Rumen fermentation parameters in cows receiving polyclonal antibodies and adapted or not to highly fermentable carbohydrates diets after an acidosis challenge

E Cassiano[1], T Barros[1], C Marino[1], R Pacheco[2], F Ferreira[1], F Perna Jr[1], M Martins[1], P Meyer[3], M Arrigoni[2], P Rodrigues[1]
[1]University of Sao Paulo, Pirassununga, Sao Paulo, Brazil, [2]University of Sao Paulo State, Botucatu, Sao Paulo, Brazil, [3]Brazilian Institute of Geography and Statistics, Pirassununga, Sao Paulo, Brazil
Email: paulamarquesmeyer@yahoo.com.br

Introduction Scientific community is continuously searching new feed additives that could prevent digestive disturbances in ruminants as well as are safe to humans who will consume the animal products. The concept of immunity as a potential tool in the manipulation of ruminal fermentation has been previously cited (Berghman and Waghela, 2004). In this scenery, the objective of this trial was to evaluate the effects of two forms (liquid or powder) of polyclonal antibodies preparation (PAP) against specific rumen bacteria *Streptococcus bovis*, *Fusobacterium necrophorum* and *Lactobacillus* on rumen fermentation parameters in ruminally cannulated cows adapted or not to highly fermentable carbohydrates diets (HFC) after an acidosis challenge.

Materials and methods The present study was carried out at College of Veterinary Medicine and Animal Science (FMVZ/USP), Brazil. Six ruminally fistulated cows were used in this trial. Experimental design was two Latin squares 3x3 in factorial arrangement of treatments 3x2 regarding two feed additives (PAP in powder form (PAPP) and PAP in liquid form (PAPL)) plus control group (CON) and two managements of diets adaptation resulting in six treatments. The first Latin square had a step-up diet adaptation: from D0 to D4 (100% of forage); D5 to D9 (30% of concentrates) and D10 to D14 (60% of concentrates). The second Latin square received 100% of forage from D0 to D14. On D15 and D16, all animals received 80% of concentrates in diet. For pH and total concentration of short-chain fatty acids (tSCFA) analysis, rumen fluid was sampled at 0 and every 3 h postfeeding totaling 36 h (D15 and D16) of challenge with a diet of 80% of concentrates. Short-chain fatty acids included acetate, propionate and butyrate. Data were analyzed by MIXED procedure 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 adaptation and time was observed for ruminal pH ($P < 0.0001$). The adapted group had lower pH values than non-adapted group until 12 h after the start of challenge (6.18 *vs* 6.55, respectively). For tSCFA concentration, an interaction between adaptation and time was also observed ($P < 0.0001$). From 0 to 9 h (109.38 *vs* 82.26 m*M*) and at 36 h (121.11 *vs* 107.62 m*M*) after the start of challenge, the adapted group had greater values compared to non-adapted group. At 24h and 27h, the non-adapted group had greater values compared to the adapted group (114.75 *vs* 127.4 m*M*). Polyclonal antibodies preparation, in both forms, did not affect these variables.

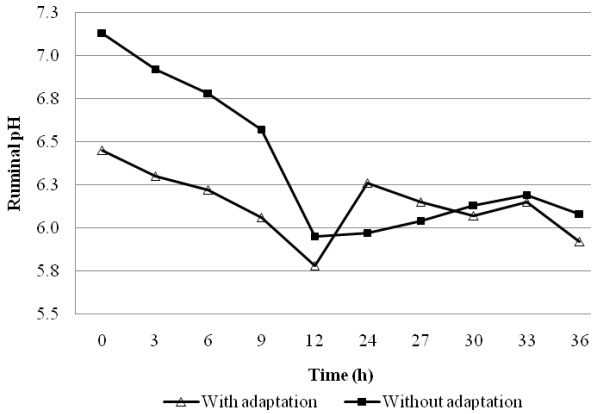

Figure 1 Ruminal pH for factors composed by different diet adaptation

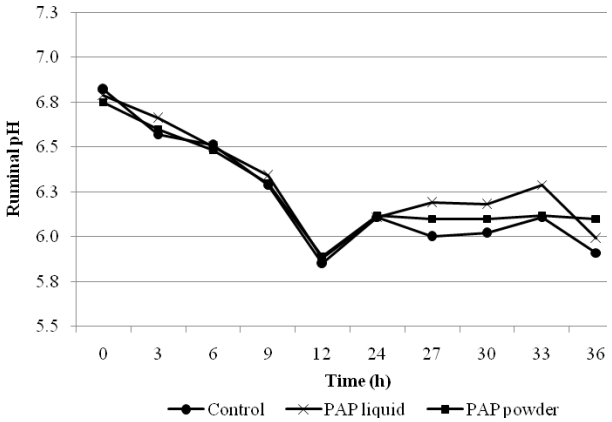

Figure 2 Ruminal pH for factors composed by different feed additives

Conclusions From these data, it is possible to conclude that step-up adaptation did not prevent the drop of rumen pH in conditions of great availability of highly fermentable carbohydrates. High total SCFA concentration is expected in adapted group due to the adaptation of rumen microbial population to the substrate, increasing their fermentative capacity. Both forms of PAP (liquid or powder) did not affect any of the variables studied.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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The impact of selection for leanness on lamb carcase composition, intramuscular fat, and muscle metabolic type, is not influenced by nutritional variation within Australian production systems.

G E Gardner[1], L Pannier[1], F Anderson[1], K R Kelman[1], A Williams[1], R H Jacob[2], A J Ball[3], D W Pethick[1]
[1]Murdoch University, Murdoch, Australia, [2]Department of Agriculture and Food WA, South Perth, Australia, [3]Meat & Livestock Australia, University of New England, Armidale, Australia
Email: g.gardner@murdoch.edu.au

Introduction Due to the nutritional importance of iron and zinc in human diets, marketing campaigns for lamb and beef are often focused on these minerals. Iron and zinc are associated with muscle aerobicity which may be diminishing in lamb meat due to selection practices targeting leanness and muscularity to increase lean meat yield (Pannier *et al.* 2010). Aerobicity of muscle has also been linked to intramuscular fat (IMF) percentage, and like-wise IMF is also depressed through selection for leanness (Gardner *et al.* 2010). Poor nutrition will also reduce carcase fatness and IMF, potentially limiting the scope for other genetic factors to impact. Therefore it seems plausible that the impact of selection for leanness will be less in a poor nutrition environment. Thus we hypothesised that selection for leanness would reduce carcase fatness and IMF, reduce aerobicity, and therefore reduce iron and zinc concentration, but these impacts will be depressed within flocks maintained on sites with poorer nutrition.

Materials and methods The Australian Cooperative Research Centre for Sheep Industry Innovation has designed an Information Nucleus Flock using Merino and crossbred ewes located at 8 research sites across Australia (Katanning WA, Cowra NSW, Trangie NSW, Kirby NSW, Struan SA, Turretfield SA, Hamilton VIC, and Rutherglen VIC) which represent a broad cross section of Australian production systems. In 2007 these ewes were artificially inseminated with semen from 93 sires representing major production types in the Australian sheep industry, with this process repeated using different sires in 2008. About 2000 lambs were slaughtered each year at a target average carcass weight of 22kg. Samples were taken from the *longissimus thoracis lumborum* muscle at 1hour post-mortem for isocitrate dehydrogenase (ICDH) activity, and at 24 hours *post mortem* to determine IMF%, as well as the concentrations of myoglobin, iron, and zinc, with methodologies described by Pannier *et al* (2010). A subset of lambs (approx. n=250) were CT scanned each year to determine the percentage of carcase fat, lean, and bone as described by Gardner *et al* (2010).
To determine the differences between sites, all traits were analysed using a linear mixed effects model with fixed effects (and their relevant interactions) for site, year, sex, birth type-rear type, dam breed within sire type, sire type and kill group within site. Sire and dam identification x year were included as random terms and hot carcass weight (HCWT) was used as a covariate for carcase fat%, lean% and bone%, and IMF analyses. After the site differences were determined, sire Estimated Breeding Value (EBV) for post-weaning C-site fat depth (PFAT) and its interaction with fixed effects (including site) was included as a covariate.

Results Decreasing PFAT EBV across the 4 unit range evident in this study, reduced ($P<0.05$) carcase fat by 3.8% units, and increased ($P<0.05$) lean and bone by 2.6% and 0.8% units. Across this same PFAT range IMF was also decreased ($P<0.05$) by 0.7% and ICDH activity decreased ($P<0.05$) by 0.48nmol/min/g tissue. None of these PFAT effects varied between sites, and neither iron, zinc nor myoglobin concentrations were affected by PFAT EBV. For all traits measured there were marked differences between sites ($P<0.05$), the magnitude of which were greater than any of the corresponding PFAT effects.

Table 1 Differences between sites, and the impact of PFAT EBV on carcase composition, and selected muscle traits.

	Site range (min, max±S.E.)	PFAT (effect per ASBV unit±S.E.)
Carcase Fat (%)	(26.2±0.28, 28.8±0.40)	0.94±0.58
Carcase Lean (%)	(54.8±0.40, 58.7±0.62)	-0.65±0.41
Carcase Bone (%)	(15.3±0.12, 16.5±0.14)	-0.21±0.13
Intramuscular Fat (%)	(3.67±0.10, 5.21±0.12)	0.17±0.05
Myoglobin Concentration (mg/g)	(5.23±0.12, 8.12±0.10)	n.s.
ICDH activity (nmol/min/g tissue)	(5.81±0.14, 6.91±0.14)	0.12±0.05
Iron Concentration (mg/100g)	(18.7±0.25, 23.9±0.25)	n.s.
Zinc Concentration (mg/100g)	(2.15±0.03, 2.98±0.03)	n.s.

