this data has been extrapolated from other species. The aim of this study was to evaluate the body changes and the growth of the gravid uterus and mammary gland of pregnant dairy goats subjected to feed restriction.

Materials and methods A total of 58 dairy goats were used (50.1 ± 8.6 kg BW) in a randomized block design in factorial 3x3x2, with three gestational age (80, 110 and 140 days of pregnancy), two breeds (Oberhasli and Saanen) and three levels of feed restriction (0%, 20%, 40%). The animals were fed *ad libitum* with a diet formulated to meet their requirements, according to NRC (2006). After confirmation of twin pregnancy, the goats of each bred were assigned to the treatments (gestational age and feed restriction). When the goats reached the specific gestational age they were slaughtered and then there was the withdrawal of the pregnant uterus, mammary gland and all organs and structures. The entire gravid uterus was weighed and then separated into an empty uterus, placenta, placentomes, placental fluid and fetuses, which were weighed separately. These components were ground and samples were freeze-dried. The mammary gland was weighed after removing the skin. The body of each animal was separated into carcass, digestive tract, abdominal fat and blood. In the samples from the fetus, uterus and mammary gland were determined the content of dry matter, ash and fat, according to AOAC (1995) and protein content was obtained by Dumas combustion method (LECO FP-528 LC). The energy content was estimated using the formula predicted by ARC (1980), Energy (Kcal) = 5.6405* protein (kg) + 9.3929 * fat (kg). The results of body changes and the development of the mammary gland, gravid uterus and its components were analyzed in a randomized block design in a 3x3x2 factorial, using the MIXED procedure of SAS (2006). The model included breed, gestational age, feed restriction, breed x gestational age, breed x feed restriction, gestational age x feed restriction and breed x gestational age x feed restriction as fixed effects and feed restriction within breed and gestational age as random effect. The interactions between gestational age and other factors were examined at each stage of pregnancy, using the slice option of MIXED procedure. Once satisfied the assumptions of normality and homogeneity of variances, data were subjected to analysis of variance and polynomial regression for the levels of feed restrictions and gestational age. The means were evaluated by Tukey's test. The correlation coefficients were obtained using the Person CORR (SAS, 2006).

Results Oberhasli fetuses were heavier than those from Saanen goats ($P < 0.05$). Feed restriction did not affect the growth of the gravid uterus and mammary gland. Placentomes and placental fluid weight varied quadratically with advances in gestational age from 80 to 140 days of pregnancy ($P < 0.05$). Nutrient deposition in the fetus, pregnant uterus and fetus weight increased exponentially throughout pregnancy. Likewise mammary gland weight increased exponentially, however its nutrient deposition increased linearly during pregnancy. Abdominal adipose depots, lungs, diaphragm and stomach weight decreased at the end of pregnancy ($P < 0.05$). Goats subjected to feed restriction showed lighter carcass as well as diaphragm and abdominal adipose depots

Conclusions Feed restriction does not change the development of the pregnancy, because goats mobilize body reserves, specially the abdominal fat depots, to meet their nutrient requirement for pregnancy.

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In vitro studies on the effects of grass silages containing < 50% of crude protein as true protein on up to now not identified substances in bovine ruminal fluid

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Introduction Presumably grass silages that contain < 50% of crude protein (CP) as true protein (TP) caused a disease called "factorial disease of dairy herds" (suspected silages). Problems that occurred in dairy farms after feeding these suspected silages were described as follows: high incidences of cases with a high milk cell count, disturbances of fertility, digestive disorders, abomasal displacements, downer cow syndrome up to sudden death (Eicken 2005). Because of varying protein composition (true protein contingent, free amino acids) in the grass silages of these farms, especially differences in the peptide fractions in rumen fluid after feeding these silages were supposed and analytical procedures adapted accordingly. The object of the present study was to investigate if there are differences in the content of rumen fluid after fermenting suspected grass silages compared to control grass silages (silages, that did not cause clinical problems in dairy cows and contained > 50% of crude protein as true protein) using the *in vitro* system RUSITEC (RUmen SImulation TEChnique).

Materials and methods Sickness causing silages (n=10) as well as control silages (n=7) were previously fermented *in vitro* using RUSITEC. A 28 days lasting test period started with an adaptation period (9 days) fermenting hay (10.5 g DM/d), followed by silage input (10.5 g DM/d) for ten days and ended with a recovering period (9 days) fermenting hay again. A constant amount of 3.4 g of a concentrate (18.0 % CP, 3.5 % crude fat, 9.5 % crude fibre, 6.0 % crude ash) was given every day. Six RUSITEC runs per silage were performed. Fermenter samples were taken on days 7-13, 16-21, 26-28 once a day before adding grass silage and analysed by RP-HPLC, which belongs to the standard methods of peptide analytics. Single substances were further examined via LC-MS and MS/MS. This tool enables a characterisation of these substances by specific mass to charge (m/z) ratios and MS/MS fractions, respectively. Additionally, all tested grass silages were analysed by RP-HPLC to compare these results with those of the fermenter samples. For statistical analyses the computer program SPSS-PC Inc Chicago® was used. Differences between the effects of suspected and control silages throughout the period of silage input were determined by *t*-test.

Results Six substances (1-6) were detectable in fermenter samples by RP-HPLC. Fermenting suspected silages led to increased quantities of substances 1-3 and 5 compared to the control silages (Figure 1). There were no differences (P>0.05) for substance 4 after the fermentation of silages with different true protein contingent. For interpretation of diagram 6 in Figure 1 it is important to know, that the amounts of substance 6 stayed the same after fermenting suspected silages, whereas quantities decreased after control silage input. None of these substances were detectable in extracts of original silages. By LC-MS and MS/MS analytics of substance 1 containing fractions this substance could be characterized via detecting molecules with following m/z ratios: 249, 295, 313, 348 and 353. Currently, further research on the identification of these substances is carried out.

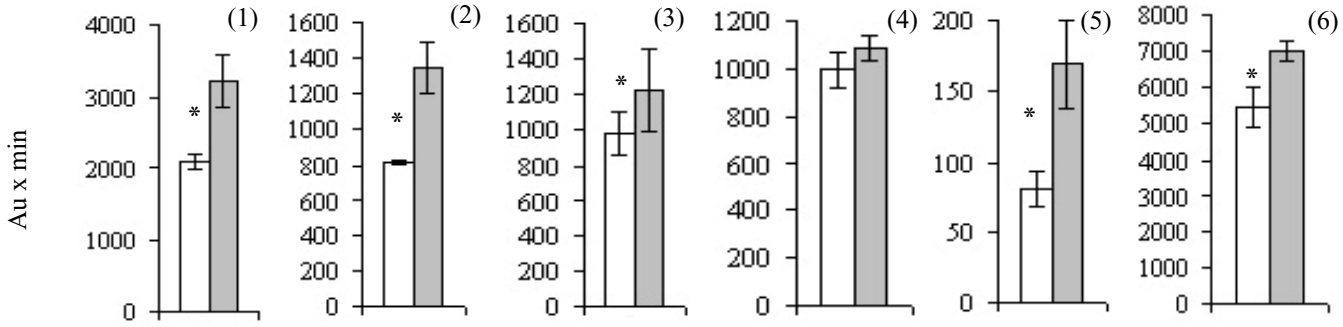

Figure 1 Means of substances 1, 2, 3, 4, 5 and 6 in RUSITEC fermenter fluid during supplementation with control (▭) and suspected (▭) grass silages. Because an identification was impossible so far, areas under the curve (AU x min) instead of concentrations are compared. Values are means ± SEM. (* = P<0.05)

Conclusions Results of this study indicate that fermentation of silages that contain < 50% of crude protein as true protein led to increased quantities of substances 1, 2, 3 and 5 compared to control silages in bovine ruminal fluid. As these substances were not detectable in extracts of original silages, it is assumed that they were formed during ruminal fermentation. Because of the fact that these substances could not be identified so far, no definitive statement about their meaning is possible. Identification of these agents could be important for further diagnostics and therapy of this disease in future.

Acknowledgement We thank Milchförderungsfonds Hannover-Braunschweig for financial support.

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In vitro studies on the effects of grass silages containing < 50% of crude protein as true protein on carbohydrate metabolism in bovine ruminal fluid

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Introduction For twenty-five years, a non-infectious disease that caused high economic losses has been observed in North German Holstein Friesian dairy herds ("factorial disease of dairy herds", Eicken 2005). The major constituent of forage that was fed in these cattle herds were grass silages (suspected silages) that contained < 50% of crude protein (CP) as true protein (TP). When these grass silages were not fed anymore the dairy cows recovered. Because of that a nutritive aetiology was assumed to exist. The object of the present study was to investigate if the fermentation of suspected grass silages in the *in vitro* system RUSITEC (RUmen SImulation TEChnique; Czerkawski a. Breckenridge 1977) leads to differences in carbohydrate metabolism in bovine ruminal fluid compared to the fermentation of control grass silages (silages, that did not cause clinical problems in dairy cows and contained > 50% of CP as TP).

Materials and methods Ten suspected grass and seven control grass silages were tested using RUSITEC. A 28 days lasting test period started with an adaptation period (9 days) during that hay was added to the fermenters (10.5 g DM/d). The adaption period was followed by silage input (10.5 g DM/d) for ten days and ended with a recovering period (9 days) throughout that hay was fermented again. A constant amount of 3.4 g of a concentrate (18.0 % CP, 3.5 % crude fat, 9.5 % crude fibre, 6.0 % crude ash) was given every day. Six RUSITEC runs per silage were performed and samples of ruminal fluid taken once a day to determine the production of volatile fatty acids (VFA; acetate C2; propionate C3; n-butyrate nC4; n-valerate nC5; caproate C6; i-butyrate iC4; i-valerate iC5) using gas-chromatography. For statistical analyses the computer program SPSS-PC Inc Chicago® was used. Differences between the effects of suspected and control silages throughout the period of silage input were determined by *t*-test.

Results (Figure 1) The production of nC4 (4% to 40%), nC5 (16% to 73%), and C6 (18% to 139%) increased when grass silages with TP contingent < 50% compared to control silages were added. The impact of suspected grass silages on C2 and C3 production was not significant (-2% to +14% and -5% to +25%, respectively). In contrast, feeding these silages strongly increased branched-chain volatile fatty acid (BCVFA) production (iC4: 14% to 65%; iC5: 4% to 97%), whereas total VFA production was not affected (1% to 28%, P>0.05).

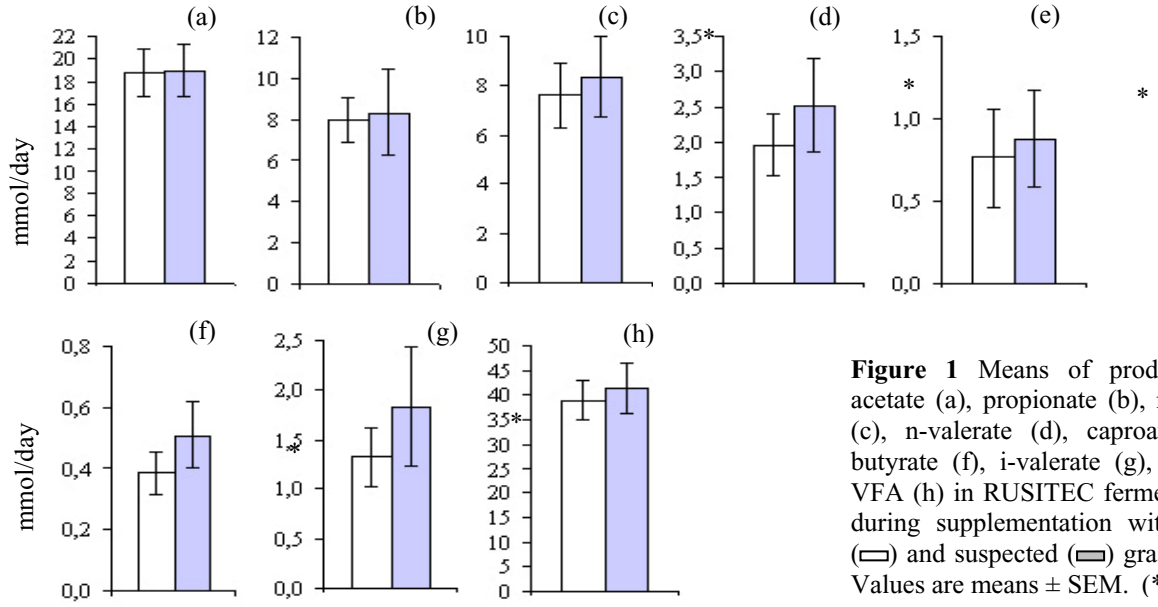

Figure 1 Means of production of acetate (a), propionate (b), n-butyrate (c), n-valerate (d), caproate (e), i-butyrate (f), i-valerate (g), and total VFA (h) in RUSITEC fermenter fluid during supplementation with control (▭) and suspected (▬) grass silages. Values are means ± SEM. (*=P<0.05)

Conclusions These results indicate that grass silages with TP contingent < 50% did not have the ability to disarrange ruminal carbohydrate metabolism *in vitro*. The increases of nC4, nC5, and C6 resulted most likely from higher activity of *Megasphaera elsdenii*. Altogether, the effects on carbohydrate metabolism were of moderate extend and were too low to induce clinical symptoms but increased BCVFA production indicate that ruminal amino acid metabolism was affected by feeding grass silages with TP contingent < 50%.

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The hedonic value of food diversity in sheep is not only a matter of choice

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Introduction Food diversity is attractive for ruminants as when they have choice opportunities they actively select mixed diets (Duncan A. *et al.*, 2003), and often increase their intake on the short-term. Food diversity is supposed to induce positive effects by allowing each individual to meet its nutritional requirements and to maintain optimal ruminal conditions; it is also supposed to have a hedonic value. We studied the hedonic value of food diversity on the short-term, and investigated whether diversity is still attractive for animals on the long term.

Materials and methods Our experiment involved rumen fistulated sheep (n=6 in the first trial and n=12 in the second trial) housed in individual pens. It also involved two hays: a lucerne hay (L; NDF = 531 and CP = 156 g/kg DM) and a mixed grass hay (G; NDF = 667 and CP = 72 g/kg DM). In the first trial, either G or L was consumed orally *ad libitum* (o) 6 hours daily, while the same or the other hay was introduced into the rumen (r) in an amount equal to the amount eaten. Consequently, Go/Lr and Lo/Gr treatments involved different sensory characteristics but similar post-ingestive consequences (Favreau *et al.*, 2010). All treatments (Go/Gr, Go/Lr, Lo/Gr, Lo/Lr) were tested according to a Latin square design. Short-term choice tests (5 min) were performed daily just after the food withdrawal, and sheep behaviour was recorded every 15 sec to assess the relative preference for the hays as the proportion of total scans devoted to ingestive behaviour. These daily data were averaged individually for each experimental period (7 days), and then analysed with a Student's t-test in order to compare them with the theoretical proportion of non-choice. In the second trial, sheep were offered *ad libitum* the two hays simultaneously, 6 hours per day, during 28 days. Their relative preferences were daily assessed as the proportion of total intake, and analysed with the Mixed procedure of SAS using the Repeated statement to account for a potential day effect.

Results In the short-term choice tests of the first trial, sheep preferred to eat the hay that was not orally consumed during the preceding 6 hours, whatever the nature of both the hay previously eaten and the one introduced into the rumen (Table 1).
In the long-term choice trial, sheep expressed a partial preference for L hay on the first day (0.74 ± 0.3) but this preference increased with time to reach 0.96 ± 0.2 at the end ($p < 0.0001$; Figure 1), including many animals that ingested the L hay only.

Table 1 First trial. Choice of sheep (n=6) for diversity (mean ± s.e.), i.e. for the hay that was not orally consumed before choice tests, depending on whether grass (G) or lucerne (L) hay was orally consumed (o) and introduced into the rumen (r)

Feeding regime	Go/Gr	Go/Lr	Lo/Gr	Lo/Lr
Choice for diversity	Choice for L		Choice for G	
Mean	0.86	0.82	0.69	0.71
standard error	0.06	0.08	0.06	0.07
comparison with 0.5 [1]	**	**	*	**

[1] Comparison with the theoretical 0.5 proportion of non-choice with Student's t-test

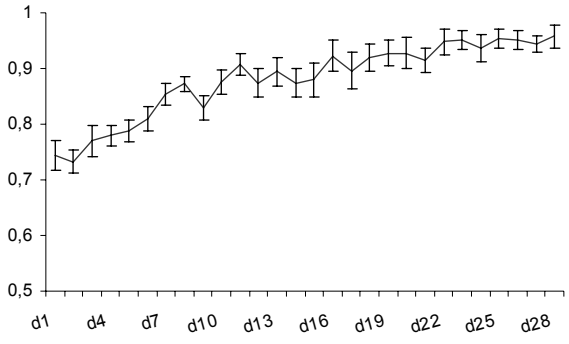

Figure 1 Second trial. Choice of sheep (n=12) for lucerne hay (mean ± s.e.) when offered in choice with mixed grass hay for 28 days

Conclusions On the short-term, sheep always looked for food diversity whatever the post-ingestive consequences they felt. This means that food diversity can induce positive effects on animals because of a hedonic value *per se*, and not just because of post-ingestive benefits. In return, animals did not appear to look for diversified diets on a longer term. This could be partly explained by the good quality of the lucerne hay that could meet all individuals' metabolic and digestive requirements by itself. Sheep may also have considered their feeding situation as monotonous as they were always offered the same hays for several days. Thus, the hedonic value of food diversity would rely more on temporal changes in foods and so on breaking of monotony than on simultaneous variety.

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Differences in the anthelmintic activity of sainfoin (*Onobrychis viciifoliae*) depend on the varieties : possible relationships with the flavonoid composition

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Introduction Gastrointestinal nematodes remain a major constraint on the health and production of ruminants. Over the last 50 years, the usual control of this parasitism relied on the repeated use of chemical anthelmintics (AHs). However these treatments are nowadays facing several limits, the most important being the increasing development and widespread diffusion of resistance to AHs within worm populations. The need to find complementary or alternative solutions is becoming urgent. Amongst those, the possible exploitation of the AH properties of some bioactive plants, like chicory (*Cichorium intybus*) (Marley *et al*, 2003) or tannin containing forage legumes (Hoste *et al*, 2006), has been shown to affect the nematode biology and thus, to reduce the reliance on chemical AHs. However, one of the main difficulties to use these plants as nutraceuticals relates to variations in efficacy because of variations in content of the suspected active compounds. By using sainfoin (*Onobrychis viciifoliae*) as a model of tannin-containing legume, the main objectives of this study were **i)** to examine the influence of genetic factors on the AH properties of sainfoin and **ii)** to analyse whether some differences in phenolic compounds and quality of condensed tannins (CTs) might relate with these variations.

Materials and methods Forty sainfoin varieties were grown in the same place (NIAB, Cambridge, UK) and collected at the same time of the year. From these 40 samples, after freeze-drying processes, were used: **i/** 38 methanolic extracts, and **ii**/ 14 acetonic extracts of divergent biochemical characterisation for proanthocyanidin contents. The samples were compared for their AH activity relying on *an in vitro* assay. Measurements of total tannins (TT), total phenols (TP) and biological activity by the radial diffusion (RD) method were performed on both types of samples. The quantification of phenolic compounds in the methanolic extracts was performed using an HPLC method (Regos and Treutter, 2010). For the 14 acetonic extracts, the condensed tannins were analysed by direct thiolysis of freeze-dried samples (Gea et al, 2011) to obtain quantitative and qualitative (mDP, cis:trans ratios, Prodelphinidin (PDs) /procyanidin (PCs) ratios) information. The *in vitro* AH activity of both methanolic and acetonic extracts was measured by use of the larval exsheathment inhibition assay (LEIA) on *Haemonchus contortus* larvae. Statistical comparisons to control values for the LEIA rates depending on two factors, i.e. the treatment and time were assessed through a general linear model (GLM) procedure. Pearson's coefficients of correlations were calculated to measure the relation between the LEIA values at 60 minutes and the measurements of the different biochemical compounds for both the methanolic (df = 36) and acetonic (df = 14) extracts. Last, two multivariate Principal Component Analyses (PCA) were performed to obtain a synthetic description of the relationships between the AH effects of the methanolic and/or acetonic extracts and their respective content of either phenolic compounds or characteristics of CTs.

Results A wide variability was observed in the *in vitro* AH activity of the 38 methanolic extracts. Compared to the control, **7** out of the 38 methanolic extracts of sainfoin tested showed a significant delay, higher than 80%, in the exsheathment rate of *H.contortus* larvae. In contrast, for 22 out of 38 samples, the delay was lower than 30 %. Coefficients of correlations calculated between the different measurements of phenols and the inhibition values reflecting the AH activities were found to be significantly and positively associated with the RD values but also with the total flavan-3ols, total anthocyanidins, total flavonols, cinnamic and coumaric acids, ($P<0,01$) but not with flavones, arbutin and caffeic acid. These strong relationships were confirmed by the multivariate PCA analysis.

Like for the methanolic samples, wide variations were observed between the 14 acetonic extracts. Two extracts showed very high (≥80%) 4 high (79-50%), 3 medium (49-30%), and 5 low (29-0%), AH activity. An overview of the relationships between the AH effects and the biochemical characteristics of tannins, both through quantitative and qualitative (such as mDP, cis-trans and PD:PC ratios) measurements was obtained by PCA . When combined Axis 1 and 2 represented nearly 90% of the total variance. The overall analyses of multicorrelations confirmed the results of the 2 by 2 analyses On the plane, the variables Inhib1200 and Inhib600 were close to the RD variable and the PC values but opposite to the PD, total tannin and mDP variable.

Conclusions A comparison of the biochemical profiles associated with the variations in AH activities confirmed a role for proanthocyanidins. The respective importance of 2 classes of condensed tannins, i.e. PDs *vs* PCs requests further investigations as well as the importance of the MW/degree of polymerization. Moreover, the possible role of other flavonoids, in particular of flavan-3- ols and flavonols was also confirmed. These results should promote the development of adapted biochemical measurements to identify sainfoin samples with higher AH properties.

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The effect of substituting the rumen degradable nitrogen of sunflower oilcake meal with urea on digestibility, intake and microbial protein yield in sheep fed low quality forage

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Introduction Research has indicated that rumen degradable protein (RDP) has a positive influence on intake and digestibility of poor quality roughage fed to ruminants (Köster *et al.,* 1996). The aim of this study was to determine if substituting the RDP of sunflower oilcake meal (SOCM) with urea [non protein nitrogen (NPN)- source] would impair the intake and digestibility of the roughage as well as the microbial protein supply to sheep fed low quality roughage diet formulated to meet to fulfil maintenance requirements.

Materials and methods Five rumen cannulated Merino wethers (average 60 kg) received a poor quality *Eragrostis curvula* hay (< 0.65% N, > 75% NDF) *ad lib* during a 14-day adaptation period in a 5 x 5 Latin square design format. A diet where the RDP portion of the SOCM was substituted by NPN source (urea) was formulated to fulfil the maintenance requirements. This was tested in a previous intake trial according to the NRC, (2007) requirements. The supplement, containing maize starch, urea and/or SOCM and a commercial premix was infused directly into the rumen at 9:00 and 15:00. The ratios of RDP from urea to SOCM, as the rumen degradable N-source, were for the 5 treatments 100:0; 75:25; 50:50; 25:75 and 0:100 respectively. During the following 9-day experimental period the sheep were transferred to individual metabolic cages. Feed offered, orts and water intake were measured, sampled and pooled within treatment. Urine was collected, sampled and pooled to determine microbial protein supply (Chen *et al.*, 1992, Chen *et al.,* 1995). During the last three days rumen fluid was collected at six hour intervals (with a 2 hour delay/day in order to get a representative daily sample), pooled and analysed for volatile fatty acid (VFA) and rumen ammonia (NH_3-N) concentrations. Rumen fluid pH was measured during these periods. The data was analysed using ANOVA (SAS 9.2, 2008) for the Latin square design. Treatment differences were determined using the F-test (Samuels, 1989).

Results There was no effect on substituting urea with SOCM on roughage DM digestibility, digestible organic matter intake per kg metabolic weight (DOMI/kg $BW^{0.75}$), rumen pH, VFA or microbial protein supply between the five treatments. Significantly higher rumen NH_3-N concentrations were observed for the 100% SOCM treatment compared to the 50:50 treatment (Table 1).

Table 1 Substituting urea for SOCM on intake, digestion and microbial protein supply to sheep receiving poor quality *Eragrostis curvula* hay.

Parameter	The rumen degradable N as provided by urea: SOCM ratios					SE
	100:0*	75:25	50:50	25:75	0:100	
DM Digestibility (%)	47.05	43.23	43.60	46.24	45.19	2.94
DOMI (g)/kg $BW^{0.75}$	24.00	21.59	21.49	20.18	21.52	1.84
Rumen pH	6.47	6.46	6.52	6.49	6.48	0.05
Rumen VFA	78.96	75.96	75.27	72.11	73.74	2.39
Rumen NH_3-N	7.84^{ab}	8.16^{ab}	7.41^{b}	8.60^{ab}	9.35^{a}	0.56
Microbial protein supply (g CP)	58.60	59.40	59.23	60.72	59.75	0.21

Different superscripts within rows differ significantly ($P < 0.05$).

Conclusions Substituting the rumen degradable fraction of SOCM with urea had no effect on dry matter intake or the digestibility of low quality roughage. In addition the rumen pH and VFA concentration did not differ between treatments. Although the rumen NH_3-N concentration of the 100% SOCM treatment was significantly higher than for the 50:50 treatment it did not result in increased microbial protein supply to the host animal. It is concluded that when low quality roughage is fed to sheep on maintenance requirements, the RDP from SOCM can be substituted safely by urea without affecting intake, digestibility, rumen fermentation activities and microbial protein supply. This in turn will decrease the cost of N supplementation.

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Accuracy of n-alkane method to estimate diet composition in sheep fed contrasting forages and concentrate diets

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Introduction The n-alkanes present in the cuticular wax of forage plants can be used to estimate intake and diet composition (Dove and Mayes, 2005). However, the accuracy of this method may decrease with increase in the number of plant components, particularly if using forages with lower alkane concentration such as maize silage in the diet (Garcia *et al.,* 2000). The aim of this study was to determine if alkanes could be used to accurately predict diet composition and therefore intake in diets with lower levels of n-alkane concentration.

Materials and methods Four diets (D1, D2, D3 and D4, Table 1) comprising different proportions of concentrate, fresh herbage, maize silage and lucerne hay were randomly allocated to sheep housed in individual metabolism crates, in a latin square design. Animals were adapted to these diets for 15-days before collecting feed, residue and faecal samples for 5 consecutive days. The concentration of n-alkanes was determined according to Mayes *et al.* (1986). To calculate diet composition using least-squares optimization procedure, n-alkanes were corrected for their faecal recovery. Different approaches were followed to compare the accuracy of estimated diet composition: first, the established faecal recoveries values of Mayes *et al.* (1986) were used; second, mean actual faecal recovery was calculated and mean faecal alkane concentration was recovery corrected; third, each faecal alkane concentration was recovery corrected by calculating individual faecal recovery of each diet in each period. With known supplement (concentrate) intake and dietary composition, intake of each feed and total dry matter intake could be calculated as per Dove and Mayes (2005). The accuracy of the three methods was tested using mean square prediction error, where values of mean prediction error between 0 to 10, 10 to 20 and >20 indicate good, moderate and poor relative agreement, respectively (Fuentes-Pila *et al.,* 1996).

Results The diet composition estimated with the established faecal recoveries values (approach 1) of Mayes *et al.* (1986) did not predict the diet composition accurately (Table 1). Lucerne hay was over-estimated while the other three components were under-estimated. In contrast, both mean and individual faecal recovery data (approach 2 and 3, respectively) accurately predicted diet composition (R^2 = 0.99). However MPE was lower for approach 2 (0.38%) than for approach 3 (4.42%). Based on these results, the estimated intake using diet composition and known concentrate intake was highly correlated with the actual intake (Figure 2).

Table 1 Actual and estimated proportion of diet components by different approaches

	Diet	Pellets	Ryegrass	Lucerne hay	Maize silage
Actual	D1	0.36	0.41	0.10	0.12
	D2	0.47	0.30	0.11	0.13
	D3	0.16	0.75	0.05	0.04
	D4	0.26	0.51	0.11	0.13
Approach 1	D1	0.25	0.00	0.75	0.00
	D2	0.15	0.00	0.38	0.47
	D3	0.12	0.00	0.88	0.00
	D4	0.23	0.00	0.77	0.00
Approach 2	D1	0.37	0.41	0.10	0.12
	D2	0.47	0.30	0.11	0.12
	D3	0.16	0.75	0.05	0.04
	D4	0.26	0.51	0.11	0.13
Approach 3	D1	0.36	0.41	0.10	0.12
	D2	0.47	0.30	0.11	0.13
	D3	0.12	0.77	0.06	0.06
	D4	0.25	0.51	0.11	0.13

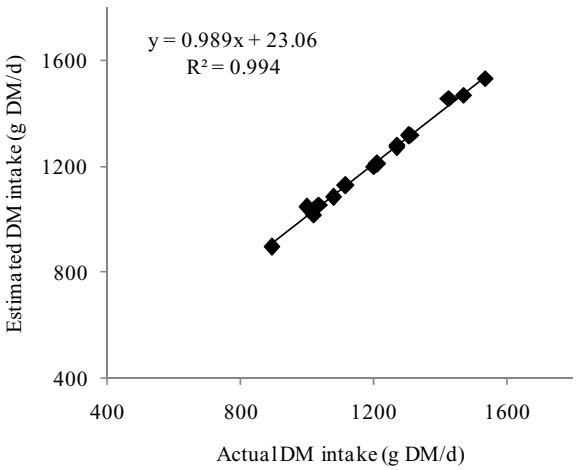

Figure 2 Relationship between actual and estimated intake by diet composition method

Conclusions The present study accurately estimated the proportions of components in the diet despite the fact that the alkane concentration of maize silage and pellets were lower compared to ryegrass and lucerne hay. The work presented here does not support the fact that accuracy of intake estimation may decrease with more complex diets. Results indicate that 'assumed' faecal recovery values cannot be applied to another study and the method of calculating faecal recovery affects the proportion of diet components. Faecal recovery data need to be 'absolute' for accurate estimation of proportion of diet components and therefore intake.

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The effect of supplementing different ratios of non-protein nitrogen to fermentable metabolizable energy on digestibility, intake and microbial protein yield in sheep

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Introduction Leng (1990) stated that the use of energy supplementation in addition to crude protein (CP) or nitrogen (N) would be beneficial in increasing the efficiency of nutrients from roughages fed to ruminants. The aim of this study was to determine if supplements containing various ratios of non-protein nitrogen (NPN) to fermentable energy (FME), would have an effect on the intake and digestibility of the roughage as well as on the microbial protein supply to sheep.

Materials and methods Five rumen cannulated Merino wethers (average weight of 50 kg) received a poor quality *Eragrostis curvula* hay (<0.65% N, > 75% NDF) *ad lib* during a 14-day adaptation period in a 5 x 5 Latin square format. The animals received one of the following five treatments as supplements: treatment 1 (control) was formulated to fulfil the maintenance requirement for N while the FME was calculated to support the maximum microbial protein synthesis for the amount of N supplied by the supplement. Treatment 2 and 3 had 15% less and more NPN than the control respectively, while treatment 4 and 5 had 15% less and more FME than the control treatment. Treatments were infused directly into the rumen twice per day at 9:00 and 15:00. During the following 9-day experimental period the sheep were transferred to individual metabolic cages. Feed offered, orts and water intake were measured, sampled and pooled within treatment. Urine was collected, sampled and pooled to determine microbial protein supply (Chen *et al.*, 1992, Chen *et al.,* 1995). During the last three days rumen fluid was collected at six hour intervals (with a 2 hour delay/day in order to get a representative daily sample), pooled and analysed for volatile fatty acid (VFA) and rumen ammonia (NH_3-N) concentrations. Rumen fluid pH was measured during these periods. The data was analysed using ANOVA (SAS 9.2, 2008) for Latin square design. Treatment differences were determined using the F-test (Samuels, 1989).

Results No significant differences were observed between treatments for both the forage digestibility and digestible organic matter intake per kg metabolic weight. The average rumen pH and VFA did not differ significantly between treatments. Although the rumen NH_3-N concentration of treatment 3 was significantly higher compared to the other treatments, it did not result in significant differences in microbial protein supply between treatments (Table 1).

Table 1 The effects of supplements differing in NPN:FME ratio compared on intake, digestibility, rumen fermentation and microbial protein supply to sheep receiving poor quality *Eragrostis curvula* hay

Parameter	Dietary treatments					SE
	T1*	T2	T3	T4	T5	
DM Digestibility (%)	48.67	50.07	51.31	48.56	52.92	1.68
DOMI (g)/kg $BW^{0.75}$	24.42	26.03	23.89	24.61	24.81	0.18
Rumen pH	6.58	6.60	6.58	6.59	6.59	0.03
Rumen VFA (mmol/L)	69.91	69.58	76.14	79.25	75.02	4.80
Rumen NH_3-N (mg/100 mL)**	8.44^{b}	7.59^{b}	11.43^{a}	9.40^{ab}	7.92^{b}	0.86
Microbial protein supply (g CP)	57.42	50.69	50.72	51.93	51.55	3.30

*For treatment 1 (T1) the NPN and FME were kept at maintenance requirements, for treatments 2 (T2) and T3 the NPN was 85% and + 115% of the maintenance requirements while FME was kept at maintenance. For treatments 4 (T4) and T5 the NPN was kept at maintenance while FME was 85% and + 115% of the maintenance requirements.
** Treatments with different superscripts within a row differ significantly ($p < 0.05$)

Conclusions Increasing or decreasing the NPN or FME to 15% above and below maintenance requirements have no effect on DM digestibility, digestible organic matter intake (DOMI), average rumen pH or the VFA production in the rumens of the sheep fed a low quality roughage. Although significant differences were detected in the rumen NH_3-N concentrations between the treatments it did not result in differences in microbial protein supply. It is concluded that supplements containing various ratios of NPN:FME (within 15% of the maintenance requirements) fed to sheep on low quality roughage will have no effect on intake, digestibility or the microbial protein supply to the animal. However, further research is necessary to determine the effect of wider ratios on especially the rumen fermentation parameters and the subsequent microbial protein supply to the host animal.

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Feeding behavior of male, female and castrated male goats subjected to different nutritional levels

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Introduction Goats are selective eaters, they present interesting characteristics related to versatility in harvesting forage and ability to survive under adverse foraging conditions which set them apart from other species of livestock (Lu, 1988). During the year, the goats raised in some regions of the world go through periods when the food availability is not enough for the animal to meet their nutrient demand. When animals are confined, the shortage of feed might also occur due to management strategies. This variation in nutritional level influences the ingestive behavior of animals. Moreover, it is possible that feeding behavior varies between intact male, castrated male and female, because of their physiological and hormonal differences. Thus, the aim of this study was to evaluate the feeding behavior of goats of different sexual categories under three nutritional levels.

Materials and methods Fifty four Saanen goats with an average body weight of 38±2,5 kg (18 intact males, 18 castrated and 18 females) were subjected to three nutritional levels (*ad libitum*, 75% and 50% food restriction). Food restriction was calculated according to the intake of animal fed *ad libitum*. All animals were fed the same diet, in 50:50 roughage:concentrate ratio. In roughage, it was used corn plant hay and concentrate was based on soybean meal and corn. The animals were housed individually with unrestricted access to water. The feeding behavior of the animals was evaluated during a period of 24 hours by two observers who performed visual measurements every five minutes, and identified the activities of standing, lying, eating, ruminating and idle. The experimental design was completely randomized in factorial (3X3). The data were analyzed using SAS statistical software and the analysis of variance, adopting the 5% level of probability, was performed using PROC GLM. Tukey test was applied at 5% probability for comparison of means.