Conclusions As expected selection for leanness reduced carcase fatness and IMF%, as well as reducing aerobicity (ICDH), however contrary to our hypothesis this did not impact on iron and zinc concentrations. Thus changes in muscle fibre type appear to be too subtle to impact on muscle mineral status. Also contrary to our hypothesis, the impact of PFAT did not differ between sites indicating that the effects of this trait are robust against nutritional interaction across variable Australian production systems.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

The effect of feeding rice straw and tree legumes on liveweight maintenance of mature Ongole cows (*Bos indicus*) in Indonesia

D Mayberry[1], R Antari[2], T Syahniar[2], D Pamungkas[2], Marsetyo[3], D Poppi[1]
[1]University of Queensland, Gatton, Queensland, Australia, [2]Beef Cattle Research Institute, Grati, East Java, Indonesia, [3]University of Tadulako, Palu, Central Sulawesi, Indonesia
Email: d.mayberry@uq.edu.au

Introduction Improving the productivity and profitability of smallholder cattle enterprises in Indonesia requires greater and more efficient utilization of existing feed resources. Rice straw is often burnt, but could meet the maintenance requirements of a cow if a small amount of extra energy and protein is provided. This could be in the form of green feed such as tree legumes. The aim of this experiment was to measure the amount of rice straw and tree legumes required to maintain the weight of a mature, non-lactating, non-pregnant Ongole cow in Indonesia. This was important in establishing a cow-calf system based on rice straw, the Straw Cow project.

Materials and methods Thirty-two multiparous, dry Peranakan Ongole cows (318 ± 12 kg (SEM) liveweight) were allocated to one of 4 treatments in a randomised block design, with 8 replicates per treatment. The 4 treatments were (A) rice straw *ad libitum*, (B) rice straw *ad libitum* plus tree legumes (2.5 g DM/kg $W.d^{-1}$), (C) rice straw *ad libitum* plus tree legumes (5.0 g DM/kg $W.d^{-1}$), and (D) rice straw *ad libitum* plus tree legumes (10 g DM/kg $W.d^{-1}$). The tree legumes used were *Gliricidia sepium* and *Leucaena leucocephala*, offered in equal portions on a dry matter basis at the designated treatment levels. The animals were housed in individual pens and had free access to fresh drinking water. Feed intake was determined daily for 20 weeks and liveweight was measured every second week. Digestibility was measured by total faecal collection over 7 consecutive days on 3 separate occasions, during weeks 3, 10 and 20 of the experimental period. Some animals were found to be pregnant during the experiment, and their liveweight was corrected for pregnancy using the equations of Silvey and Haydock (1978). Metabolisable energy (ME) required for maintenance and ME intake from the diets were estimated using the equations in CSIRO (2007). Differences between the treatments were analysed using ANOVA with Tukey's pairwise comparisons in Genstat (13th edition).

Results There was no difference in total feed intake between the 4 treatment groups ($P>0.05$). Intake of tree legumes was higher when more was offered ($P<0.05$), but cows did not consume their targeted legume supplement level. Cows substituted tree legumes for rice straw (Table 1). The inclusion of tree legumes in the diet had no significant effect on organic matter digestibility, liveweight gain or estimated energy balance of the cows ($P>0.05$).

Table 1 Average feed intake, digestibility, liveweight gain and energy balance of mature Ongole cows fed rice straw and tree legumes

Parameter	A	B	C	D	SEM
Rice straw intake (g DM/kg $W.d^{-1}$)	17.4^{a}	16.4^{ab}	15.3^{ab}	13.9^{b}	0.528
Tree legume intake (g DM/kg $W.d^{-1}$)	0^{a}	2.1^{b}	3.3^{c}	5.2^{d}	0.350
Total feed intake(g DM/kg $W.d^{-1}$)	17.3^{a}	18.2^{a}	18.7^{a}	19.0^{a}	0.498
OM digestibility (g/kg)	531^{a}	535^{a}	546^{a}	556^{a}	4.92
Liveweight gain (kg/d)	-0.11^{a}	-0.07^{a}	0.02^{a}	-0.03^{a}	0.027
Estimated ME maintenance (MJ/kg $W.d^{-1}$)	0.11^{a}	0.11^{a}	0.10^{a}	0.10^{a}	0.001
Estimated ME intake (MJ/kg $W.d^{-1}$)	0.11^{a}	0.13^{a}	0.13^{a}	0.14^{a}	0.004

Means within each row with different letters are significant ($P<0.05$)

Conclusions Our results demonstrate that it is possible for a non-pregnant, non-lactating Ongole cow to maintain weight on a rice straw based diet with the addition of a small amount of green feed. The ME requirement for maintenance of cows on treatment C was estimated to be 0.57 MJ/kg $W^{0.75}.d^{-1}$. Regression of daily weight gain and ME intake for all cows in the experiment also predicted maintenance requirements of 0.57 MJ/kg $W^{0.75}.d^{-1}$. This is higher than the value published by Chizzotti *et al* (2008) for genotypically similar Nellore cattle in Brazil (0.47 MJ/kg $W^{0.75}.d^{-1}$) and that estimated using the CSIRO equation for maintenance (0.44 MJ/kg $W^{0.75}.d^{-1}$; CSIRO 2007). The calculation relies on an estimate of ME content of the diet which was calculated from *in vivo* OM digestibility, but the high ash content of the rice straw (240 g/kg DM) may result in an overestimate of ME content. It may be concluded that cows can maintain weight on rice straw especially if a small amount of tree legume at approximately 3 g DM/kg $W.d^{-1}$ is included, which would also ensure that adequate N was supplied to rumen microbes.
Although the consumption of up to 5 g DM/kg $W.d^{-1}$ of tree legumes tended to increase the energy intake and weight gain of cows, this effect was not significant. Across all treatments, none of the cows ate all of the gliricidia or leucaena offered to them, which was unexpected. It appears unlikely that cows will consume enough tree legumes to improve digestibility, energy intake and daily liveweight gain unless rice straw intake is restricted.

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

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Effects of ruminal infusion of garlic oil on ruminal fermentation dynamics of goats

Z Zhu, W Zhu
College of Animal Science and Technology, Nanjing Agricultural University, Nanjing, China
Email: zhuzhi612@163.com

Introduction Some *in vitro* studies showed that garlic oil reduced the proportion of acetate and increased the proportion of propionate and butyrate (Busquet *et al*. 2005a, 2005b, 2006). But others reported that garlic oil had no effect on total VFA concentration and the molar proportions of acetate, propionate and butyrate (Cardozo *et al*. 2004) or had pH-dependent effects on *in vitro* 24-h ruminal fermentation of high-concentrate diet for beef cattle (Cardozo *et al*. 2005). This study aimed to investigate the effects of garlic oil on *in vivo* ruminal fermentation dynamics of goats.

Materials and methods Six wethers fed individually in pens were assigned to two groups for cross-over design with 14-d intervals. Goats were fed a basal diet without (control) or with garlic oil infusion (0.8 g/d) via ruminal fistulas for a period of 30 days. Ruminal contents were collected before (0 h) and at 2, 4, 6, 8 and 10 h after morning feeding on d 28, 29 and 30. The commercial garlic oil (stored at 4°C) produced by steam distillation contained $C_6H_{10}S_3$ (29.3%) and $C_6H_{10}S_2$ (31.3%) as its main components was used. Measurements included pH, NH_3-N, microbial crude protein (MCP) and volatile fatty acid (VFAs).

Statistical Analysis Data were analyzed using repeated measures of PROC MIXED procedure of SAS. Terms in the model with the covariance type auto-regressive order 1 contained effects of period, treatment, sampling day and time, and the interaction of treatment with time. The goat of the run was considered a random effect. The effect of sampling time was repeated measures. Means were separated by using the PDIFF option in the LSMEAS statement.

Results During ruminal fermentation, garlic oil reduced pH ($P \leq 0.05$) and total VFAs concentration ($P = 0.056$), increased ($P < 0.01$) NH_3-N and MCP concentrations compared with the control. No differences ($P > 0.1$) in the proportions of acetate, propionate and butyrate, and the ratio of acetate to propionate were observed between the treatments. But the interaction of treatment with sampling time had significant effects ($P \leq 0.05$) on total VFAs concentration, propionate proportion and ratio of acetate to propionate. The dynamic features showed total VFAs concentration (Figure 1) and propionate proportion (Figure 2) reached maximum at 2 h after morning feeding in the control, whereas at 6 h in the garlic oil treatment. The ratio of acetate to propionate was not reduced sharply after morning feeding by garlic oil infusion compared with the control (Figure 3).

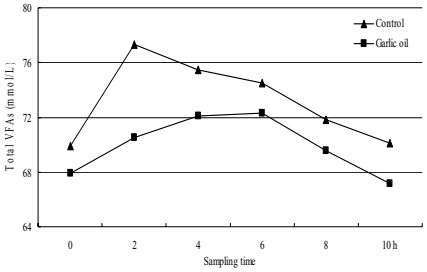

Figure 1 Temporal change of total VFAs concentration

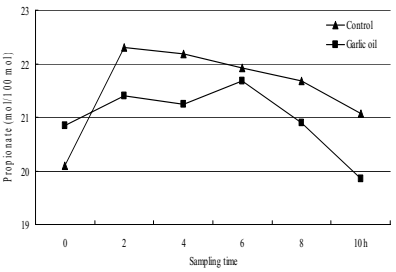

Figure 2 Temporal change of propionate proportion

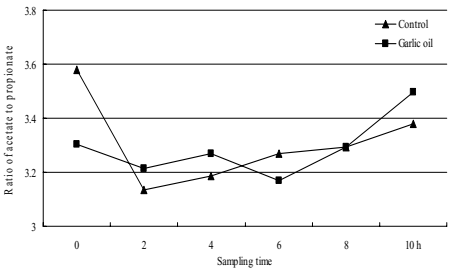

Figure 3 Temporal change of ratio of acetate to propionate

Conclusions Garlic oil infusion reduced ruminal pH and increased NH_3-N and MCP concentrations. It had no significant effects on ruminal total VFAs concentration and individual VFA proportion, but the alleviated changes occurred after feeding suggested a role of garlic oil in maintaining the rumen environment.

Acknowledgements This work was supported by a grant from the Key Scientific and Technological Project of Jiangsu Province (Q200710).

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Could *Quinella*-like microorganisms be involved in the formation of saturated oxygenated fatty acids in the rumen of lactating sheep fed diets containing sunflower oil and marine algae?

A Belenguer[1], P G Toral[1,2], K J Shingfield[2], G Hervás[1], V Toivonen[2], P Frutos[1]
[1]Instituto de Ganadería de Montaña (CSIC-Universidad de León), Grulleros, León, Spain, [2]Animal Production Research, MTT Agrifood Research Finland, Jokioinen, Finland
Email: a.belenguer@csic.es

Introduction Dietary supplements of sunflower oil (SO) in combination with marine lipids (fish oil or marine algae; MA) are known to increase the concentration of potentially beneficial bioactive lipids, including cis-9, trans-11 conjugated linoleic acid in ovine milk (Toral *et al.* 2010a). Changes in milk fat composition are related to diet-induced alterations in ruminal biohydrogenation (BH) of unsaturated fatty acids (FA) and associated ruminal microbiota. Inclusion of SO with fish oil also promotes the accumulation of oxygenated 18-carbon saturated FA in ruminal digesta in sheep (Toral *et al.* 2010b). However, the bacterial species responsible for the formation and accumulation of keto and hydroxyl FA in the rumen are not well characterised. In the current study, ruminal fluid from lactating ewes fed a diet supplemented with SO plus incremental amounts of MA was used to investigate the association between the formation and accumulation of 18-carbon saturated keto FA and the variations in the rumen bacterial community.

Materials and methods Twenty-five lactating Assaf ewes were allocated to 5 experimental treatments: a total mixed ration (TMR) without lipid supplementation (Control diet) or supplemented with 25 g SO/kg DM plus 0, 8, 16 or 24 g MA/kg DM (SO, $SOMA_1$, $SOMA_2$ and $SOMA_3$ diets, respectively). After 28 days on treatments, and 3 hours after removing the morning meal, samples of ruminal fluid were collected from each animal using a stomach tube. Aliquots of ruminal fluid for DNA extraction and FA determination were immediately frozen at -80ºC, freeze-dried and stored at -80ºC. Ruminal fluid FA composition was determined by GC and HPLC (Toral *et al.*, 2010b). Microbial DNA extraction and terminal restriction fragment length polymorphism (T-RFLP) analysis of the total bacterial community were conducted as reported previously (Belenguer *et al.* 2010). Data were analysed by one-way ANOVA, with the MIXED procedure of the SAS software package Version 9.1 (SAS Inst. Inc., Cary, NC, USA).

Results Four 18-carbon keto acids were detected in ruminal fluid (9-, 10-, 13- and 15-O-18:0). Relative abundance of all 18-carbon keto acids was low in ruminal fluid for the Control and SO treatments. However, inclusion of MA in the diet increased the accumulation of 10-O-18:0 in ruminal fluid of up to 3% of total FA (Figure 1A). The T-RFLP analysis revealed that certain fragments (T-RF) compatible with *Quinella*-like bacteria were present at a higher relative frequency in ruminal fluid of sheep on SOMA treatments after *HhaI* (98 bp) or *MspI* (150 plus 268 bp) digestions (Figure 1B). A concomitant accumulation of 10-O-18:0 and increased proportion of *Quinella*-like bacteria in ruminal fluid was also observed in ewes fed diets containing SO plus fish oil (Belenguer *et al.* 2010; Toral *et al.* 2010b).

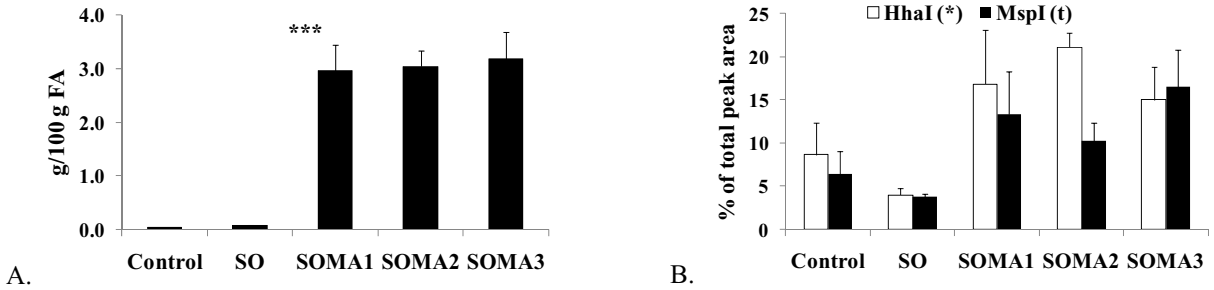

Figure 1 Concentration of 10-O-18:0 (A) and relative proportions of T-RF compatible with Quinella-like bacteria (B) in ruminal fluid of lactating ewes fed a TMR containing no additional lipid, SO or SO plus MA (t= P<0.10; *= P<0.05; ***= P<0.001).

Conclusions Metabolism of *Quinella ovalis* is similar to that of *Selenomonas ruminantium* (Krumholz *et al.* 1993), a ruminal bacterium known to produce 10-OH-18:0 in the ovine rumen (Hudson *et al.* 1995). Higher relative abundances of T-RF compatible with *Quinella*-like bacteria in the sheep fed SO plus MA is consistent with a possible role in the hydration of unsaturated 18-carbon FA in the rumen. However, the function of these microorganisms in the rumen remains unclear and their involvement in other pathways of ruminal BH cannot be excluded.

Acknowledgments This work was supported by the Spanish Ministry of Science and Innovation (MICINN; AGL2008-04805-C02-02) and the Spanish National Research Council (CSIC; 200940I034). P. G. Toral was granted by the CSIC (I3P Program).

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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The structural diversity of condensed tannins in willows (*Salix* spp.): a first screening to improve the nutritional quality of ruminant products

L Falchero[1], R H Brown[1], A Karp[2], S Hanley[2], I Shield[2], I Mueller-Harvey[1]
[1]Agriculture Department - University of Reading, Reading, UK, [2]BBRSC Rothamsted Research, Harpenden, UK
Email: l.falchero@reading.ac.uk

Introduction Condensed tannins (CT) are polyphenols widely investigated in recent plant and animal research. Forage plant CT can in fact interact with animal physiological processes and with the metabolism of the microbial population in the rumen. Both these processes can generate useful benefits for ruminant nutrition and health, productivity, and product quality (Vasta *et al*., 2010). However, some tannins can also have dose-dependant negative nutritional effects. Thus, all tannins were often mistakenly described in the past as anti-nutritional factors.
Actually, plants produce a wide range of tannin structures, differing enormously between plant species, varieties and plant parts. This explains why some tannin-containing forages are much more effective than others. Therefore, investigations into the beneficial or detrimental effects of CT need to consider all aspects of their structural variation.
Within the EU project 'Tannin StrACTure QTLs', willow (*Salix* spp.) has been selected as model plant to investigate the biological effects of plant CT structural traits. The UK National Willow Collection, maintained at the Rothamsted Research Institute, is a worldwide and unique willow germplasm collection that currently includes about 1300 accessions representing over 100 different willow species. Willows contain CT (2-3% of leaf dry matter), and their leaves and twigs have been used in the past as ruminant feeds and feed supplements in extensive systems in Northern Europe (Waller *et al*., 2001) and Bhutan (Roder, 1992), while researchers from New Zealand have investigated their potential as substitutive feed to deal with summer drought (Moore *et al*., 2003). As a first step in order to evaluate the potential use of willows as novel sustainable feed and the biological effect of the different CT structural traits, this work reports the CT composition of 18 diverse accessions of the UK National Willow Collection, determined by direct thiolysis. This method is suited to quantify the variable proportions as terminal or extension units of the CT monomers, named flavan-3-ols (catechin, epicatechin, gallocatechin, and epigallocatechin), that define CT structural traits.

Materials and methods Leaves from 18 accessions of willow were collected on July 2010 from the National Willow Collection (BBSRC Rothamsted Research, Harpenden, Hertfordshire, UK), then freeze-dried for 48 hours, ground to pass a 0.5 mm sieve, and stored in the darkness. The thiolysis extraction was performed on the dried-leaves samples (200 mg each) after prewashing in acetonitrile to remove free catechin. Then, after HPLC separation, CT structural information (Total extracted condensed tannins, mean Degree of Polymerization – mDP, Procyanidin/Prodelphinidin ratio - PC/PD, and *cis/trans* ratio) were obtained according to Gea *et al*., 2011.