Results There was no significant effect (P> 0.05) of the interaction between nutrition level and sex condition on eating behavior, which demonstrates that the studied factors have independent effects. Also, no significant effect (P> 0.05) of sexual category was found on the variables of feeding behavior, which can be explained by the fact that the animals were approximately at the same age, and they were in confinement. On the other hand, the nutritional level influenced (P <0.05) the feeding behavior of animals. Compared to animals under 50% restriction, those fed *ad libitum* spent more time in activities of feeding and rumination, because they had more food available for consumption. The animals subjected to a 50% food restriction remained longer standing and less time lying, compared to animals without food restriction (Table 1). Probably, this pattern of behavior can be explained by the discomfort of animals managed with greater food restriction. In fact, it was observed that some animals at higher food restriction showed agonistic behaviors in the standing position, which also justifies a longer time observed for the animals in idle.

Table 1 Mean values of time spent in daily activities by intact male , female and castrated male goats subjected feed restriction

	Nutrition Level (%)		
Time (minutes/day)	100	75	50
Standing	591,97 b ± 31,42	628,61 ab ± 22,30	709,72 a ± 29,58
Lying	844,55 a ± 31,71	802,78 ab ± 24,80	720,83 b ± 32,63
Eating	116,37 a ± 11,01	81,11 b ± 8,67	80,56 b ± 11,60
Rumination	347,59 a ± 18,77	344,17 a ± 20,32	212,50 b ± 16,15
Idle	947,57 b ± 24,25	1010,83 b ± 23,94	1167,50 a ± 23,16

* For each characteristic, means followed by different letters in the line differ by Tukey test (P <0.05).

Conclusions Feeding behaviour in Saanen goats is not influenced by gender. Saanen goats subjected to food restriction show differences in time spent eating and ruminating.

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Fast-gas chromatography analysis: Perspectives on the resolution of odd- and branched-chain fatty acids

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Introduction In the last decade, milk odd- and branched-chain fatty acids (OBCFA) have taken on new importance because of their potential as diagnostic tools of rumen function (Vlaeminck *et al*. 2006). Gas-chromatography (GC) is currently the most popular tool for elucidating milk FA profile from ruminants. However, depending on the degree of separation required, GC analysis of milk FA takes 80 min or more. For laboratories where many routine samples are analysed on a daily basis and where a short time-to-result is needed, it becomes imperative to speed up these analyses. The objective of the current trial was to assess the potential of a highly polar and a non polar capillary column to obtain a fast but yet precise resolution of OBCFA in milk fat.

Material and methods Milk samples (n = 56) were randomly selected from a previous study performed at Lanupro, Ghent University, Belgium. Lipid extraction of milk samples and the extracted lipids were methylated according to Stefanov *et al.* 2010. Conventional chromatography using two T° programs on a 100-m CP-Sil 88 capillary column was used as the reference method and co-elution was based on fractionation of methyl esters by silver-ion-thin layer chromatography (Ag^+-TLC). Composition analyses of FA were carried out with a GC-FID equipped with a fused-silica 100-m CP-Sil 88 capillary column; a fused-silica 10-m BPX70 capillary column; or a 30-m HP-5 capillary column. The T° programs used with the different capillary columns are summarised in Table 1. Injection and detection T° were kept at 250 °C and 280 °C, respectively. Effect of type of column was determined using GLM procedure of SAS and means were separated using Fisher's least square difference.

Table 1 Capillary columns and T° programs used for the analysis of milk OBCFA.

GC Column	T° program		Time (min)
100-m CPSil-88, i.d. 0.25mm film thickness, 0.20µm	a)	70°C (4 min) ─ 10°C/min ─ 150°C ─ 1°C/min ─ 165°C (20 min) ─ 2°C/min ─ 170°C (10 min) ─ 4°C/min ─ 215°C (30 min)	100.8
	b)	150°C (4 min) ─ 10°C/min ─ 180°C (20 min) ─ 15°C/min ─ 215°C (30 min)	59.3
10-m BPX-70, i.d. 0.10mm film thickness, 0.20µm	c)	50°C (0.3 min) ─ 85°C/min ─ 135°C ─ 5°C/min ─ 150°C ─ 10°C/min ─ 175°C ─ 25°C/min ─ 220°C (1.0 min)	9.6
	d)	150°C ─ 120°C/min ─ 200°C (1.2 min) ─ 50°C/min ─ 220°C (1.0 min)	3.0
30-m HP-5, i.d. 0.32mm film thickness, 0.25µm	e)	50°C (0.5 min) ─ 65°C/min ─ 140°C ─ 5°C/min ─ 165°C ─ 20°C/min ─ 170°C ─ 40°/min ─ 220° □2 mi□)	10.3

Results and discussion As for the 100-m CPSil-88, combination of two GC programs was necessary for resolution of milk OBCFA when using a fast 10-m BPX-70. Because the T° program (c; Table 1) led to co-elution of iso15:0 and *c*9 14:1, a second program (d) was used to establish the respective proportions of these FA. The overall time for combined T° programs was 12.6 min. Fractions obtained from Ag^+-TLC (*cis*, saturated, and *trans*) revealed that with program c), iso17:0 and anteiso17:0 were clearly separated from *c*9 16:1, but were co-eluting with other minor isomers of 16:1, i.e. *c*11 16:1 and *c*13 16:1, respectively. On the other hand, when using the HP-5 non-polar column, Ag^+-TLC fractions revealed that milk OBCFA can be clearly separated from the other milk FA, except for iso18:0, as partial co-elution with C18 PUFA might result in a slight overestimation of its concentration. These results were obtained in a single T° run of 10.3 min (e; Table 1). Despite significant effect of columns on some individual OBCFA concentrations, the amplitude of differences remains modest and should not impair the precision required to detect biologically relevant differences.

Table 2 OBCFA (mean with standard deviation in subscript) composition (mg/g) of 56 milk samples achieved by GC using different capillary columns and T° programs.

GC column	i13:0	i14:0	i15:0	ai15:0	15:0	15:1	i16:0	i17:0	ai17:0	17:0	17:1	i18:0
CPSil-88	$0.22_{0.06}$	$0.57^{b}_{0.18}$	$1.5^{b}_{0.2}$	$3.2_{0.6}$	$8.1_{1.6}$	$0.50^{a}_{0.11}$	$1.8_{0.3}$	$2.5^{c}_{0.4}$	$3.4^{b}_{0.8}$	$5.4^{c}_{1.0}$	$3.1_{1.1}$	$0.53^{b}_{0.24}$
BPX-70	$0.20_{0.05}$	$0.66^{a}_{0.13}$	$1.2^{c}_{0.3}$	$3.4_{0.7}$	$8.0_{1.5}$	$0.47^{a}_{0.14}$	$1.7_{0.3}$	$3.6^{a}_{0.5}$	$3.9^{a}_{0.6}$	$6.3^{a}_{0.9}$	$3.5_{1.0}$	0.62^{b}_{024}
HP-5	$0.19_{0.06}$	$0.58^{b}_{0.09}$	$1.6^{a}_{0.3}$	$3.4_{0.6}$	$8.1_{1.6}$	$0.42^{b}_{0.14}$	$1.8_{0.3}$	$3.0^{b}_{0.4}$	$3.9^{a}_{0.4}$	$5.8^{b}_{1.0}$	$3.4_{1.1}$	$0.81^{a}_{0.32}$

[1]Different letters within a column denote differences ($P < 0.05$)

Conclusion These results support the potential of fast-GC for routine analyses of milk OBCFA composition, with a non polar 30-m HP-5 column currently identified as most appropriate as it allows the separation of all the OBCFA within a single run of 10.3 min.

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Comparison of Mobile bag starch disappearance *versus* glycaemic response for studying starch digestion in horses

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Introduction Horses have a limited capacity for digesting high quantities of starch in the small intestine. The reported upper limit varies according to the botanical origin, physical processing of the grains and the method of investigation (Zeyner, 2008). Two methods are commonly used for estimating foregut starch digestion: the mobile bag technique and the glycaemic response to a starch meal. Both techniques have limitations. The first method measures the extent of starch disappearance from a nylon bag which overestimates the production of glucose since part of the ingested starch is fermented to organic acids within the gastric content (Varloud *et al*. 2006). The second one is based on the hypothesis that post-prandial blood glucose concentration correlated with starch digestibility in the small intestine. These two approaches have never been used in the same trial. We hypothesized that they would not give comparable results when tested a large variability in starch digestion.

Materials and methods Four mature caecally fistulated geldings (449 ± 41 kg) were used in a 4x4 Latin square design. They were fed a 62:38 hay:concentrate (20% barley) diet at 1.7 kg DM/100 kg BW. The barley was given in four different physical forms: whole grain(), 2-mm ground (◊), steam-flaked (Δ) and pelleted (o). Five bags of each barley form (previously 4-mm ground) were intubated through a nasogastric tube right after the ingestion of a third of the morning pelleted barley meal and were captured with a magnet through the caecal cannula (de Fombelle *et al*., 2004). After washing and drying, residual starch was analyzed. In addition, starch losses occurring through the pores of the nylon were measured in a domestic washing machine. Blood was collected via a jugular catheter just before the morning meal of concentrate, and thereafter at 30-min intervals for 4 h and, then at 60-min intervals for the next 4 h. Two measurements were made, without or with the morning meal of hay. Total blood glucose was analyzed with a blood glucose meter (One Touch® Ultra 2, LifeScan Inc., USA) right after collection. Mean concentration, time to peak, peak were calculated and area under the curve (AUC) using the trapezoidal method. All variables were analyzed using the Mixed Procedure (SAS). Fixed effects were barley form, for starch disappearance, and period, barley form, type of method (with or without hay) and their interactions, for glucose variables. Horse was tested as random effect. Pearson correlation coefficients were calculated for the comparisons between glucose variables and the intake of precaecal digestible starch (PDSI) (starch intake x precaecal digestibility).

Results The starch intake in the morning meal averaged 267 (± 16) g starch/100 kg BW. Bag mean retention time in prececal tract was 5.9 (± 0.1) h. Starch disappearance depended (P<0.0001) on the physical form (from 55.1 to 97.4 % for whole and ground, respectively (SEM=3.9)). The extent of variation could be partly related to variable washing starch losses (from 10.3 (whole) to 52.4 (ground) %). Time to reach glucose peak was shorter (P=0.019) when hay was given in the morning meal (Figure 1). Except for AUC (P=0.07), mean glucose concentration, peak value and time to peak did not differ (P>0.05) according to the physical form. For a same PDSI, a wide variability in AUC was determined according to process and horses (Figure 2). Also, there were no significant correlation between any of the glycaemic parameters and PDSI.

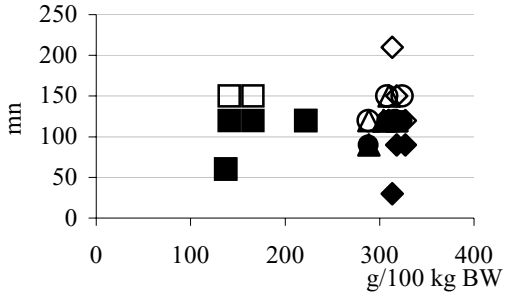

Figure 1 Relationship between time of peak for the four physical of barley with (dark) or without (blank) hay and PDSI.

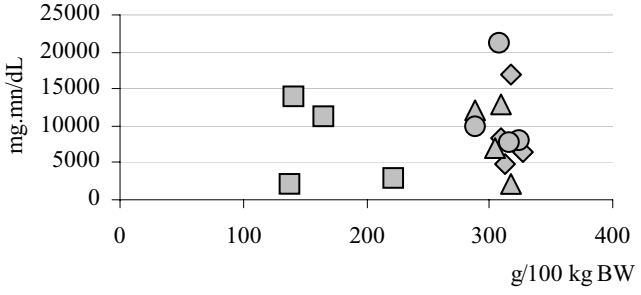

Figure 2 Relationship between AUC for the four physical forms of barley and PDSI.

Conclusion There was no relationship between glycaemic response and precaecal starch digestion measured by mobile bags in this study. Although technical adaptations may be needed, the mobile bag technique should be preferred to estimate the amount of undigested starch and potentially responsible for hindgut acidosis. The glycaemic and insulinaemic responses to starch meals should be preferentially focused on endocrine glucose regulation studies. In order to prevent the risk of microbial and endocrine diseases in horses, both could be used to improve the estimates of upper critical limits of starch intake which currently vary between 100 to 400 g /100 kg BW/meal.

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A quantitative approach to representing variation in rumen acidity

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Introduction The concentration of volatile fatty acid (VFA) in the rumen is the prime determinant of the acidity of luminal contents (Counotte *et al*., 1979). However, relationships to predict lumen acidity (pH) from rumen VFA concentration is weak (Dijkstra *et al*., 2011). Bicarbonate has added been addressed in attempting to understand variation in rumen pH (Kohn and Dunlap, 1998; Imamidoost and Cant, 2005). The present study aimed to develop a method that is capable to represent the effect of any factor identified as influencing rumen pH, besides the factors affecting VFA concentration (production and clearance of VFA; Bannink *et al*., 2008).

Materials and methods A model was constructed that represents the effect of rumen concentration of weak and strong acids and bases, of the partial gas pressure of carbon dioxide and of the buffering characteristics of rumen contents on pH (Figure 1). These independent factors affecting acid-base equilibrium are separated from factors as pH, dissociation of acids and bicarbonate concentration which are considered dependent.

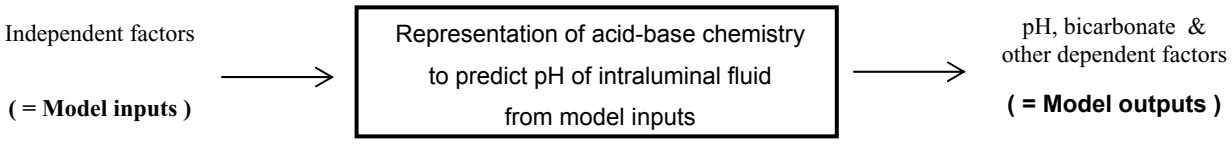

Figure 1 Schematic representation of the distinction between independent (causal) factors and dependent (affected) factors in a model that represents intraluminal buffering mechanisms and acidity.

Results Considering the physiological range of factors affecting rumen pH, variation in the concentration of VFA (generally between 75 and 150 mmol/l) was the strongest determinant (case of acetate, Figure 2). The concentration difference between strong cation-anions (meq/l) has in principle a similar marked effect (Figure 2 *versus* Figure 3) but in the physiological range rumen variation is less (between 130 and 150 meq/l) than for VFA concentration. In the simulations, next to VFA concentration, saliva production with a positive cation-anion concentration difference showed to have the strongest modifying effect on rumen pH. Cation exchange by cell walls (forages) affected rumen pH as well, but remained quantitatively less important. The remaining factors (ammonia, phosphate, partial gas pressure carbon dioxide) only had a minor role. Simulation results indicate, however, that it may prove to be necessary to take most of these factors into account when aiming to predict rumen pH dynamics with high precision.

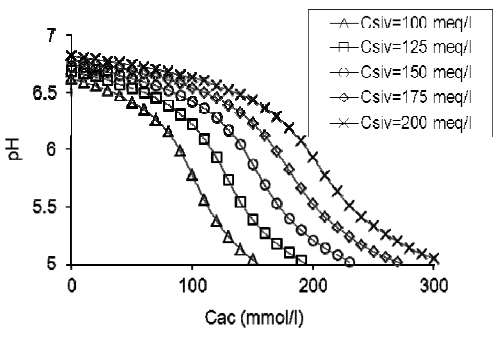

Figure 2 Effect of concentration of acetate (Cac; mmol/l) and of strong cation–anion difference (Csiv; meq/l) on pH.

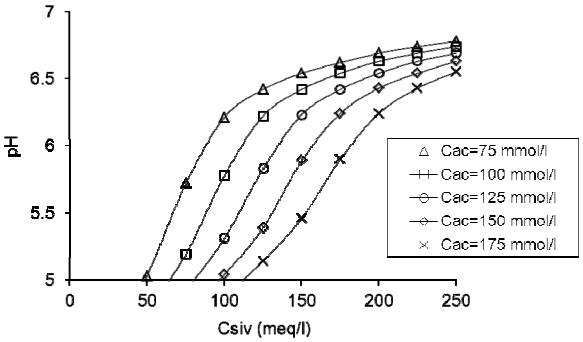

Figure 3 Effect of concentration of strong cation-anion difference (Csiv; meq/l) and acetate (Cac; mmol/l) on pH.

Conclusions A quantitative method was developed, based on concepts of acid-base theory, which allows the inclusion of any factor thought to be relevant to explain variation in rumen pH. The method aids in delineating how other factors affect variation in rumen pH, besides rumen VFA concentration as most important determinant factor. Although the impact of these other factors is small, it is of the size needed to predict rumen pH accurately and to prevent the suboptimal trajectory of rumen pH by nutritional measures.

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The effects of sward pre-grazing herbage mass on dairy cow rumen function

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Introduction The optimal use of grazed grass is identified as a key component of profitability in Irish dairy production systems (Shalloo *et al.,* 2004). Pre-grazing herbage mass (HM) affects herbage quality and can influence herbage intake at grazing. O'Donovan and Delaby (2008) illustrated that swards with higher HM reduced the feeding value of grass and thus reduced DMI. However, it is also suggested that grazing high quality pastures can lead to low rumen pH (Gibbs *et al.,* 2007), which is an important factor related to milk fat %, fibre degradation, nutrient absorption and overall cow health and welfare (Kleen *et al.,* 2003). Altered rumen pH is often accompanied by altered rumen volatile fatty acid (VFA) profiles (Kleen *et al.,* 2003). Hence, the objective of the current study was to investigate the effects of three different HM treatments on dairy cow rumen pH and VFA, lactic acid and ammonia concentrations.

Materials and methods A systems study with 3 separate farmlets was established and 20 dairy cows were allocated to each treatment. The three treatments were i) low HM [LHM] (1200kg DM/ha), ii) medium HM [MHM] (1600kg DM/ha) and iii) high HM [HHM] (2200kg DM/ha). The experiment took place in June to July 2009. Grass was allocated on a daily basis and no supplementary feed was offered. Six lactating rumen-cannulated dairy cows were arranged into two 3x3 latin squares. Each period was 2 weeks in duration. Rumen pH was measured continuously on days 10 and 11 of each period by means of an indwelling rumen pH probe. The data were logged at 60-second intervals over the 48-hour period. Average rumen pH across the day was calculated. Rumen fluid samples were taken on days 13 and 14 of each period at 8 am and 12 pm. Samples were strained through three layers of synthetic cheesecloth and frozen as-is for subsequent analysis of lactic acid and with 50% TCA for subsequent analysis of ammonia and VFA. The data were analysed as a 3x3 latin square using the mixed procedure (PROC MIXED) of SAS (SAS Institute, 2003) with pre-grazing herbage mass treatment, experimental period, square, cow and their interactions included in the model.

Results There was no difference in the average rumen pH of dairy cows grazing grass of different HM ($P>0.05$), but average daily pH was low at 6.10. There was also no effect of treatment on either D- or L-lactic acid concentrations ($P>0.05$), but again, on average, these values were low (1.67 and 1.30 mmol/l for D- and L-lactic acid respectively). However, HHM swards gave rise to significantly lower molar proportions of isobutyric and isovaleric acid ($P<0.05$). This can reflect a reduction in excess rumen degradable protein, which agreed with the lower rumen ammonia concentrations found on the HHM treatment ($P<0.05$).

Table 1 The effects of sward pre-grazing herbage mass on rumen volatile fatty acid and ammonia concentrations at 8am and 12pm

	8:00 AM					12:00 PM				
	LHM	MHM	HHM	s.e.	Sig	LHM	MHM	HHM	s.e.	Sig
Total VFA (mmol/l)	205	207	201	4.8	ns	205	220	216	9.2	ns
Acetic (propn)	0.69	0.69	0.71	0.007	ns	0.68	0.68	0.68	0.005	ns
Propionic (propn)	0.18	0.17	0.17	0.004	ns	0.18	0.18	0.19	0.004	ns
Butyric (propn)	0.11	0.10	0.10	0.004	ns	0.11	0.10	0.10	0.002	ns
Iso-butyric (propn)	0.009^{b}	0.009^{b}	0.008^{a}	0.0002	<0.01	0.009^{ab}	0.009^{b}	0.008^{a}	0.0003	<0.05
Valeric (propn)	0.011	0.012	0.009	0.0015	ns	0.010	0.012	0.010	0.0010	ns
Iso-valeric (propn)	0.012^{b}	0.012^{b}	0.010^{a}	0.0003	<0.01	0.012^{ab}	0.013^{b}	0.011^{a}	0.0005	<0.05
Ammonia (mmol/l)	19.1^{b}	15.3^{ab}	12.6^{a}	1.39	<0.05	18.8	21.6	16.2	1.64	ns

Conclusions Rumen fermentation profiles are important in interpreting grazing system studies effects on dairy cows. Further work is needed to explore the mechanisms which lead to the observation of low rumen pH values in combination with low lactic acid and altered VFA profiles in grazing systems.

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Ingestive behaviour and nutrient digestibility of Holstein cows fed diets containing maize silage from silos with different covering methods

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Introduction The plastic sheet used to cover silage has oxygen permeability and small amounts of air will penetrate the silage. Thus, the use of different plastic polymers or covering materials over the plastic sheet may avoid the aerobic deterioration of silage (Ashbell and Lisker, 1988) and nutritive value losses during the storage. Many farmers are particularly resistant to cover horizontal silos with soil, due to the labor and handling of soil used to weigh the plastic. Moreover, the use of soil can also be a source of contaminants to silage, mainly during the silo feedout. Therefore, the use of sugarcane bagasse may an alternative to reduce aerobic deterioration in the peripheral zone of the maize silage silos.The objective of this trial was to study the effectiveness of covering methods to reduce the losses in maize silage and their response in ingestive behavior of dairy cows and rations digestibility.

Materials and methods The trial was carried out in Piracicaba, Brazil (22°42′S, 47°38′W). Twenty-four multiparous and sixteen primiparous, lactating Holstein cows (33.0 kg/d milk production) were e assigned in a randomized complete block design with 4 treatments and 10 replicates. Treatments were defined according to the maize silage covered method adopted (52.8% of maize silage inclusion in the total dietary DM): oxygen barrier film (OB) 45 μm thick + black-on-white polyethylene film (BW) 200 μm thick over the OB film; black-on-white polyethylene film (BW) 200 μm thick; black polyethylene film (B) 200 μm thick; recycled black polyethylene film (RB) 200 μm thick + sugarcane bagasse over the RB film. Diets were formulated to meet NRC (2001) recommendations and provided similar concentrations (g/kg DM) of DM (462.0), CP (162.9), NDF (369.7), EE (21.0). Diets were fed as total mixed rations twice and animals were allowed *ad libitum* access to feed and fresh water. The ingestive behavior was measured preceding the digestibility trial. Eating, ruminating, chewing and resting activity (h/d) were monitored for each animal. The activities were recorded by direct observation at 10-min intervals throughout 24 h. During the digestibility trial, total feces were collected, during three days, across alternate times. Data was analyzed using the MIXED procedure of SAS (1999), according to the model: $Y = \mu + T_i + B_j + e_{ij}$, where μ = overall mean, T_i = treatment effect (i = 1 to 4), B_j = block effect (j = 1 to 10), and e_{ij} = residual error.The LSMEANS option was used to generate individual treatment means and effects declared significant at $P < 0.05$ using the Tukey test.

Results There was no difference (P>0.05) across treatments on dry matter and NDF intake (Table 1). The time spent to eating, ruminating, and chewing were similar (P>0.05) among treatments. The values were 4.3, 8.7 and 13.1 h/d, respectively. These results were expected in respect of the same dry matter intake and the NDF contents of the diets.

Table 1 Intake and nutrient digestibility of dairy cows fed diets containing maize silage from silos with different covering methods

Items[1]	Treatments[2]				SE[3]	Effect[4]
	BW+OB	BW	B	RB+SCB		
	Intake, kg/d					
DM	22.25	22.24	21.03	21.84	1.10	NS
NDF	8.05	8.10	7.89	7.91	0.27	NS
	Digestibility, g/kg MS					
DM	649.8[a]	589.4[b]	592.4[b]	674.8[a]	16.4	**
OM	728.6[a]	675.4[b]	693.1[b]	740.9[a]	14.9	**
NDF	495.0	401.5	382.2	469.9	37.8	NS

[1]DM; dry matter; NDF: neutral detergent fiber; OM: organic matter. [2]BW+OB: black-on-white+oxygen barrier film; BW: black-on-white film; B: black film; RB+SCB: recycled black film+sugarcane bagasse. [3]SE: standard error of means. [4]NS: no significance; ***P*<0.05.

The Holstein cows fed BW+OB and RB+SCB diets showed higher DM and OM digestibilities when compared to BW and B diets. Probably, the higher digestibilities values might be attributed to the effect of low oxygen permeability of the OB film and/or the gas transmission rate would be reduced by the presence of sugarcane bagasse over the film preventing the losses of nutritive value.

Conclusions The use of OB film or SCB were effective on minimizing silage losses and keep high digestibilities.

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Effects of adding a mycotoxin-sequestering agent on milk aflatoxin M_1 concentration and the performance and immune response of dairy cattle fed an aflatoxin B_1 contaminated diet

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Introduction Hydrated sodium calcium aluminosilicates (HSCAS) have been recommended as practical and effective additives that can prevent negative effects of aflatoxin on dairy animals and products (Phillips, 1999). The objective was to examine effects of adding two doses of a montmorillonite - based HSCAS mycotoxin adsorbent, on milk aflatoxin M_1 (AFM_1) concentrations and the performance and innate immune response of dairy cows fed an aflatoxin B_1 (AFB_1)-contaminated diet.

Material and methods Eight multiparous lactating Holstein cows in late lactation (DIM 295) were housed in an open sided, free stall barn bedded with sand and equipped with Calan gates (American Calan Inc). Treatments included the following: 1) Control diet (C); 2) Toxin diet (T) containing C and 75 µg/kg of AFB_1; 3) Low-clay (LC) diet containing T and 0.2% Calibrin A (Amlan International, Chicago, IL); and 4) High-clay diet (HC) containing T and 1% Calibrin A. Milk production and DMI were recorded daily, and milk was sampled twice daily on d 5, 9, 10, 11, and 12 in each of 4, 12-day period. Blood samples were collected on d 5 and 9 of each period.

Statistical analyses The experiment consisted of a duplicated Latin square design with 4 treatments x 4 periods lasting 12 days each. The MIXED procedure of SAS (Version 9.2 SAS Institute Inc., Cary, NC) was used to analyze the data and the Fisher's F-protected Least Significant Difference test was used to separate least square means. Contrast statements were used to directly compare the control treatment to the others. The model included treatment, square and period effects and significance was declared at $P < 0.05$ and tendencies were declared at $0.10 > P > 0.5$.

Results Dietary treatments did not affect DMI, milk yield, or feed efficiency. Feeding T instead of C tended to reduce 3.5% FCM yield (19.0 *vs*. 20.8 kg/d; SE = 0.79, P = 0.08) and reduced milk fat yield (0.67 *vs*. 0.74 kg/d; SE = 0.03, P = 0.04) and milk protein concentration (3.28 *vs* 3.36%; SE = 0.03, P = 0.01). Concentrations of AFM_1 in milk of cows fed the T and LC diets were similar (0.63 and 0.65 µg/kg; SE =0.05, P = 0.69) and greater than those of cows fed the HC diet (0.48 µg/kg; SE = 0.04, P < 0.01), but cows fed C had trace levels (0.03 µg/kg; SE = 0.04). Haptoglobin concentration was greater (22.0 *vs*. 14.4; SE = 1.9, P = 0.01) and β2-integrin expression (220 *vs*. 130;; SE = 32, P = 0.1) tended to be greater in cows fed diet T instead of C, but values for cows fed LC, HC and C did not differ.

Conclusion Adding the sequestering agent to the toxin diets prevented the tendency for an increase in the innate immune response and a decrease FCM yield caused by the toxin, but milk AFM_1 concentration was only reduced by feeding the high dose of the sequestering agent.

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Effect of ethanol and acetic acid on ingestive behaviour and selective consumption by high producing dairy cows

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Introduction Ethanol and acetic acid are common end-products from fully fermented silages. The objective of this study was to determine whether ethanol and acetic acid may affect the intake, chewing activity, and feed sorting by dairy cows.

Material and methods Thirty lactating Holstein cows averaging 40 kg/d of milk at beginning of trial were grouped in ten blocks and fed either: Control (33% Bermuda hay + 67% concentrates); Ethanol (control diet + 5% ethanol); or Acetic acid (control diet + 5% acetic acid, DM basis) diets, during seven weeks. Ethanol and acetic acid were diluted in water (1:2) and sprayed onto total mixed ration twice daily before feeding. The same amount of solution was replaced with water in the control diet. During the 1st week the cows received half-dose of these chemical compounds. Dry matter intake (DMI), chewing activity, and feed sorting were monitored for each cow on 6th week of trial. Time spent to eat during 4 h following morning feeding was recorded during 1st, 2nd and 6th weeks. Oral activity of each animal was recorded by direct observation at 10-min intervals. Particle size distribution of diets and orts were determined by using Penn State particle size separator adapted with a top screen (38 mm pore size), resulting in four fractions (long, medium, short and fine particle size). Sorting was calculated as the observed intake of each particle size fraction expressed as a percentage of the predicted intake (as fed basis). Values <100% indicate selective refusal, >100% indicate preferential intake, and =100% no sorting (Leonardi and Armentano, 2003). Data were analyzed by MIXED procedure of SAS. Fixed effects were block and diet. Model for sorting index also includes screen and diet*screen effects. Week and diet*week effects were included for analysis of eating during 4 hours after morning feeding.

Results Dry matter intake and time spent on eating, ruminating and chewing were similar across treatments. However, eating during 4 hours following morning feeding was lower for acetic acid diet (Table 1). It means that acetic acid change eating pattern, but cows compensated the delay to start eating throughout the 24-h period. In fact, Hutchinson and Wilkins (1971) also observed changes on eating pattern of sheep fed perennial ryegrass silage supplemented with acetate. As well sorting behaviour was altered by acetic acid in the present trial (Figure 1). While cows fed control and ethanol diets sorted mainly against the longest particles, animals fed acetic acid diet prefered longest particles (hay) instead of shortest ones (concentrate). It might be a metabolic response due to the total mixed ration acidity associated to a low fiber content of diets, since the acetic acid concentrations across particles length (hay *vs* concentrate) were similar both in the ration and orts (not showed).

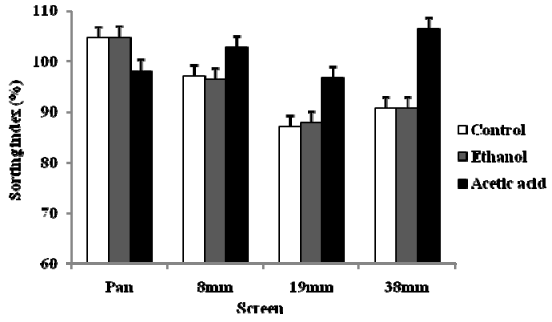

Figure 1 Sorting index of lactating dairy cows supplemented with ethanol or acetic acid over seven weeks. P < 0.01 for diet*screen interaction.

Table 1 Ingestive behaviour of high producing dairy cows

Parameter	Control	Ethanol	Acetic acid	SE	*P* Diet
Eating (min)	245	233	231	17.0	0.77
Ruminating (min)	418	435	451	19.0	0.38
Chewing (min)	663	668	682	25.0	0.81
Chewing/Intake (min/kg DM)	31.3	29.2	31.9	1.3	0.33
Eating during 4h after morning feeding (min)	98.7a	95.1a	71.7b	7.1	0.02†

† *P* = 0.65 for Diet*Week interaction

Conclusion Ethanol unchanged ingestive behavior, feed selection and intake, however, acetic acid altered eating pattern and feed sorting without modifying dry matter intake.

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Milk odd- and branched-chain fatty acids: biomarkers to optimize microbial metabolism by ruminants?

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Introduction Determination of OBCFA in milk could be a useful tool to measure the microbial flow to the duodenum in nonfistulated cows. A clear relationship has been established between the duodenal flow of bacterial nitrogen and secretion of OBCFA in milk although differences between experiments were observed in degree of correlations, which might be due to confounding factors e.g. lactation stage, endogenous OBCFA synthesis, fatty acid absorption in the small intestine, transport through different blood plasma lipid classes. The objective of this study was to validate the relationships using datasets from two experiments with urinary excretions of purine derivates as the reference indicator of *in vivo* microbial nitrogen flow into the duodenum (Tas and Susenbeth, 2007).

Materials and methods Nine multiparous lactating cows were used in the first experiment. The experimental period was divided into 3 periods, in which the animals got 3 different diets, randomly assigned. Each period consisted of 7 weeks: 4 weeks as adaptation period, the remaining weeks for sampling, of which total urine collection and milk sampling took place in week 5. Diet 1 was deficient in rumen available nitrogen. Diet 2 had balanced amounts of rumen available nitrogen through urea supplementation, diet 3 6g niacin was added per cow per day. In the second experiment six multiparous cows were assigned to a 3x3 Latin square, in which the animals got 3 diets: the first diet had a maize silage/ pre-wilted grass silage ratio of 40/60, the second diet a ratio of 70/30, in the third diet with maize silage/ grass silage at ratio of 70/30, additionally 15kg pressed beet pulp per day was added. Each period consisted of 3 weeks with the first 2 weeks for adaptation. Milk fatty acids were extracted (Chouinard *et al*., 1997), methylated and analysed by GC (Stefanov *et al*., 2010). Daily milk OBCFA flow was calculated as the sum of iso C14:0, C15:0, iso C15:0, anteiso C15:0, iso C16:0, C17:0, iso C17:0, anteiso C17:0 and cis-9 C17:1 and multiplied with milk yield. Purine derivates allantoin, uric acid and hypoxantine were determined in urine as described by Fievez *et al*. (2001) from which daily microbial N flow in the duodenum was calculated as described by Tas and Susenbeth (2007).

Results Total daily milk production was comparable between experiments (28.6 ± 2.43 kg/day and 30.4 ± 5.62 kg/day for exp. 1 and 2 respectively). Figure 1 shows a similar relationship between the milk OBCFA secretion and the microbial N flow at duodenum level as found by Vlaeminck *et al*. (2006), with a N/OBCFA ratio of 9.18 (8.99 in Vlaeminck *et al*. (2006)). This relation is mainly induced by a difference between data of experiment 1 and 2 with the former showing relatively lower and the latter higher urinary excretion of purine derivates and milk OBCFA. However, it is of more interest to assess whether differences between cows and periods are reflected. Indeed, Figure 2 illustrates much more variation between the different observations for the different cows and diets. Within exp. 1, calculated duodenal microbial N flow range from 150 to 250 g/d and milk OBCFA between 15 and 30 g/day. In exp. 2, duodenal microbial N flow is between 250 and 420 g/day and milk OBCFA between 15 and 45 g/day. Regressions within experiments or for both experiments together are not better when milk OBCFA is expressed as g/100g milk FA instead of daily milk OBCFA flow (g/day) (data not shown).

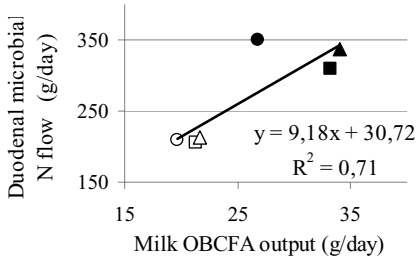

Figure 1 Relationship between milk secretion of OBCFA and duodenal flow of microbial N(6 dietary treatments)

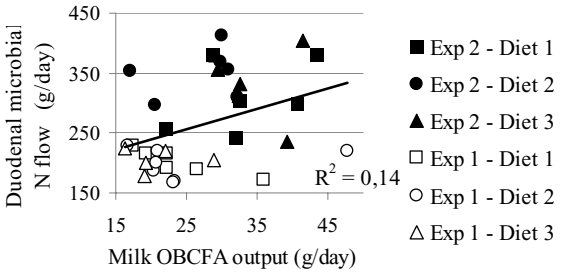

Figure 2 Relationship between milk secretion of OBCFA and duodenal flow of microbial N (all data points)

Conclusions More knowledge about different confounding factors is necessary, because large variation among the different observations indicates that measuring microbial N flow of individual animals, based on total milk OBCFA production, is not possible.

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Ruminal fermentation parameters in ruminally fistulated cows fed polyclonal antibodies and adapted or not to highly fermentable carbohydrates diets

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Introduction Highly fermentable carbohydrates (HFC) diets have been used in feedlot cattle, but it is associated with disturbances in rumen fermentation, such as acidosis. In order to control these disorders, it is often necessary to use protocols for diet adaptation and administration of feed additives (Millen *et al*., 2009). However the use of additives is currently controversial, because consumers demand for animal products without residuals that could endanger human health. Polyclonal antibodies preparation (PAP) is a new option of additive and uses passive immunity against specific bacteria to modulate rumen fermentation. The objective of this study was to evaluate the effects of two different forms (liquid or powder) of PAP against specific ruminal bacteria *S. bovis*, *Fusobacterium necrophorum* and *Lactobacillus* on rumen fermentation parameters in animals adapted or not to diets with high proportion of HFC.

Materials and methods In this experiment, 6 ruminally fistulated cows were used. The experimental design was two Latin squares 3x3 in factorial arrangement of treatments 3x2 regarding two feed additives (PAP in powder (PAPP) or PAP in liquid (PAPL) presentation) plus control group (CON) and two schemes of diet adaptation resulting in 6 treatments. This trial had three 17-day experimental periods, with two 10-day washout period among them. The first Latin square received a step-up adaptation diet: from D0 to D4 (100% forage); D5 to D9 (30% of concentrates) and D10 to D14 (60% of concentrates). The second Latin square received 100% forage from D0 to D14 (no adaptation). On D15 and D16 all animals received a challenge diet that consisted of 80% of concentrates. Rumen fluid was sampled for pH and short-chain fatty acids (SCFA) analysis from D0 to D16 at 3 h postfeeding. Data were analyzed by MIXED procedure of SAS with a significance level of 0.05. In the model, the effects of treatments and time were considered fixed factors. Period and animal nested in square were considered random factors.

Results An interaction between time and adaptation (P<0.0001) was observed for ruminal pH (Figure 1). On the days 2 and 4-15, non-adapted group had higher values of ruminal pH compared to adapted group (6.80 *vs* 6.34, respectively). On day 16, the adapted group had higher values than the non-adapted group (6.15 *vs* 6.04, respectively). A feed additive effect was verified for ruminal pH (P=0.0432), independently on time or adaptation, i.e. PAPL had greater values (6.62) than PAPP (6.57) and control (6.56) , as shown in Figure 2. An interaction between time and adaptation was observed for total SCFA concentration (P < 0.0001). From D1 to D15, the HFC-adapted group had higher values of tSCFA when compared to the non-adapted one (101.62 *vs* 78.01 m*M*, respectively). On D16 the non-adapted group showed higher concentration of tSCFA than the adapted group (121.18 *vs* 107.89 m*M*). An interaction between time and adaptation was observed for Ac:Pr ratio (P < 0.0001), i.e. the adapted group had lower values than non-adapted group on D2 (2.08 *vs* 2.33) and from D5 to D7 (1.92 *vs* 2.28). However, from D12 to D16, there was an inversion of these values and the adapted group showed higher values when compared to the non-adapted group (2,87 *vs* 2.41, respectively).

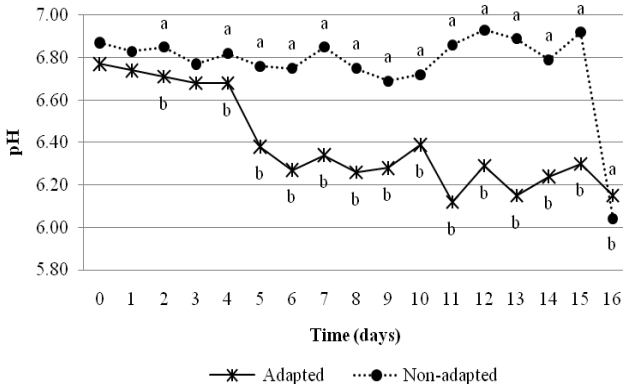

Figure 1 Ruminal pH for factors composed by different diet adaptation.

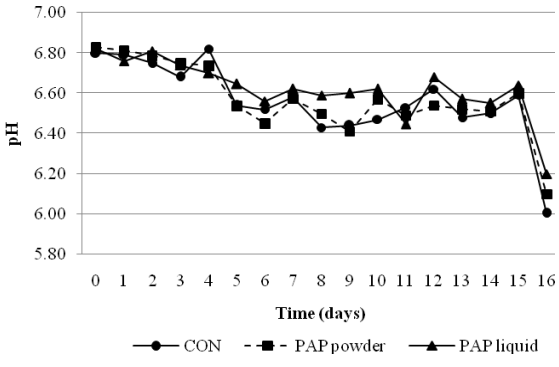

Figure 2 Ruminal pH for factors composed by different feed additives.

Conclusions From these results, it is possible to conclude that step-up adaptation reduced rumen pH and increased total SCFA, as expected by the increase of total highly fermentable carbohydrate available. However, these effects presented an inversion when animals were submitted to a challenge of HFC diet. Polyclonal antibodies in liquid presentation was efficient to avoid the reduction of ruminal pH at 3 h postfeeding, when compared with PAP in powder presentation and control group, proving to be a potential feed additive for ruminants.

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Impact of dietary nitrogen supply on renal electrolyte excretion in growing goats

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Introduction Unlike other species, ruminants are able to use highly developed adaptive renal recycling mechanisms to conserve nutrients in times of restrictive dietary intake. Especially renal tubular reabsorption of urea by urea transporters and of phosphate (Pi) by sodium-dependent Pi transporters guarantees an effective recycling of nitrogen (N) and Pi. It is well known that dietary N restriction led to adaptation of renal N recycling in ruminants allowing the animals to cope with adverse N-deprived environmental conditions. However, in humans and monogastric animals a link between dietary protein supply and bone mineral metabolism was discussed (Peters & Martini 2010; Bourrin *et al*. 2000). Dietary protein restriction caused bone loss and osteoporosis by mechanisms involving renal electrolyte handling. Hypothetically, in ruminants dietary protein restriction has less influence on renal function and mineral metabolism due to constitutively expressed, highly adapted recycling mechanisms. Aim of the study was to examine renal Pi transporters, respective regulatory hormone receptors and their intracellular signaling pathways influenced by restrictive dietary N intake in growing goats.

Materials and methods In this experiment, twenty growing male white Saanen goats were divided into two feeding groups. One group was fed a reduced N (RN) diet containing 8% crude protein, the other group was fed an adequate N (AN) diet containing 14% crude protein. The dietary conditions were maintained for 7 weeks. Blood and urine samples were taken for the analysis of hormones, urea and electrolyte concentrations. After slaughtering, renal cortex samples were obtained. Semiquantitative analysis of sodium-dependent Pi transporter (NaPi IIa) and parathyroid hormone receptor (PTHR) on mRNA level was performed by Northern Blot analysis. Protein amounts of PTHR, vitamin D receptor (VDR), NaPi IIa and extracellular signal regulated kinases 1/2 (ERK1/2) were detected semiquantitatively by Western Blot analysis. All data were statistically analysed by unpaired Student's t-test using software Graph Pad prism4. Differences were considered significant with $P < 0.05$.

Results In RN fed goats, plasma urea concentration and urinary fractional excretion of urea decreased showing that the N-reduced diet led to strong adaptive responses in the kidney of growing goats. Calcitriol plasma levels decreased while plasma Pi and calcium (Ca) concentrations were not affected between the two feeding groups. Balance studies obtained by a similar experiment resulted in unaffected daily urinary Ca excretion, but a decrease in daily urinary Pi excretion. The mRNA amount of NaPi IIa and PTHR did neither show any difference between the two feeding groups nor did protein amount of VDR. However, the tubular protein amount of NaPi IIa, the major renal transport system for Pi reabsorption, increased. In accordance, the protein amounts of the receptor for PTH (PTHR) and ERK1/2, known as negative regulators of NaPi IIa, both decreased under reduced N intake. These data showed that renal Pi excretion and its regulation were strongly affected by reduced dietary N supply in growing goats.

Conclusions The decrease in renal Pi excretion under an N-reduced diet was associated with an increase in NaPi IIa whereby PTHR and ERK1/2 protein expression was down-regulated. Adaptation of this molecular pathway indicates the existence of a link between N and mineral homeostasis in growing goats. The molecular identity of a respective molecular connection is unclear yet.

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Modulation of electrolyte homeostasis by dietary nitrogen intake in growing goats

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Introduction In ruminant feeding, the reduction of dietary protein is a common approach to decrease the excretion of environmental pollutants like nitrogen (N). In lactating cows, the total dietary N intake determines the total N excretion as manure (Castillo *et al*. 2001). In monogastric animals and humans a close relationship between protein metabolism and electrolyte homeostasis was observed. Alterations of plasma calcidiol concentration and urinary excretion of calcium (Ca) and phosphate (P_i) were detected in protein restricted fed rats (Orwoll *et al*. 1992). During times of protein scarcity, it is expected that in goats, because of their ability to recycle N, the described interaction between protein metabolism and electrolyte homeostasis will be less pronounced and therefore animal's health will be maintained. The aim of the present study was to characterise the effects of dietary N reduction on mineral and bone metabolism in young goats.

Materials and methods Young male white Saanen goats with an average age of three months were used. The animals were fed either with a reduced (RN, 8%, n =10) or an adequate (AN, 14%, n = 10) N diet for about 7 weeks. All diets were isoenergetic containing 11.35 kJ metabolizable energy/kg diet. Saliva and plasma samples were collected weekly while urine, abomasal and ruminal fluids were obtained at time of slaughter. Plasma urea was measured as an indicator of protein status with a commercial kit. Concentrations of P_i and Ca in caprine body fluids were determined colorimetrically by standard spectrometric techniques. Plasma calcidiol concentrations were determined by using a commercial ELISA, while calcitriol concentrations were measured by a commercial RIA (Immundiagnostik, Bensheim). The concentration of the bone resorption marker carboxyterminal cross-linked telopeptide of type I collagen (CTX) was detected in plasma using a Serum CrossLaps ELISA. The activity of total plasma alkaline phosphatase (AP) was measured with a colorimetric standard assay. Total plasma insulin-like growth factor 1 (IGF-1) was determined by an IRMA kit. The concentration of growth hormone (GH) was measured by an ELISA in plasma. Significance of differences between N-reduced fed goats and their controls were assessed by unpaired Student's t test. A difference was considered significant when $P < 0.05$.

Results Under a reduced N feeding regime, the concentration of P_i was decreased in saliva, abomasal and ruminal fluid in growing goats while plasma and urinary P_i concentrations were not changed. The decline in P_i concentrations in saliva ($r = 0.684$, $P<0.001$), ruminal ($r=0.503$, $P<0.05$) and abomasal fluid ($r=0.715$, $P<0.001$) was positively related with decreasing plasma urea concentrations. Renal excretion of Ca was elevated, while plasma Ca concentrations were not affected. Concentrations of calcidiol and calcitriol were both significantly reduced in RN fed goats. The amount of CTX and the total AP activity were both significantly elevated indicating some bone remodelling processes taking place during a reduced N diet in growing goats. A decrease in plasma IGF-1 concentrations was observed in the RN fed animals while GH concentrations were not affected.

Conclusions The electrolyte homeostasis of growing goats was influenced by changes of dietary N level like in monogastric animals despite efficient N recycling mechanisms. A link between protein metabolism and electrolyte homoeostasis is postulated for ruminants. In monogastric animals and humans, the calcium-sensing receptor (CaR) could be such a linking candidate (Conigrave *et al*. 2007). The CaR is able to respond to altered Ca and/or amino acid concentrations. Additionally, this receptor is modulated by calcitriol, a hormone which is involved in the regulation of electrolyte homeostasis. These properties of the CaR may be the molecular basis for the crosstalk between protein metabolism and electrolyte homeostasis. Therefore, more research is needed to find the balance between reduction of environmental N pollution by reducing dietary N in ruminant feeding and maintaining the animal's health.

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Effect of genome shuffling on endo-β-1,4-glucanase production in *Bacillus licheniformis* JK7 isolated from the rumen of Korean native goat

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Introduction Bacillus licheniformis, facultative spore forming Gram positive microorganism, has been extensively utilized for the industrial production of various enzymes, antibiotics and biochemical agents. In feed industry, Bacillus licheniformis is also used as a direct-fed microbials (DFM) to improve feed utilization, animal production and health. Genome shuffling accelerates entire genome recombination by recursive protoplast fusion between multi parent strains so that various phenotype strains are obtained (Zhang et al., 2002). We have isolated Bacillus licheniformis JK7 from the rumen of Korean native goat and tried to improve their endo-β-1,4-glucanase production by using genome shuffling with a final aim of producing a strain having higher cellulolytic enzyme activity.

Materials and methods To isolate facultative cellulolytic bacteria from the rumen of Korean native goat, appropriately diluted ruminal digesta were inoculated to endo-β-1,4-glucanase screening agar plate anaerobically. Finally, cellulolytic microorganisms which could survive in aerobic condition, were selected and their 16s rDNA sequences were analyzed using software V-NTI (Invitrogen Co. Ltd., USA) and blasted on NCBI database (http://www.ncbi. nlm.nih.gov). Then selected strain, *Bacillus licheniformis* JK7 was mutagenized with UV irradiation(30cm * 10min) to obtain initial parent library, and 4 mutated strains having increased endo-β-1,4-glucanase activity were selected. Genome shuffling was conducted using modified recursive protoplast fusion method (Hida *et al*., 2007). Random amplified polymorphic DNA (RAPD) PCR technique was used to identify genotype variation between wild type and shuffled strains. Endo-β-1,4-glucanase activity was determined using 3, 5-dinitrosalicylic acid (DNS) reducing sugar assay (Ghose, 1987). One unit of enzyme activity was defined as the amount of enzyme releasing 1μM of reducing sugar per minute.

Results Genome shuffling requires various mutant parent strains to accelerate genetic diversity. We isolated four strains (UV3, UV9, UV33, UV50) showing higher endo-β-1,4-glucanase activity(0.76, 0.65, 0.78, 0.78 units/ml, respectively) over wild type (0.60 units/ml). After first protoplast fusion, GS1-3, GS1-11, GS1-19 strains were founded. Their activity was 0.85, 0.92, 0.87 units/ml, respectively. Totally, 3 rounds of genome shuffling were conducted to obtain isolates that had further improved endo-β-1,4-glucanase activity. GS3-8, GS3-20 isolates were selected after 3 rounds of protoplast fusion, and they showed enzyme activity of 1.23 and 1.24 units/ml which was 2-fold higher compared to wild type strain (Figure 1). To compare genotype variation between wild type and shuffled strains, RAPD PCR technique was used. Total genomic DNA of wild type and shuffled strains were amplified by PCR with random oligoprimer (B4 primers : 5' TACCTAAGCG 3'). Shuffled strains showed specific fragment about 2kb suggesting that genome shuffling induced genotype variation.

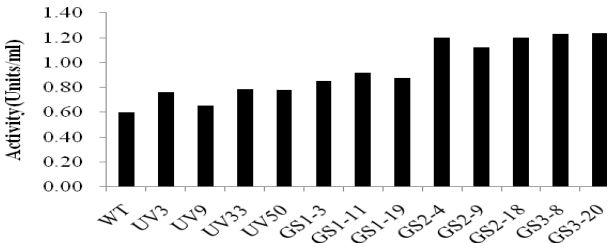

Figure 1 Endo-β-1,4-glucanase activities of wild type(WT), U.V. mutated strains(UV3, UV9, UV33, UV50) and genome shuffled strains(GS1-3,. GS1-11, GS1-19, GS2-4, GS2-9, GS2-18, GS3-8, GS3-20).

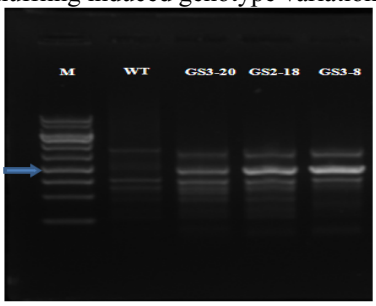

Figure 2 RAPD banding patterns of WT strain and GS strains using B4 (5' TACCTAAGCG 3') primer.

Conclusion Genome shuffling was efficient method to produce of Bacillus licheniformis JK7 strains having increased endo-β-1,4-glucanase activity. As this method did not use genes originated from other species, shuffled strains are not considered genetically modified organism (Zhang *et al*., 2002). Therefore, the strains may become good candidates for DFM to improve forage digestion by herbivores.

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Changes in performance parameters, hepatic and endocrine regulation of metabolism during a negative energy balance at two different stages of lactating dairy cows

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Introduction The onset of lactation is associated with profound changes in dairy cows. After parturition, lactation in dairy cows is accompanied by a negative energy balance (NEB) as a result of insufficient feed and thus energy intake while milk production simultaneously increases steeply. This lactation-induced NEB is associated with a considerable mobilization of body reserves to cover nutrient demands. However, health disorders (e.g. ketosis, displaced abomasum, and laminitis) are closely related to high-yielding dairy cows with a NEB. Later in lactation, a NEB may occur during insufficient quality and supply of feed. The liver plays a central role in metabolic regulation of the somatotropic axis and the insulin system to adapt to these changed physiological and environmental conditions. In order to study performance parameters and metabolic reactions during adaptation processes at two different stages of lactation, effects of an induced NEB by feed-restriction at around 100 days in milk (DIM) were compared with the responses due to the NEB in early lactation. The focus was set on hepatic gene expression and the endocrine system of the somatotropic axis and related parameters.

Material and methods Fifty multiparous cows (3.2 ± 0.2 parities) were studied in three experimental periods (1 = week 3ante-partum (a.p.) up to week 12 post-partum (p.p.), 2 = feed restriction for 3 weeks beginning at around 100 DIM with a feed-restricted (n=25) and control group (n=25), 3 = realimentation period for the feed-restricted group for 8 weeks). Throughout the study, all animals, except feed-restricted cows during period 2, had free access to a partial mixed ration 1 (PMR 1; 33.7% grass silage, 44.9% corn silage, 6.5% hay and 14.9% concentrate (DM basis)). When milk yield was above 21 kg/d, additional concentrate (CONC, based on barley, wheat, corn kernels, soybean meal, dried sugar beet pulp with molasses) including a vitamin-mineral premix was fed individually according to milk yield. In order to induce an energy deficiency of at least 30% of cows` requirements at the start of period 2, feed-restricted cows received a limited amount of a similar diet as control cows, but mixed with additional hay (PMR 2), and a reduced amount of CONC. Nutrient values of PMR 1, PMR 2 and CONC are described in Gross *et al*. (2011). Individual feed intake (PMR and CONC) and milk yield were recorded daily, body weight weekly. Energy content of the diets and milk as well as the energetic requirement for maintenance were determined according to the German Society of Nutrition Physiology. The difference between energy intake by feed and energy expenditure for maintenance and milk production results in the energy balance of the individual cow.
Blood samples were taken in week 3 a.p., week 1 (on day 3), week 2, 4, 8, and 12 p.p. (period 1), weekly during period 2 and in week 1, 2, 4, and 8 during period 3. Liver biopsies were taken at week 3 a.p., week 1 (on day 3) and week 4 p.p. (period 1), before feed restriction in week 0 and week 3 of period 2. Data analysis was performed by using a mixed model of SAS with group and parity as fixed effects and the individual cow as repeated subject. Differences between the groups were detected by the Bonferroni t-test. P-values < 0.05 were considered to be significant. Data are presented as means ± SEM.

Results In period 1, dairy cows experienced a NEB for the first 8 weeks being most negative in week 1 p.p. (-46.1 ± 3.4 MJ NEL/d) and decreasing simultaneously with increasing feed intake thereafter. The NEB in early lactation was accompanied by a loss of body weight, while milk yield increased up to 39.5 ± 0.8 kg/d in week 6 p.p.. In period 1, plasma growth hormone (GH) concentration reached a maximum in early lactation, whereas insulin-like growth factor-I (IGF-I), leptin, the thyroid hormones T3 and T4, insulin and the revised quantitative insulin sensitivity check index (RQUICKI) increased gradually after a nadir in early lactation. In week 1 p.p., mRNA abundance in liver of GH receptor 1A (GHR 1A), IGF-I, IGF-I receptor (IGF-IR) and IGF binding protein-3 (IGFBP-3) were decreased, whereas mRNA of IGFBP-1 and -2 and insulin receptor (INSR) were up regulated compared to week 3 a.p.. In period 2, feed-restricted cows had a more NEB compared to the NEB in early lactation. During period 2, feed-restricted cows showed a decrease in milk yield, body weight and plasma concentrations of IGF-I and leptin compared to control cows ($P < 0.05$). RQUICKI was lower for feed-restricted cows (period 2) than for control cows ($P < 0.05$). Compared to the NEB in period 1, the changes due to the induced NEB (period 2) in performance and endocrine parameters were less pronounced. At the end of the 3-wk feed-restriction period, mRNA abundance of IGF-I, IGFBP-1, -2, -3 and INSR was increased as compared to the control group ($P < 0.05$).

Conclusions Despite a deliberately induced NEB by feed-restriction that was even higher than the NEB in early lactation, alterations of the studied parameters were smaller compared to the changes in early lactation. The different effects of a NEB at the two stages in lactation show that the endocrine regulation changes qualitatively and quantitatively during the course of lactation.

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Comparative analysis between microscopic count and molecular-based DGGE approaches to examine ruminal protozoal community diversity

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Introduction Ruminal ciliate protozoal studies have been widely based on classical microscopic observations. Recently, PCR-based methods and several protozoan-specific 18S rRNA gene primers have been used in nutritional and diversity studies. Some agreements between microscopic counts (MC) and denaturing gradient gel electrophoresis (DGGE) analyses have been noted (Karnati *et al*. 2007). The aim of this study was to investigate the relationship between MC and DGGE analysis for the comparison of protozoal communities in cattle fed differing diets.

Material and methods Twelve Brahman crossbred steers, 480 ± 50 kg BW, were randomly allocated to three treatment groups of four animals. The treatments were: i) low quality hay (LQH); ii) high quality grass (HQG); iii) grain-based diet. The animals on grain and LQH diet were assigned to two collective pens. Animals on the grain diet were fed 1 kg of Mitchell grass hay (MG) plus 6 kg barley-based concentrate offered as equal amounts twice daily. Animal on LQH were fed *ad libitum* with MG. HQG animals were grazed on a paddock of regrowth Bermuda grass (*Cynodon influencis*) pasture. The experiment period was 28 d. Rumen contents were collected from each animal following slaughter at a commercial abattoir and fractioned into liquid and solid phases. From the liquid phase, a 4 mL aliquot was added to 16 mL of formal saline solution for MC and 1.5 mL was taken and frozen at -20°C for molecular analysis. Protozoans were counted and classified to the level of genera based on morphological characteristics using a counting chamber (Dehority 1993). Total gDNA was extracted by the repeated bead-beating protocol. DGGE through a 30–60% gradient and primers 316f and 539r-modified (Huws *et al*. 2009) were used. Clone libraries of thirteen excised DGGE bands were constructed using the PCR TOPO TA Cloning kit (Invitrogen), following the manufacturer's instructions and sequenced using Sanger DNA sequencing. A dendrogram of digitalized DGGE profiles was constructed with BioNumerics (v6.01). Principal component analysis (PCA) and ANOVA were performed using GenStat statistical software.

Results The mean ciliate protozoan numbers were significantly different between diets. The lower concentration was on grain-based diet (0.74 x 10^4/mL) than in HQG (2.96 x 10^4/mL) and LQH (12.23 x 10^4/mL). *Entodinium* spp. corresponding to one of the highest proportions in all the treatments, accounting for 32 to 56% of the total. *Diplopastron* were in high proportions on HQG and LQH. *Ostracodinium* spp. were detected in high densities in the LQH group. *Epidinium* spp. and *Diplopastron* were not observed on the grain-based diet. All the 18S rRNA gene sequences had a high percent BLASTn similarity to species of rumen protozoa (above 98%). A significantly lower community diversity was seen in the grain-based group with both MC and DGGE methods (Table 1). Richness and Shannon-Wiener index were positively correlated ($P<0.01$) between methods (0.75 and 0.79, respectively). DGGE profiles from animals clustered according to the treatments (Figure 1). A single animal (LQH2) clustered with the HQG group. The first component of PCA analysis explained 44.5% and 49.3% of the variation in similarities (diet effect) for MC and DGGE, respectively.

Table 1. Ruminal protozoal community diversity indexes for three diets using microscopic count and DGGE data

	Treatment			
	LQH	HQG	Grain	SEM
Microscopic count				
Number of genera	9.5[a]	6.8[a]	3.0[b]	0.97
Shannon–Wiener	1.62	1.31	0.68	0.26
DGGE gel				
Number of bands	9.0[a]	9.5[a]	4.8[b]	0.74
Shannon–Wiener	2.04[a]	2.13[a]	1.35[b]	0.12

Means with different superscripts differ significantly ($P<0.05$)

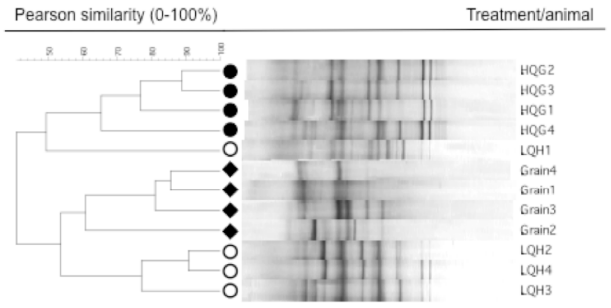

Figure 1. Dendrogram based on Pearson similarities correlation and UPGMA algorithm from DGGE profiles of the ruminal protozoal community of 12 steers on three diets

Conclusions Comparative analysis between MC and DGGE approaches demonstrated comparable descriptions of the ruminal protozoal community. Comparable patterns in PCA analysis, a significant positive correlation on diversity indices and similar taxonomic groups were detected between the two techniques. Therefore, DGGE and DNA sequence analysis are both useful tools to accurately describe the ruminal protozoal community.

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Biohydrogenation intermediates of linolenic acid are formed by different mechanisms during incubations with bovine ruminal fluid

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Introduction Recent studies have shown that the reduction of *cis*-9 18:1, *trans*-11 18:1 and 18:2n-6 during incubations with ruminal fluid or pure cultures of certain ruminal bacteria involves several different mechanisms (Wallace *et al*., 2007; McKain *et al*., 2010). There are no similar reports for linolenic acid (LNA; 18:3n-3). Samples of bovine ruminal fluid were incubated with 5.0 mg of LNA in buffer containing deuterium oxide to investigate the mechanisms and intermediates formed during biohydrogenation of LNA.

Materials and methods Incubations were performed in 100 mL glass flasks using an *in vitro* batch culture method. Five mg of LNA was incubated with McDougall buffer containing deuterium oxide and strained bovine ruminal fluid collected from four cows fed a grass silage-based diet (forage:concentrate ratio 60:40) in triplicate for 0, 1.5, 3 and 12 h. At each designated time point, flasks were placed in cold water and the contents were stored at -20°C. Lipid was extracted from freeze-dried samples and non-esterified fatty acids were recovered and converted to fatty acid methyl esters (FAME) and 4,4-dimethyloxazoline (DMOX) derivatives. Fatty acid structure, location of double bonds and enrichment of n+1, n+2 and n+3 isotopomers were determined by gas-chromatography mass-spectrometry of FAME and DMOX derivatives. Enrichment in water was determined by gas isotope ratio mass spectrometry. Data were analysed by ANOVA for repeated measures, with the MIXED procedure of the SAS software package Version 9.1 (SAS Inst. Inc., Cary, NC, USA).

Results Incubations of LNA with strained ruminal fluid resulted in a time-dependent accumulation of numerous 18:1, 18:2 and 18:3 intermediates and 18:0 (data not presented). *C***is-**9, **trans-**11, *cis*-15-18:3 and Δ8, 12, 15-18:3 represented the main 18:3 products to accumulate. Other 18:3 fatty acids including Δ5, 12, 15-18:3, Δ7, 12, 15-18:3, *c***is-**9, **trans-**11, *trans*-13-18:3 and **trans-**9, **trans-**11, *cis*-13-18:3 were also formed. Mass spectrometry indicated an enrichment of deuterium in the n+1 isotopomer of *c***is-**9, **trans-**11, *cis*-15-18:3 as a result of labelling at C-13, whereas the Δ8, 12, 15-18:3 contained minimal enrichment (Table 1). Concentrations of other 18:3 intermediates were too low to make an assessment of the labelling pattern. *Cis*-12, *cis*-15-18:2, **trans-**11, *cis*-15-18:2 and *trans*-11, *trans*-13-18:2 represented the main 18:2 intermediates to accumulate during incubations of LNA with strained ruminal fluid. **Trans-**11, *cis*-15-18:2 was enriched at n+1 and n+2 due to the incorporation of deuterium on carbon atoms 9 and 13. The n+2 isotopomers of *cis*-12, *cis*-15 18:2 and *trans*-11, *trans*-13-18:2 were also found to be enriched (Table 1) but the location of ^{2}H could not be determined.

Table 1 Enrichment of n+1, n+2 and n+3 isotopomers of 18:2 and 18:3 intermediates formed after 3 h incubations of 5 mg of linolenic acid with strained ruminal fluid collected from four cows.

	n+1		n+2		n+3	
MPE ratio[1]	Mean	SEM	Mean	SEM	Mean	SEM
Δ8, 12, 15 18:3	0.238	0.0050	0.094	0.0645	-	-
cis-9, *trans*-11, *cis*-15 18:3	0.813	0.0480	0.087	0.0230	-	-
cis-12, *cis*-15 18:2	1.180	0.0088	1.019	0.0168	0.458	0.0677
trans-11, *cis*-15 18:2	0.965	0.0958	0.715	0.0399	0.125	0.0148
trans-11, *trans*-13 CLA	0.872	0.0163	0.446	0.0193	0.098	0.0142

[1]MPE, moles percent excess. The MPE ratio indicates MPE in fatty acid isomer/MPE in water. Mean 56.6 % ± 1.33% MPE enrichment in deuterated water. Values are least square means and SEM for 12 observations.

Conclusions Incubations of LNA with strained bovine ruminal fluid resulted in the formation *cis*-9, *trans*-11, *cis*-15-18:3 via a mechanism that appears identical to that responsible for the conversion of 18:2n-6 to geometric isomers of 9, 11 18:2 by ruminal bacteria. Hydrogenation of LNA also resulted in the accumulation of geometric isomers of 9, 11, 13 18:3. These data also provide the first indication of an alternate mechanism of biohydrogenation of LNA that results in the formation of non-conjugated 18:3 intermediates via a mechanism that differs from the synthesis of conjugated 18:3 isomers.

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Effects of L-tryptophan i.v. infusion on the serum gastrointestinal hormone concentration and α-amylase activity in Korean native steers

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Introduction Recent study has shown that melatonin or its precursor; L-tryptophan, stimulates pancreatic enzyme secretion, when given parenterally to the rats (Jaworek *et al*., 2004). Above pancreatic secretory effects of melatonin or L-tryptophan are indirect and probably depend on the CCK release. However it has not been documented whether L-tryptophan is able to affect pancreatic enzyme secretion when applied into gastrointestinal hormones in ruminant. The aim of this study was to investigate the influence of L-tryptophan given intravenous (i.v.) on pancreatic α-amylase secretion in the Korean native steer with and to assess the involvement of cholecystokinin (CCK).

Materials and methods Three Korean native steers (362±22.9kg) fitted with catheter in intravenous were used in a 3x3 Latin square design to investigate effects of *i.v.* infusion of L-tryptophan (TRP) on gastrointestinal hormone secretion and α-amylase activity in blood. The experiment was a 3x3 Latin square design whereby each group was infused with saline (control), saline + TRP 27.9mg/kg (T1), and saline + TRP 57.8mg/kg (T2) into the jugular vein for 120 min. Blood and duodenum samples were collected for serum GI hormones and the α-amylase activity at -60, 0, 30, 60, 90, 120, 150, 180, 240, 300 hours from the infusion of L-tryptophan or saline solution. The serum ghrelin, CCK, and secretin concentrations were determined by an enzyme immunoassay (human EIA kit for ghrelin (EK-031-30), secretin (EK-067-05) and CCK-8 (EK-031-30)) using a secondary antibody coated plate. Insulin was determined using a bovine insulin ELISA kit (catalog No. 10-1201-01) (Mercodia). The serum α-amylase activity was analyzed by an EnzyChrom ™ a-Amylase Assay Kit (ECAM-100). Samples were compared by ANOVA using SPSS 14.0k for Windows. By using the New multiple range test of the Duncan's, they performed the significance test.

Results The level of serum CCK in the saline + TRP 57.8mg/kg (T2) treatment was higher than in the other treatments ($p<0.05$) (Figure 1), although there was no effect of L-tryptophan treatment on the serum ghrelin, secretin and insulin levels in steers. The α-amylase activity in the blood was greater in the L-tryptophan treatment than the control diet, although there is no significant difference.

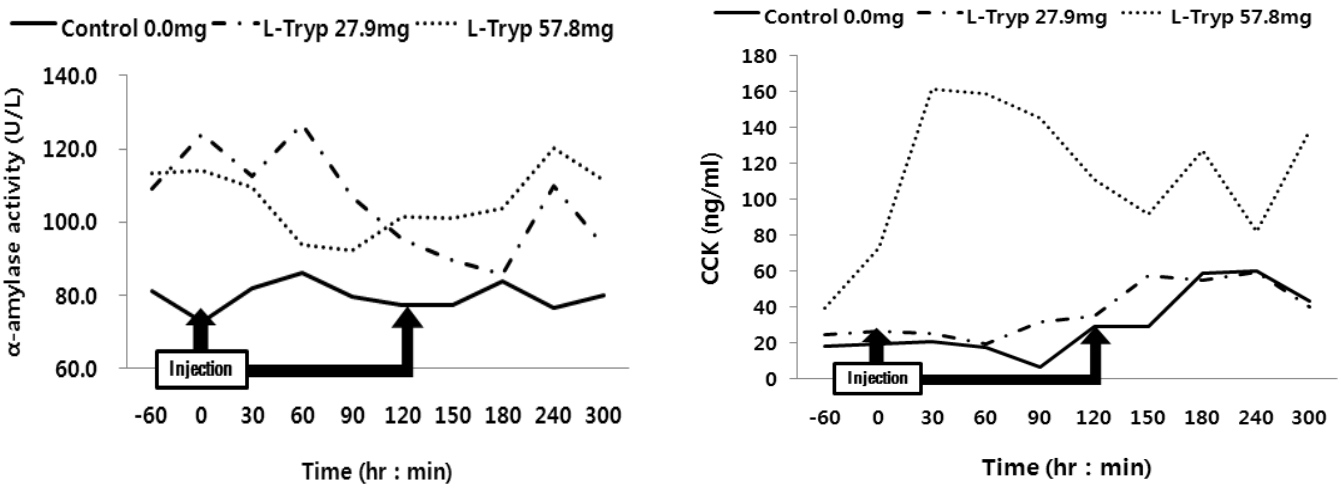

Figure 1 Response of serum CCK and α-amylase to L-tryptophan *i.v.* infusion in Korean native steers.