Results Results showed a great and partially unexpected range of variability for each tannin trait among the accessions. Total CT content varied from 0.30 (accession n:1000, *Salix myrsinifolia*) to 2.50 g\100g DM (n:449, *S. appendiculata*). mDP, related to CT molecular weight, varied from 4.1 (n:1000, *S. myrsinifolia*) to 26.1 (n:987, *S. hookeriana*). PC/PD ratio varied from 14.8/85.2 (n:99, *S. triandra*) to 97.7/2.3(n:956, *S. eriocephala*). Also *cis/trans* ratio showed large variations among accessions, ranging from 1.7/98.3 (n:99, *S.triandra*) to 81.3/18.7 (n:945, *S. myricoides*).

Conclusions This screening has demonstrated that a wide range of different combinations of CT traits exists in willow germplasm. This structural richness is coupled to a CT concentration that is close to the *optimum* considered for feed to potentially obtain benefits for animal health and nutrition (Dixon *et al*., 2005).
Further investigations are therefore planned in the project "Tannin StrACTure QTLs" to link willow CT structural traits and their biological activity. Future results will be useful for the researchers investigating how to optimize in a sustainable way novel feeds for ruminant nutrition, in order to improve animal health, productivity, and the quality of derived products.

Acknowledgements The research project 'Tannin StrACTure QTLs' is funded by the European Union (FP7/2007-2013) under grant agreement n PIEF-GA-2009-253905.

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Morphological and chemical composition of hand-plucked samples of Tifton-85 pasture managed under different herbage allowance grazed by sheep in rotational stocking system

C M Battiston Loureiro, A C Ruggieri, T T Berchielli, A Garcia Silva Sobrinho, W Ladeira da Silva, R Andrade Reis
Universidade Estadual Julio de Mesquita Filho, UNESP, Jaboticabal, Sao Paulo, Brazil
Email: rareis@fcav.unesp.br

Introduction Sheep have a characteristic of feeding habit, which is resulted of a greater selection in grazing conditions. These animals use their lips to the seizure of forage. They prefer species of medium to low height, prostrate growth habit as Tifton-85, a hybrid species of the Cynodon genus. The objective of this trail was to evaluate the quality of Tifton-85 managed under different herbage allowance, in a rotational grazing system by sheep.

Materials and methods The experiment was conducted at the Animal Science Department, of São Paulo State University, Jaboticabal, São Paulo State, Brazil, in the summer of 2008. The treatments were 5, 7 and 10% of herbage allowance in relation to body weight, distributed into nine paddocks with areas 200 m^2 to 300 m^2 and 400 m^2, respectively. There were used for grazing sheep wooless, weighing 40 kg, which grazing during eight hours per day at the experimental pasture. The rotational grazing system, with fix rest period of 21 days, and grazing by eight hours was used. The determination of herbage mass in each paddock was taken, one day before the grazing (pre-grazing), and in post grazing condition, using the double sampling method (Sollenberger & Cherney, 1995). To estimate forage chemical composition, samples were collected from Tifton-85, according to the hand plucking method proposed by Sollenberger & Cherney (1995). A sub-sample was taken for evaluation of morphological composition, and after the separation of leaves and stems, the sub samples were weighed and dried at 55oC for 72 hours, and processed in a Wiley type mill, with 1 mm mesh sieve. Grass samples were analysed for dry matter and nitrogen, according to the procedures described by AOAC (1995). Cell wall composition was analysed by the methods proposed by Robertson & Van Soest (1981).The experimental design was a randomized block design with repeated measures (three grazing periods), with three replications. Statistical analysis was performed analysis of variance and Tukey test to compare averages, and regression analysis for height and grazing periods, using the System for Statistical Analysis of Agronomic Trials (AGROESTAT).

Results There was no effect (P> 0.10) of herbage allowance on the leaf and stem proportions in the different grazing cycles (Table 1). However, the proportion of leaf increased, and the stem decreased in the last evaluation (Table 1). There are no effect (P> 0.1) of the herbage allowance in the levels of CP, MM, NDF, ADF, LIG and CEL. The chemical composition of forage was not affected (P> 0.1) by the levels of herbage allowance, and grazing cycles (Table 2). Samples of pasture were collected by hand plucking technique, which represents the forage harvested by the animals in grazing condition. If the herbage allowance did not limit the grazing selection process, the animals consumed forage with similar chemical composition throughout the experimental period.

Table 1 Percentage of leaves and stems of the simulated grazing samples of Tifton-85 managed in a rotational stocking by sheep in different periods and forage allowance

Cycle	Allowance		
	5%	7%	10%
Leaves %			
1 (January)	87,8A	90,77A	91,49A
2 (February)	71,84AB	81,65A	74,51AB
3 (March)	57,78B	61,31B	66,57B
Stems %			
1 (January)	12,19B	9,22B	8,50B
2 (February)	28,15AB	18,34B	25,48AB
3 (March)	42,21A	38,69A	33,42A

Table 2 Chemical composition of the Tifton-85 sampled by hand plucking, managed under rotational stocking system with sheep in different forage allowance and grazing cycles

Allowance	CP%	NDF%	ADF%	LIG%	CEL%
5%	7,80A	77,04A	33,85A	3,47A	30,38A
7%	7,87A	76,35A	32,69A	3,33A	29,35A
10%	7,51A	77,40A	33,31A	3,30A	30,00A
Cycle					
1 (January)	8,35A	76,82A	31,56A	3,99A	28,66A
2 (February)	7,38A	76,98A	33,82A	3,22AB	30,59A
3 (March)	7,46A	76,98A	34,47A	3,99A	30,48A

Means followed by same letters in columns do not differ statistically by Tukey test at 10% probability.

Conclusions Tifton-85 pasture management at 5, 7 and 10% of herbage allowance resulted in similar pasture structure and chemical composition around the experimental period.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Responses of growth performance and nutrient digestibility to partial or total substitution of corn grains with non-cereal by-products in the diet of growing crossbred bulls

F Shi, Q Meng, H Wu, L Ren
China Ariculture University, BeiJing, China
Email: shifenghua1984@163.com

Introduction The shortage of animal feed resources and increasing cost of corn grains used as animal feeds have stimulated the utilization of non-cereal by-products as feeds for ruminants. Currently, the application of by-products on livestock has gained more attention in China, because non-cereal byproducts are abundant there. Take molasses, potato meal and so on for example, molasses and potato meal can be used as silage fermentation stimulants and included in concentrate mixtures up to 20% of the fresh weight (Schneider *et al*., 1985). Vander Pol *et al*. (2006) reported that 40% wet distillers grains can be included in the diet of finishing cattle. But the research regarding using by-products to replace cereal grains in the diet of beef cattle and to reduce feed cost is still rare. In this experiment, the non-cereal by-products such as molasses, cottonseed cake, brewer's grains, bean curd residue, Chinese Data and soybean hull were used, and the objective the experiment was to study the responses of growth performance and nutrient digestibility to partial or total substitution of corn grains with non-cereal by-products in the diet of growing crossbred bulls.

Materials and methods Forty-five Limousin×Local Yellow bulls were blocked by initial BW (412±21kg) and allotted randomly to three groups, with 15 bulls in each group. Each group of animals received one of three diets: non-substitution treatment (NST), partial substitution treatment (PST), and total substitution treatment (TST). The contrast group (NST) contained 45.4% corn, 3% cottonseed cake, 11% brewer's grains, 8.9% bean curd residue, 0.6% limestone, 0.7% $CaHPO_3$, 0.3% salt, 0.1% beef additives and 30% corn silage. Partial substitution treatment (PST) contained 15.1% corn, 14.7% brewer's grains, 13.8% bean curd residue, 20% Chinese Data, 5% molasses, 0.3% limestone, 0.7% $CaHPO_3$, 0.3% salt, 0.1% beef additives and 30% corn silage. Total substitution treatment (TST) contained 0% corn, 11.2% brewer's grains, 9.8% bean curd residue, 27% Chinese Data, 5% molasses, 15.7% soybean hull, 0.2% limestone, 0.7% $CaHPO_3$, 0.3% salt, 0.1% beef additives and 30% corn silage. All the diets were formulated to contain a minimum of 7.14Mkal/kg ME, 16% CP, 0.49% Ca, and 0.26% P. All bulls were tethered using neck straps in tie stalls and were individually fed the diet twice a day (0600 and 1700).Water freely accessed to bulls. The trial was lasted 98 days, including 7 days for pre-feeding and 91 days for data collection. Daily gain and feed intake were recorded during the trial and feces samples were collected at the end of the trial for measurement of nutrient digestibility. Statistical analyses were conducted using the GLM procedure (SAS Institute Inc., Cary, NC), and the differences among means were tested using Duncan's New Multiple Range Test.

Results The results (Table 1) showed that ADG was higher (p□0.01) for NST than PST and TST. The DMI expressed as absolute amount per day were lower (P<0.01) for NST than PST and TST. The feed conversion was not different between NST and PST, while TST was significantly higher(p□0.01) than NST and PST. The cost per kg gain (RMB) of PST and TST was 0.65 and 0.74 RMB lower than NST, respectively. No significant differences in digestibilities of DM, OM and CP among three groups were observed different (Table 1).