Conclusions We conclude that L-tryptophan infusion stimulates pancreatic α-amylase secretion via stimulation of CCK release in Korean native steer.

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Bacterial protein breakdown by holotrich rumen protozoa

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Introduction The presence of a mixed population of rumen protozoa has a negative effect on dietary N utilization by the ruminant as a consequence of their bacterial predation activity. The presence of protozoa increases bacterial N recycling by 38% and rumen ammonia concentration by 42% leading to a 10% decrease in the microbial N flow to the duodenum (Eugene *et al.,* 2004). Nevertheless, the protozoal species that contributes most negatively to rumen N utilization, and to what extent this occurs, are not known yet. A substantial decrease in the deaminating activity of rumen microbes in holotrich monofaunated animals compared with normally faunated animals has been observed (Ivan *et al.,* 2000), suggesting limited bacterial predation by holotrich protozoa. Nevertheless, the holotrich population in ruminants comprises two genera of different sizes and hypothetically different activities.
The aim of this *in vitro* experiment was to quantify the capacity of bacterial protein breakdown by holotrich protozoa. A differential fractionation procedure and several expression forms were used to evaluate how the protozoal genus and/or size modify their activity.

Material and methods Holotrich activity was measured by the breakdown of ^{14}C leucine-labelled rumen bacteria *in vitro* as described Wallace and McPherson (1987) in two consecutive batches. Rumen fluid was obtained by oesophageal tubing before the morning feeding from eight holotrich monofaunated sheep fed on a maintenance diet. Rumen bacteria were isolated and labelled by overnight growing on medium no. 2 of Hobson with ^{14}C-leucine (1.44 μ Ci/tube) at 39ºC. Then bacteria were harvested, washed and resuspended in simplex type salts solution (STS) containing ^{12}C-leucine to prevent re-incorporation of released ^{14}C-leucine. Bacterial protein was determined with Folin reagent. Two litres of rumen fluid were diluted with four litres of STS buffer and placed in a sedimentation funnel at 39ºC for 1h to settle the protozoal cells. The sediment was filtered in anaerobic conditions through 250μm pore nylon mesh to remove plant material and the filtrate was then fractionated through 6 nylon meshes (80, 60, 45, 35, 20 and 5μm pore). Every fraction was washed and diluted with 50 ml of STS. Protozoal pellets obtained after filtration with 60 and 45μm mesh sizes were chosen as representative of the *Isotricha* pool while 20 and 5 μm fractions were regarded as being representative of the *Dasytricha* pool. Subsamples of these fractions were taken and pooled to reconstitute the initial protozoal population and one negative control was included. Every protozoal fraction was incubated in quadruplicate in Hungate tubes at 39ºC after addition of labelled-bacteria. Tubes were sampled every hour, samples were fixed with trichloroacetic acid, centrifuged (11,000 × *g* for 5 min) and the supernatant was diluted with scintillation fluid to determine the radioactivity released by liquid-scintillation spectrometry. The bacterial breakdown at each incubation time was calculated from the acid-soluble radioactive label and expressed as a percentage of the total radioactivity present in the labelled bacteria suspension. The degradation rate per hour was calculated as the difference from the linear portion of the degradation curve. Concentration and size of every holotrich species was determined in each tube by optical microscopy. ANOVA was carried out to make comparisons between fractions (blocking by batch) while multiple linear regression was used to predict the activity of the protozoal groups studied.

Results Protozoa of different sizes were successfully separated by the fractionation procedure. *Isotricha* and *Dasytricha* populations almost entirely constitute the fractions of 60 or 45, and 20 or 5μm, respectively. *Isotricha prostoma* and *Isotricha intestinalis* had 20 and 14 times greater volume than *Dasytricha ruminantium* respectively, although estimations of bacterial protein breakdown per ciliate showed that *Isotricha* had only between 1.4 and 1.6 times greater activity than *Dasytricha*. Thus, when data were corrected according to the ciliate volume, *Dasytricha* had 13 to 14-times greater bacterial breakdown activity than *Isotricha*.

Table1 Protozoal fractionation and activity

Fraction	Initial	F60	F45	F20	F5	SEM	*P*
Proportion of cells							
Isotricha, %	14.0	99.5	96.3	0.9	0.5	0.89	<0.001
Dasytricha, %	86.0	0.5	3.7	99.1	99.5	0.89	<0.001
Proportion of volume							
Isotricha, %	74.7	100	99.8	12.9	5.5	2.42	<0.001
Dasytricha, %	25.3	0	0.2	87.1	94.5	2.42	<0.001
Bacterial breakdown							
%/10^5ciliates·h	1.7	3.3	1.9^b	1.4	1.1	0.13	<0.001
%/100mm^3 ciliate·h	6.6	2.1	1.2^c	15.7	13.5	1.10	<0.001
pg protein/ciliate·h	6.6	13.6	7.7^b	5.9	4.2	0.57	<0.001
ng protein/mm^3 ciliate·h	22.8	7.1	4.3^c	54.6	47.1	3.84	<0.001

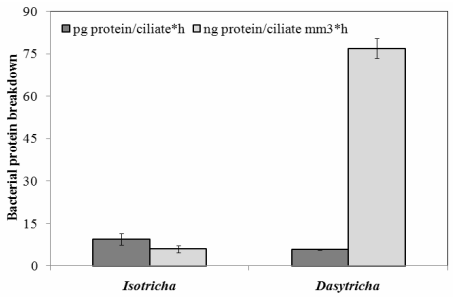

Figure 1 Bacterial breakdown by holotrich

Conclusions Our results showed that holotrich protozoa have different activities of bacterial protein breakdown depending on the genus. While *Isotricha* had a slightly higher activity per cell than *Dasytricha*, the later specie had a substantially higher activity per unit volume than the former.

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Variations in microbial population and fermentation traits along the digestive tract of grass- or browse-fed goats

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Introduction The degradation of plant fibres in the ruminant's digestive tract is accomplished by various *Bacteria* and *Protozoa*, and is maintained through the removal of hydrogen by the methanogenic *Archaea*. The composition of the microbial population is expected to differ concomitantly with the stratification of the rumen contents and along the digestive tract. These variations are also expected to depend on the type of forage fed. The aim of the present study was to test these hypotheses by assessing the microbial population and fermentation at various sites of the digestive tract of goats, a herbivore species which is able to feed both on grass and browse. The freezing-in-standing technique was applied which was essential to prevent any mixing of rumen content during slaughter.

Material and methods 10 adult female Saanen goats were fed either grass hay (n=5) or dried browse (poplar, raspberry and chestnut leaves in a ratio of 1:1:1) (n=5) for at least 20 days as the only diets in indoor enclosures. Afterwards, the animals were euthanized (app. 12 hours after last feeding) and frozen at -20°C in standing position. Samples of gastrointestinal contents were taken from eight different sampling sites in the rumen (dorsal, central, central close to rumen wall, ventral; each caudal and cranial) and from caecum and colon. pH and ammonia concentration in gastrointestinal fluid were measured in squeezed and filtered samples using respective electrodes connected to a potentiometer (model 713; Methrom AG, Herisau, Switzerland). For microbial analyses, the gastrointestinal content was lyophilised; DNA was extracted according to Guertler *et al*. (2009), but dichlormethane was used instead of chloroform, and DNA was collected via centrifugation at 16000 *g* for 2 min instead of using columns, and washed with 70-% ethanol. DNA was eluted in water and stored at -20°C. Primers for *Bacteria* were from Denman and McSweeney (2006), for *Protozoa* from Sylvester *et al*. (2005), and for *Ruminococcus albus* from Koike and Kobayashi (2001). Primers for *Archaea*, *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* were newly designed. Quantitative PCR was performed in 10µl reactions using the Fast SYBR Green Master Mix on a 96-well 7500 Fast Real-Time PCR System (Applied Biosystems). Microbial 16S rDNA copy numbers were calculated using standard curves with purified PCR products for each gene. Data was analysed using the MIXED procedure of SAS with diet and sampling site and the interaction of diet and sampling site as effects, and multiple comparisons among means were carried out by Tukey`s method. Differences among means were considered significant at $P < 0.05$.

Results In the digestive tract of both grass and browse fed goats, the DM content was highest in the colon (21.2%), lower in dorsal (14.2%) and central (12.2%) rumen and caecum (13.4%), and lowest in the ventral rumen (6.7%). The ventral rumen content contained more fluid in browse-fed goats (3.7% DM) than in grass-fed goats (9.7% DM). The pH and the concentration of ammonia were similar in grass- and browse-fed goats but differed along the digestive tract; the pH increased from the dorsal rumen to the hindgut and was significantly higher in caecum (7.9) than in the dorsal and central rumen (5.9) of the browse fed goats. The ammonia concentration was similar along the digestive tract of browse-fed goats (11.6 mmol/l), but in grass-fed goats, it was higher in caecum (18.9 mmol/l) and colon (29.7 mmol/l) than in the rumen (4.1 mmol/l). The bacterial concentration in the content of the digestive tract was similar in grass- and browse-fed goats; it was highest in the dorsal rumen (1.1×10^{11} 16S rDNA copy numbers/ g wet weight) and lowest in the caecum (3.9×10^{10}/g) and colon (6.3×10^{10}/g). The ruminal concentrations of *F. succinogenes* and *R. flavefaciens* were higher in browse-fed goats (5.0×10^{8}/g and 6.0×10^{8}/g, respectively) compared to grass-fed goats (7.9×10^{4}/g and 2.5×10^{8}/g, respectively), whereas no significant differences were found with *R. albus* in the rumen of grass- (1.9×10^{8}/g) and browse-fed (7.9×10^{7}/g) goats. In the caecum and colon of both groups of goats, the three fibre degrading bacterial species investigated were found in similar and rather small concentrations (2.5×10^{5}/g to 7.5×10^{7}/g). A higher protozoal concentration was present in the rumen of browse-fed (1.1×10^{9}/g) compared to grass-fed goats (2.5×10^{8}/g). The *Archaea* were more abundant in the central rumen of browse fed (3.3×10^{9}/g) than of grass fed (1.5×10^{9}/g) goats. In browse-fed goats, archaeal numbers were higher in the dorsal and central rumen (on average 2.9×10^{9}/g) than in the ventral rumen (4.0×10^{8}/g), caecum (4.6×10^{8}/g) and colon (5.6×10^{8}/g). A similar pattern, on a lower level, was observed for the *Archaea* in the gastrointestinal tract of grass-fed goats, but differences between digestive tract sampling sites were not significant.

Conclusions The adaptation of the goats to the browse diet was accompanied by an increasing abundance of *F. succinogenes* and *R. flavefaciens,* which are able to degrade more resistant fibre (Akin and Rigsby, 1985), and of methanogenic *Archaea*, probably due to a higher degree of fibre degradation and hence hydrogen production. Furthermore, the results confirm that microbial populations and fermentation pattern vary from the rumen to the hindgut, and between dorsal and ventral rumen irrespective of the type of forage and highlight that rumen contents should be sampled from defined rumen compartments.

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Microbial detoxification of fluoroacetate in herbivores

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Introduction Microbial degradation of fluoroacetate (FA), particularly in anaerobic environments is important due to the toxic nature of the compound. Globally, fluoroacetate is found in over 40 plant species and is responsible for intoxication of commercial livestock species (McIlroy, 1982). Due to this problem with fluoroacetate-accumulating plants, dehalogenase genes have also been used to develop transgenic rumen bacteria (Gregg *et al.*, 1994) which detoxify fluoroacetate within the gastrointestinal tract of ruminant livestock (Gregg *et al.*, 1998; Padmanabha *et al.*, 2004). The use of dehalogenases and other microbial enzymes has also been proposed for bioremediation of contaminated soil and water. Concerns over the release of these transgenic bacteria, and the lack of information on fluoroacetate-degrading anaerobes prompted us to search for naturally-occurring anaerobic microorganisms that could reduce fluoroacetate toxicity in ruminants. Despite the interest in the microbial degradation of fluoroacetate, there are few reports of fluoroacetate being degraded naturally under anaerobic conditions. We have been able to isolate fluoroacetate degrading bacteria from the gut of cattle, kangaroos and emus in Australia. These bacteria all belong to the phylum Synergistetes and are 92% similar (16S rRNA gene) to the 'leucaeana detoxifying bacterium' *Synergistes jonesii*.

Methods A PCR assay which was specific for the 16S rDNA sequence of the fluoroacetate degrading bacteria was designed and used in an environmental survey. A representative strain (MFA1) of the fluoroacetate degrading bacteria from the rumen was examined in relation to its growth requirements and a draft genome sequence assembled.

Results and Conclusions These gut bacteria are widely distributed in herbivorous animals but present in relatively low numbers (< 10^6/g digesta). In some animals the putative FA degraders were the dominant members of the resident Synergistetes phylum. Growth of the FA degraders was stimulated by amino acids and peptides with greater quantities of amino acids metabolized in the presence of fluoroacetate but sugars were not fermented. Hydrogen and formate produced *in situ* were consumed during dehalogenation. Results on the growth characteristics of strain MFA1 associated with fluoroacetate metabolism indicated that the bacterium may gain energy via reductive dehalogenation. This is the first study to identify a bacterium that can anaerobically dehalogenate fluoroacetate.

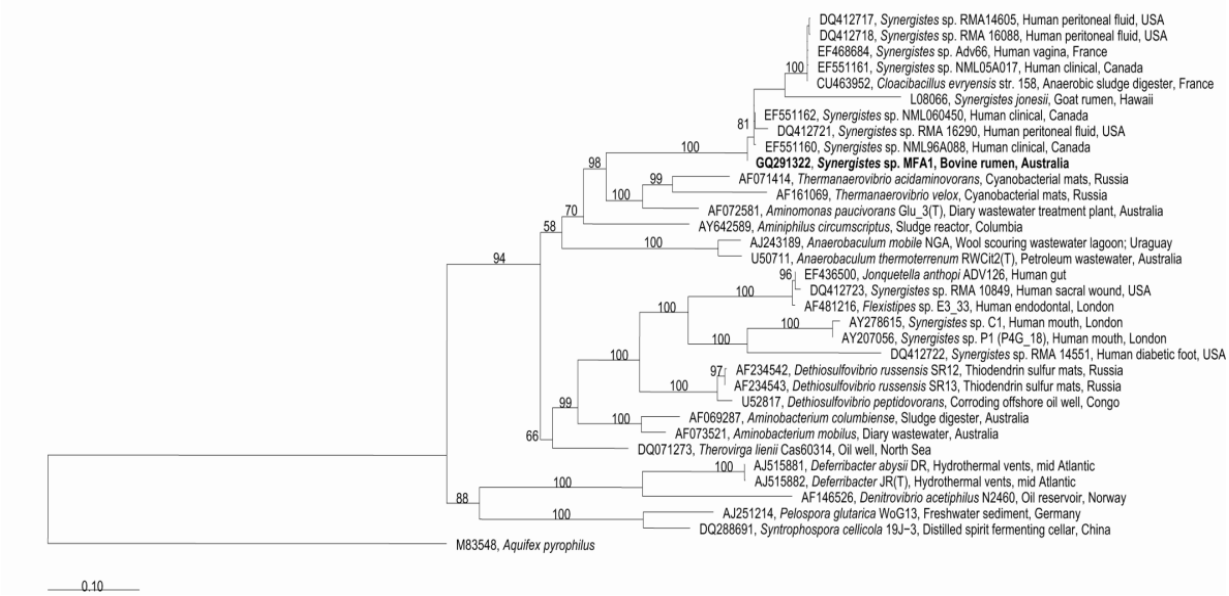

Figure 1 16S rRNA gene distance tree of the phylum *Synergistetes* and other related species. Stain MFA1 is highlighted in bold.

The draft genome of strain MFA1 and preliminary annotations have also provided new insights into the metabolism of this unique organism. From the genome of 3.2Mb, a large proportion of the gene annotations (approximately 13%) are assigned to amino acids metabolism and peptide transport, while only 6% of the genes annotations are for carbohydrate metabolism. These findings correlate with other *Synergistetes* bacteria, which have a higher abundance of genes for amino acids metabolism (Hugenholtz *et al*, 2009). The gene encoding fluoroacetate dehalogenation has not been identified from this process which indicates that it is likely to be novel.

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Genomic analysis of *Anaerovibrio lipolytica* strain 5S, a rumen lipolytic bacteria

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Introduction The relationship between dietary fat and coronary heart diseases is well established, and many studies have contributed to the advice that saturated fatty acids (SFA) should not supply >0.10% of total energy intake, polyunsaturated fatty acids (PUFA):SFA should be >0.45, and n-6:n-3 PUFA should be <4 for the whole diet. The ruminant diet is rich in PUFA, yet ruminant products are rich in SFA due to bacterial lipolysis and subsequent biohydrogenation of ingested PUFA within the rumen (Scollan *et al.*, 2006). Understanding lipolysis, the first step in rumen lipid metabolism, is key to reducing subsequent biohydrogenation, thereby improving the PUFA content of ruminant products. *Anaerovibrio lipolytica* is a well and long known rumen lipolytic bacterium (Hobson and Mann, 1961), nonetheless the lipases of this organism have not been characterised nor is the genome of this bacterium or indeed its lipase gene(s) currently sequenced. Our aim within this study was to characterise the lipase gene(s) of *A. lipolytica* in order to increase our understanding of rumen lipolysis.

Material and methods *A. lipolytica* strain 5S was anaerobically incubated at 39°C and grown overnight in Hobson's medium, the genomic DNA was extracted using the QIAmp® DNA stool mini kit (Qiagen Ltd, Crawley, UK). The draft nucleotide sequence of the bacterium was established by a shotgun sequencing approach carried out on a Genome Sequencer FLX system (454 Life Sciences, Roche) using the Titanium sequencing chemistry. Assembly of the reads was done using the gsAssembler software (Roche). The reads were then blasted against a database of lipase gene sequences retrieved from NCBI, and the matches combining ≥300 bp and e-value $\leq 10^{-30}$ were chosen for further analysis. These positions were translated with the software BioEdit v7.0.5.3 (Hall, 1999) and phylogenetically classified alongside 50 bacterial lipases and esterases (Liu *et al.*, 2009) using the software ClustalW.

Results The pyrosequencing yielded 284 reads with about 2.83 Mbp total sequence information. The assembly resulted in 247 large contigs (>500 bp) with a total size of 2,816,384 bases. BLASTN of the contigs against the lipase sequence database produced 13 alignments of interest (length alignment from 302 to 1,599 bp and e-value from $3x10^{-32}$ to $4x10^{-138}$), best matching with the uncultured bacteria from rumen metagenome Rlip2 (FJ529694), *Lactococcus lactis* (AF059739) and *Ralstonia solanacearum* MolK2 (NW_00219653). Phylogenetic analysis of the translated contigs showed that four of them were related to putative lipases and esterases. Nine other contigs clustered in a separate branch, with no close relationship with known lipases and esterases. Indeed, bioinformatic analysis for these nine contigs did not identify lipases, matches were with various proteins related to the genera *Mitsuokella*, *Selenomonas* and *Veillonella*: peptide chain release factor 2, preprotein translocase SecA subunit, glycine hydroxymethyltransferase, transketolase, translation elongation factor G, ATP synthase F1 α and β subunits, an ATP-binding protein and hypothetical proteins.

Conclusions *A. lipolytica* 5S draft genome was retrieved using 454 pyrosequencing. Putative genes of interest for the expression of the lipase of *A. lipolytica* 5S were identified as well as other putative genes that did not matched with known lipase or esterase genes. Future work will be to clone them in an expression vector with different frameshifts to characterize features of the protein, as well as determine the physiological function of the proteins within the bacterium.

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Novel lipolytic activity isolated from bovine rumen bacteria metagenomic libraries

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Introduction Most research on lipid metabolism in the rumen has mainly focused on biohydrogenation of polyunsaturated fatty acids and conjugated linoleic acids due to the potential of these molecules in human health. On the contrary there is a dearth of available data on ruminal lipolysis, the first step in rumen lipid metabolism (Lourenço *et al.*, 2010). Indeed, though bacteria are considered to be the most active among ruminal microorganisms in lipolysis, there are only a few culturable isolates known for lipolytic activity (Jarvis and Moore, 2010), and only two lipases have been retrieved so far from the rumen bacterial metagenome (Liu *et al.*, 2009). Our aim within this study was to characterize lipolytic activity from the rumen metagenome in order to get a better understanding of the rumen lipases and the bacteria that possess them.

Material and methods Metagenomic DNA was extracted using the Bio101 Fast DNA Spin kit for soil (Qbiogene, Cambridge, UK) from bovine rumen content fractionated into strained ruminal fluid (SRF), solid-attached bacteria (SAB) and liquid-associated bacteria (LAB) and subsequently cloned into the pCC1FOS^{TM} fosmid vector (Epicentre, Cambio Ltd., Cambridge, UK) hosted by *Escherichia coli* EPI300^{TM}. Transformants were selectively screened for lipolytic activity onto spirit blue agar plates containing 1 % (v/v) tributyrin, and incubated at 37°C for 48 h. A blue precipitate formed around positive colonies. The plasmids were extracted from the positive clones using the QIAprep® Spin Miniprep kit (Qiagen Ltd., Crawley, UK) and their sequences were analyzed using the Genome Sequencer FLX system (454 Life Sciences, Roche). Contigs were assembled using the gsAssembler software (454 Life Sciences, Roche) and their sequences analyzed using BLASTN, ORF-Finder and BLASTP on NCBI. Maps of the plasmids were realised with the software Geneious v4.8.5 (Drummond *et al.*, 2011).

Results The libraries consisted of a total of 23,872 clones with an average insert size of approximately 30 to 35 kbp. Five clones from the SAB library and four from the LAB library exhibited lipolytic activity on spirit blue agar whereas no clones were identified as showing lipolytic activity from the SRF library. The size of the plasmids ranged within 20 and 40 kbp. Three plasmids were successfully sequenced into single contigs, and six others were assembled into multiple contigs. Putative lipase and esterase genes have been identified for six of the plasmids, matching (75 to 93% similarity) with the lipid hydrolase gene from uncultured bovine rumen bacterium (DQ788540), the ester hydrolase gene from uncultured marine prokaryote (AJ811969) and *Prevotella ruminicola* 23 genome (CP002006). The putative lipase genes are to be cloned in an expression vector to purify and characterize the proteins.

Conclusions Nine fosmid clones expressing lipolytic activity were identified from SAB and LAB metagenomic libraries. The putative lipase and esterase genes were identified. Future work will be purification and characterization of the features of the proteins, as well as establish which bacteria possess them in the rumen.

Acknowledgments The authors would like to thank Mrs Hilary Worgan and Dr Susan Girdwood for their valuable technical assistance.

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Comparison of bacterial populations from different regions of the large intestine of horses using terminal restriction fragment length polymorphism and 454 pyrosequencing

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Introduction The horse has a diverse bacterial large intestinal population which is required for efficient fermentation and utilisation of fibre based dietary energy. The microbiota also plays an important role in the health of horses and disturbances may trigger metabolic disease such as laminitis or colic (Millinovich *et al*., 2008). However, there is little published information pertaining to the quantification, characterisation, and metabolic activity of this microbial population. In addition, although changes in the faecal microflora are commonly accepted to be representative of changes within the large intestine, very little attention has been given to how closely the faecal microbial population reflects other sections within the equine large intestine in particular the caecum (Hastie *et al*., 2008). The objective of this study was to use next generation sequencing technology, 454-pyrosequencing, to compare the microbial population in different regions of the large intestine from horses' and ponies' euthanized for non-research purposes.

Material and methods Samples were taken of luminal gut contents from the terminal ileum and 7 regions of the large intestine (Caecum, right ventral colon (RVC), left ventral colon (LVC), left dorsal colon (LDC) right dorsal colon (RDC), small colon (SC) and rectum) from five thoroughbred horses (5-9 years old) and five native breed ponies (7-19 years old) euthanized for non-research purposes. Pre-euthanasia the horses and ponies had been maintained out on pasture and came from two groups one consisting of all the horses and one of all the ponies. There was no known history of any intestinal disease (including colic) or laminitis within the previous six months and they were considered to be healthy (pers comm. Horse/pony owners).
Prior to extraction of nucleic acids, samples were freeze dried before being disrupted by bead beating. Genomic DNA was then extracted from all samples using QIAGEN QIAamp® DNA stool mini kits (Qiagen Ltd.,UK). For 454-pyrosequencing amplification of the V1-V3 regions of 16SrDNA was performed using the primer pair 27F and 357R (Liu *et al*., 2007) incorporating Roche/454 adaptors and a sample specific barcode tags allowing samples to be multiplexed. Amplicon sequencing was performed with the GS FLX system using titanium chemistry (454 Life Sciences, Roche). Data trimming and analysis was carried out using the Mothur software package (Schloss *et al*., 2009). Using Mothur sequences from all samples were classified and assigned into operational taxonomic units (OTU's). Diversity indices (Shannon-Weiner and Simpson's indices) were calculated and compared by ANOVA (with significance at $P<0.05$). A phylogenetic tree was also created (not shown).

Results Sequences produced in each gut region were for the ileum; 5820, caecum; 6185, RVC; 18751, LVC; 19701, LDC; 3652, RDC; 4237, SC; 7990 and rectum; 6592.These sequences were assigned to 107, 322, 822, 812, 156, 169, 394 and 318 operational taxonomic unit's (OTU's) respectively. Shannon-Weiner diversity indices were calculated (see table 1). Phylogenetically the microbial population appears to differ according to the region with the ileal samples from all animals showing tight clustering whereas the caecal samples tend to cluster together with the ventral colon samples. The dorsal colon and rectal samples also tend to cluster together. It can also be noted that within this grouping of gut regions the ponies tend to cluster together with each other and similarly the horses.

Table 1 Shannon-Weiner diversity indices for different regions of the horse large intestine

	Ileum	Caecum	RVC	LVC	LDC	RDC	SC	Rectum	S.E.D.
Shannon-Weiner	3.284^{a}	5.376^{c}	6.2^{d}	6.03^{d}	4.428^{b}	4.411^{b}	5.431^{c}	5.152^{d}	0.383^{***}

Different letters (a-d) denote significant differences.
***= $P<0.001$

Conclusions Phylogenetic clustering of the caecum and proximal regions of the large intestine could be expected due to predicted alterations in the microbial population, in parallel with changes to the flow and consistency of digesta as it moves through the tract. Sequence and OTU numbers varied according to the region which could reflect differences in fermentative capacity. The Shannon-Weiner index increases where the number of different bacterial species increase and the relative numbers of each representative of those species increase (species richness and evenness). Table 1 shows that according to this index there were significant differences ($P<0.001$) between regions and; the ventral colon and the rectum are similar, the caecum and the small colon are similar and the left and right dorsal colon are similar. These observations demonstrate the variability of the microbiota in the large intestine of the horse and suggest the need for more detailed sequencing to establish more precise information regarding its composition.

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Effect of pasture site and stocking density on milk yield and composition of yak-cattle crossbreds (*Bos grunniens* x *Bos taurus*) along a transhumance route of the Eastern Himalayan Mountains of Nepal

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Introduction Yak-cattle crossbreds are the choice of genotype in yak rearing areas where also lower altitudes are grazed. Livestock systems with yaks and their crossbreds are also found on traditional routes of transhumance in the mountainous regions of Nepal. In this migration system, the herders use different pastures during the course of the year covering altitudinal levels between 1500-5000 m a.s.l. (Dong *et al.*, 2009). High grazing pressure may result in declining pasture productivity on the alpine and subalpine grasslands, making the stocking density (SD) an important issue as well in economic as also ecological terms. The present study was conducted for assessing milk yield and milk composition of yak-cattle crossbreds (called *chauries* in Nepali) at different altitudes kept at two different SD along a transhumance route in the Kanchenjunga Conservation Area of the Eastern Himalayan Mountains of Nepal.

Materials and methods Following one transhumance route, five pastures (i.e. sites) at altitudes of 3200 m, 4000 m, 4500 m, 4000 m and 2600 m a.s.l. were investigated successively in a controlled grazing experiment conducted between May and November 2010. Twelve multiparous female *chauries*, which had calved in a short time interval in April 2010, were selected and allocated to four paddocks stocked with either two or four animals (low and high SD, respectively) by balancing groups for pre-experimental average daily milk yield and body weight. Paddock size was equal across groups, which was adjusted at every site to animal requirements in the high SD groups based on estimated biomass availability (Wiener *et al., 2003*). The groups stayed together throughout all five experimental sites. The calves had been separated one week after parturition. The *chauries* were milked in the morning and evening. Daily milk yield was recorded and the milk composition was measured at each milking event using a portable milk analyzer (Ultrasonic Lactoscan, Milkotronic Limited, Bulgaria). The data on milk yield and composition were averaged across the last three sampling days per site per cow, treating the first six days as adaptation period. The mixed procedure of SAS (version 9.2) was used for analysis of variance considering site, SD and the interaction of site and SD. Significant differences among means were established by Tukey's method.

Results The effects of site and SD on daily milk yield were highly significant ($P<0.001$). Concerning site, there was a progressing decline of milk yield with time, with 2637 g, 2616 g, 2118 g, 1830 g and 1146 g across both SD. Milk yield was generally higher with low SD (2413 g on average of all sites) in comparison to the high stocking density (1726 g). The highest daily milk yield (3134 g) was found for low SD at 4000 m a.s.l. in July, whilst the lowest daily milk yield (876 g) was recorded for high SD at an altitude of 2600 m a.s.l. in November. The site effect was highly significant ($P<0.001$) for the contents of fat, lactose and protein. Milk fat content was lowest at 3200 m a.s.l. in May (5.48% *vs*. 5.31% for high and low SD), while it was highest at the lowest pasture at 2600 m a.s.l. in November (7.05% and 7.12% in high and low SD, respectively). The protein content in milk was highest at 3200 m (4.22% and 4.16% for high and low SD, respectively) and the lowest at 2600 m in November (3.38%and 3.26% for high and low SD). The highest lactose percentage (4.97% *vs*. 4.95% for high and low SD) was observed at 3200 m in May. SD did not influence the milk contents. Concerning the daily amounts of milk constituents, the effects of SD were similar to that on milk yield. The milk yield decreased with progressing experimental duration which resulted in a reduction of daily fat and protein amounts. In detail, the daily amount of fat was highest (182.19 g, low SD) at 4000 m a.s.l. in July. The highest daily protein yield (122.06 g) was measured for low SD at 4000 m in July.

Conclusions The present study confirmed that not only site but also SD can have a significant effect on daily milk yield of yak-cattle crossbreds in the transhumant system. The depression in milk yield with ongoing experimental duration was likely an effect of progressing lactation and of declining forage quality when moving downwards. The enhancement in milk fat content and corresponding depression in milk protein content might be an effect of pasture quality at high altitude as shown from studies with transhumant systems made in the European Alps (Leiber *et al*., 2006). The role of the nutritional value of the herbages available in the seasonal/altitudinal gradient has to be studied in detail to explain the found effects.

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Characteristics of fallow lands extensively grazed by Polish Heath Sheep in North-West Poland

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Introduction Agricultural lands of high productivity for both intensive plant and animal farming are used, whereas the worst areas are abandoned or simply fallowed. Therefore pasturing of small ruminants may be a useful tool to maintain low productivity lands with their unique landscape values and high biodiversity (Groberek *et al*. 2003). The aim of this research was to investigate environmental conditions and nutritional potential of fallow lands used for extensive grazing by Polish Heath Sheep - a breed of primitive type.

Materials and methods The research was carried out during three consecutive years (2006-2008) on the one farm allocated in the North-West part of wielkopolskie voivodeship in Poland. Polish Heath Sheep flock consisted of approx. 100 ewes/yr with lambs was pastured extensively during vegetative period (May-October) on 50 ha area (0.2 LSU/ha) fallowed for 10 yrs where no fertilisation had been used since then. Pasture was a basic fodder during vegetation, whereas during the rest of year grass silage, sugar beet pulp as well as the crushed wheat and ray were used. In 2006-2008, June, July and August were the hottest months (17.5, 20.0 and 17.6°C, respectively), whereas the highest rainfalls were observed in May, June and August (63.33, 61.67 and 90.00 mm) in average. Approximately 5% of total area (3 ha) was randomly chosen for the research purposes. Places of plant and soil samplings were located via GPS device concerning data of longitude (53°11'62''-53°13'39''), latitude (16°37'39''-16°30'02'') and altitude (102-108 m). On the whole, 24 soil samples were collected. In order to investigate chemical composition (Cu, Ca, Mg, P, Zn, Na, K) and pH of the soil each place was sampled 2 times a year (May and turn of September/October). For botanical, chemical, nutritional and production examination of the herbage 48 samples were collected 4 times a year (May, June/July, August, September/October) from 4 researched areas. For statistical analysis general linear model considering effects of place of collection, year and month of vegetation as well as the double-factors' interactions was used. To investigate relation between level of elements in soil and botanical composition Pearson's correlation were estimated.

Results Place of collection statistically affected contents of Ca (0.54%, SEM:0.08, $P<0.05$), Cu (4.34.mg/kg, SEM: 0.14, $P<0.01$) and P (0.41 g/kg, SEM: 0.02, $P<0.01$), whereas effect of the year was observed in contents of K (1.12 g/kg, SEM: 0.05, $P<0.01$), Mg (0.99 g/kg, SEM: 0.03, $P<0.01$) and Na (38.21 mg/kg, SEM: 1.36, $P<0.01$). Month of soil collection influenced concentration of all examined elements, excluding Ca and Cu. pH value of soil ranged from 5.37 to 7.53 in May and 5.08-7.30 in September/October. Percentage distribution of *Asteraceae* (1.55%, SEM: 0.19, $P<0.05$), other species of *Dicotyledoneae* (5.62% SEM: 0.38, $P<0.01$) and *Equisetaceae* (0.21%, SEM: 0.07, $P<0.05$) in herbage were statistically influenced by the place of collection. Year of experiment influenced share of *Poaceae* (87.43%, SEM: 0.76, $P<0.01$), *Equisetaceae* (0.21%, SEM: 0.07, $P<0.01$) as well as *Cyperaseae et Juncaceae* (4.68%, SEM: 0.71, $P<0.01$) in samples. Month of vegetation affected only distribution of *Poaceae* and *Asteraceae in herbage*. None of examined factors influenced share of *Fabaceae* (0.51%, SEM: 0.1) in all analyzed plants. Pasture yield (1.29 of d.m./ha, SEM: 0.11, $P<0.01$) was low and varied due to the month of vegetation.
Contents of dry matter (30.81%, SEM: 1.01, $P<0.05$), crude protein (3.48%, SEM:0.07, $P<0.05$), crude fibre (8.1%, SEM: 0.41, $P<0.05$) and N-free extract (15.37%, SEM: 0.46, $P<0.01$) in pasture herbage were statistically influenced only by month of vegetation. Neither effect of place nor collection and year of experiment were observed. All examined fibre fractions (ADF–12.38%, SEM: 0.43, $P<0.01$; NDF–18.17%, SEM: 0.62, $P<0.05$; and ADL–2.21%, SEM:0.06, $P<0.01$) were influenced by month of vegetation, whereas year of experiment affected only content of ADF fraction. In case of trace elements, contents of Ca (0.81%, SEM: 0.03, $P<0.01$) and Zn (32.6 mg/kg, SEM: 1.46, $P<0.01$) were affected by place of collection, whereas year of experiment influenced Cu (4.4 mg/kg, SEM: 0.25, $P<0.01$), K (0.89%, SEM: 0.04, $P<0.01$), Mg (2.69%, SEM: 0.19, $P<0.01$), Na (382.55 mg/kg, SEM: 56.82, $P<0.01$), P (0.6%, SEM: 0.06, $P<0.01$) and Zn (32.6 mg/kg, SEM: 1.46, $P<0.01$) contents. Significant and negative correlations were observed between Cu percentage in soil and distribution of *Poaceae* (-0.46, $P<0.01$) and *Asteraceae* (-0.51, $P<0.01$) in herbage. However, positive and significant correlation was observed between Cu content and other species of *Dicotyledoneae* (0.49, $P<0.05$) as well as between P and this group of plant (0.49, $P<0.05$). Phosphorus content was negatively and significantly correlated with share of *Asteraceae* (-0.48, $P<0.05$) in pasture herbage.

Conclusions Chemical composition of soil as well as chemical composition and its fibre fractions of pasture herbage were statistically influenced by month of vegetation. Nevertheless, none of studied trace element in pasture herbage was affected by this factor. Month of vegetative period also influenced botanical composition on pastures especially in case of *Poaceae* and *Asteraceae* percentages as well as pasture yield. Multitudinous correlations were found between some elements in soil and some groups of plant families on pastures grazed by sheep. Obtained results indicated sufficient grazing conditions for Polish Heath Sheep to maintain that area.

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Effects of two forage-based nutritional regimens on intake and weight gain of three genotypes of young red deer (*Cervus elaphus*) during autumn, winter and spring in New Zealand.

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Introduction The farming of red deer is a major enterprise in New Zealand agriculture. Recent developments in the industry have seen the inclusion of a range of genotypes from different regions of the world. There is potential for each of these genotypes to respond differently to seasonal signals of day length and temperature, and to nutritional regimen, based on their evolutionary circumstance. The experiment aims to improve the understanding of this variation to develop profitable farming systems that reflect this variation.

Materials and methods One hundred and twenty six 5-month-old red deer were assigned to one of two nutritional regimens in early autumn. High and Low nutritional regimens were imposed using a leader – follower rotational grazing system, with the High treatment being allocated 1 kg barley/head/d plus approximately 0.4 kg DM as lucerne hay during autumn and winter, while the Low treatment were allocated 0.5 kg barley/head/d plus 0.2 kg DM as meadow hay during autumn and winter. During spring the leader – follower grazing system was used without supplement. Within each nutrition treatment were 21 deer of three genotypes – NZ Red (*Cervus elaphus scoticus*), Eastern European (*C. e. hippelaphus*) and Elk cross (2/3 NZ Red x 1/3 Elk *C. e. nelsoni*). In each season seven animals of each genotype were used to determine the forage intake and digestibility for each nutritional regimen using the alkane dilution technique as described by Dove and Mayes (1991). Liveweights were measured every two weeks and liveweight gain assigned to each season based on the calendar months March to May (Autumn), June to August (Winter) and September to November (Spring). Pasture yield and composition was determined at each allocation of fresh pasture. Data for each season was analysed separately by ANOVA as a 2 x 3 factorial randomised plot design with individual animals being used as replicates. The experiment was approved by the AgResearch Invermay Animal Ethics Committee in accordance with New Zealand animal welfare regulations.

Results The amount of pasture on offer differed significantly between the nutritional treatments (8.0 and 5.1 kg DM/head/d, SED: 1.7, $P<0.05$, for High and Low respectively) but not between seasons (7.2, 5.3 and 7.8 kg DM/head/d, SED: 2.3, for Autumn, Winter and Spring respectively). Pasture composition when measured as the proportion as grass leaf, clover and dead material did not differ significantly throughout. The residual pasture cover was 2060 and 1290 kg DM/ha after the High and Low treatments had grazed the pastures ($P<0.01$). During autumn no significant differences were found in deer growth rate (mean=145 g/d), though estimated forage intake was significantly lower (1.65 and 2.27 kg DM/head/d, SED: 0.15, $P<0.001$) for the High and Low treatments respectively). The addition of barley to the diet substituted for the forage, leading to similar metabolisable energy intakes on both treatments. No differences were found between the liveweight gains of the genotypes in autumn. The NZ Red and Eastern European deer were similar weights (60.6 and 57.2 kg respectively) while the Elk cross were heavier at 72.5 kg (SED: 0.18; $P<0.001$). There was an interaction between nutrition and genotype in the proportion of forage intake as hay (SED: 103, $P<0.01$) indicating that the NZ Red deer ate similarly low proportions of hay on either nutritional regimen (117 and 65 g/kg forage DM eaten on the High and Low treatments respectively), and Eastern European deer having the greatest increase in hay intake on the Low nutritional regimen (231 and 646 g/kg forage DM eaten on the High and Low treatments respectively). The Elk cross had an intermediate increase in hay intake on the Low nutrition treatment. During Winter significant differences were found in growth rate, average live weight, forage intake, and hay intake between the High and Low treatments. The NZ Red and Elk cross deer had higher growth rates than the Eastern European deer in winter (129, 134 and 92 g/d, SED: 17, $P<0.05$, respectively). The Elk cross were the heaviest ($P<0.001$) and had the highest forage intake ($P<0.001$), greatest hay intake ($P<0.05$) and metabolisable energy intake ($P<0.001$), while the NZ Red and Eastern European deer were similar for these measurements. In Spring significant interactions emerged between nutrition and genetics. NZ Red deer grew at a similar rate on both High and Low treatments (250 and 251 g/d respectively), while the Elk cross deer responding the most to the High treatment compared to the Low treatment (368 and 260 g/d respectively) with the Eastern European deer being intermediate (311 and 259 g/d, SED: 29, $P<0.05$ for High and Low respectively). This was reflected in forage intake ($P<0.05$) and metabolisable energy intake ($P<0.05$).

Conclusions The red deer genotypes currently being used in New Zealand deer farming have different forage use habits and exhibited different growth profiles in different seasons. Few differences were found in Autumn, but by Winter the NZ Red and Elk cross genotypes grew faster than the Eastern European genotype. The only interaction between nutrition and genotype in the Autumn was in the proportion of hay in the diet indicating some variation in forage use habits. No interactions were evident in Winter. In Spring, however, the NZ Red genotype did not respond to increasing feed availability, while the Eastern European and Elk cross deer increased growth rate by approximately 50 g/d and 100 g/d respectively in response to increased feed availability. This was matched by increases in forage and metabolisable energy intake. Understanding these interactions provide the researcher and farmer with information that will enable tailored feeding systems to be developed to improve on-farm resource use efficiency.

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Effect of goat grazing on tedera pasture(*Bituminaria bituminosa*)

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Introduction The tedera (*Bituminaria bituminosa*) is a traditional forage species in the Canary Islands where it is used as hay (Méndez *et al*., 2006); the goat is the most interesting livestock of the islands, mainly due to the importance of their cheeses. After the sixties, animal production have evolved to intensive systems and in addition in the last 20 years several laws have been designed to protect biodiversity contributing to the reduction of the extensive systems. On the other hand approximately 30% of cultivated lands have been abandoned in recent decades, these are areas with suitable soils for cultivation with a potential interest for the production of forage so scarce in this region. This study aims to evaluate the response to grazing goats of an sown meadow established in an abandoned crop land with tedera as main constituent and barley and other anuals species. It is known that tedera plants are able to tolerate heavy sheep grazing, 3 or 4 times per year depending on growing conditions (Real, unpublished) and rotational grazing with cattle seemed to be appropriate for the species (Sternberg *et al*. 2006).

Material and methods A goat grazing experience was carried out on 3000 m^2 of a sown meadow on an abandoned crop land of Mediterranean semiarid climate, with tedera as perennial species and barley and other accompanying annual species (least mallow and other forbs). The grazing area was divided into six paddocks of 500 m^2 each and a rotational grazing management was established during spring and early summer. One of the paddock remained as control plot and was not grazed by goats. All of them were sampled before and after the grazing period. Three linear transect in each paddock was carried out to asses plant cover (C) of each species (tedera, least mallow, barley and forbs) according to Daget and Poissonet (1971), at four different times: before the start of experience, one day before grazing, one day after grazing and regrowth period. Also five plants were selected in each paddock for biovolume estimation (B) and utilization rate (UR) according Etienne *et al.* (1996). A discriminant analysis was used to evaluate F and C. Bivariate correlation test was carried out to evaluate the relationship between regrowth evolution (height, horizontal growth and biovolume) and UR (SPSS 11.0.1).

Table 1 Matrix structure of the discriminant functions (grazed and control plots)

		Function			
		1	2	3	4
		p<0.05	p<0.05	p>0.05	p>0.05
Grazed	least mallow	**0.790***	**-0.029**	-0.157	**-0.592**
	barley	**0.255**	**0.676***	**-0.646**	**0.245**
	tedera	**0.111**	**0.121**	**0.813***	**0.558**
	forbs	0.410	-0.440	-0.374	0.706*
		1	**2**	3	-
		p<0.05	**p>0.05**	p>0.05	-
Control	least mallow	**0.826***	**-0.507**	-0.223	-
	barley	**0.170**	**-0.561***	0.510	-
	tedera	**0.059**	**-0.119**	-0.748*	-
	forbs	**0.250**	**0.665**	0.647*	-

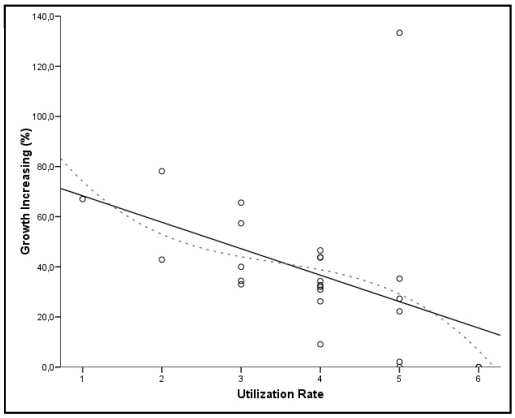

Figure 1 Scatter Plot Utilization Rate-Growth Increasing
(*****largest absolute correlation between each variable and any discriminant function)

Results Results showed that changes in grazed and control plots are due to the C value of annuals species (function 1 and 2 of grazed plots analysis and function 1 of control plots analysis) (Table 1) along the experience, which explain 99,2 % and 66,1% of the variance respectively. However tedera C value was not affected by time or grazing. Tedera C value remained stable during grazing and regrowth period, whereas annual species reduce coverage, maintaining this downward trend (barley) or increasing the coverage significantly (least mallow). On the other hand, measurements on individual plants of tedera showed that the species was well grazed, its heigth had a negative and signifficant correlation with UR so the plants with higher consumption rates decreased in height at regrowth (Figure 1). UR above 5 (more than 90% of the green matter consumed) did not allow an adequate plant regrowth and UR between 3 and 4 (more than 90% buds consumed and 50-90% of the green matter consumed) were the best value to achieve a balance between harvesting and plant regrowth.

Conclusions Tedera had a good response to grazing goats and a good regrowth when plant consumption was under 90% of the green matter available. Results suggest the interest of tedera as grazing perennial species that could contribute to maintain and even extend the grazing period in mixtures with annual species in areas of semiarid Mediterranean climate.

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Evolution of the land use efficiency by Brazilian bovine husbandry since 1970, based on official databases

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Introduction Brazil is a leading player in the beef, poultry and pork world markets. It has expanded its national herd 24% since 1994, with consumption per capita rising 13% over the same period and has also increased the exports, up over 450% in volume and 385% in value. For this reason, Brazil is now the world's leading exporter. This great change has occurred because of the continued availability of natural resources and a favourable exchange rate (Steiger, 2006). For this reason, the objective of this research was to evaluate the land use efficiency by Brazilian bovine herd since 1970, using the Census data available, by calculating the annual average growth rate, in order to understand how this development has occurred in the different regions through the last three decades.

Material and methods Data were collected at Digital Databases "Sidra" (IBGE, 2010), including all the Husbandry Census available years (1970, 1975, 1980, 1985, 1995, 2006). Variables collected (per year and per region) were: bovine herd size, area of natural pasture and area of cultivated pasture. Herd size was divided by total pasture area, creating the ratio of head/ha of total pasture in order to evaluate the pasture use efficiency. Brazilian states were aggregated in regions: North, Northeast, Southeast, South and Midwest. The annual average growth rate (%/year) was calculated using regression analysis, obtaining the slope between natural logarithm of each variable and the correspondent year (SAS, 1999). Then, the anti-logarithm of the slope minus 1 was taken, according to the following equation: $V_t=V_0 (1+r)^n$, where: V_t=growth at time t; V_0=initial growth; r=annual average growth rate; n=number of years. Finally, this number was transformed in %, by multiplying per 100.

Results All the results are shown in Table 1. In 1970, Brazil had 78.6 million bovine heads, raised on 124.4 million hectares of natural pasture and 29.7 million ha of cultivated pasture, with a ratio of 0.51 head/ha of total pasture. In 2006, Brazilian bovine herd went up to 171.6 million heads, decreasing the use of natural pasture to 57.3 million ha and increasing the cultivated pasture area to 101.4 ha, with a ratio of 1.08 head/ha of total pasture. From 1970 up to 2006, Brazilian bovine herd increased at 2.04%/year, the total pasture at 0.07%/year, the area with cultivated pasture at 3.5%/year and the ratio of head/ha of total pasture at 1.97%/year, while the area with natural pasture decreased at 2.26%/year. The Southeast, the most technologically developed region, used to have the largest bovine size (34.17% of the total herd) in 1970. But, in 2006, the Midwest region, where predominates the savanna biome, became the largest producer with 33.52% of the total Brazilian herd. It also showed the highest growth (3.22%/year) in efficiency (from 0.31 in 1970 up to 0.98 head/ha of total pasture in 2006) and the 2nd highest increase in herd size (3.25%/year). The North region, the location of the Amazon forest and an agricultural border, showed the highest increase in bovine herd size (8.96%/year), in natural pasture area (2.07%/year - while the other regions decreased), in cultivated pasture area (9.95%/year) and also in total pasture (5.67%/year). The Northern efficiency increased from 0.39 in 1970 to 1.18 heads/ha of total pasture in 2006 (the 2nd highest growth of 3.12%/year).

Table 1 Annual average growth rate of bovine herd, natural pasture area, cultivated pasture area, total pasture area, ratio of heads/total pasture in different regions of Brazil from 1970 to 2006

	Relative herd[1] in 1970	Relative herd[2] in 2006	Bovine herd[3]	Natural pasture[4]	Cultivated pasture[5]	Total pasture[6]	Head/ha in 1970[7]	Head/ha in 2006[8]	Head/ha[9]
Region	(%)	(%)	(%/year)	(%/year)	(%/year)	(%/year)	(ratio)	(ratio)	(%/year)
North	2.17	18.26	8.96	2.07	9.95	5.67	0.39	1.18	3.12
Northeast	17.57	14.76	1.41	-0.99	2.47	0.14	0.50	0.83	1.27
Southeast	34.17	19.85	0.41	-3.29	1.44	-1.36	0.60	1.24	1.80
South	24.12	13.61	0.55	-1.32	0.89	-0.77	0.88	1.50	1.33
Midwest	21.96	33.52	3.25	-3.79	4.41	0.03	0.31	0.98	3.22
Brazil	100.00	100.00	2.04	-2.26	3.50	0.07	0.51	1.08	1.97

[1] relative bovine herd in 1970 (% of Brazilian herd), [2] relative bovine herd in 2006 (% of Brazilian herd), [3] annual average growth rate of bovine herd (%/year), [4] annual average growth rate of natural pasture area (%/year), [5] annual average growth rate of cultivated pasture area (%/year), [6] annual average growth rate of total pasture area (%/year), [7] ratio of bovine head/ha of total pasture in 1970, [8] ratio of bovine head/ha of total pasture in 2006, [9] annual average growth rate of ratio of bovine head/ha of total pasture area (%/year).

Conclusion Brazilian bovine production system has become more efficient, as the herd size continues to increase and the pasture area remains stable. If, on the one hand, the pasture areas have been migrating towards the Midwestern and Northern regions of Brazil, which is undesirable, on the other hand the efficiency of pasture use has increased, due to the replacement of natural pasture with cultivated pasture, which is desirable.

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Feeding and grazing behavior of Angus beef calves associated to residual feed intake

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Introduction Differences in feeding patterns between high and low residual feed intake (RFI) animals have been observed in many species. The reasons for the variation in RFI in cattle are still not fully understood but are likely to include differences in feeding behavior in confinement trials (Richardson and Herd, 2004). Furthermore, grazing behavior probably explains a greater proportion of the variation in RFI as it is a more complex event than feeding behavior in confinement. Two experiments were carried out to determine RFI at the "Dr. M. A. Cassinoni" Experimental Station of the Faculty of Agronomy (32.5°SL and 58°SW; Paysandú-Uruguay), during 2010. In Experiment 1(E1) the objective was to characterize the diurnal feeding behavior of Angus beef calves in confinement, which differed in the presence of allelic variants of candidate genes related to RFI. In Experiment 2 (E2) the objective was to evaluate the diurnal grazing behavior in the same Angus beef calves previously evaluated for RFI in E1.

Materials and methods In E1, 36 calves (initial BW = 186.2 ± 32 kg and 262 ± 27 days of age) were in a confinement RFI trial and allocated to individual pens; feed intake was estimated daily by difference between feed offered and refused. Half of the calves carried 3 "favorable" alleles simultaneously (V, validation group) while the other half carried 3 "unfavorable" alleles (C, control group). Calves were fed twice daily of an *ad libitum* mixed diet (60:40 as fed, concentrate: chopped alfalfa hay) and had *ad libitum* access to fresh water during 56 days (from 24 May to 9 August, autumn and winter seasons).In E2, 12 heifers (average initial BW= 283.3± 38 and 396 ± 27 days of age) from each group were selected based on the RFI obtained in E1. A grazing RFI trial was conducted from 5 October to 16 December on an improved first year pasture of fescue, birdfoot trefoil and white clover. Calves from each group (C and V) were blocked by BW and RFI and allocated to graze in 4 paddocks, each divided in 2 plots (1.5 ha). Medium to high forage availability was maintained in the 4 paddocks (average initial availability: 4337 ± 690 Kg DM/ha), and, fresh water and a mineral supplement were offered continuously throughout the trial; n-alkane method was used to estimate intake. Feeding behavior in E1 was recorded by visual observation as eating (E), ruminating (R) or idling (I) activities every 5 minutes in the daylight hours (exceptin midday hours) for each animal during 2 days in week 1 and 7. Grazing behavior in E2 was collected with the same methodology that E1 but considering midday and was characterized as grazing (G), R or I activities for each animal during 2 days at the end of the RFI grazing trial (December, summer season).Data was analyzed as a completely randomized design with repeated measures over time using GENMOD procedure of SAS (Ver. 9.0 SAS Institute, Cary, NC, USA) with the binomial distribution option.

Results In E1, neither group nor group by week interaction affected the pattern of E, R and I (P> 0.05), however session (am, pm), week and day within week, significantly (P <0.05) affected E, R and I (Figure 1a). In E2, G, R and I activities were similar between groups and between days (P> 0.05). However, there was a significant (P <0.05) effect of session (early morning, midday, late evening) in G, R and I activities, as well as, group within session in G activity. Heifers of V group presented less probability of G activity in the midday session than heifers of C group (Figure 1b). No effect of group by day interaction was observed (P> 0.05) neither in G nor in R but it was observed in I activity (P <0.05).

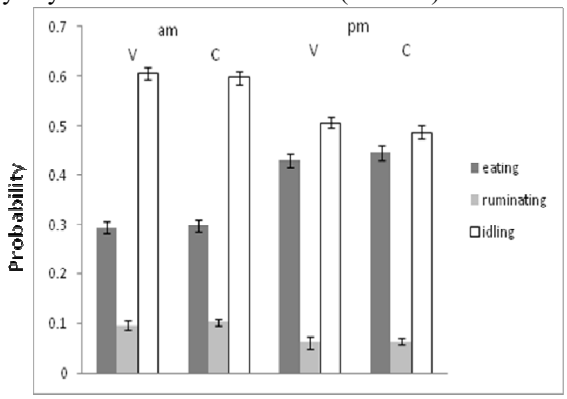

Figure 1a Feeding behavior of groups of calves (V, C) in two diurnal sessions

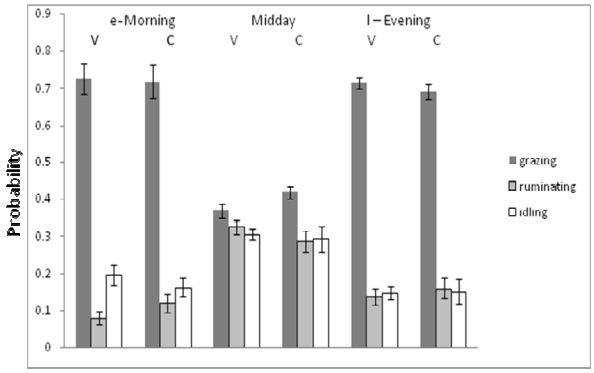

Figure 1b Grazing behavior of groups of heifers (V, C) in three diurnal sessions

Conclusions Although this study is only a preliminary analysis of feeding patterns, our results suggest that grazing behavior could be more related with efficiency than feeding behavior in confinement. Therefore it is possible that the activity associated with observed difference in grazing behavior during midday session contributed to energetic differences associated with variation in RFI. As grazing pattern is the product of complex decisions made by animal responding to multiple variables, further research in feeding behavior, including night hours and bite rates records, is required to improve our understanding in the association between feeding behavior and feed efficiency.

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Grazing down process in tropical grasslands: effect on the short-term intake rate of heifers

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Introduction During grazing down, the variation on sward structure is the main determinant of the reduction on intake rate of grazing animals (Baumont *et al*., 2004). Based on data of short-term intake rate (STIR) of heifers during grazing down of two contrasting tropical swards, the aim of this study was to identify the proportion of grazing down in which the STIR is maintained constant.

Materials and methods For the models, there were used data from two experiments performed at tropical grasslands (*Sorghum sp.* And *Cynodon sp.*). In both experiments, the grazing down was determined using a proportion of the pre-grazing sward height that maximize the STIR. These values were established in previous protocols. For *Sorghum sp*. The value of pre-grazing sward height that maximized the STIR was 50 cm (Fonseca 2011), while in *Cynodon sp.* Was found 19 cm (Mezzalira in preparation). For *Sorghum sp.,* the proportions of grazing down tested were 16, 33, 50, 67 and 84% of pre-grazing sward height. For *Cynodon* the proportions of grazing down were 20, 40, 60 and 80% of pre-grazing sward height. In both experiments, the sward height was estimated using a *sward stick* (expressed in cm). The STIR was determined according to Gibb *et al*. (1997) and it was expressed in g DM min^{-1} kg LW^{-1}. The dependent variable STIR was analyzed using linear and nonlinear regressions of JMP version 9 (SAS Inst. Inc., Cary, NC). The fits were compared using the coefficient of determination (r^2) and the probability value (P=). The best model adjusted was a broken line ($P<0.05$).

Results In both experiments (*Sorghum sp.* and *Cynodon sp.*), the STIR remained constant until 40% of grazing down (Figure 1) and we observed the same value of plateau for both forage species (0.16 g DM min^{-1} LW^{-1}). This value of STIR was defined by the structural characteristics that were common for *Sorghum* sp. and *Cynodon sp.* (e.g. leaf lamina proportion, bulk density of the grazed stratum). After this proportion of grazing down (40%), there was observed a linear decline on STIR of heifers, however, in distinct intensities. On *Cynodon* this decline was higher than *Sorghum.* If we consider the paddock of rotational grazing as a *patch* (see definition of Illius & Gordon, 1999), we could infer that the optimized use of the paddock by the grazing animal occur while the STIR is maintained constant, according to the theoretical assumptions of efficiency. With the reduction on STIR, the animal would probably move to another *patch* (Roguet *et al*., 1998).

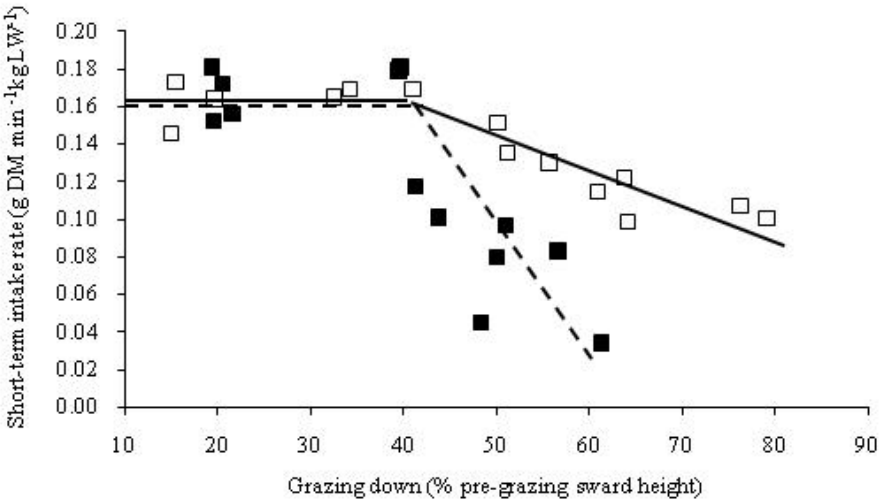

Figure 1 Intra-experiment relationship between STIR and grazing down in tropical grasslands (□ Sorghum: y=0.16 if x<40 and y= 0.16 + 0.002(40-x) if x>40;r^2=0.86; SE = 0.01;P<0.01; ■ Cynodon: y=0.16 if x<40 and y= 0.16 + 0.006(40-x) if x>40; r^2=0.73; SE = 0.03; P<0.01)

Conclusions To maximize the short-term intake rate of heifers in tropical grasslands, the proportion of grazing down should not exceed 40% of pre-grazing sward height, independent of the forage specie. Sward management's targets should focus on depletion without depression of sward structure.

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Effects of the flavonoids quercetin and rutin on ruminal fermentation *in-vitro*

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Introduction Flavonoids, a large group of secondary plant metabolites occuring ubiquitously in feed plants, are discussed as possible feed additives due to their health promoting effects. With regard to oral use of flavonoid supplements in ruminants, the aim of this *in-vitro* trial was to investigate whether flavonoids have an impact on the fermentation of organic matter (OM) in the rumen.

Materials and methods In the present study, the impact of the flavonoids quercetin (Q) and rutin (R, quercetin-3-O-rutinoside) on gas production (GP) which is highly correlated to fermentation of OM was tested. Therefore, blanks, grass hay (8 % crude protein, 5.3 MJ net energy), a compound feed (18 % crude protein, 6.7 MJ net energy), and a mixed ration (50 % grass hay and 50 % compound feed) were incubated in the Hohenheim gas test for 120 hours with or without Q or R (0, 1, 10, and 50 µmol/l, respectively); GP was measured after 0, 1, 2, 4, 6, 8, 10, 12, 24, 36, 48, 60, 72, 82, 106, and 120 h. Cumulative GP and kinetic parameters of GP were calculated using a modified Gompertz equation. The calculated parameters were analysed in a one-way anova for each substrate with dose as variable with subsequent comparison of group means using the Tukey test. Dose effects were analysed using orthogonal polynomials.

Results The flavonoids had no influence on GP of the blanks. The addition of 50 µmol/l Q enhanced GP from all substrates starting after 10 h of incubation. Gas production was enhanced up to 29 % from compound feed and up to 19 % from grass hay and the mixed ration. The maximum rate of GP (MGP) was increased by Q (50 µmol/l) compared to the groups without Q in grass hay and the mixed ration. Furthermore, the exponential phase of GP (Exphase), and the time from incubation until the inflexion point of the graph (TIP) were shortened with grass hay and elongated with the compound feed (Table 1). In contrast to its aglycon Q, an addition of 50 µmol/l R had no influence on GP in all feedstuffs.

Discussion and conclusions We speculate that the positive influence of Q on GP might be caused by its degradation product phenylacetic acid, which functions as a precursor for biosynthesis of phenylalanine by certain strains of cellulolytic bacteria (e.g. *Ruminococcus albus* 8, *Ruminococcus flavefaciens* and *Bacterioides succinogenes*). This could reduce energy requirements for protein synthesis and thereby lead to advanced bacterial growth and enhanced cellulolytic activity. The fact, that the addition of R did not enhance GP may be due to the slow release of Q from this glycoside due to a low α-rhamnosidase activity or to different pathways in the metabolism of R by ruminal microorganisms.

Table 1 Effect of quercetin supplementation on GP and kinetic parameters of GP (means ± SD).

Substrate	Quercetin (µmol/l)	Gas production (ml/g DM)	MGP (ml/h)	Lag-time (h)	Exphase (h)	TIP (h)
grass hay	0	290 ± 10 ^{a}	9.3 ± 0.8^{a}	0.46 ± 0.49	22.4 ± 1.1^{a}	11.7 ± 0.4^{a}
	1	318 ± 11^{ab}	11.4 ± 0.9^{b}	0.82 ± 0.27	20.2 ± 1.0^{b}	10.9 ± 0.6^{ab}
	10	328 ± 16 ^{b}	11.8 ± 1.0^{b}	0.72 ± 0.30	20.1 ± 0.8^{b}	10.8 ± 0.5^{b}
	50	345 ± 28 ^{b}	12.5 ± 0.6^{b}	0.83 ± 0.33	19.9 ± 1.1^{b}	10.8 ± 0.4^{b}
P-value	linear	0.0003	<0.0001	0.2944	0.0180	0.0810
	quadratic	0.0468	0.0061	0.6821	0.0265	0.0436
	cubic	0.0253	0.0008	0.1106	0.0022	0.0279
grass hay:	0	299 ± 5^{a}	12.2 ± 0.8^{a}	-1.18 ± 0.20^{a}	17.6 ± 1.0	7.6 ± 0.6
compound feed	1	337 ± 23^{b}	14.1 ± 1.1^{b}	-0.50 ± 0.21^{b}	17.3 ± 0.7	8.1 ± 0.5
50: 50	10	332 ± 15^{ab}	14.5 ± 0.9^{b}	-0.55 ± 0.18^{b}	16.5 ± 0.5	7.7 ± 0.3
	50	355 ± 28^{b}	14.5 ± 0.6^{b}	-0.55 ± 0.13^{b}	17.7 ± 0.9	8.3 ± 0.5
P-value	linear	0.0020	0.0144	0.0104	0.3868	0.0884
	quadratic	0.3237	0.0081	0.0040	0.0154	0.3819
	cubic	0.0077	0.0045	<0.0001	0.5993	0.0977
compound feed	0	299 ± 11 ^{a}	17.4 ± 1.8	-0.75 ± 0.19	12.5 ± 1.4^{a}	5.5 ± 0.7^{a}
	1	345 ± 35^{ab}	17.8 ± 1.1	-0.51 ± 0.19	13.9 ± 0.7^{ab}	6.5 ± 0.2^{ab}
	10	357 ± 36^{ab}	17.7 ± 1.1	-0.57 ± 0.39	14.5 ± 1.2^{ab}	6.7 ± 0.4^{b}
	50	385 ± 50 ^{b}	17.0 ± 1.1	-0.80 ± 0.63	16.4 ± 3.1^{b}	7.4 ± 1.0^{b}
P-value	linear	0.0029	0.3635	0.3524	0.0025	0.0003
	quadratic	0.1364	0.6886	0.5437	0.3657	0.1034
	cubic	0.0620	0.6329	0.3203	0.2414	0.0286

Different letters indicate significant differences within columns ($P \leq 0.05$); n=6 per treatment.

Chemical composition and *in vitro* digestibility of date-palm leaflets treated with *Neurospora intermedia* fungi

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Introduction Changes in climate conditions and shortage of water resources have increased costs of animal feeds in many countries. Therefore, better utilization of non-conventional feed resources, which do not compete as human foods, is important. Agro-industrial by-products, such as date-palm leaflets, could form an important component of ruminant diets. In Iran, large amounts of date-palm leaflets are produced. Their use as animal feeds may be an effective means of utilizing crop by-products, which, if allowed to accumulate could lead to environmental pollution (Huber, 1980). Physical, chemical and biological methods have been used to improve the nutritive value of agricultural by-products. Treating citrus pulps with *Neuspora sitophila* fungi has increased the concentration of crude protein (CP) and nutrient digestibility coefficients (Nazem *et al.*, 2009). The objective of this work was to assess the effects of treating date-palm leaflets with *Neurospora intermedia* fungi upon nutritive value, measured *in vitro*.

Materials and method *N. intermedia* fungi (PTCC 5591) were maintained at 4°C on potato dextrose agar (PDA) slants. Date-palm leaflets were dried at 90°C overnight and then hammer-milled and sieved to 60-gauge mesh. Inoculum was prepared in 250 ml conical flasks containing 100 ml medium of the following composition (per l): glucose 10.0 g; yeast extract 2.0 g; $(NH_4)_2SO_4$, 0.47 g; urea, 0.86 g; KH_2PO_4, 0.714 g; $MgSO_4{\cdot}H_2O$, 0.2 g; $CaCl_2$, 0.2 g; $FeCl_3$, 3.2 mg; $ZnSO_4{\cdot}7H_2O$, 4.4 mg; $CuSO_4{\cdot}5H_2O$, 0.78 mg; $MnCl_2{\cdot}4H_2O$, 0.144 mg. The pH was adjusted to 5.5 after sterilization at 121°C for 30 min. Date-palm leaflets were the carbon sources. The solid state fermentation was conducted in 500-ml flasks and a column (6×25 mm) (Moo-Young *et al.*, 1992) with medium of untreated ground Date-palm leaflets. The inoculum size was based on 10% (w/w) of wet media. Methods to determine the concentrations of organic matter (OM), protein and fibre fractions were carried out according to AOAC (1990) and phenolic compounds analysed by methods described by Makkar (2000). Organic matter digestibility (OMD) was determined as described by the *in vitro* method of Menke and Steingass (1988). Neutral detergent fibre digestibility (NDFD) was determined using the Tilley and Terry (1963) method, by measuring the amount of NDF in the original sample and that remaining after 48 h anaerobic incubation. The nutritive value measurements of treated and untreated date-palm leaflet materials were compared using the Student's t-test, in 5 replicates.

Results As shown at Table 1, CP and true protein content were significantly ($P<0.05$) increased in treated date-palm leaflet in comparison with untreated date-palm leaflet. However, organic matter (OM), NDF, acid detergent fibre, lignin (sa) (lignin determined by solubilisation of cellulose with sulphuric acid), water soluble carbohydrates, total phenolics and total tannins, as well as OMD and NDFD were decreased ($P<0.05$), which is probably due to the reduction in the concentration of OM.