Table 1 Growth performance and Nutrient digestibility of growing crossbred bulls fed the three different diets

Item	Dietary treatment			SEM	P
	NST	PST	TST		
Growth performance					
Average daily gain (ADG, kg)	1.78[a]	1.65[ab]	1.47[b]	0.08	p□0.01
Dry matter intake(DMI, kg)	8.64[b]	9.18[a]	9.02[a]	0.06	p□0.01
Feed conversion (F/G)	4.84[a]	5.57[ab]	6.15[b]	0.01	p□0.01
Cost per kg gain (RMB)	6.93	6.28	6.19	-	-
Comparing with NST (RMB)	-	-0.65	-0.74	-	-
Nutrient digestibility					
DM digestibility (%)	58.63	57.92	64.08	2.31	0.1533
OM digestibility (%)	63.68	60.28	66.57	2.11	0.1436
CP digestibility (%)	42.38	43.21	51.97	3.68	0.1588

Conclusions While the traditional diet was the best group considering growth rate, the diets substituted partially or totally with byproducts were economically acceptable because of their low cost and similar digestibilities of DM, OM and CP. The increased DMI and similar nutrient digestibilities of PST and TST diets may compensate their potentially decreased growth performance of beef cattle.

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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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Nitrogen balance in dairy heifers fed with sugar cane combined with different protein sources

R C Canesin[1], D A Mota[2], G Fiorentini[1], J D Messana[1], B L Rosa[1], T T Berchielli[1]
[1]FCAV/UNESP, Jaboticabal, São Paulo, Brazil, [2]UFAM, Parintins, Amazonas, Brazil
Email: betacanesin@hotmail.com

Introduction The lower cost of including sugar cane in animal feeds has enhanced viability. Otherwise, sugar cane has low protein content. So it should not be the only food source to the animals, being necessary an additional source of protein added to the diet. Alternative protein sources different from the soybean meal have been targeted by researchers in recent years. However, it must be considered that the inclusion of alternative sources of protein in diets for growing heifers can cause changes in basal metabolism, especially in the rumen metabolism, and may modify the microbial flora as well the utilization of nutrients. The study aimed to evaluate the effect of different protein sources in nitrogen compounds in dairy heifers fed diets based on sugar cane.

Materials and methods Eight Holstein x Zebu dairy heifers crossbred, averaging 202.12 kg body weight and 18 months of age, cannulated in the rumen, were used. The sugar cane was used as exclusive forage, it was hand picked and chopped daily to provide to the animals particle size of about 2.0 cm. The diets were composed by different protein sources: soybean meal (60% sugar cane, 15% soybean meal, 23.5% corn grain, 1% mineral and 0.5% urea); cottonseed meal (60% sugar cane, 14.2% cottonseed meal, 23.8% corn grain, 1% mineral and 1% urea); peanut meal (60% sugar cane, 13% peanut meal, 25.3% corn grain, 1% mineral and 0.7% urea); and sunflower meal (60% sugar cane, 16.2% peanut meal, 21.75% corn grain, 1% mineral and 0.85% urea); and 60:40 diet ratio (forage:supplement) having 13% CP. The animals were kept in metabolic crates to collect data. The nitrogenous compounds were obtained by difference between total nitrogen intake and total nitrogen excreted in feces and urine. During the five days of each experimental period the total feces were collected, where feces were removed daily from trays in the morning, weighed, homogenized and sampled. Concomitantly, there were the total urine samples using Folley probes. Containers used to receive the urine contained 300 mL of sulfuric acid 20%. After measuring, mixing, and filtrating, aliquots were taken for further analysis. In this same period, daily samples of diet and orts were made for later determination of nutrient intake. The partial samples of feces, diet, and orts were dried in forced air ventilation at 55°C and powdered in a knife mill with a sieve of 1 mm sieves and mixed to form a composite sample per animal at each period. The determination of nitrogen (N) in total food, feces and urine was performed according to AOAC (1990). The statistical design used was a double 4x4 latin square. Data were subjected to analysis of variance by PROC GLM of SAS (2004) using the Tukey test.

Results Protein source did not affect any of the measure parameters in the various components of nitrogen balance. The nitrogen balance showed values of 44.78 g/day and 43.55% nitrogen intake. According to Hoffman *et al*. (2001), there is a linear relationship between nitrogen intake and nitrogen excretion in feces and urine.

Table 1 Nitrogen balance of crossbred dairy heifers fed with diets based on sugar cane and several protein sources

	Diets				*P*	*CV*
	SBM[1]	SFM[2]	PNM[3]	CTM[4]		
DMI (kg/day)	5.18	5.11	5.21	5.02	0.735	9.13
N intake (g/day)	104.53	101.95	100.69	104.59	0.827	24.71
N fecal (g/day)	23.59	24.10	27.77	24.10	0.444	15.05
N Urinary (g/day)	32.23	32.23	32.70	32.09	0.876	21.86
N balance (g/day)	48.70	45.62	44.59	40.22	0.404	16.00
N balance (% intake N)	46.60	44.72	40.22	42.69	0.326	8.92

[1]Soybean meal, [2]Sunflower meal, [3]Peanut meal, [4]Cotton meal. P= significance; CV= variation coefficient.

Conclusions The supply of different protein sources is presented as economically viable alternative to feed dairy heifers in growth, whereas did not affect the balance of nitrogen compounds in crossbred dairy heifers fed diets based on cane sugar.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Validation of Nutrient Requirements of Dairy Cattle model in a Brazilian Dairy Farm

J Souza, M Osmari, A Branco, J Barreto, F Granzotto, S Teixeira
Universidade Estadual de Maringá, Maringá - PR, Brazil
Email: mileneosmari@yahoo.com.br

Introduction Dairy production has contributed to environmental pollution with high production of wastes contaminating water, soil and air, with emissions of ammonia and nitrates (Arriaga *et al*., 2009). The absence of information to adequately quantify the intake, retention and excretion of nitrogen in Brazilian dairy herds contributes to underestimate the true impact of dairy farming on the environment. The objectives of this study were to quantify the intake and excretion of N in a dairy herd with intensive production and counteract the observed values with those estimated by the NRC (2001) model to verify the possibility of use NRC (2001) as a tool to estimate N balance in dairy farms.

Material and methods Sixteen lactating Holstein cows over 60 days in milk were used, belonging to a farm located in Castro, Paraná State, Brazil. The experiment lasted 21 days and diet was balanced to 16% CP, 1.55 Mcal of NE_L/kg DM, 55% NDF and 20% ADF and the cows yielded on average 30 kg of milk/day. Dry matter intake (kg/day) was calculated by the amount of feed (kg of DM/day) provided to the lot of cows discounting orts (kg of DM/day) and divided by the number of cows (16). Observed values of net energy of lactation (NE_L, Mcal/kg DM) were obtained from *in vitro* organic matter digestibility (IVOMD, %). Values of IVOMD were used as diet TND (%). These TND values were corrected for intake level as multiple of maintenance using NRC (2001) equations. Estimated values of diet NEL (NRC, 2001) were obtained from feed composition. Milk samples were collected at the time of milking and were preserved using Bronopol (2-bromo-2-nitropropane-1,3-diol) under refrigeration (4ºC) until analyzes. Faecal samples were collected directly from the rectum. The faecal dry matter output (kg) was estimated from the observed dry matter intake (kg) multiplied by the percentage of dry matter indigestible obtained from *in vitro* dry matter digestibility. Nitrogen intake was calculated using DMI (g) and % of N in the diet. The excretion of N in milk was calculated from the values of milk yield and milk protein. Faecal N (g) was determined by faecal DM output (g) and faecal N (%) for each cow. The efficiency of N use was obtained by N in milk/N intake x 100. For each cow, individually, were collected (observed) data for N intake, N excreted in faeces, dry matter intake, milk yield permitted by energy intake, milk yield permitted by metabolizable protein intake,diel NEL, intake of metabolizable protein, diet metabolizable protein and efficiency of N utilization. Cow characteristics and diet composition were used to perform a simulation using NRC (2001) model to obtain the estimated data. Analyses of variance were performed considering the observed values as a treatment and the estimated values by the NRC (2001) model as another treatment. Results were analyzed by ANOVA and means compared by Tukey test at 5% of probability.

Results The dry matter intake observed (DMI) was not properly estimated ($P<0.01$) by NRC (2001) model, and in the same way the milk yield permitted by metabolizable protein intake (MY/MPI) ($P<0.05$), diet NE_L (Mcal/kg DM) ($P<0.01$), diet MP (g/kg) ($P<0.01$), faecal N (g/cow/day) ($P<0.01$) and efficiency of N use (ENU,%). However, the variables, milk yield permitted by energy intake (MY/NE_L), intake of NE_L (Mcal/day), intake of MP (kg/day) and nitrogen intake (g/cow/day), were well estimated by NRC (2001) model because the observed and estimated values were similar ($P>0.05$) (Table 1).

Table 1 Observed and estimated dependent variables

Characteristics	Observed	Estimated	P
DMI (kg/d□y)	19.64	23.18	S[1]
MY/NE_L (kg/day)	30.26	29.21	NS[2]
MY/MPI (kg/day)	30.26	25.22	S
INE_L (Mcal/day)	31.52	31.85	NS
DNE_L (Mcal/kg DM)	1.58	1.37	S
MP (kg/day)	2.17	2.16	NS
DMP (g/kg)	98.48	93.68	S
NI (g/cow/day)	502.71	532.77	NS
FN (g/cow/day)	142.47	64.76	S
NEU (%)	32.35	23.62	S

[1]S – $P<0.05$ by Tukey test. [2] NS – $P>0.05$ by Tukey test.