Table 1 Table 1 Chemical composition and digestibility coefficients (g/kg DM) in leaflet date-palm treated and un-treated with *Neurospora intermedia.*

	OM	CP	TP	WSC	ADF	NDF	Lignin	TPH	TT	OMD	NDFD	TDM	DML
Untreated	883	50	44	65	483	613	120	55	44	388	240	1000	
Treated	864	91	82	26	445	517	126	36	29	372	230	912	8.8
SED	0.3	0.4	0.1	0.3	0.8	0.8	12.7	0.1	0.3	4.2	4.5	-	
Sig.	**	**	**	**	**	**	ns	**	*	*	*	-	-

OM: organic matter; CP: crude protein; TP: true protein; WSC: water soluble carbohydrates; ADF: acid detergent fibre; NDF: neutral detergent fibre; TPH: total phenolic; TT: total tannin; OMD: organic matter digestibility; NDFD: NDF digestibility; TDM: total dry matter; DML: dry matter lost (%); **: $p<0.01$; *: $p<0.05$; ns: not significant.

Conclusion Except for the protein content, treatment of date-palm leaflets with the fungus, *Neurospora intermedia,* did not improve the nutritive value of date-palm leaflets, measured *in vitro*. The possible improvement in nutritive value of date-palm leaflets using other processing methods should be investigated.

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Tiller population density and tillering dynamics in mulato palisade grass subjected to strategies of rotational stocking management

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Introduction Tillers represent the growth unit of forage grasses, and swards are comprised of a population of tillers. Sward persistency and productivity depends on the balance between the appearance and death of tillers throughout the year, processes that happen according to variable rates and are dependent on seasonal variations in climatic conditions and grazing management. Against that background, the objective of this experiment was to evaluate the tiller population density and tillering dynamics of mulato palisade grass (hybrid between *Brachiaria brizantha* cv. Marandu (CIAT 6297) and *Brachiaria ruziziensis* (clone 44-6) (CIAT, 2001) subjected to strategies of rotational grazing management.

Materials and methods The experiment was conducted at E.S.A. "Luiz de Queiroz" (ESALQ), University of São Paulo, Piracicaba, SP, Brazil (22°42' S, 47°37' W and 550 m a.s.l.) on a mulato palisade grass pasture (*Brachiaria* hybrid, cv Mulato – CIAT 36061) from January 2008 to March 2009. Treatments corresponded to combinations between two post-grazing heights (15 and 20 cm) and two pre-grazing conditions (95% and maximum light interception by sward canopy – LI), and were allocated to experimental units (1200 m^2 paddocks) according to a 2x2 factorial arrangement and a randomised complete block design, with four replications. The following response variables were evaluated: tiller population density (TPD) and rates of tiller appearance (TAR) and death (TDR). Analysis of variance was performed using the Mixed Procedure of SAS® (Statistical Analysis System). The choice of the covariance matrix was made using the Akaike Information Criterion (AIC) (Wolfinger, 1993), and analysis performed considering post-grazing height, light interception pre-grazing, season of the year and their interactions as fixed effects and blocks as a random effect (Littel *et al*., 2000). When appropriate, treatment means were calculated using the "LSMEANS" statement, and comparisons made with "PDIFF" based on a Student t test and a 5% significance level.

Results In general, larger values of TPD were recorded during both summers (2008 and 2009) and late spring relative to autumn/winter/early spring. Higher TPD were associated with high rates of tiller appearance and death, characterising a fast turnover in tiller population, a conditions that was mainly associated with swards grazed at 95% relative to maximum canopy LI (99%) and was augmented by the favourable climatic conditions of late spring and summer. Autumn/winter/early-spring is a low temperature, dry period of the year, when plants drastically reduce their growth and all associated processes (e.g. tillering). Overall, the effect of post-grazing height on TPD, TAR and TDR was small compared to the effect of LI pre-grazing (grazing frequency), but there was an indication that, during autumn/winter/early spring and summer 2009, the post-grazing height of 15 cm, when associated with the pre-grazing conditions of maximum LI (99%), could result in reduced tiller population, consequence of the lower TAR and TDR.

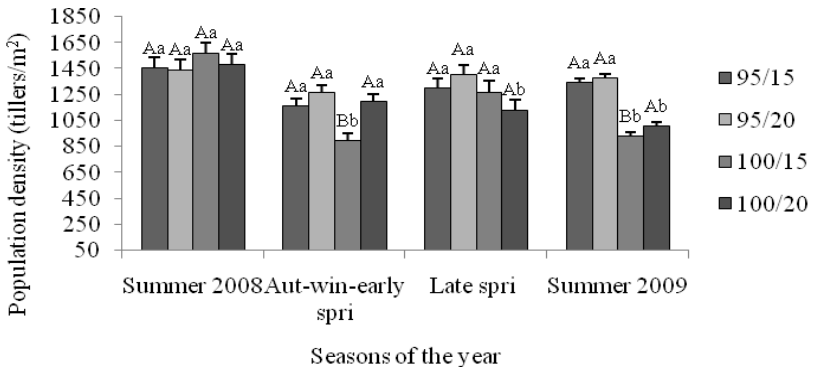

Figure 1 Tiller population density of mulato palisade grass subjected to strategies of rotational stocking management

Conclusions Mulato palisade grass has a clear seasonal pattern of tiller turnover in tiller population, which is mainly concentrated during early to late spring, suggesting that to be a critical period for management purposes since it corresponds to the transition between the end of the dry and the beginning of the rainy seasons. Grazing at 95% LI provides favourable condition for intense tillering, but when managed at maximum LI post-grazing the post-grazing height of 15 cm may severely depress tillering, with potential negative effects on sward longevity and productivity.

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Herbage utilisation efficiency on mulato palisade grass swards subjected to strategies of rotational stocking management

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Introduction Restoration of sward leaf area comprises two concomitant and antagonist processes, growth and senescence. The relationship between these two processes determines the utilisation efficiency of the produced herbage (Bircham & Hodgson, 1983). One of the processes, growth or senescence, may become more evident during regrowth depending on the grazing strategy used, since the growth rhythm of plants varies with frequency and severity of defoliation, use of fertilisers and irrigation according to season of the year, year and geographical localisation. Understanding of the relationship between those two processes and their pattern of variation during regrowth is important for monitoring and defining efficient grazing management practices. Against that background, the objective of this experiment was to evaluate the utilisation efficiency of herbage on mulato palisade grass swards subjected to strategies of rotational stocking management defined as combinations between canopy light interception and sward height as pre and post-grazing targets.

Materials and methods The experiment was conducted at E.S.A. "Luiz de Queiroz" (ESALQ), University of São Paulo, Piracicaba, SP, Brazil (22°42' S, 47°37' W and 550 m a.s.l.) on a mulato palisade grass pasture (*Brachiaria* hybrid, cv Mulato – CIAT 36061) from January 2008 to March 2009. Treatments corresponded to combinations between two post-grazing heights (15 and 20 cm) and two pre-grazing conditions (95% and maximum light interception by sward canopy – LI), and were allocated to experimental units (1200 m^2 paddocks) according to a 2x2 factorial arrangement and a randomised complete block design, with four replications. The following response variables were evaluated: growth, senescence and utilisation efficiency of the produced herbage ([1-(senescence/growth)] x 100) (Bircham & Hodgson, 1983). Analysis of variance was performed using the Mixed Procedure of SAS® (Statistical Analysis System). The choice of the covariance matrix was made using the Akaike Information Criterion (AIC) (Wolfinger, 1993), and analysis performed considering post-grazing height, light interception pre-grazing, season of the year and their interactions as fixed effects and blocks as a random effect (Littel *et al*., 2000). When appropriate, treatment means were calculated using the "LSMEANS" statement, and comparisons made with "PDIFF" based on a Student t test and a 5% significance level.

Results Utilisation efficiency was affected by LI pre-grazing (P=0.0317), season of the year (P=0.0002) and LI pre-grazing x season of the year interaction (P=0.0028). Swards managed at maximum LI (99%) were characterised by lower utilisation than those managed at 95% LI during autumn-winter-early spring and summer 2009, with no difference between LI treatments recorded in the remaining seasons of the year (Table 1). In general, larger utilisation was recorded during both summers (2008 and 2009) relative to the other seasons of the year.

Table 1 Herbage utilisation efficiency (%) on mulato palisade grass swards subjected to strategies of rotational stocking management characterised by the pre-grazing conditions of 95% and maximum canopy light interception during regrowth

Season of the year	Canopy light interception (%)		SEM
	95	Max. (99)	
Summer 2008	62.8 Ab	66.3 Aa	4.5
Autumn-winter-early spring	49.9 Abc	34.5 Bc	4.5
Late spring	44.6 Ac	45.7 Abc	4.5
Summer 2009	72.6 Aa	58.5 Bab	4.5

Means followed by the same upper case letters in rows and lower case letters in columns are not different (P>0.05).
SEM= standard error of the mean.

Conclusions The lower utilisation efficiency during autumn-winter-early spring and late spring is associated with limiting environmental conditions to growth, affecting herbage accumulation. Herbage utilisation efficiency is maximum at 95% canopy light interception during regrowth, a condition equivalent to a sward height of 30 cm. Under rotational stocking management, a pre-grazing target of 30 cm should be used in combination with post-grazing heights of 15 or 20 cm.

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Effect of herbage allowance and grass silage supplementation on grazing behaviour of dairy cows in autumn pasture

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Introduction In Southern Chile, milk production is predominantly based on perennial pastures as pasture based milk production systems are more cost effective than indoor concentrate-based systems (Pulido *et al*. 2010). Grazing low-mass pastures is almost inevitable when extending the grazing season into late winter to reduce feed costs and a reduction in pasture intake and milk production is expected (Pérez-Prieto *et al*. 2010). The response to supplementary forages depends on herbage availability, the relative nutritive values of grazed herbage and the supplementary forage, and the duration of access to each feed (Phillips, 1988). This study was undertaken to evaluate the influence of daily pasture allowance (PA) and grass silage supplementation level (SS) on dry matter intake and grazing behavior of fresh calving dairy cows grazing an autumn pasture.

Materials and methods The study lasted 56 days between April and June using 24 Holstein Friesian dairy cows from the University herd (milk yield 23.9 ± 0.99 kg/d; BW 526 ± 11.9 kg; parity 2.4 parity; DIM 37.8 ± 1.5). They were randomly assigned to four dietary treatments resulted from the combination of two pasture allowance measurements at ground level (low, 17 *vs*. High, 25 kg of dry matter/cow per day) and two grass silage supplementation levels (4.5 and 9 kg/cow per day). Total dry matter intake of individual cows was estimated indirectly from animal performance results, individual measurement of feeding behaviour was carried out in two opportunities, recording the grazing, ruminating, laying, standing, walking, and milking activities every ten minutes during 24 hours. Cows were supplemented twice a day and managed under strip grazing system on pasture consisting mainly of perennial rye grass. Food intake and grazing behaviour were analysed using a randomized block by means of repeated measurements.

Results The nutritive value of the two pastures offered were similar and contained in average 17.9 % DM, 20.4 % CP, 2.54 Mcal ME, 44.5 de NDF and 28.9 % FDA. Grass silage contained 24.9 % DM, 17.0 % CP, 2.41 Mcal ME, 49 % NDF, 10% NH3-N and pH 4.0 %. The pastured offered had pre-grazing DM availability averaging 2220 kg and 2133 kg DM/ha, for high and low pasture allowances. Pasture dry matter intake (PDMI) was similar for both pasture allowances (P>0.05) but decreased from 9.2 for the low grass silage supplementation to 4.6 for the high grass silage supplementation. Total dry matter intake (TDMI) was not affected either by pasture allowance or grass silage supplementation (P>0.05). Grazing time was not decreased by increasing herbage allowance or grass silage supplementation (P>0.05). However supplement eating time increased (P<0.05) by decreasing pasture allowance with a mean time of 125 min/d for the low pasture allowance (P<0.05). Rumination time was not significantly affected by any factor studied.

Table 1 Dry matter intake (DMI) (kg DM/cow per day) and grazing behaviour (min/cow per day) of dairy cows grazing an autumn pasture

	Herbage allowance		Grass silage		Sig.		
Ítem	High	Low	High	Low	PA	SS	PA*SS
TDMI	16.6	15.9	16.2	16.3	0.634	0.636	0.989
PDMI*	7.2	6.6	4.6	9.2	0.634	0.002	0.809
GSDMI	6.8	6.8	9.0	4.5	-	-	-
CDMI	2.6	2.6	2.6	2.6	-	-	-
Total bites (bites/d)	20637	21336	21657	30315	0.557	0.262	0.677
Rate of pasture DM intake (g DM/min)	28.7	32.3	47.0	33.0	-	-	-
Grazing time (min/d)	311	330	314	328	0.289	0.420	0.231
Supplement eating time (min/d)	99	125	130	94	0.000	0.000	0.239
Ruminant time (min/d)	479	500	500	480	0.332	0.361	0.878
Idling time (min/d)	523	508	495	536	0.535	0.090	0.594

DMI estimating by animal performance

Conclusions For cows grazing low-mass pastures in autumn, the increasing in PA does not affect PDMI or TDMI due to incapacity of the cows to increases grazing time. Silage supplementation in dairy cows grazing low herbage allowance reduces motivation for pasture intake and increases supplementation time.

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Structural characteristics of Elephant grass cv. Napier swards under different grazing severities

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Introduction Recent studies have indicated that sward targets for stopping regrowth are at the ideal point for initiating the defoliation of tropical grasses when 95% of incident light is being intercepted (Da Silva & Nascimento Júnior, 2007). At this stage, leaf accumulation is at a maximum, while the accumulation of stems and dead material are at a minimum. In addition, defoliation severity must be adjusted to leave a remaining leaf area that will allow a rapid and efficient regrowth without impairing organic reserves or sward structure. Therefore, studies of sward structures can help better our understanding and definition of sward targets for proper management. This work was undertaken to determine the influence of post-grazing height on the structural characteristics and forage production of swards of *Pennisetum purpureum* cv. Napier under different defoliation frequencies under conditions of 95% light interception.

Materials and methods The experiment was carried out with the Elephant grass between February and May of 2009 in an area of the Animal Science Department of Federal University of Vicosa (UFV) at Vicosa-MG, Brazil (20º 45' S, 42º 51' O, 651 m.a.s.l.). Three post-grazing heights (30, 50 and 70 cm), were assigned to experimental units of 400 m^2 according to a completely randomised block design, with three replications that were grazed by 220 kg crossbreeding Zebu steers. Grazing was allowed when the sward had reached 95% light interception (LI), as determined by a canopy analyser (LAI 2000, LI-COR, Lincoln, Nebraska, USA*)*. Herbage accumulation (kg DM/ha) was evaluated along with morphological composition, foliage area index, foliage angle, residue light interception and percentage of weeds. To identify factors that best described Elephant grass cv. Napier, the data were statistically processed by factor analysis, using the Varimax Rotation Method and factor orthogonalization using the SAS statistical package (SAS Institute, 2009).

Results All variables were reduced to four factors (forage accumulation, non-foliar components, light interception at post-grazing and plant competition) that explained 74.82% of the total data variation, with commonality values between 25.1 and 92.3%. Paddocks managed with a post-residue height of 30 cm presented smaller factorial scores of forage accumulation (-0.5276), non-foliar components (-0.5273) and light interception at post-grazing (-0.7996) (Table 1). Therefore, this post-grazing height resulted in lower forage accumulation, leaves and non-foliar components, foliage area index and post-grazing light interception (Table 1). Furthermore, paddocks grazed to 30 cm residue presented higher plant competition (0.1523), with a higher percentage of weeds and foliage angles at post-grazing (Table 1). This pattern indicates that the growth and development of Elephant grass managed with a post-grazing height of 30 cm were beyond its potential in relation to the post-grazing heights of 50 and 70 cm, with a negative influence on forage production and possibly its persistence under grazing.

Table 1 Factorial score means of four factors and structural characteristics of swards of Elephant grass cv. Napier under different grazing severities

	Post-grazing height (cm)		
	30	50	70
Factorial scores			
Forage accumulation	-0.5276	-0.2992	0.2494
Non-foliar components	-0.5273	-0.2317	0.4549
Post-grazing light interception	-0.7996	-0.0362	0.4794
Plant competition	0.1523	-0.1729	-0.0961
Structural characteristics			
Forage accumulation (kg DM/ha)	4,341	5,204	6,403
Leaf accumulation (kg DM/ha)	2,676	3,279	4,031
Non-foliar component accumulation (kg/ha of DM)	1,568	1,651	2,251
Foliage area index at post-grazing	1.25	1.65	1.89
Post-grazing light interception (%)	62.8	73.9	77.4
Post-grazing foliage angle (degrees)	51	46	46
Post-grazing % of weeds	7.03	4.29	3.33

Conclusions A post-grazing height of 30 cm it is not recommended for the management of Elephant grass cv. Napier using 95% light interception as sward target.

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Performances of two handheld spectrometers to analyse grass silage in order to improve their valorisation in herbivore diets

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Introduction Optimisation of rough forage implementation in herbivore diets will allow improve economical and environmental performances of herbivore based livestock farming systems. In order to reach such a target, it is necessary to quantify and qualify rough forages resources. These products are characterised by a high level of heterogeneity (Lecomte *et al*., 1998). To take into account this variability in diet definition, there is a need to perform numerous analyses with, sometimes, important delays before to obtain the results, reducing their usefulness. To face this problem, we tested the performances of two handheld spectrometers (Polychromix Phazir1624 and Phazir1018) that would be used to qualify grass silage on farm (Fernández Pierna *et al*., 2010).

Materials and mthods Twenty two grass silage samples were collected on farm before being scanned, fresh, with the two Phazir spectrometers. To do so, each sample was first divided in two sub samples, each sub sample being scanned twice with each of the spectrometers. Each spectrum is the average of ten scans performed on the subsample analysed. Each subsample was then dried, to quantify their DM content, and analysed with the Foss NIRSystem 5000 instrument for which successful calibrations are already available for ash (N = 2055; Mean = 10.8; SD = 2.71; SECV = 1.35), crude protein (N = 1997; Mean = 14.6; SD = 3.87; SECV = 0.85), cellulose contents (N = 1939; Mean = 27.6; SD = 4.28; SECV = 1.37) and digestibility quantification (N = 739; Mean = 70.9; SD = 10.38; SECV = 2.99). So, each of the 44 subsamples was scanned four times, two times with each handheld spectrometer.
Polychromix Phazirs are integrated handheld spectrometers using micro-electro mechanical system technology (MEMS). They combine a digital transform spectrometer, rechargeable batteries, integrated computer, color LCD display and software into one unit that can be used remotely, such as in field applications. The spectra are acquired within a wavelength range of 1600 to 2400 nm and 1000 to 1800 nm, with a spectral resolution of 8 nm, respectively for Phazir 1624 and Phazir 1018 (Fernández Pierna *et al*., 2010).

Results and conclusions The first results (Table 1; Figure 1) underlined the potential offered by such handheld spectrometers to assess grass silage. Nevertheless, with the exception of the DM content, SD/SECV ratio remained under 3. So calibrations developed allowed to classify silage samples in 'good', 'average' or 'bad' classes but not to analyse them in a quantitative way. To reach such a target additional samples have to be included in the calibration to cover a wider range of variation, as only 22 silages were initially sampled. Moreover, these performances could be improved if laboratory reference methods are mobilised instead of reference values obtained through another NIRS technique. Indeed, in our approach there is interference between the error linked to the handheld spectrometer and the error of the NIRS analysis performed on dried material.

Table 1 Performances of the calibrations developed to analyze grass silage with phazir (1624) and (1018) spectrometers.

Parameters	Mean	SD	SEC (1624)	SECV (1624)	R^2 (1624)	SEC (1018)	SECV (1018)	R^2 (1018)
DM (%)	44.9	11.7	2.9	3.4	0.92	2.5	3.2	0.93
Ash (% DM)	15.2	2.5	1	1.1	0.54	0.9	1	0.65
Crude Protein (% DM)	11.1	1.6	1.3	1.4	0.71	1.6	1.8	0.55
Cellulose (% DM)	25.2	2.1	1.6	1.6	0.38	1.3	1.8	0.39
Digestibility (% DM)	74.4	4.7	3.5	3.8	0.38	2.8	3.8	0.36

SD : Standard Deviation, SEC : Standard Error in Calibration, SECV : Standard Error in Cross Validation, N = 88.

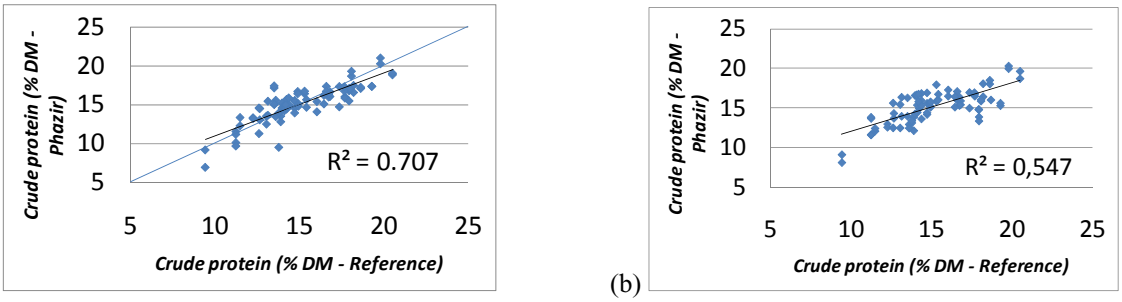

Figure 1 Regression of crude protein content (% DM) quantified with Phazir spectrometers against crude protein content quantified with reference method (Foss NIR 5000). (a) Phazir 1624 - (b) Phazir 1018

Acknowledgment Thanks to Polychromix for the Polychromix Phazir instruments setup for use at CRA-W.

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Differentiation between true methanogen inhibitors and different sinks for hydrogen using a new automated batch culture system

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Introduction In recent years many studies have been carried out using natural plant components to modify rumen fermentation to decrease methane emissions in ruminants. *In vitro* batch culture systems are the method of choice as a screening tool, since they are low cost and straightforward to operate. However most automated *in vitro* systems only determine gas production over time and gas composition has to be determined by manual sampling (Beuvink *et al*. 1992; Davies *et al*. 2000). In the present study we show the use of a fully automated batch culture system for the measurement of gas volume and composition that allows for differentiation between organic or inorganic compounds that act as hydrogen (H_2) sinks and true methanogen inhibitors.

Materials and methods Several pure compounds were incubated in an automated incubation system where gas volume is measured by pressure in the bottles before gas composition is analysed by gas chromatography. The model compounds used were bromoethane sulphonate (BES, 30 μM) as a true inhibitor of methanogens, nitrate (NO_3, 160 μM) as an inorganic sink of H_2 and L-rhamnose (Rh, 60 μM) as an organic sink of H_2. The compounds were incubated with ryegrass, which was dried and ground to pass a 1mm sieve. Ryegrass was incubated at a level of 10 mg/ml in 60 ml of buffered rumen fluid (20% rumen fluid) in 100 ml serum bottles in duplicate. The bottles were connected to the pressure sensors and an array of valves that controlled gas flows via a dedicated computer. Incubation was terminated after 48 hours and endpoint samples for the analysis of short chain fatty acids (SCFA) were taken and analysed in a gas chromatograph.

Results BES and Rh only slightly decreased gas production (Figure 1a) compared to the control, while the addition of NO_3 reduced gas production by around 10%. Therefore the results of CH_4 and H_2 are given as a percentage of total gas production [%GP]. All model compounds reduced CH_4 by around 25 to 30% compared to control at the end of the incubation period (Figure 1b). The inhibitor (BES) led to an accumulation of H_2, but only minor amounts of H_2 accumulated when Rh or NO_3 was present (Figure 1c).

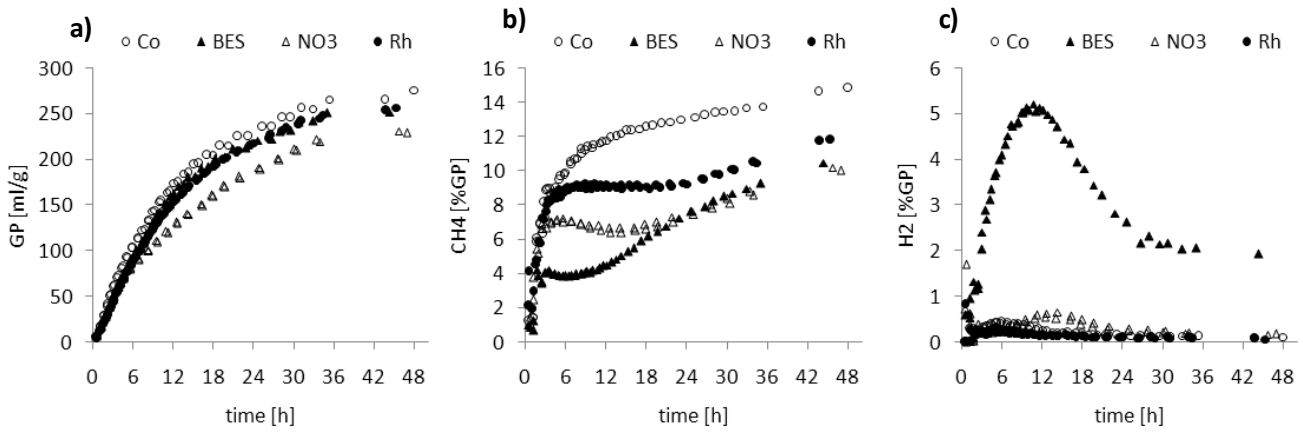

Figure 1 Total gas production of ryegrass (Co) incubated with BES, NO_3 and Rh (a) and the proportion of methane (b) and hydrogen (c) produced over time when incubated with ryegrass.

Although a lower gas production was observed when nitrate was included there was no change in total SCFA concentration or the proportion of individual fatty acids compared to the control. Fermentation of Rh had a similar SCFA production relative to the control but the proportion of propionate was doubled. Inhibition of methane by BES led to a 5% decrease in total SCFA produced and increased the production of propionate and butyrate by 10 and 9% respectively.

Conclusion The analysis of gas and SCFA composition *in vitro* enables us to identify whether a plant contains a true methanogen inhibitor or if hydrogen is utilised by either the production of specific fermentation products or metabolism of inorganic components. This will help narrow down the possible targets in the search for an active component in the plant. This system has been tested by screening 100 plants grown in New Zealand and has already identified the type of methane inhibition in two of the samples tested.

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Passage characteristics of ^{13}C-labelled carbohydrate fractions in dairy cows fed high and low levels of concentrates

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Introduction Within the Dutch protein evaluation system (DVE/OEB system; van Duinkerken *et al*., 2011) fermentation behaviour of rumen resistant protein and starch is described by feed specific fractional degradation rates and fixed fractional passage rates. Digestion of feed is not a static process though. It is therefore necessary to take into account variation in fractional rumen passage rates related to diet composition, to change from an animal requirement-based system to an animal response-based system. This enables to determine responses of dairy cows to diet changes and predict nutrient availability and utilisation within the digestive tract more accurately (Dijkstra *et al*., 2007). Fractional passage rates are conventionally determined by using indigestible external markers, which have been often criticised for behaving differently from feed particles (e.g., Tamminga *et al.*, 1989). Internal markers like stable isotopes (e.g., ^{13}C) are truly associated with the fraction under investigation and hence, would be more representative of the *in vivo* situation. This research aims at quantifying fractional passage rates of cell wall and non-cell wall carbohydrate fractions of concentrates fed at high *vs*. low levels of concentrates. An increase of concentrate levels up to 50% in the diet was found to increase fractional passage rates of concentrates (Owens and Goetsch, 1986). Cr mordanted fibre (Cr-NDF) was used as an external marker and ^{13}C as internal marker. In the current study, the principle of higher ^{13}C abundance in C_4-plant species (e.g., maize) as compared to C_3-plant species (e.g., rye grass silage) was used (Südekum *et al*., 1995). By exchanging part of the C_3-plant components by a pulse dose of C_4-plant components in the diet, a shift in ^{13}C enrichment will be created. Passage kinetics can then be obtained from changes in the ^{13}C:^{12}C-ratio in the undigested carbohydrate fractions in the faeces.

Material and methods Six dairy cows fitted with rumen fistulae were paired according to milk production and within pairs randomly assigned to one of the two concentrate levels in a cross-over design. Cows were fed whole barley and grass silage (1:1 on product base) supplemented with concentrates at a high (9.2 kg DM/day; HC) or low (4.0 kg DM/day; LC) level. Concentrates were solely composed of C_3 plants and concentrate levels chosen to constitute 52% (HC) or 23% (LC) of the total diet (DM basis). Each measurement period was preceded by two weeks of adaptation. At time t=0 h of each measurement period, animals received a pulse dose of 9.0 kg pelleted maize bran to create a measurable contrast in ^{13}C enrichment, and an external marker (90 g Cr-NDF). Faecal spot samples were collected frequently after pulse-dosing during a 5-day period, resulting in 21 samples per cow and treatment. The samples were analysed for Cr-content and ^{13}C-enrichment in the dry matter (^{13}CDM), neutral detergent residue (^{13}CNDR) and neutral detergent soluble (^{13}CNDS) fraction. Fractional passage rates (k_p) were calculated using the multicompartmental model of Dhanoa *et al.* (1985). Total mean retention time and moment of peak concentration were derived from the estimated passage parameters. An analysis of variance (Proc GLM, SAS Inc., 2008) was carried out to test the effect of the concentrate level on marker passage.

Results Feed intake, NDF intake and NDF degradability were not affected by the concentrate level. Accuracy of the model in predicting fractional passage rates of faecal marker excretion was similar between markers (mean prediction error of 0.12-0.14 on average). Fractional passage rates of the slowest compartment (k_{p1}; rumen-reticulum) differed between markers (P<0.001); ^{13}CNDR and ^{13}CNDM had a higher k_{p1} than any other markers (Table 1). The second slowest compartment (k_{p2}; large intestine) showed the opposite pattern with higher k_{p2} values for ^{13}CNDS and Cr (P=0.044). Therefore, rumen and total tract residence times of ^{13}CNDR and ^{13}CDM were shorter. Peak concentration of ^{13}CNDS was reached at an earlier stage, compared to any other markers. No significant effect of the concentrate level on k_{p1} values was observed. For the second slowest compartment, k_{p2} values tended to be higher with increased levels of concentrates (P=0.08).

Table 1 Fractional passage rates of the slowest (k_{p1}) and second slowest (k_{p2}) compartment at high (HC) and low (LC) level of concentrates

Markers	k_{p1} (/h)		k_{p2} (/h)	
	HC	LC	HC	LC
Cr	0.037	0.040	0.398	0.366
^{13}CDM	0.054	0.057	0.358	0.323
^{13}CNDR	0.059	0.064	0.321	0.296
^{13}CNDS	0.043	0.039	0.474	0.363
SEM	0.004		0.035	

Conclusions The cell wall fractions (NDR) and DM fractions of maize bran passed out quicker from the entire gastro-intestinal tract than the non-cell wall fractions (NDS) and Cr. Furthermore, this study suggests that fractional passage rate are not influenced by the concentrate level in the diet. The fractional passage rate of ^{13}CDM for concentrates matches with the assumed value in the Dutch DVE/OEB system (0.06/h; van Duinkerken *et al*., 2011), while rumen passage rates based on the external marker Cr-NDF seem to underestimate the fractional passage rates for both the DM and cell wall fractions.

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Potential mineral deficiencies for Ndama cattle grazing *Urochloa* sp. based tropical pastures in the Bas-Congo province of the Democratic Republic of Congo

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Introduction Intensification of cattle production using artificial pastures can considerably increase carrying capacity of tropical rangelands as well as productivity per head by supplying adequate energy and protein to sustain animal requirements all year long. However, artificial pasture are labour-intensive and costly to implement so when alleviating macro nutrient provision, to maximise the return on investment, the grazier must make sure no other limiting factor such as mineral contents still limit animal's performances. Mineral deficiencies in forage depend on the species and the season of grazing, but also on the location through soil characteristics and rain distribution pattern. In this study, intake of nutrients including mineral content of cattle grazing *Urochloa* sp.-based pastures were measured during two different seasons and compared to requirements (IEMVT-CIRAD, 1990).

Materials and methods Three Ndama steers (260 kg of liveweight; LW) and 3 5-years old Ndama cull cows (303 kg LW) were followed up, within a herd of 30 animals, on artificial *U. decumbens* (*U. decumbens* : 46.3%; *Calopogonium mucunoides* : 23.2% and *Mimosa pudica* 5.2%; other : 25%); and *U. ruziziensis* pastures (*U. ruziziensis* : 41.3% ; .*C mucunoides* : 25.7%; *Panicum maximum* : 7.3 %; and *M. pudica* : 6.6%; other : 19.1%) located in Kolo-Fuma (Bas-Congo, D.R.Congo). The experiment was replicated during two grazing seasons : (1) from end February until end March (short dry season) and (2) during April (short rainy season) 2009. Each pasture was divided in 3 paddocks that were grazed consecutively for 4 days during each season before moving to the next paddock. Intakes of each steer and cow during the different grazing seasons on each paddock were estimated using NIRS on faecal samples collected during 3 consecutive days (Decruyenaere *et al*., 2009). These 3-days also corresponded to observation periods (from 8 AM until 5 PM) when hand plucking was used to sample the forage consumed mimicking the animals' grazing behaviour. All forage samples were analysed for energy (calorimeter), crude protein (AOAC 981.10) and ash (AOAC 923.03) contents. Energy value (fodder units, FU) and digestible crude protein (DCP) contents were estimated using Dijkstra (1957). Forage samples of the 3 animals of a same category (steer *vs*. cows) were pooled prior to mineral analysis by Inductively coupled plasma atomic emission spectroscopy (ICP-AES) (2 categories × 2 pastures × 3 paddocks × 2 seasons). Animal potential performances expected on the basis of DM intake and feeding values were estimated according to Rivière (1991) and mineral contents were compared to deficiency limits (IEMVT-CIRAD, 1990).

Results and conclusions With a nutritive value of 0.701 ± 0.036 FU kg^{-1} DM and 4.78 ± 1.04 % DCP, and intake levels of 66 ± 4,3 g kg^{-1} $LW^{0.75}$, both pastures would allow daily liveweight gains (DLG) of above 550 and 350 g day^{-1} for steers and cull cows, respectively, which is close to the highest DLG expected with Ndama cattle. Even if DCP did not differ between both pastures (P = 0.40), the CP content of *U. decumbens* pasture was significantly higher (P < 0.01) during short dry and short rainy season. This explains the higher intakes recorded for both seasons on *U. decumbens* (69 g kg^{-1} $LW^{0.75}$) compared to *U. ruziziensis* (63 g kg^{-1} $LW^{0.75}$). Higher DLG by 150 and 200 g, for steers and cows, respectively, were thus expected on *U. decumbens* than *U. ruziziensis*.

Table 1 Deficiency limits and animal's diet mineral contents when grazing improved pastures based, respectively, on U. decumbens and U. ruziziensis (P, Ca, Mg, K, Na in g kg^{-1} DM; Fe, Mn, Zn, Cu in mg kg^{-1} DM)

	Pasture	P	Ca	Mg	K	Na	Cu	Zn	Mn	Fe
Dry season	*U. decumbens*	2.64	3.66	2.35	15.07	0.08	0.99	26.58	158.20	174.23
	U. ruziziensis	2.47	3.97	2.38	14.03	0.11	0.56	31.62	184.48	215.84
Rainy season	*U. decumbens*	2.68	2.96	2.07	17.54	0.08	1.81	29.70	154.55	201.76
	U. ruziziensis	2.72	3.65	2.26	17.46	0.09	2.49	42.98	163.99	236.81
	P-value	NS^1	S^*, P^{NS}	S^*, P^{NS}	S^*, P^{NS}	NS	S^*, P^{NS}	$S^*, P \times S^*$	NS	NS
	Deficiency limits	2	2	0,7	3,2	0,6	7	45	45	5

[1]NS, not significant; *, P<0.05; P, Pasture; S, Season.

Whatever the season or the pasture, macro-nutrients contents did not limit production, with the exception of Na, while Cu and Zn contents were severely deficient for both seasons and pasture types (Table1), as usually observed in the Tropics. It can be concluded that, in order to maximise the valorisation of both artificial pastures, a mineral supplement providing Na, Cu and Zn is required to reach the DLG allowed based on energy and protein supplies and prevent fur and hoof malformations.

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Preference of Pelibuey hair sheep for the ground pods of five different tropical tree species

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Introduction Feeding of ruminants under extensive conditions in the tropics is based on the grazing of tropical grasses and the intake of a variety of foliages and fruits fallen from trees and shrubs. Tropical regions posses a variety of trees and shrubs such as *Enterolobium cyclocarpum, Acacia pennatula, Guazuma ulmifolia, Samanea saman* and *Mucuna pruriens* which represent nutritional alternatives for sheep given their crude protein content and high rumen degradability. Food preference is given by the interactions among the senses (smell, taste) and chemical composition, which leads to selection and intake. Sheep is an intermediate selector and can select preferred feedstuffs, thus avoiding ingestion of toxic compounds (Provenza, 1995). Even when ruminants consume pods from different tree species, published work has focused mainly on the utilization of foliage. There is little information on the preference of sheep for the intake of different pods from tropical trees. The aim of the present work was to assess the preference of hair sheep for the intake of ground pods of five different tropical tree species.

Materials and methods The experiment was carried out at the School of Veterinary Medicine, University of Yucatan in South Mexico. Climate in the region is Aw0 (hot tropical and sub-humid with summer rains). Mean annual temperature fluctuates among 26 to 27.8 °C and rainfall ranges between 940 to 1,100 mm. Six Pelibuey sheep with a mean liveweight of 26 kg ± 1 were randomly housed in pairs in three pens. Treatments were ground pods (meal) of *E. cyclocarpum, A. pennatula, G. ulmifolia, S. saman* and *M. pruriens*, which were manually collected from below the trees and dried in a forced-air oven at 60 °C for 48 h and passed through a mill of 3 mm. The study lasted for 25 days (10 days adaptation and 15 days data collection) during which the ground pods were offered daily for 60 minutes, with daily rotation of feeders to avoid conditioning (food learning), among the species. Basal ration consisted in *Brachiaria brizantha* hay fed *ad libitum* sprayed with a molasses-urea mixture plus minerals. A randomized block design was employed to assess the preference for the intake of the ground pods. Data was analyzed by analysis of variance using the SAS statistical package, means were tested with the Tukey test at $P < 0.05$ level.

Results *Guazuma ulmifolia* was the most preferred pods by sheep (Fig. 1) followed by *M. pruriens* and *A. pennatula* ($P>0.05$). Least ground pods consumed were those of *S. saman* and *E. cyclocarpum* without differences between them ($P>0.05$) but different relative to the other pods studied ($P<0.05$; Fig. 1). Total intake of ground pods increased with time, resulting in a reduction in hay intake (substitution effect). It can be observed that sheep showed an adaptation stage to regulate intake probably to satisfy nutritional requirements and/or reduce ingestion of secondary compounds (Fig. 2).

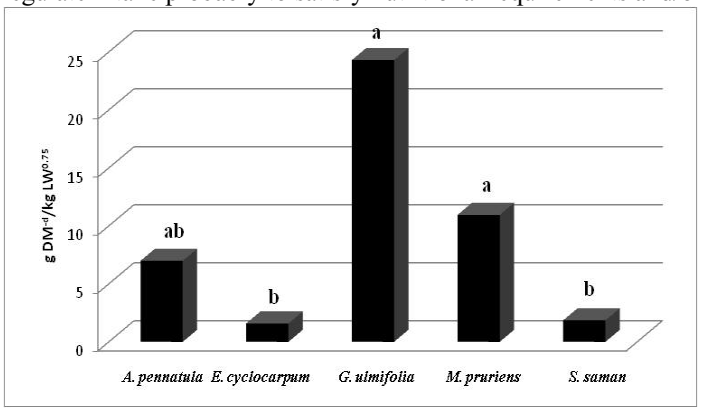

Figure 1 Preference of hair sheep for ground pods of five different tropical trees (g DM/kg $LW^{0.75}$)
Rows with different literal differ significantly ($P<0.05$); SEM

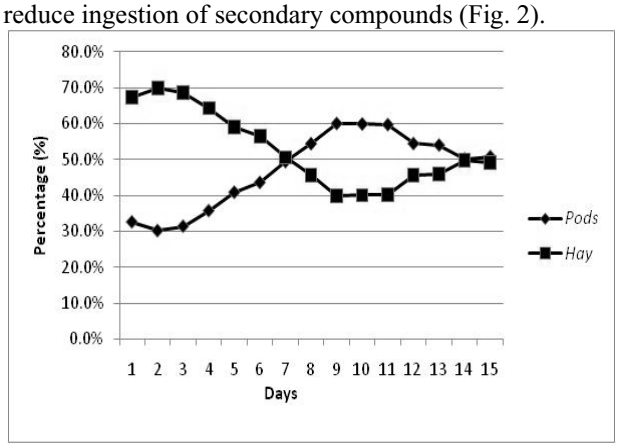

Figure 2 Total daily DM intake by hair sheep (hay + pods; g DM/kg $LW^{0.75}$)

Conclusion Ground pods of *G. ulmifolia* followed by *M. pruriens,* were the most preferred by Pelibuey sheep. Sheep were able to substitute up to 50% of total DM intake after eight days of exposure to the ground pods of tropical resources after one hour per day. More work is needed to understand factors affecting food preference by sheep in order to relate it to nutritional quality (digestibility) which may be useful in designing supplementation strategies for ruminants under practical conditions in the tropics.

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Tillering dynamics in spring of signal grass deferred on variable heights in early autumn

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Introduction To overcome the negative effects of seasonality of forage production from tropical pastures, deferring the pasture stands out as a relatively easy and inexpensive management strategy. Accordingly, management actions, such as adequate pasture height at the beginning of the period of deferment may be made to improve plant productivity and animal performance on deferred pasture. Nevertheless, it should be considered that these management actions also change the pattern of regrowth of pastures during the growing season ahead. Thus, this study was aimed to evaluate the dynamics of tillering during the regrowth in the spring in pastures of *Brachiaria decumbens* cv Basilisk deferred on four heights.

Material and methods The experiment was conducted during April-December 2009, in the Department of Animal Science of the Federal University of Vicosa (UFV), located in Minas Gerais state, Brazil. The experimental area is located 20 ^{o} 45 'S, ^{42} o 51' W and 651 m of altitude. The annual precipitation is around 1,340 mm. The maximum and minimum temperatures are 22.1 and 15 ^{o} C, respectively. The experimental area was divided into eight plots (experimental units) of 0.30 ha each. Four sward heights at the beginning of deferred period (10, 20, 30 and 40 cm) were evaluated (early and late spring) in early and late spring. The split plot scheme, with the height of the grass in plots and the two evaluation periods in the subplots (early and late spring), was used, according to a randomized block design with two replications. In April, after the establishment of the goals regarding the heights of the grass, began the period of deferment of pastures. After 70 days of deferral, in July, all pastures were grazed under continuous stocking with stocking rate fixed (3 AU / ha) until September. After the occupation period of the deferred pastures, the evaluation began and the pastures were grazed under continuous stocking with 25 cm high, adopting a variable stocking rate with crossbred steers of 200 kg until the end of December, when the experiment was closed. Each experimental unit was delimited in three places, with an area of 0.0625 square meters each, representing the average initial condition of the pasture. These locations were demarcated using a metal frame painted in white and shaped like a square with sizes of 25 cm. At the beginning of the evaluation, all tillers contained within the frames were counted and marked with colored plastic coated wire. Every 30 days, all tillers were counted again and the new tillers were marked with wire of different color. With these data the rates of appearance, mortality and survival of tillers were calculated, according to Carvalho *et al*. (2000).The data relating to times were compared by Tukey test (10%), while those related to sward heights were subjected to regression analysis.

Results The rates of appearance and tiller mortality were higher in early spring. The average values of appearance rate and the mortality rate of tillers were respectively 53.5% and 39.3% in early spring, and 36.8% and 11.7% in late spring. These results characterize the high turnover of tillers in *Brachiaria decumbens* pastures in the spring, resulting in more young tillers in the pasture. The survival rate of tillers was greater in late spring (88.3%) compared to early spring (60.7%). In late spring, in order to offset the reduction in the rate of appearance, the tillers survived longer. Nevertheless, in early spring, the appearance rate was higher and the tillers had lower longevity. This tradeoff between appearance and tiller survival contributes to the stability of the population of tillers in the pasture under specific environmental conditions. The deferred pasture with higher height showed lower tillering (43.6%) in early spring, when compared to the lower height (63.5%). This indicates that the lower pasture at the beginning of the deferral period has better regrowth in the following season, probably due to higher incidence of light at the base of plants, which stimulates tillering (Sackville Hamilton *et al*., 1995). There was no effect of height on the appearance rate of tillers in late spring, which showed a mean value of 36.84%. However, the sward height influenced linear and positively the tillers mortality rate during spring ($Y = 23.729 + 0\ 621818X$, $R^2 = 0.73$). It is possible that the tillers that remained alive during the occupation of the deferred pastures died in spring more markedly in those pastures deferred with higher height. The survival rate of tillers decreased linearly with sward height in both periods evaluated ($Y = 76.271-0.621818\ X$, $R^2 = 0.73$), with values ranging from 70.0% and 51.4% in deferred pastures with 10 and 40 cm respectively.

Conclusion The grass height at the beginning of the deferral in early autumn period influences the dynamics of tillering of *Brachiaria decumbens* cv. Basilisk during the spring; so that the deferred pastures with 10 cm and 20 cm have a higher rate of regrowth in spring.

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Respirometry, net energy requirements and methane production of Holstein, Gir and crossbred Holstein-Gir dairy heifers fed tropical grass

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Introduction In Brazil we formulate diets for dairy cattle using NRC (2001) date because we don´t know the nutrient requirements of dairy zebu cattle and their crossbreds, especially for heifers. The objective of this research was to find the nutritional requirements of Holstein-Gir heifers fed tropical grass hay, by using respirometric technique.

Materials and methods Standard procedures were estimated in order to calibrate a respirometric chamber for large animals at Metabolism and Calorimetry Laboratory of Veterinary Scholl of Federal University of Minas Gerais, Brazil. Indirect calorimetry system was used. Correction factors were calculated for oxygen, carbon dioxide and methane gases (Lachica, 1995), and values were 1.0001, 0.8972 and 1.0755, respectively. Eighteen heifers were used: six Gir, six Holstein and six crossbred. The average live weight was 300 Kg. The statistical delineation used was completely randomized, with Tukey test used at 5% of probability. Animals were considered adapted when the dry matter intake inside the chamber was similar to outside. The diet was *Cynodon dactylon* hay plus mineral supplement. In order to get the net energy for maintenance, animals were submitted to 72 hours fasting period. Third day data into respirometric chamber were used for fasting metabolism calculation. Brower (1965) equation "HP = (16.18 * VO_2) + (5.02 * VCO_2) – (2.17 * VCH_4) – (5,99 * Nur)" was used to estimate the heat production (HP), where VO_2 is oxygen volume, VCO_2 is carbon dioxide volume, VCH_4 is methane volume and Nur is urine nitrogen. Relationship between methane production, DMI and neutral detergent fiber was studied in order to compare greenhouse effect in bovines of different genotypes feeding tropical grass.

Results Feed intake was controlled in order to keep the daily gain between 0.2 and 0.4 Kg/d. Holstein heifers had the highest dry matter intake (DMI, 4.41 Kg/d) and Gir heifers had the lowest DMI (3.72 kg/d). The NEm (Table 1) for F1 Holstein x Gir animals was higher (102.3 Kcal/$LW^{0.75}$). Gir heifers had lower NEm (85.2 Kcal/$LW^{0.75}$). The values were compared with AFRC (1993), NRC (2001) and CSIRO (2007) tables. NEm for Gir heifers was very similar to AFRC (1993) prediction (83,5 Kcal/ $BW^{0.75}$) and to CSIRO (2007) prediction (80.25 Kcal/$LW^{0.75}$). Crossbred animals didn´t have good predictions by these tables. The daily methane production was measured. Methane production was similar between heifers. Values obtained were 33.7L of methane for kg of dry matter intake and 41.9L of methane for Kg of neutral detergent fiber intake.

Table 1 Net energy for maintenance (Kcal/ $BW^{0.75}$) and daily methane production of Gir, Holstein and F1 Holstein x Gir crossbred heifers

Racial group	NEm	CH_4/DMI	CH_4/NDFI
Gir	85.2^{b}	31.7^{a}	41.4^{a}
Holstein	96.4^{ab}	35.6^{a}	43.1^{a}
F1 Gir x Holstein	102.3^{a}	33.7^{a}	41.1^{a}
VC	11	10	9

Different letters in the column differ for test Tukey (P< 0,05). VC: variation coefficient. NEm: net energy for maintenance. CH_4/DMI: daily methane production to dry matter intake. CH_4/NDFI: daily methane production to neutral detergent fiber intake.

Conclusions We can conclude that Gir heifers have lower NEm than F1 Gir-Holstein, and the methane production of heifers fed tropical grass is similar between different genotypes.

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Evaluation of the fermentative and nutritive characteristics of the silage made from banana (*Musa acuminata cola*, sub. *cavendish*) by-product feeding for ruminants

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Introduction Canary Islands is the most important producer of bananas in Europe (over 345,000 tons of bananas per year). Banana by-products consist of the banana crop residues (stem and leaves) and the classification and packaging process (cluster and bananas fruits) and are often used fresh. 10% of the weight of banana fruits (40-50 kg) can be considered waste product in the packaging, conditioning and classification process before banana commercialisation. Of these 5 kg, about 3 kg would correspond to the cluster and 2 kg to banana waste. Therefore, the production of banana packaging by-products is estimated around 35 million kg of fresh matter. Ensilage could be the most appropriate way for preserving such by-products for long periods. This study was carried out to ascertain the ensiling capacity and the chemical composition of fresh banana packaging industries by-products.

Material and methods The ability of a product to be silage depends on the values of dry matter, soluble carbohydrates, buffering capacity and nitrate. Five combinations representing the product (100% green banana (GB), 100% cluster (C), 30% GB-70% C, 50% GB-50% C and 100% ripe banana (RB)) were taken from various banana packaging industries. The following measurements were made after grinding and homogenizing the product: pH, dry matter, soluble carbohydrates, buffering capacity, nitrite and nitrate, determining also the ratio of fermentability. According to these results three combinations were selected (100% GB, 50%GB-50%C, 50%GB-50%C + additives) in order to assess the quality and stability over time of the experimental banana microsilages. Molass and beet pulp were the additives used in the third combination. Microsilages model used in this experiment was based on that defined by Martinez (2003). Chemical composition and silage suitability was evaluated after 45 days, 3 and 6 months. The containers were individually opened and three samples were taken from each microsilage. The fermentation process was evaluated from final metabolites in silage juice by HPLC performed in the Animal Nutrition laboratory in SERIDA (Asturias): pH, soluble-N and ammonia-N, water soluble carbohydrates (WSC), lactic acid, volatile fatty acids (VFAs), acetic, propionic and butyric acid. The chemical composition was determined after sample liofilization for DM, OM, ash, CP, ADF, NDF and ADL. Silage metabolites and chemical composition data were analysed by analysis of variance performed with Tukey test using the statistical package SPSS 15.0 for Windows Release.

Results As expected, the combination with better results was GB/C+additives (Table 1). Soluble-N and ammonia-N values below 50% and 10% respectively, denoted an adequate protein degradation in the fermentative process. Morover high amounts of lactic acid in these samples indicate an optimal conversion of carbohydrates, contribuiting to the reduction of pH and the stability of silage. Finally the absence of butyric acid and a low content of volatile fatty acids characterize this combination as an excellent quality silage with a high stability over time.
The other two combinations presented excessively high rates of ammonia-N, VFAs and specially butyric acid. These results clasified these combinations near the limit considered as poor quality silage (Martinez *et al*. 1999). On the contrary, their stability over time was considered acceptable as the values remain reatively constant throughout the conservation process.

Table 1 Final metabolites in silage juice of banana by-product (two combinations) for 6 ensiling months

Comb.	Sampling day	pH	Sol-N (% totN)	NH3-N (% totN)	WSC	Lactic Acid	Acetic Acid	Propionic Acid	Butyric Acid	VFAs
			(mgN/100ml)		(mg glucose/100ml)	(mg/100ml)				
GB/C + additives	45d	3,99 ±0,04	43,94 ±2,47	6,75 ±0,34	2,71 ±0,74	6,75 ±0,21	1,48 ±0,05	0	0	1,48 ±0,05
	3m	3,98 ±0,02	45,24 ±1,95	6,90 ±0,20	1,96 ±0,12	6,45 ±0,22	1,68 ±0,03	0	0	1,68 ±0,03
	6m	3,96 ±0,03	45,91 ±4,17	5,95 ±0,98	2,90 ±0,70	6,39 ±0,38	1,87 ±0,01	0	0	1,87 ±0,01

Conclusion The banana packaging industry by-products showed a good aptitude for ensiling specially when some additives were added to the raw material to improve dry matter and soluble carbohydrates content. Its fermentative composition was acceptable and low changes in chemical composition during silage were detected although its moderate nutritional value recommend a limited inclusion in ruminant diets. However, further studies are required in order to assess palatability and to observe feed intake. In addition the influence of banana silage diets on milk and cheese production must be checked.

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Effects of additive on aerobic stability and nutritive value of maize silage stored during different time periods when harvested at advancing maturity stages

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Introduction The use of maize silage in Swedish feed rations to cattle increases. However, the quality of maize silage varies considerably between herds and farmers often experience problems with silage heating after opening of the silo (Nadeau *et al*., 2010). Variations in silage quality are related to differences in maturity stages at harvest and ensiling technique. The aim of this experiment was to study the effects of additive on aerobic stability and nutritive value of maize silage stored during different time periods when harvested at advancing maturity stages.

Materials and methods Whole-crop maize was harvested at the dough (280 g DM/kg, September 24), the dent (370 g DM/kg, October 20) and at the physiological maturity stage (410 g DM/kg, November 2) in 2009 in south-west Sweden (58°23'N, 13°29'E). The maize was chopped to a mean particle length of 13 ± 0.9 mm and packed in 1.7-l mini silos, equipped with fermentation traps, to a density of 225-250 kg DM/m^3. The crop was ensiled for 28 and 110 d. Silages treated with KOFASIL® STABIL, 2 l/ton herbage (sodium benzoate and potassium sorbate; Addcon Europe GmbH, Germany) and ProMyr® XR 680, 4 l/ton herbage (formic acid, propionic acid and formate; Perstorp Inc., Sweden) were compared to a control treatment without additive at both storage times. In addition, maize, which was ensiled for 110 d., was treated with KOFASIL® LIFE "M", 1*10^5 cfu of *Lactobacillus buchneri* DSM 13573/g herbage (Addcon Europe GmbH, Germany). Application rates of the additives were recommended rates by the manufacturers. Silages were sampled for chemical composition, total yeast counts and aerobic stability at opening of the silos. Aerobic stability of the silages was measured as the number of days before the temperature in the silage reached 2°C above the ambient temperature of 20°C during a two-week period. *In vitro* organic matter digestibility (IVOMD) of the silages was measured before and after the aeration period. The experiment had a completely randomized design with four replicates (=silo) per treatment. Data on chemical composition of herbage and silage, yeast counts and aerobic stability of silage were analysed in PROC GLM, SAS (ver. 9.1), whereas IVOMD before and after aeration of the silages was analysed in PROC MIXED, SAS. When the F-value was significant, pair-wise comparisons between LSMEANS were done with Tukey´s t-test.

Results Fresh herbage contained 269, 323 and 356 g starch (P<0.05), 88, 21 and 12 g water soluble carbohydrates (P<0.001) and had IVOMD values of 0.83, 0.79 and 0.77 (P<0.01) at the dough, dent and physiological maturity stage, respectively. The NDF content of the herbage ranged from 385 to 441 g and the crude protein was 72 g/kg DM. All silages were well fermented (pH 3.8-4.6) with stimulation of lactic acid production in the untreated silage (39 g/kg DM) and silage treated with KOFASIL® STABIL (34 g/kg DM) at all maturity stages. Silage treated with KOFASIL® LIFE "M" showed decreased lactic acid (10 g/kg DM) but increased acetic acid contents (25 g/kg DM) at the two later maturity stages. ProMyr® XR 680 restricted lactic acid production to 11 g/kg DM, when averaged over maturities. Small amounts of yeasts were found in untreated silage at all maturities and opening times (log 2.5 cfu/g) and in inoculated silage at the dough stage (log 3.3 cfu/g), which might have contributed to the less stable silages during aeration (Table 1). Silage treated with KOFASIL® STABIL was more stable against heating than the control silage at all maturity stages in both storage periods, whereas silage treated with ProMyr® XR 680 was more stable than the control silage in four of the six opening times. Silages treated with the chemical additives had similar IVOMD, which did not decrease during aeration (0.81 *vs*. 0.80, P>0.05). However, IVOMD of untreated silage decreased from 0.83 to 0.76 during aeration, when averaged over maturity stages (P<0.05). Likewise, IVOMD of inoculated silage decreased from 0.84 to 0.80 (P<0.05), with the decrease originating from the silage harvested at the dough stage.

Table 1 Number of days before the silage temperature reached 2°C above ambient temperature of 20°C during a 13.7-day period

Maturity stage at harvest	Untreated	KOFASIL® LIFE "M"	KOFASIL® STABIL	ProMyr® XR 680	SEM	P-value
28 d. of storage						
Dough stage	8.7	not determined	≥ 13.7	≥ 13.7	1.43	0.057
Dent stage	6.2^b	not determined	≥ 13.7^a	12.7^a	0.67	<0.001
Physiological maturity	6.4^b	not determined	12.0^a	9.9^{ab}	1.03	<0.05
110 d. of storage						
Dough stage	5.7^b	6.3^{ab}	≥ 13.7^a	11.0^{ab}	1.68	<0.05
Dent stage	5.4^b	≥ 13.7^a	≥ 13.7^a	10.3^a	0.97	<0.001
Physiological maturity	4.7^b	11.4^a	10.5^a	10.6^a	1.42	<0.05

[a,b]Means with different superscripts differ significantly (P<0.05).

Conclusion Additives are needed to prevent or delay heating of maize silage after opening of the silo, with KOFASIL® STABIL being most effective, when compared to the control. All silages showed good fermentation characteristics regardless of maturity stage and length of storage period.

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Effect of crude fibre on digestion of woody plants by roe deer (*Capreolus capreolus*)

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Introduction Changing environment conditions of free living herbivorous animals in conditions of cultural landscape in Central Europe induce requirement for an additional feeding. Choice of suitable food for different herbivorous animals requires knowledge concerning their real nutritional needs in different species. The physiology of nutrition and requirements in basic nutrients in farm animals are relatively well known, but in free living animals some aspects of nutrition are yet to be investigated. For example, Hoffman (1995) divided European ruminants into the following nutritive types on the basis of natural food consumption. Roe deer belong under the concentrate selectors. The knowledge concerning nutritive value of natural food consumed by the particular species should be the basic criterion for its supplementary feeding. Roe deer is the most ubiquitous free living herbivore species in Slovakia. In winter these animals consumate the distal parts of wood twigs. The aim of our studies was to examine the digestibility of selected woody plants by roe deer.

Materials and methods We studied the digestibility of nutrients of woody plants by four tame roe deer females during December-March. The animals were housed individually in balance boxes. The digestibility was assessed on the basis of balance of received and excreted nutrients. The animals received twigs for nipping once every 24 hours. Faeces were sampled 2 times in 24 hours and delivered to the laboratory. The results were evaluated for each individual animal and each day. After period of adaptation (21 days) the consumption of twigs was studied over 10 days. In our experiments we have examined the consumption of willow (W), oak (O) and beech (B). The control food was completed feed mixture (C). C contained 90% of concentrates and 10% of alfalfa hay. We tested the preference for each woody plant in laboratory conditions, where the experimental animals had *ad libitum* access to different woody plants. We measured precisely the length of ends of nipping twigs of each species. At the basis of these characteristics we assessed the digestibility of parts of the wood which was consumed by roe deer. In the experiments we used terminals of twigs from willow 10cm long, beech 10cm and oak 4cm long. In this material we determined the content of basic nutrients. Statistical analyses in this work were performed by means of ANOVA, SAS 2002, v.1.9.

Results On the basis of the analyses we proposed that the greater the distance from the end (terminal) of twig, the higher the proportion of less digestible part, and there is less proportion of crude fibre, fat and the rate of total energy metabolized by animals. The subsequent chemical analyses demonstrated that the average content of crude fibre in dry matter was in W 293.31 $g.kg^{-1}$, in B 410.01 $g.kg^{-1}$, in O 374.13 $g.kg^{-1}$ and in C 112.10 $g.kg^{-1}$. Average content of crude protein in dry matter was with W 133.7 $g.kg^{-1}$, with B 85.9 $g.kg^{-1}$, with O 76.4 $g.kg^{-1}$ and with C 186.45 $g.kg^{-1}$. Content of crude protein correlated negatively with the content of fibre in woody plants (Pearson's correlation coefficient, R=-0.83). We found high association between the digestibility of organic matter and the content of crude fibre in the studied woody species (R=-0.97, P=0.0003). The highest digestibility of organic matter in our experiments was detected in W (53.9 %), the lower digestibility was observed in B (30.6 %), in O (33.4 %) and in C (73.72 %). Differences in digestibility of organic matter between W *versus* B and O was highly significant (P<0.001). We found no significant differences between the digestibility of organic matter in B and O. W had significantly higher digestibility of crude protein 59.9 % and fibre 24.2 % than other species (P<0.001). In B the digestibility of crude protein was 44.6 %, of fibre 15.3 %. In O the digestibility of crude protein was 17.3 %, and the digestibility of fibre was 19.6 %. %. In C the digestibility of crude protein was 66.82 %, and the digestibility of fibre was 32.62 %. The digestibility of ash was 40.17 % in C, 23.72 % in W, 7.75 % in beech, whilst in O we have found even negative values of ash balance. Digestibility of ashes in W was significantly higher than in B and O woody plants (P<0.001). Extreme results of ash digestibility in B and O were caused by high proportion of fibre on one hand and by low content of ashes (mainly phosphorus) in dry matter of twigs, which was not able to meet the requirements of animal organism on the other hand. We proposed, that the excretion of calcium and phosphorus from the skeleton of animals occurs. This hypothesis explains the fact, that measured content of phosphorus in faeces was several times higher, than the amount of phosphorus received from the food, i.e. nutrients balance of phosphorus was negative.

Conclusions The digestibility of woody plants by roe deer is generally low because of high content of fibre. Woody plant W reaches the highest content of basic nutrients among the studied woody plants. At the same time W had also the highest digestibility of organic matter and ash. W had also the highest preference for consummation by roe deer in comparison with other woody species. W does not belong to the most economically important wood products. Therefore it is recommended to use W as supplementary woody plant to increase the nutritional capacity of biotopes for roe deer and other free living herbivores. Increased nipping of W twigs by wild ruminants reduces damage to other woody plants of higher economic significance.

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Effects of microbial inoculants on the quality and stability of bermudagrass haylage

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Introduction Ensiling is an alternative method of forage preservation to making hay that requires relatively lower dry matter concentrations in the forage for successful preservation. Tifton 85 bermudagrass is an improved tropical grass cultivar that is widely used in southern US dairy systems because it has greater NDF digestibility compared with other grasses adapted to the region (Hill *et al*., 1993). However, ensiling bermudagrass is challenging due to its low concentration of readily fermentable carbohydrates (Kunkle *et al*., 1988). Inoculants have been applied to forage to improve their fermentation by rapidly reducing the pH thereby reducing losses of dry matter or to improve aerobic stability by inhibiting the growth of spoilage-causing yeasts. Little information exists on inoculant effects on bermudagrass silage. The objective of this study was to compare the efficacy of four bacterial inoculants at improving the fermentation and aerobic stability of bermudagrass haylage.

Materials and methods Bermudagrass (4-wk regrowth) was harvested, chopped (approximately 2 cm) and treated with 1) deionized water (CON); 2) Buchneri 500 (B500) containing 1 x 10^5 of *Pediococcus pentosaceus* and 4 x 10^5 *Lactobacillus buchneri* 40788, 3) Biotal Plus II (BPII) containing 1.2 x $10^5$of *P. pentosaceus* and *Propionibacteria freudenreichii*; 4) Silage Inoculant II (SI) containing 1 x 10^5 of *L. plantarum and P. pentosaceus* and 5) SiloK (SK), containing 1 x 10^5 of *L. plantarum, Enterococcus faecium*, and *P. pentosaceus*, respectively. Four replicate round bales (441 ± 26 kg) per treatment were wrapped with 7 layers of plastic and stored for 112 d. Four additional bales per treatment were prepared and analyzed for pH after 3, 7 and 30 d of ensiling. The experiment had a completely randomized design and data were analyzed with the Mixed model procedure of SAS. Temperature was recorded every 30 min. for 14 d. Aerobic stability was denoted by the time (h) before a 2ºC rise in silage temperature above ambient temperature (23ºC).

Results The pH of Control and inoculated d 3 silages was similar (P > 0.05) but B500 had lower pH than SK by d 3, and B500 and BPII had lower pH (P < 0.001; 5.79 ± 0.07 *vs*. 6.16 ± 0.07; 5.02 ± 0.23 *vs*. 5.69 ± 0.23, respectively) than other treatments by d 7 and 30. Treatments B500, BPII, and SI had lower pH than the Control (P = 0.003; 4.76 ± 0.16 *vs*. 5.2 ± 0.16) after 112 d but SK had similar pH to other treatments. No difference (P > 0.05) was found among treatments in NDF digestibility, DM losses, DM, lactic and acetic acid concentrations, and yeast and coliform counts. Other VFA were not detected. Treatments B500, BPII, SI, and SK improved (P < 0.001) aerobic stability by 195%, 161%, 162%, and 75%, respectively compared to the Control (273 $\pm$ 36 *vs*. 110 ± 36 h). Treatment B500 and SI had lower mold counts than other treatments (P = 0.02; 2.19 $\pm$ 1.01 *vs*. 3.68 ± 1.01 cfu/g), while SK had lower clostridia counts than the Control (P = 0.02; 1.15 ± 0.43 *vs*. 2.42 ± 0.43 cfu/g).

	pH				Aerobic stability
Treatments[1]	Day 3	Day 7	Day 30	Day 112	(hours)
CON	6.37^{ab}	6.18^{a}	5.6^{a}	5.2^{a}	110.2^{c}
B500	6.23^{b}	5.73^{b}	5.03^{b}	4.67^{b}	325.0^{a}
BPII	6.28^{ab}	5.85^{b}	5.0^{b}	4.85^{b}	287.7^{a}
SI	6.38^{ab}	6.08^{a}	5.58^{a}	4.77^{b}	288.3^{a}
SK	6.44^{a}	6.21^{a}	5.89^{a}	4.92^{ab}	192.3^{b}
SEM	0.09	0.07	0.23	0.157	35.6
P value	0.036	<.0001	0.0002	0.0032	<.0001

Means with different superscripts in the same column differed, P< 0.05
[1]CON = control, no inoculant; B500 = *Pediococcus pentosaceus* and *Lactobacillus buchneri;* BPII =*P.* and *Propionibacteria freudenreichii*; SI = *L. plantarum and P. pentosaceus;* SK = *L. plantarum, Enterococcus faecium, and P. pentosaceus.*

Conclusions All treatments improved the fermentation and aerobic stability of bermudagrass haylage to varying extents.

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Comparative investigations of food comminution in herbivores – faecal particle size and its relation to body mass

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Introduction The major factor of food comminution in herbivores is grinding down the lignified plant particles by chewing. Chemical and microbial digestion are considered to be less relevant for particle size reduction (e.g. Poppi *et al*. 1980). Therefore faecal particle size admits a direct inference on food comminution by chewing. In this study, the chewing efficiency of different ruminant and non-ruminant herbivores was estimated based on equal grass hay rations. Furthermore it was examined if there is an influence of body mass (BM) on faecal particle size.

Materials and methods Faecal samples of 17 species/breeds were collected. Species (n) included: springbok (2), domestic goat (6), domestic sheep (3), blue wildebeest (5), sable antelope (3), oryx antelope (3), waterbuck (2), forest buffalo (2), Bactrian camel (4), domestic cattle (3), warthog (1), Shetland pony (3), Przewalski´s horse (4), domestic horse (6), Grévy´s zebra (3), white rhinoceros (7), African elephant (6). All animals had *ad libitum* access to grass hay (composition (g/kg dry matter): crude protein, 95.5; neutral detergent fibre, 635; acid detergent fibre, 333) for an adaption period of 14 days before sampling. Water was available all the time. Faeces were wet sieved in triplicate with sieve mesh sizes of 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 and 0.063 mm. Weighted Average (WA) [mm] was calculated according to the particle distribution on the sieves. The BM of the animals ranged from 28 kg (springbok) to 6500 kg (African elephant). Comparison of WA in ruminants and non-ruminants was done by *t*-test. Non-linear regression was used to evaluate relations between WA and BM with the general allometric formula aBM^k (a=constant, k=allometric exponent). All statistical analyses were performed with GraphPad Prism version 5.00 for Windows.

Results Ruminants had smaller faecal particles than non-ruminant herbivores (P=0.0064). The WA [mm] (species means) within the group of the non-ruminants ranged from 1.07±0.11 (Shetland pony) to 6.35±2.03 (white rhinoceros) (equids alone: 1.07±0.11 (Shetland pony) -1.48±0.43 (Przewalski´s horse)). The range for ruminants and camels was 0.25±0.02 (domestic sheep) to 0.58±0.10 (Bactrian camel). No relationship between faecal particle size and BM was found in the group of ruminants and camels ($0.54\ BM^{-0.05}$, residual standard deviation (RSD)=0.12); in non-ruminants the exponent was rather high ($0.10\ BM^{0.50}$, RSD=1.14). However if only the group of equids was examined no increase of faecal particle size with BM was evident ($1.11\ BM^{0.01}$, RSD=0.33).

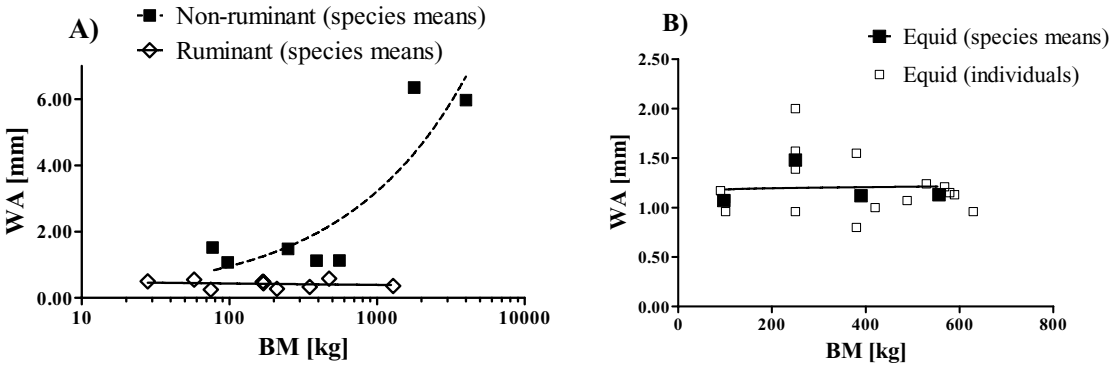

Figure 1 A): Faecal particle size and BM in ruminants and non-ruminants (BM log 10) and B): equids alone (regression line for species means).