Conclusion NRC (2001) estimated reasonably some dependents variables, but for some others did not. More Brazilian dairy farms should be studied and in different situations to adopt NRC (2001) as a tool for nitrogen balance.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Quantification of ruminal bacterial groups in wethers fed only pasture: effect of feeding schedule and bacterial dynamics according to the time from the beginning of the meal

A Pérez-Ruchel[1], J L Repetto[2], P Zunino[3], M Fraga[3], K Perelmuter[3], C Iturria[1], W Saavedra[1], C Cajarville[1]
[1]Departamento de Nutrición Animal, Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay, [2]Departamento de Bovinos, Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay, [3]Departamento de Microbiología, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay
Email: anapevet@gmail.com

Introduction In South America ruminant feeding is based mainly on direct grazing. In semi-intensive production systems temperate pasture grazed represents the main part of the diet, and often the daily time of access is restricted.
The efficiency of ruminants to utilize a wide variety of feeds is due to a highly diversified and complex rumen microbiota. Microbial fermentation action is particularly important for the bioconversion of lignocellulosic feeds, such as forages, into volatile fatty acids. The study of the bacterial biota in the ruminal ecosystem is difficult using conventional techniques. A large proportion of rumen microbes is non-culturable, so it is necessary to use molecular techniques (Kamra, 2005). The use of direct fluorescent labels of bacteria cells can provide actual numbers of viable, dead or total bacteria in fluid samples (Lascano *et al*., 2009).
Fluorescence *in situ* hybridization (FISH) seems to provide a method to directly evaluate different microbial populations. The aim of this study was to evaluate the effect of the restriction of access to forage and bacterial dynamics along time since the beginning of the meal on the rumen microbiota of animals consuming pastures.

Materials and methods Six fistulized wethers consuming forage from a temperate pasture (*Lotus corniculatus*, 13% CP, 44% NDF, DM basis) were housed in metabolic cages and separated into 2 groups. One group had *ad libitum* access to forage throughout the day (24H) while the other group had unlimited access to forage during 6h per day only (6H). To evaluate the effect of the restriction access to forage, rumen content of every animal (fluid+solid mix, 82.6% OM) was collected 4 h after the begining of the meal from the ventral rumen using permanent probes. To study the ruminal bacterial evolution along the day, rumen content was obtained from every animal of the 6H group at times 0, 4 and 8h. Ruminal contents were mixed with PBS and fixed with 50% ethanol (4°C for 24h and stored at -20°C). To study selected groups of microorganisms, FISH was performed. Probes targeting the Domain Bacteria, cellulose-degrading bacteria (*Ruminococcus albus*, *Ruminococcus flavefaciens)*, lactate-producing bacteria (*Streptococcus* spp.) and lactate-consuming bacteria (*Megasphaera elsdenii*, *Propionibacterium* spp., *Selenomonas ruminantum)* were used. DAPI was used to stain every cell. Numbers of the different groups (cells/mL) were calculated, transformed to $\log_{10}$ and analyzed using PROC MIXED (SAS®).

Results The restriction of access to forage affected the ruminal microbiota composition. Total DAPI counts, *R. albus, R. flavefaciens* and *S. ruminantum* counts were higher for animals of the 24H group compared to animals of the 6H group (Table 1A). In animals fed 6 h per day (6H group) the effect of time since the beginning of the meal only was significant in total DAPI counts and tended to affect Bacteria counts which showed lower counts at 4h (P = 0.077, Table 1B).

Table 1 A: Ruminal microorganisms counts (mean $\log_{10}$) in animals fed fresh forage during all day (24H) or 6h per day (6H). B: Ruminal microorganisms counts (mean $\log_{10}$) in animals fed 6 h (6H) at times 0, 4 and 8h since the beginning of the meal.

A	Restriction of access to forage		SEM	P
	24H	6H		
Total cells (DAPI)	10.9	10.6	0.07	<0.001
Bacteria	10.4	10.1	0.13	0.145
M. elsdenii	9.63	8.76	0.36	0.166
Propionibacterium spp	8.98	8.64	0.14	0.215
R. albus	9.13	8.74	0.10	0.052
R. flavefaciens	9.08	8.71	0.09	0.041
S. ruminantum	8.89	8.37	0.08	0.008
Streptococcus spp.	7.86	7.88	0.14	0.924

B	Times since beginning of the meal			SEM	P
	0h	4h	8h		
Total cells (DAPI)	10.73[a]	10.57[b]	10.65[ab]	0.05	0.003
Bacteria	10.44	10.09	10.20	0.08	0.077
M. elsdenii	8.83	8.76	9.07	0.27	0.723
Propionibacterium spp	8.85	8.64	9.18	0.24	0.302
R. albus	8.96	8.74	9.10	0.21	0.527
R. flavefaciens	8.90	8.71	9.14	0.35	0.716
S. ruminantum	8.91	8.37	8.91	0.30	0.408
Streptococcus spp.	7.87	7.88	8.11	0.24	0.747

SEM: standard error of means; for each row different letters between means were significantly different (P≤0,05).

Conclusions Feeding schedule affected the ruminal microbiota. The restriction of access to forage affected the numbers of cellulolytic and lactate consuming-bacteria compared to animals that received the same diet *ad libitum* along the day. In animals that were fed for 6 h per day (6H), counts of the different bacterial groups did not significantly varied along time. These results suggest that feeding restriction may affect forage utilization although bacterial evolution along the day did not significantly vary.

Acknowledgements PDT-DICyT (78/12) and CSIC.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Oxidative stress and inflammatory response in dairy cattle in an induced subacute acidosis protocol

H Golder[1,2], I Lean[1,2], A Rabiee[1,2], R King[3], P Celi[1]
[1]The University of Sydney, Faculty of Veterinary Science, Camden, New South Wales, Australia, [2]SBScibus, Camden, New South Wales, Australia, [3]Dairy Australia, Southbank, Victoria, Australia
Email: heleng@sbscibus.com.au

Introduction The effects of subacute acidosis on oxidative stress and inflammatory response are relatively unknown. The subacute acidosis protocol used in this study is capable of significantly decreasing rumen pH and altering rumen fermentation products. The greatest effects on rumen fermentation were observed in treatment groups containing fructose (Golder *et al.,* 2010). The aim of this study was to determine the effects of grain, fructose and histidine on oxidative stress and inflammatory response in dairy cattle during an induced subacute acidosis protocol. To achieve this plasma samples were analysed for advanced oxidation protein products (AOPP), glutathione peroxidase (an endogenous antioxidant), ceruloplasmin (an inflammatory enzyme), antioxidants and free oxygen radicals (oxidants).

Materials and methods Holstein-Friesian heifers (n = 30) were randomly allocated to 5 treatment groups; 1. Control (no grain), 2. Grain (1.2% liveweight (LW) rolled triticale)(GR), 3. Grain (0.8% LW) + fructose (0.4% LW)(FR), 4. GR + histidine (6g/head) (HIS) and 5. FR + HIS in an incomplete factorial design. Feed was withheld for 14 hours before challenge day, on which heifers were fed 200g of lucerne hay, and immediately after their treatment diet. At 5 and 215 minutes after consumption of treatment diets blood was collected in lithium heparin blood tubes via jugular venipuncture and centrifuged at 5^{o}C at 3000rpm for 15 minutes. Advanced oxidation protein products (AOPP) were estimated according to Witko-Sarsat *et al*. (1998). Glutathione peroxidase was estimated by spectrometry according to kit instructions (Cayman, Ann Arbor, Michigan, USA, Item No. 703102). Ceruloplasmin concentrations were determined according to the methods described by Sunderman and Nomoto (1970). Antioxidants were measured using the colourimetric determination of biological antioxidant potential (BAP) test (Diacron International, Grosseto, Italy). Free oxygen radicals were measured using the concentration of reactive oxygen metabolites (ROMs) as determined by a colourimetric assay (d-ROMs Test, Diacron International). The effects of time and treatment group, time by treatment group interaction, means and standard error of the mean were analysed for all variables by a repeated measures ANOVA in PASW Statistics 18 (SPSS Inc., Chicago, Illinois, USA).

Results The interaction between time and treatment group and the effect of treatment group was not significant for any of the measured variables (Table 1). Ceruloplasmin concentrations were greater ($P < 0.001$) 5 minutes after treatment consumption in comparison to 215 minutes (0.19 ± 0.07 and 0.09 ± 0.01 g/L respectively). Concentrations of dROMs were increased at 215 minutes ($P = 0.002$), compared to concentrations 5 minutes after ingestion (123.5 ± 3.3 and 108.7 ± 4.1 U/dL respectively). The ratio of dROMs to BAP was also increased at 215 minutes ($P = 0.009$), compared to concentrations 5 minutes after ingestion (0.041 ± 0.002 and 0.048 ± 0.003 respectively). The effect of time was not significant for AOPP, BAP and glutathione peroxidase concentrations (Table 1).

Table 1 Means, SEM and effects of oxidative stress and inflammatory response markers in a subacute acidosis protocol

	Group						P-value		
Variable	Control	GR	FR	GR + HIS	FR + HIS	SEM	Time (T)	Group (G)	T x G
Ceruloplasmin (g/L)	0.120	0.122	0.160	0.162	0.121	0.180	<0.001	0.266	0.830
AOPP (chloramine-T equivalents)	49.40	48.02	43.89	45.72	48.16	2.94	0.060	0.723	0.076
GSPx (nmol/min/ml)	32.71	32.65	27.57	30.20	30.53	3.24	0.240	0.840	0.757
BAP (µmol/L)	2523.24	2725.34	2646.06	2846.63	2918.74	141.52	0.117	0.107	0.234
dROMs (U/dL)	119.25	114.42	113.5	125.67	122.67	8.08	0.002	0.809	0.619
dROM:BAP	0.053	0.044	0.044	0.045	0.042	0.006	0.009	0.605	0.389

Conclusion The results of this study suggest that changes in ruminal metabolism in this subacute acidosis protocol may not have been sufficient to induce a marked oxidative stress or inflammatory response in these dairy heifers. The observed decrease in ceruloplasmin over time, may reflect a response to challenge, however, increased concentrations would reflect a more typical response. The increase in dROMs at 215 minutes may indicate a mild subacute acidosis. Further investigations into oxidative stress and inflammatory response in dairy cattle challenged with diets high in rapidly fermentable carbohydrates are required and possibly for a more extended time period after challenge.