Conclusions As a result of the faecal particle size distribution three groups can be distinguished: ruminants with the finest faecal particles followed by equids/warthog and white rhinoceros/African elephant (megaherbivores) with the largest particles. The process of particle size reduction is an interaction of several factors. For comparable diets, differences in faecal particle size reflect differences in physiology and chewing. Finer faecal particles of ruminants are caused by digestive strategy of rumination and selective particle retention in the rumen (Udén and van Soest 1982). Independent of size of the rumen, the mechanism to retain particles is the same in different ruminant species. This may explain why no indication for a change of faecal particle size with BM was found in ruminants. In the group of the non-ruminants there is an increase of faecal particle size with BM for the whole groupe of species investigated, while within a closer taxonomic unit like equids no tendency could be detected.

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The management of common pool resources, case of collective grasslands, inventory and characterization of their management in Steppe's region of Hadj Mechri, Laghouat (Algeria)

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Introduction After 1962, the juridical status of steppe lands was obtained from application of the land Act -April 22, 1863- (*Senatus consults*) which shared the steppe to the public land and - use rights are precarious and revocable - and Arch lands which were collectively owned by the tribes and were managed by a board (Djemaa) (Boukhabza, 1982). Since, collective grasslands have suffered the adverse effects of recurrent drought, a growing population and failures of various policies of CPR common pool resources management. For this purpose, taking advantage of unclear juridical status, a process of private appropriation of collective grasslands was initiated by the powerful agropastoralists to the detriment of collective management (Bédrani and Mouhous, 2008). All this environment put the collective grasslands in a transition situation which relates to rules of organization management.
Currently, what are the management methods of the grasslands? What are agropastoralists operating strategies?

Materials and methods An exhaustive survey was conducted in steppe's region of Hadj Mechri, in 2009. To identify the collective grasslands, we used a GPS device (global system position). . After this, a focus group was conducted with two or three agropastoralists. . For treatment and analysis of data, we used ACM method (analysis of correspondence multiple), in view of the qualitative and quantitative data, for a typology of collective grasslands.

Results The results show that collective grassland exist in different juridical status (table 1) and in different levels of degradation. They are managed by two operating modes, namely the collective mode and individual mode (table 2). The use of these collective grasslands is limited only to sedentary agropastoralists who are installed in the periphery. When operating mode is individual, it means that there was a tacit division of the collective grassland between the agropastoralists concerned. To affirm the appropriation of grasslands, agropastoralists uses some practices such as ploughing and renting of these lands.

Table 1 Total area of grasslands.

juridical status	Total area (ha)	%
Arch (tribe)	2 917.6	18
Crown	6 735.0	40
Municipal	7 012.8	42
Total	16 665.4	100

All respondents say that access to the grasslands is not free. It is limited only to residents who use this Grassland. Reasons are cultural and traditional values;
Rih Bladi: By tacit agreement between agropastoralists, each one use part of the grassland that makes the continuation of the space reserved for the house
Horma: Distances between houses must be remote in order to not recognize a woman when she is outside the house

Table 2 The juridical status and operating mode of management (%)

juridical status	Global recovery of vegetation %	Mode of grasslands management			
		Collective	Individual	Coll/ind	total
Arch (tribe)	Rather bad	0	11	0	11
	Rather medium	5	0	0	5
	Rather good	11	5	0	16
Crown	Rather bad	0	5	5	11
	Rather medium	5	11	0	16
	Rather good	0	5	0	5
Municipal	Rather bad	0	0	0	0
	Rather medium	16	11	0	26
	Rather good	11	0	0	11
Total		48	48	5	100

Conclusion The collective grasslands management seems to take new forms that are linked not only to the conditions of sedentary populations and population's growth, but also the unclear status of grasslands. When using some social values (*Horma* and *Rih Bladi*), agropastoralists puts in place new rules of collective grasslands management. This excludes any agropastoralist that has no direct access to the grassland. However, collective management of grasslands is still operating rules more stringent. The implementation of these rules is influenced by the importance of parental relationships that exist between the agropastoralists. When family ties are strong, grasslands management become collective with a few rules to respect. When family ties are weak, collective management tends to individual mode by the application of rules and social organizations already mentioned.

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Performance of beef suckler cows and growth of their calves where grass is grazed to contrasting residual sward heights

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Introduction Feed is the largest variable cost on beef farms and efficiently managed grazed grass is the cheapest feedstuff available to farmers in Ireland (Finneran *et al*., 2009). Accordingly, maximising animal performance from grazed grass should be the basis of sustainable beef systems. There is conflicting evidence that grazing to a lower residual sward height (4.1 *vs*. 5.9cm, Lee *et al.,* 2008; Humphreys, 2009; 3.4 *vs*. 4.5 cm, Ganche *et al*., 2011) improves performance of lactating dairy cows. Most research examining post-grazing sward height (PGSH) in beef cattle is confounded with stocking rate. Consequently, there is a deficit in information on this grazing practice for beef production systems. The objective of this experiment was to evaluate the effects of two PGSH on performance of beef suckler cows and their calves during the grazing season.

Materials and methods A total of 112 spring-calving (16 March; s.d. 26.6 days (d)) primiparous, lactating beef suckler cows were allocated at random within breed type to one of two grazing systems: a PGSH of either 4.0 or 5.5 cm. There were two replications of each grazing system resulting in four groups of 28 cows + their calves. The stocking rate was 2.9 LU/ha equivalent to ~220 kg of organic nitrogen/ha for each grazing system. The experiment was undertaken from early May to mid-October 2010 during which, cows and their calves were rotationally grazed on predominantly perennial ryegrass swards. Fresh herbage was allocated to each system once the target PGSH was achieved. Herbage, surplus to grazing requirements (i.e. when farm cover exceeded requirements), was removed from the rotation by harvesting relevant paddocks for silage. At the end of the grazing season (early November), animals were housed indoors. Cows and calves were offered grass silage *ad libitum* and additionally, calves received supplementary concentrates. Cow and calf live weight, and cow body condition score (BCS, 0-5) and milk yield (weigh-suckle-weigh technique, day 120 of lactation) was measured. To permit adjustments in gut fill, a final live weight 21 days post-housing was used. Grassland measurements included, pre and post-grazing compressed sward heights (rising plate meter) and herbage mass (>4 cm - lawnmower). Animal variables were statistically analysed using PROC MIXED in SAS. The model contained effects for grazing system and dam breed type, and data pertaining to progeny had additional terms for gender and sire. Cow age and calving day were included as covariates in all models.

Results Pre-grazing sward height and herbage mass did not differ (P>0.05) between grazing systems with mean values of 10.1 cm and 1845 kg DM/ha, respectively. By design, PGSH (4.1 *vs*. 5.3 cm) and herbage mass (475 *vs*. 720 kg DM/ha) was lower (P<0.001) for the 4 cm than the 5.5 cm PGSH system. Cow live weight gain at pasture was lower (P<0.01) for the 4 cm than the 5.5 cm PGSH, but live weight gain from the start of the experiment to post-housing did not differ (P>0.05) between systems (Table 1). Cow BCS gain tended to be lower to post-housing and calf live weight gain was lower (P<0.05) both at pasture and to post-housing for the 4 cm than the 5.5 cm PGSH. Milk yield did not differ (P>0.05) between systems.

Table 1 Performance of beef suckler cows and calves grazing to two residual sward heights

		Post-grazing sward height			
		4.0 cm	5.5 cm	SED	Significance
Cow: Initial live weight (kg): May		560	561	9.83	NS
Live weight change (kg)	May to October	63	92	5.0	P<0.01
	May to post-housing	95	95	5.9	NS
Initial body condition score (BCS: 0-5)		2.99	2.97	0.036	NS
BCS change	May to October	0.11	0.16	0.036	NS
	May to post-housing	0.14	0.21	0.039	P=0.05
Milk yield (kg /day)		6.7	7.3	0.46	NS
Calf: Initial live weight: May (kg)		95	96	2.61	NS
Live weight change (kg)	May to October	169	178	3.9	P<0.05
	May to post-housing	202	212	4.7	P<0.05

Conclusions Grazing to a lower residual sward height (4.1 cm) had negative effects on performance of beef suckler calves.

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In vivo effect of plant species on gastrointestinal nematodes of sheep

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Introduction Anthelminthics have been used as an effective control for nematode parasites in sheep for decades. However, parasites are developing resistance in number of countries (Schnyder *et al*., 2005; Cringoli *et al*., 2007). In addition, high costs and limited availability of anthelminthics (Akhtar and Malik, 2000), gives a clear idea that control strategies based exclusively on their use are not sustainable. Currently, screening of medicinal plant alternatives is of increasing interest. The aim of this study was to evaluate the effect of ethanolic extracts from five medicinal plants *Ananas comosus, Aloe ferox, Allium sativum, Lespedeza cuneata* and *Warburgia salutaris* on egg production and L3 larvae of nematodes in sheep.

Materials and methods This study was conducted using 48 sheep with initial live weight 33.7 ± 11kg. Animals were sorted by sex, initial eggs per gram of faeces and initial weight to six groups of eight sheep each. Each group was then randomly assigned to six treatments; combination of Abamectin and Praziquantel (CPA) (positive control), ethanol extract of *Ananas comosus* (AC group)*, Aloe ferox* (AF group)*, Allium sativum* (AS group)*, Lespedeza cuneata* (LC group) and *Warburgia salutaris* (WS group)*.* CPA was drenched using hypodermic syringes (2.5ml $10kg^{-1}$) and the plant extractions were drenched on a weekly base for 6 weeks using stomach tube. CPA treatment was repeated on day 35 when one sheep died of haemonchosis symptoms. Experimental animals were allowed to graze freely on planted contaminated Kikuyu pasture (*Pennisetum clandestinum*) with other animals. Larval contamination of pasture was determined in four paddocks. The larval infestation ranged from 315 to 677 L_3 larvae kg^{-1} of dry herbage with a mean of 425 ± 174 L_3 larvae kg^{-1} of herbage. No feed supplement was given to the experimental animals. Faecal samples were taken on days 0, 7, 15, 21, 28, 35 and 42 post-treatment for nematode egg counts using McMaster Technique (Hansen and Perry, 1994). Faeces were pooled and mixed per group, sub-sampled, and incubated for 12 days at 27 °C. Baermann technique was used to count infective larvae (Hansen and Perry, 1994) under 100 magnification. Data were analyzed using the General Liner Model (GLM) procedure of SAS (2000), following the model: $Y_{ijkl} = \mu + W_i + T_j + (W*T)_{ij} + G_k + L_l + e_{ijkl;}$ Where: Y_{ijkl} = individual weekly observation; μ = overall mean; W_i = weekly effect; T_j = effect of treatment; $(W*T)_{ij}$ = interaction between week and treatment; G_k = co-variate effect of initial egg count, L_l = co-variate effect of initial live weight and e_{ijkl} = error of mean.

Results Following administration of treatments, egg per gram of faeces (EPG) for the control (CPA group), dropped to a trough 7 days after administering the medicine. Beyond day 7 EPG recovered rapidly for CPA until day 35, then start dropping after giving another dose of CPA, thus lower EPG was observed on day 42 (Figure 1). EPG consistently decreased with time for the rest of the plant extracts. Analysis of the area under the curve confirms that LC and AC groups constantly had the lowest egg production. Figure 2 shows total number of larvae recovered from faecal culture. Proportions of total larvae were relatively similar to the pattern of EPG. The effect of treatments was significant ($P<0.001$) on day 35.

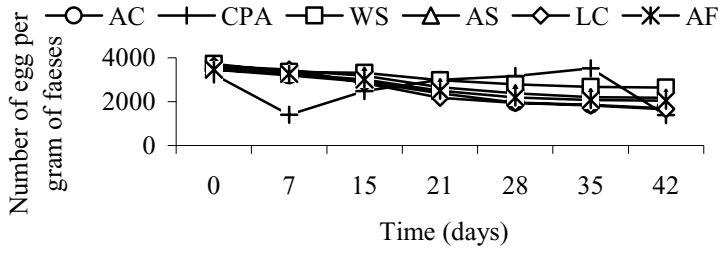

Figure 1 The effects of five plant species on gastrointestinal nematodes egg count

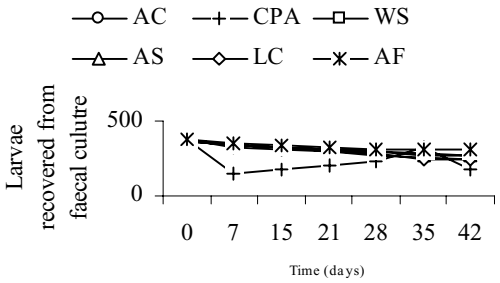

Figure 2 The effects of five plant species on nematodes larvae count

Conclusion Ethanol extract of *Ananas comosus, Aloe ferox, Allium sativum, Lespedeza cuneata* and *Warburgia salutaris* reduced nematode egg production and infective larval yields of sheep. Continuous treatment or increase dosage with these plants could further reduce nematode parasites and increase host immunity.

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Consequences of feeding management on body condition and reproductive performance in primiparous Charolais cows

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Introduction Beef cattle livestock systems mainly rely on forage resources. Due to environmental constraints, forage stocks may not be sufficient for the whole winter period and cattle might have to undergo feed restriction. Body condition at critical times and its variation around the year may affect female reproductive performances (Richards *et al*. 1986), and thus farm economic viability. The aim of our study was to determine the influence of two postpartum feeding strategies on body condition and reproductive performance of primiparous Charolais cows. Reproductive efficiency was evaluated by both physiological and behavioural criteria.

Material and methods Two experiments (Trial 1: n=14 and Trial 2: n=16) were successively carried out using primiparous Charolais cows. In each trial two energy level diets (High: 125% energy requirements (H1: trial 1; H2: trial 2) *vs*. Low: 65% energy requirements (L1: trial 1; L2: trial 2) were applied from calving to turn out. Diets were formulated with pasture hay (90% in H and 95% in L diets) and concentrate (10% in H and 5% in L diets). Cows were reared in groups (n=7 or 8) in a loose-housing system from mid-November to mid-May. Weight and body condition score (BCS, scale 0-5) were respectively measured once a week and once a month (trial 1) or twice a week and twice a month (trial 2). Measures of adipose cell diameter at calving and turn out were used to assess change in body composition during the postpartum period (Robelin *et al*. 1981). Cyclicity was studied through plasmatic progesterone profiles (two samples per week). Ultrasonographic examination was performed at 195±16 d (trial 1) and 198±10 d (trial 2) after calving to determine cows' pregnancy status. Oestrus expression was analyzed from continuous video camera records that were studied using The Observer® software. Effect of feeding management on body condition change, oestrus behaviour and cyclicity was analyzed using ANOVA (SAS software).

Results BCS at calving was 2.4±0.1 in trial 1 and 2.0±0.2 in trial 2. Postpartum BCS changes were -0.2±0.2 (L1) *vs*. 0.2±0.2 (H1) and -0.4±0.2 (L2) *vs*. 0.1±0.2 (H2). In the L groups, despite underfeeding and lipid mobilization, growth was not totally inhibited as protein depots were observed (Figure 1). Within each experiment, BCS change and lipid variation during postpartum period, were significantly different between L and H groups (Figure 1). Protein depots significantly differed between L and H cows in trial 2 but not in trial 1. Postpartum underfeeding did not affect calving to first oestrus interval (76±22d (L1) *vs*. 75±13d (H1) and 100±13d (L2) *vs*. 97±12d (H2)). Pregnancy rates were lower in L cows in trial 1 (43±53% (L1) *vs*. 86±38% (H1), P<0.05) but not in trial 2 (88±35% in both groups). In both trials, standing to be mounted represented between 3% and 5% of all sexual behaviours expressed during oestrus and was not significantly different between L and H groups. Oestrus intensity (sum of all sexual behaviors expressed during oestrus) was higher in L group in trial 2 (317±194 (L2) *vs*. 159±108 (H2), P<0.05) but not in trial 1 (287±175 (L1) *vs*. 204±97 (H1) (Figure 2)). In both trials, oestrus duration (time between first and last standing to be mounted) was not influenced by postpartum energy level (8±6h (L1) *vs*. 4±4h (H1) and 9±4h (L2) *vs*. 5±7h (H2)).

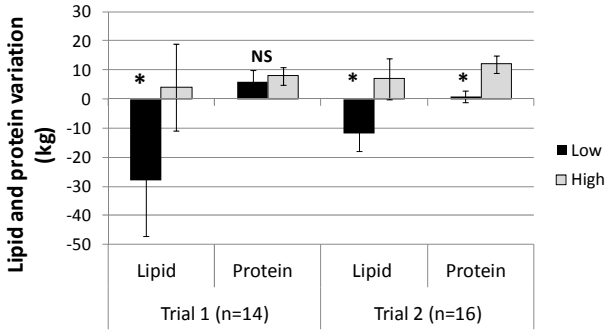

Figure 1 Lipid and protein variation from calving to turn out. *Treatment effect P<0.05, NS : Non Significant

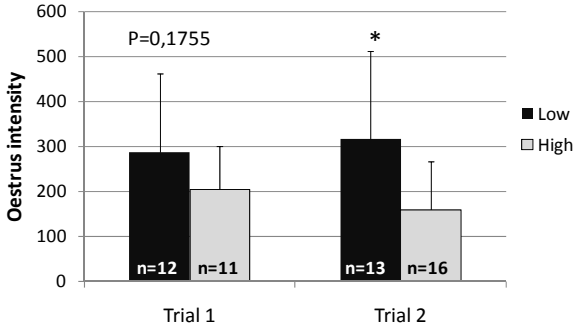

Figure 2 Oestrus intensity. *Treatment effect P<0.05

Conclusions In the present study postpartum nutritional level did not affect calving to first oestrus interval as shown by Richards *et al*. (1986). Data from trial 2 were in agreement with Richards *et al*. (1986) showing that in cows losing body condition after calving, pregnancy rate may not be significantly depressed if calving condition is good to moderate. The significant decrease in pregnancy rate of L cows in trial 1 might be explained by the higher level of lipid mobilization observed from calving to turn out. In agreement with results of Ciccioli *et al*. (2003), oestrus duration was not affected by postpartum nutritional level in trials 1 and 2. Feed restriction as tested in this study did not totally affect growth and reproductive performance when lipid mobilization remained moderate. Our results showed the ability of well-developed primiparous cows to undergo periods of moderate feed restriction.

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Effect of vitamin E and Selenium supplementation on the oestrus responses and the diameter of dominant follicles in boer crosses

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Introduction Vitamin E is an antioxidant has a biological function related to Selenium (Se). Studies on using vitamin E and Se have been conducted to evaluate its effect on meat quality of sheep (Juniper *et al*. 2008) and lambs (Yu, 2008); milk production and during late gestation in cattle (Moeini *et al*. 2008). This trace mineral and vitamin E also are able to act directly on the reproductive organs (Ganabadi *et al*. 2010; Koyuncu & Yerlikaya, 2007). In male goats, Se supplementation increased the percentages of spermatids and decreased the percentages of spermatozoa in the seminiferous tubules (Ganabadi *et al*. 2010). An increase in the incidence of oestrus, fertility and prolificacy were reported in ewes that received vitamin E together with Se (Koyuncu & Yerlikaya, 2007). There is a lack of information in goats on the effect of vitamin E and Se on the female reproductive system. The objectives of this study were to evaluate the oestrus response and size of dominant follicles in Boer cross goats fed diets supplemented vitamin E and Se.

Materials and methods Thirty-nine mature female Boer Crosses with bodyweight ranges between 20 to 25 kg were used. All does were kept in a group at Field Two farm, Universiti Putra Malaysia. The does were fed on basal diet (80% concentrate consisting 15% corn grain milled, 60% palm kernel cake, 23.5% rice brain, 1% limestone, 0.5% salt : 20% grass; free access to salt lick and water) during 14 days of adaptation period. The does were randomly divided into four treatment groups and received either i) 0.0IU vitamin E and 0.0mg/kg DM Se (VitE0-Se0 or Control; n=10); ii) 3.0IU vitamin E and 0.0mg/kg DM Se (VitE3-Se0; n=10); iii) 0.0IU vitamin E and 2.0mg/kg DM Se (VitE0-Se2; n=9); or iv) 3.0IU vitamin E and 2.0mg/kg DM Se (VitE3-Se2; n=10). The does were fed on the dietary treatments for 3 months prior to administration of $PGF_{2\alpha}$ (Cloprostenol, 125µg synthetic analogue of $PGF_{2\alpha}$) intramuscularly twice and given at 10 days apart. A fertile buck was introduced to the does twice daily (morning and evening), 12 hours after the second injection of $PGF_{2\alpha}$ for an hour to detect does with the onset of oestrus. The time of onset of oestrus was defined as the time elapsed from the second injection of $PGF_{2\alpha}$ to the first sign of oestrus; and the duration of oestrus was defined as the time elapsed from the first sign of oestrus until the last shown sign of oestrus. Ovaries were examined once daily for 5 days using an ultrasound machine (Mindray DP6900-VET, China) equipped with a multi-frequency transrectal linear transducer (7.5MHz) after the second injection of $PGF_{2\alpha}$. The diameter and the number of large follicles and dominant follicles (≥ 5mm) were measured, counted and recorded. Due to failure to locate the ovaries, the diameters of dominant follicles from nine does were excluded from the analysis. Data on the oestrus responses, number of large follicles and the diameter of dominant follicles were analysed using GLM procedure (SPSS, Version 17.0; Chicago, IL).

Results There was no significant different ($P>0.05$) between treatment groups on the time of onset of oestrus and the duration of oestrus. The mean time of onset of oestrus for VitE0-Se0, VitE3-Se0, VitE0-Se2 and VitE3-Se2 were 35.2±8.28, 26.7±1.33, 25.0±1.00 and 25.6±1.67 hours, respectively. The mean duration of oestrus for VitE0-Se0, VitE3-Se0, VitE0-Se2 and VitE3-Se2 were 79.0±1.00, 77.0±1.46, 74.9±5.14 and 78.4±1.06 hours, respectively. The percentage does that showed oestrus responses was 100% in VitE0-Se2 and VitE3-Se2 groups; 80% in VitE0-Se0 and 88.9% in VitE0-Se2. There were no significant differences ($P>0.05$) in the diameter of dominant follicles between treatment groups ($P>0.05$; 5.8±0.29, 6.6±0.42, 6.8±0.57 and 6.2±0.26, for VitE0-Se0, VitE3-Se0, VitE0-Se2 and VitE3-Se2, respectively). However, there were a significantly higher ($P<0.05$) number of larger follicles (>5mm) in VitE0-Se2 (1.8±0.37) compared to VitE0-Se0 (1.0±0.00), VitE3-Se0 (1.6±0.29) and VitE3-Se2 (1.6±0.20). This result was supported by that of Cerri *et al*. (2009), where supplementation of selenium at 0.3mg/kg DM did not improve fertilization and early embryo development in heifers.

Conclusion Vitamin E and Se supplementation shows no effect on the oestrus responses and the diameter of dominant follicles of Boer crosses. However, there was an increase in the number of large follicles (≥ 5mm) in Boer cross goats fed on a diet supplemented with 0.0IU vitamin E and 2.0mg/kg DM Se**.**

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Different forage types and their influence on digestive variables in goats

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Introduction In some situations, herbivores can choose between different types of forages like grass and browse. For wild herbivores, gastrointestinal morphology has been shown to change corresponding to forage p**References**, e.g. a uniform papillation of the rumen in browsers compared to an uneven distribution with little ventral and dorsal papillation in grazers (Hofmann 1989). While there is also good evidence for adaptive differences in aspects of digestive physiology of feeding types, it is much less clear how forage types may change such aspects directly. It was the intention of this study to quantify the effect of different forages (grass, lucerne, browse) when they are fed to an intermediate feeding type (goats).

Material and methods Eight German White-Improved castrated male goats (body mass (BM): 71 ± 8 kg) had *ad libitum* access to either a first cut grass hay (grass 1), a second cut grass hay (grass 2), a lucerne hay or a browse hay (2:2:1 mixture of ash, aspen and chestnut) in a 4 x 4 Latin Square arrangement (2 animals/cell). All forages were at the lower end of quality in terms of nutrient content. A transition period of 7 days was followed by an adaptation period of 14 days and an 8 day trial period (quantification of intake, orts and faecal output). Retention time markers (Cr-mordanted fibre 1-2 mm – small particle fraction; Co-EDTA – fluid fraction) were given in pulse dose and mean retention time (MRT) was calculated from faecal excretion. Dry matter gut fill was estimated according to Hollemann and White (1989). Faecal particle size was quantified via wet sieving (9 sieves, mesh sizes 16 - 0.063 mm), and mean particle size (MPS) calculated as in Kovács *et al*. (1997). Statistical analysis was done via ANOVA (forage and period as fixed and animal as random effects); Tukey-Kramer adjustment was used for post-hoc comparisons. Analyses were performed in SAS.

Results The nutrient composition (Table 1) confirmed the poor quality of all the forages used. Intake was acceptable (Table 2). It was higher in browse and lucerne compared to grasses, and organic matter (OM) digestibility was highest in lucerne (Table 2). Digestible OM intake (DOMI) was highest in lucerne, and higher in browse than in grasses. No difference was found in $MRT_{particle}$ and MRT_{fluid}. Gut fill was higher in browse than in the other forages, while no difference was found in MPS. However, during sieving obvious differences in average particle shape could be detected, with more longish particles in the grasses, more polyedric particles in browse and intermediate shapes in lucerne.

Table 1 Nutrient composition of forages (Mean ± SD)

	Grass 1	Grass 2	Lucerne	Browse
Crude protein, g/kg DM	62.5 ± 7.1	71.8 ± 5.7	145 ± 14.0	123 ± 16.8
Neutral detergent fibre, g/kg DM	708 ± 17.5	616 ± 33.3	549 ± 34.8	443 ± 11.3
Acid detergent lignin, g/kg DM	83 ± 1.1	67 ± 4.1	105 ± 10.1	157 ± 26.1

Table 2 Digestive variables for the different forages (LS means) and results of ANOVA (significance of factors)

	Grass 1	Grass 2	Lucerne	Browse	SEM	Forage	Period
DM intake, g/kg $BM^{0.75}$	60^{a}	62^{a}	86^{b}	80^{b}	2.9	< 0.0001	0.0039
OM digestibility, %	49^{a}	51^{a}	60^{b}	49^{a}	0.8	< 0.0001	0.4549
DOMI, g/kg $BM^{0.75}$	28^{a}	30^{a}	48^{c}	36^{b}	1.5	< 0.0001	0.0031
$MRT_{particle}$, h	52	53	42	49	3.2	0.0613	0.9499
MRT_{fluid}, h	40	35	34	35	2.1	0.1647	0.7968
DM gut fill, g/kg $BM^{0.75}$	79^{a}	79^{a}	74^{a}	91^{b}	3.4	0.0017	0.0438
MPS, mm	1.21	1.19	1.36	1.36	0.12	0.5874	0.1260

Values with different superscripts are significantly different

Conclusions Among the most remarkable results of this study was the considerably higher intake of browse compared to grasses, despite comparable OM digestibility and gut MRT. The correspondingly estimated higher DM gut fill during browse feeding is likely to be linked to differences in physical intake limitation (e.g. via rumen fill); the strikingly different particle shapes can be interpreted as facilitating a higher DM gut fill in animals feeding on browse, as postulated by Troelsen and Campbell (1968) for lucerne compared to grass.

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Influence of source of protein in supplements, urea or soybean meal, and level of supplement intake on rumen kinetics in cattle fed low quality hay

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Introduction Dry matter intake by cattle fed low-quality forages can be restricted as result of low passage rate of solids, high ruminal retention time, low digestion rate, and low ruminal disappearance rate and recycling (Pereira *et al*., 2002). The use of protein supplements can increase nitrogen supply for microorganisms and improve the digestion rate and nutrient use. Thus, the objective was to evaluate the effect of protein supplements with two sources of nitrogen at two levels of intake on parameters of rumen kinetics.

Materials and methods We used five Nellore steers of 366 kg ± 55 kg body weight and ruminally cannulated. The animals were allocated to 5 experimental diets, based on chopped signalgrass hay (*Brachiaria humidicola* cv. Llanero) and receiving protein supplements with two protein sources and two levels of intake, in a Latin square 5 x 5. Treatments were: hay without supplementation (HWS); hay + 1 g of urea supplement/kg BW (1U); hay + 2 g of urea supplement/kg BW (2U); hay + 1 g of soybean meal supplement/kg BW (1SM) and hay + 2 g of soybean meal supplement/kg BW (2SM). The animals were weighed at the beginning of each period, in order to adjust the amount of supplements to be fed to steers. The kinetics of liquid phase were determined by ruminal infusion of 30 g of Co-EDTA diluted in 500 mL of distilled water. The solution of Co-EDTA was infused before the first meal as a pulse dose to determine the passage rate for liquids (Udén *et al*. 1980). The liquid phase parameters were calculated according to Colucci *et al*. (1990). To determine the kinetics of the solid phase the technique of rumen evacuation described by Rinne *et al*. (1997) and Khalili and Huhtanen (2002) was used. The data were analyzed by ANOVA and by orthogonal contrasts, as following: 1 = HWS x 1U, 2U, 1SM, 2SM, 2 = 1U, 2U x 1SM, 2SM; 3 = 1U X 2U; 4 = 1SM x 2SM.

Results Protein supplements increased (P <0.05) the passage rate of liquid phase (Kpl) and probably increased microbial turnover, improving the digestion rate and consequently the passage rate of the solid phase (Kps). Supplementation decreased the rate of recycling (RR) in the rumen probably because there had been a significant increase in the digestion rate (Kd). Supplemented animals also had a higher (P <0.05) flow rate of liquids (FlowR) and higher ruminal disappearance rate (Kt), influenced by the higher Kd, when compared to non-supplemented animals. Despite the increase in passage rate of the solid phase (Kps) in the case of supplemented animals, it is still considered low, but consistent with the type of diet offered to the animals (low-quality roughage). Animals fed supplements containing urea had a higher (P <0.05) passage rate of the liquid phase (Kpl) than those supplemented with soybean meal.

Table 1 Parameters of ruminal kinetics

	Treatments					CV(%)	Contrasts			
	HWS	1U	2U	1SM	2SM		1	2	3	4
Kpl						26.49	*	*	NS	NS
RV	3	9	3	4	5	23.87	NS	NS	NS	NS
RetT	2			2	8	28.53	*	NS	NS	NS
FlowR						18.02	*	NS	NS	NS
RR						32.44	NS	NS	NS	NS
Kps						22.95	*	NS	NS	NS
Kt						22.39	*	NS	NS	NS
Kd						44.69	*	NS	NS	NS

Kpl = Passage rate of liquid phase (/h); RV = Ruminal volume (l); RetT = Retention time (h); FlowR = Flow rate of liquid (l/h); RR = Recycling rate (times/d); Kps = Passage rate of solid phase (/h); Kt = Disappearance rate (/h); Kd = Digestion rate (/h). * P<0.05.

Conclusions Protein supplements improved the parameters of rumen kinetics in cattle fed low quality hay. Parameters of rumen kinetics were not influenced by source of protein in supplements, urea or soybean, and level of supplement intake, 1 or 2 g/kg of BW.

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The effect of supplementing highly wilted grass silage with water soluble carbohydrates and starch rich feeds on degradation of the diets and efficiency of microbial protein synthesis in the rumen of sheep

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Introduction Lack of fermentable energy and asynchronous release of protein and energy in the rumen is characteristic of grass silage (GS)-based diets. Consequently, efficiency of microbial protein synthesis (EMPS) in the rumen is lower than it could be. It has been suggested that synchrony of energy and protein release in the rumen and EMPS may be improved by the supplementation of GS with maize silage (MS), fodder beet (FB) or molasses (M). FB and M are both characterised by high content of water soluble carbohydrates (WSC) while MS contains high proportion of starch.

Materials and methods The *in vivo* experiment was carried out with four nine months old wethers (local breed Jezersko-Solčavska). Diets were composed of highly wilted grass silage (GS, 100 %), a mixture of grass silage and maize silage (GS-MS, 60:40), a mixture of grass silage and fodder beet (GS-FB, 83:17) and a mixture of grass silage and molasses (GS-M, 86:14) on dry matter (DM) basis. Diets were made isonitrogenous by the supplementation of urea. Animals were assigned to a balanced Latin square design (four diets × four animals × four periods). Urine and faeces were collected using the total collection method to estimate whole body nitrogen balance by difference from nitrogen intake. Microbial protein synthesis was assessed on the basis of urinary purine derivative excretion (allantoin, uric acid, xanthine and hypoxanthine) proposed by Chen *et al*. (1990). *In sacco* degradabilities of experimental feeds were determined by the use of the nylon bag technique (Ørskov and McDonald, 1979) using three wethers fitted with a ruminal cannulae. Bags with fresh forage samples were introduced into the rumen of each sheep in quadruplicates for 3, 6, 12, 18, 24, 48 and 72 h. For molasses and urea which were not incubated in the rumen it was assumed that crude protein (CP) and organic matter (OM) were immediately soluble and completely degradable in the rumen. Effective degradability was calculated using a two compartment model of Hvelplund and Weisbjerg (2000).

Results GS was characterised by high DM and WSC content (539 g kg^{-1} and 86 g kg^{-1} DM). In comparison to GS diet the concentration of WSC in GS-MS diet was lower (58 g kg^{-1} DM) while in GS-FB and GS-M diets it was much higher (162 and 171 g kg^{-1} DM). The estimated EMPS varied from 34.0 g microbial nitrogen (MN) kg^{-1} organic matter apparently digested in the rumen (OMADRI) in GS-M to 36.6 in GS diet. Supplementation of GS with MS, FB and M showed no significant effect on the EMPS. The highest nitrogen retention was obtained in the diet of GS-MS but there were no significant differences between diets. Supplementing GS with MS, FB and M significantly ($P < 0.05$) increased the effective degradability of OM and CP (EDG_{OM} and EDG_{CP}). Synchrony of protein and organic matter release in the rumen, which is described by a synchrony index (I_S), was increased only in the case of M supplementation. The absence of an effect of MS, FB and M supplementation on I_S was due to the supplementation of GS-MS, GS-FB and GS-M diets with urea.

Table 1 Dry matter intake (DMI), microbial protein synthesis estimated on the basis of urinary purine derivative excretion (in grams microbial nitrogen), nitrogen retention, effective degradability of organic matter (EDG_{OM}) and crude protein (EDD_{CP}) and synchrony index (I_S) of experimental diets

Parameter	GS	GS-MS	GS-FB	GS-M	SEE	Significance
DMI (g per day)	1031	1040	1048	1033	12.7	NS
Microbial nitrogen (in g per)						
kg DMI	15.8	16.1	15.7	15.3	0.83	NS
kg OMADRI	36.6	35.1	34.7	34.0	1.99	NS
N retention (in g per kg N intake)	107	116	100	98	26	NS
EDG_{OM} (g per kg)	563^{a}	577^{b}	593^{c}	618^{d}	2.31	$P < 0.05$
EDG_{CP} (g per kg)	756^{a}	800^{c}	778^{b}	814^{d}	1.99	$P < 0.05$
I_S	0.75^{b}	0.67^{a}	0.67^{a}	0.79^{c}	0.014	$P < 0.05$

OMADRI, intake of organic matter apparently digested in the rumen;

Conclusion It was concluded that highly wilted GS containing a high concentration of WSC supports high EMPS in the rumen and that it was not improved by the supplementation with starch or WSC rich feeds.

References

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