Acknowledgements This project was funded by Dairy Australia, SBS*cibus* , I&I NSW and The University of Sydney.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Improved growth performance and nutrient digestibility in pre-ruminant calves supplied with bee pollen and its polysaccharides

J-H Yan[2], Y Tu[1], G-F Zhang[1], Q-Y Diao[1], N-F Zhang[1], C-G Jiang[1], L-H Zhao[1]
[1]Feed Research Institute of Chinese Academy of Agricultural Sciences, Beijing, China, [2]Bee Research Institute of Chinese Academy of Agricultural Sciences, Beijing, China
Email: tuyan@mail.caas.net.cn

Introduction Application of natural products and preparations as feed and nutritional supplementation or substitute antibiotics has gained increasing attention in recent years. Among them, honey bee-derived apicultural products, such as pollen, have been applied for centuries in traditional medicine as well as in feed diets and supplementary nutrition due to their nutritional and physiological properties (Linskens *et al*., 1997). One important group of bioactive substances in pollen are its abundant polysaccharides, which are the effective natural and functional dietary feed supplement based upon their nutritional-physiological implications and health promoting effects (Lothar *et al*., 2005). Currently, trials on pigs and chickens showed that polysaccharide substances could improve animal growth and feed utilization (Pan *et al*., 2006). Research about bee pollen polysaccharides focused on extraction process, as well as immune function in animals, but application of bee pollen and its polysaccharides on the performance of calves has not been conducted. This study was conducted to understand the effects of bee pollen and its polysaccharides on calf growth performance and nutrient digestibility and to provide a scientific basis for the advantage and practical guidance of its industrial application.

Materials and methods Twenty-five neonatal Chinese Holstein female calves were randomly allocated to 5 groups, and each group was offered a milk replacer (MR) supplemented with 0 (control, C), 10 (10BP), 25 (25BP) or 50 g (50BP) bee pollen/kg MR, or 5 g (5PS) polysaccharides/kg MR respectively for 63 d. The MR, containing 20.41 MJ digestible energy/kg, 26.16% crude protein (CP) and 15.62% ether extract (EE), was fed at 11.0% of live weight of the calves, and a starter ration was offered *ad libitum* from d 28 thereafter. Average daily gain (ADG), average daily feed intake (ADFI) and feed/gain ratio (F/G) were measured fortnightly. A three-day digestion trial by total collection of feed refusals, feces, and urine was conducted from d 26 to 28 and from d 47 to 49, respectively. The apparent digestibility of dry matter (DM), CP, EE, Ca and total P was calculated. The data were analysed using the GLM procedure of SAS software (version 8, SAS Inst. Inc., Cary, NC).

Results Compared with group C, ADG was significantly higher in the calves from group 25BP or group 5PS (656.6 vs 808.7 or 797.5 g/d, $P<0.05$); F/G was decreased by 12.85% in the calves from group 25BP (1.79 vs 1.56,$P<0.05$); there was no significant differences in ADFI among the groups. The apparent digestibility of DM during 26 to 28 d was increased by 8.38% and 7.66% respectively in the calves from groups 25BP and 5PS (79.02% vs 85.64% and 85.07%,$P<0.05$); the apparent digestibility of CP was increased by 18.63% in the calves from group 25BP (66.35% vs 78.71%, $P<0.05$). No differences in the apparent digestibility of the nutrients were detected among the groups during 47 to 49 d($P>0.05$).

Table 1 Apparent digestibility of nutrients (%)

Items	Age(d)	Treatments				
		C	5PS	10BP	25BP	50BP
DM	21～28	79.02±2.26^{a}	85.07±1.46^{b}	83.51±0.88^{ab}	85.64±2.87^{b}	80.14±3.72^{a}
	42～49	67.56±4.06	73.44±10.60	65.43±2.54	75.20±5.34	68.34±4.86
CP	21～28	66.35±4.48^{a}	75.63±5.40^{ab}	71.22±3.71^{ab}	78.71±7.63^{b}	70.88±10.36^{ab}
	42～49	62.69±6.60	70.07±9.04	65.91±8.48	71.02±8.4□	66.47±5.75
EE	21～28	91.46±6.19	93.89±1.73	91.84±2.64	92.03±2.58	93.16±0.02
	42～49	89.29±3.87	89.40±5.05	90.97±1.26	92.84±3.34	89.38±3.05
Ca	21～28	49.20±6.52	59.34±4.13	62.84±0.97	65.63±9.85	54.02±10.79
	42～49	35.93±7.16	43.98±1.04	47.21±3.09	51.95±2.□5	34.94±3.23
TP	21～28	68.39±6.36	77.17±4.41	74.30±1.32	70.98±8.10	67.81±7.43
	42～49	52.89±6.73	62.59±11.06	62.87±4.45	63.33±5.09	55.64±9.17

Note: Values with the same small letter superscripts in the same line mean no significant difference (P>0.05). Values without superscripts in the same line mean no significant difference (P>0.05).

Conclusions Feeding bee pollen and polysaccharides at 5 g/kg MR to calves increased weight gain, feed conversion rates and apparent digestibility of DM and CP. As a consequence of this positive effect on growth performance and nutrient digestibility of the calves, the optimal amount was 25 g pollen per day .

Acknowledgements This study was supported by fund from basic scientific research of central authorities (Bee pollen polysaccharides extraction and its application in the production of safety animal products. No.0032007224).

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Forage quality as affected by two grazing dairy cows rates in a plain pasture of north eastern Italy

F Rimi[1], M Serena[1,2], S Macolino[1], U Ziliotto[1]
[1]Department of Environmental Agronomy and Crop Production, University of Padova, Legnaro (PD), Italy, [2]Department of Plant and Environmental Sciences, New Mexico State University, Las Cruces (NM), USA
Email: filippo.rimi@unipd.it

Introduction The dairy industry of the Po valley, northern Italy, is mainly based on intensive systems contrasting with the Communitarian Agricultural Policy, which promotes a low-impact system of livestock production. In order to allow an efficient shift to extensive grazing management systems, a deep understanding of the pasture characteristics is needed. Therefore, the aim of the present study was to sudy the effect of different stocking rates on the pre-grazing herbage quality.

Materials and methods The experiment was carried out from April to October 2003 at the experimental farm of Padova University, northeastern Italy, on a plain unirrigated established pasture. The pasture was grazed by Italian-Friesian cows and contained *Festuca arundinacea*, *Dactylis glomerata*, *Lolium perenne*, *Trifolium pratense*, *Lotus corniculatus*, and minor amount of other species. In a rotational stocking system, four paddocks (80 by 60 m) had an average stocking rate of 3.8 cows ha^{-1} (high stocking rate HR) and four had 2.2 cows ha^{-1} (low stocking rate LR). Whitin each paddock, two randomly selected quadrats (150 by 10 cm) were harvested at 5 cm and at ground level before each grazing event (7 days). In the upper 5 cm layer, senescent tissues were separated from the green material and classified as dead forage portion. Plant material was dried at 65°C for 48h and concentrations of CP, NDF, ADF, ADL, ether extract, and ash were determined. Forage nutritive value (UFL = French milk forage units) was also estimated on a dry matter (DM) basis (INRA, 2007) and per unit area, using yield data reported by Carlassare (2004). The experimental design was a split plot with stocking rates as whole plots and date as subplots. The subplot date represented the average date of grazing all four paddocks of both stocking rates. Nutrient concentrations, UFL, and UFL per unit area were statistically analysed using a repeated measures analysis of variance with SAS Proc Mixed (version 9.2; SAS Institute, Cary, NC). Fisher's protected LSD test was used at the 0.05 probability level to identify significant differences among means.

Results Stocking rate and date affected quality of the offered herbage in the 0–5 cm layer and in the green portion of the upper 5 cm layer. In the 0–5 cm layer, a decrease of ADF and ADL and an increase ash content and UFL (0.66 vs 0.69 UFL kg^{-1} DM) were observed as the stocking rate increased. In the green portion of the upper 5 cm layer, the HR paddocks had lower ADF and NDF, higher CP and ether extract contents, and 5.6% more UFL (0.72 vs 0.76 UFL kg^{-1} DM) compared to LR paddocks. Overall, greater quality values were found in correspondence of the spring (April and May) for all the three forage portions studied, regardless of the stocking rates. Moreover, stocking rate, date and their interactions were significant for the forage units per hectare in the 0–5 cm layer and in the dead portion of the upper 5 cm layer. In the 0–5 cm layer, UFL ha^{-1} for the LR were 43% more than for the HR on average, with the greater difference between the two stocking rates observed in July (Figure 1). In the upper 5 cm layer, UFL ha^{-1} of dead material was 78% greater in the LR paddocks on average, with the greater difference between the two stocking rates in October (Figure 1). On average, UFL yield showed a peak in July for the 0–5 cm layer and the green portion of the upper 5 cm layer (Figure 1), and in May for the dead material of the upper 5 cm layer.

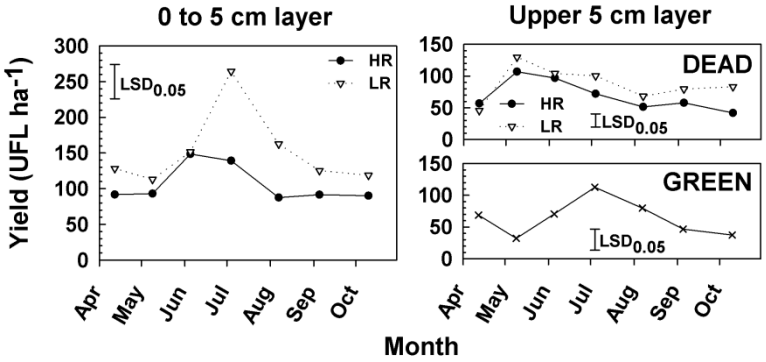

Figure 1 Pre-grazing herbage yield (1 UFL = 7.115 MJ net energy value for milk) in the 0 to 5 cm layer and dead material vs green forage portion in the upper 5 cm layer of a plain pasture under high stocking rate (HR) and low stocking rate (LR)

Conclusions Increasing the stocking rate improved quality of the offered pre-grazing herbage, regardless of the time of grazing. However, a seasonal trend of nutritive value per unit area revealed a large advantage in favour of the lower stocking rate.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Associations between plasma leptin, IgA and food intake in parasite-naive or immune lambs of two genotypes following *T. circumcincta* infection

K Zaralis[1,2], B J Tolkamp[1], I Kyriazakis[3], A R G Wylie[4], J G M Houdijk[1], M Stear[5]
[1]SAC Animal Health Group, Edinburgh, UK, [2]Swedish University of Agricultural Sciences, Skara, Sweden, [3]School of Agriculture, Food and Rural Development, Newcastle University, Newcastle upon Tyne, UK, [4]Agri-food and Biosciences Institute, Belfast, UK, [5]Faculty of Veterinary Medicine, University of Glasgow, Glasgow, UK
Email: konstantinos.zaralis@slu.se

Introduction Genotypic differences in sheep intrinsic capacity for growth are frequently associated with differences in the ability to cope with gastrointestinal infections, with genotypes selected for high growth potential being more susceptible to infection as evidenced by faecal egg counts (FEC) and immune response indicators, such as immunoglobulin A (IgA). The degree of anorexia (i.e. reduction in voluntary food intake) following nematode infection of parasite-naive or immune lambs can also be related to host production potential (Zaralis *et al* 2008). It is possible that nematode infection in lambs results in an increase in plasma leptin concentrations (PLC) but whether elevated PLC is responsible for the occurrence of anorexia (Zaralis *et al* 2008) is unknown. Our study tested the hypotheses that i) the degree of anorexia will be greater in parasite-naive or immune lambs selected more intensively for growth following nematode infection and ii) nematode infection will increase PLC in lambs and positively correlate with IgA response to infection and the degree of anorexia.

Materials and methods Parasite-naive lambs, 48 Suffolk × Greyface (S) and 48 Scottish Blackface (B), were used in experiment I that lasted 10 weeks. Half of S and B lambs were trickle infected with 21,000 L3 larvae of the nematode *Teladorsagia circumcincta* per week and were fed *ad libitum* (INF). Twelve control (not infected) lambs of each genotype were fed *ad libitum* to allow estimation of anorexia in INF lambs (AL). The remaining 12 lambs in each genotype were pair-fed (PF) with 12 lambs of the INF treatment. In experiment II, only the infected lambs from experiment I (24 S and 24 B) were used (after dosing with anthelmintic) and randomly allocated to three treatments of 8 lambs per genotype. Lambs in all treatments were fed *ad libitum* and re-infected (same protocol as experiment I) either 4 weeks (4R) or 8 weeks (8R) after the end of primary infection or not at all (C). In both experiments, animals were fed grass pellets and food intake and FEC were recorded twice weekly. Blood samples were taken weekly and blood plasma was analysed for IgA and leptin concentrations (as described by Zaralis *et al* 2008). All data were analyzed by ANOVA using the MIXED procedure of SAS (SAS 9.1.3; SAS Institute Inc., Cary, NC, USA). Data are reported as least square means and their standard error (SE). Prior to statistical analysis FEC data were logtransformed according to log10(x+1), in order to normalise residuals and are reported as back-transformed means (according to 10^{α}, with $\alpha = \mu + 0.5 \times \sigma^2$).

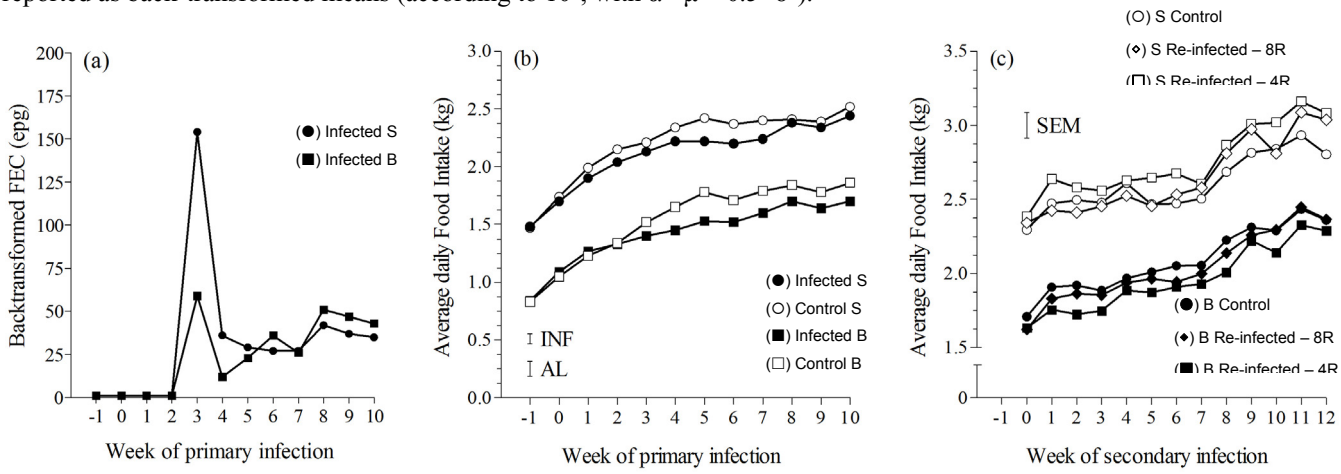

Figure 1 Average weekly faecal egg counts of infected lambs during experiment I (panel a) and average daily food intake during experiment I (panel b) and experiment II (panel c).

Results and conclusions S lambs were more susceptible to primary infection than B lambs, as judged from their higher FEC (panel a) and lower IgA response (data not shown) in experiment I. These differences were less pronounced in experiment II, as both genotypes showed similar and low FEC and broadly similar IgA response. These data support the notion that differences in intrinsic capacity for growth are associated with differences in the ability to cope with infection in growing lambs. The results showed that a primary infection is associated with anorexia in young lambs (panel b); however, the degree of anorexia was not affected by genotype, as hypothesised. Re-infection at 4 or 8 wks after the end of the primary infection did not result in renewed anorexia (panel c). These data suggest that anorexia is associated with the acquisition of immunity in previously naive lambs but not with the expression of immunity in immune lambs. Neither primary nor secondary infection increased leptin concentrations in lambs, even when PLC was accounted for variation in food intake. Therefore, it seems unlikely that leptin is associated with *T. circumcincta*-induced anorexia in growing lambs. The degree of anorexia also did not correlate with the IgA response to infection.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

The effect of casein, branch chain amino acids plus phenylalanine, and non-protein nitrogen on microbial protein production in cattle fed a low quality tropical forage

T Panjaitan[1,2], K Turnbull[2], S Quigley[2], S McLennan[3], T Swain[3], D Poppi[2]
[1]Balai Pengkajian Teknologi Pertanian (BPTP), Nusa Tenggara Barat, Indonesia, [2]Schools of Animal Studies and Veterinary Science, University of Queensland, Gatton, Qld, Australia, [3]Queensland Primary Industries and Fisheries, Department of Employment, Economic Development, Yeerongpilly, Qld, Australia
Email: tanda_panjaitan@yahoo.com

Introduction Cattle consuming forages low in crude protein and digestibility are known to have low microbial protein (MCP) production and a low efficiency of MCP production (EMCP). Branch chain fatty acids (BCFA) are one of the main nutrients produced from the degradation of true protein or branch chain amino acids (BCAA) and are required by rumen microbes for growth and MCP production (Hume *et al*., 1970; Dijkstra *et al*., 1998). Phenylalanine is likely to be the first limiting amino acid for microbial growth in the rumen of steers fed diets low in protein and high in non-protein nitrogen (NPN) (Salter *et al*., 1979). The objective of this experiment was to compare the response of MCP production and EMCP to increasing and high amounts of urea-ammonium sulphate (US), casein (CAS) and BCAA plus phenylalanine in the same proportion and amount as that supplied in the CAS treatments (USAA) with adequate rumen degradable protein (RDP) supplied with US.

Materials and methods Thirteen cannulated *Bos indicus* crossbred steers (219±9 kg liveweight) were randomly allocated to one of three supplement types (CAS, USAA, US) plus a control, in an incomplete latin square design. The experiment was conducted over 3 runs, each of 26 days duration. Each supplement was provided at four increasing intakes, with 1 replicate per supplement intake, plus 1 control replicate per run. The US treatment was supplied in increasing amounts to provide 130, 170, 210 and 250 g RDP/kg DOM. The CAS treatment was supplied in increasing amounts to provide 130, 170, 250 and 300 g RDP/kg DOM. The USAA treatment consisted of US, supplied at 170 g RDP/kg DOM, and the BCAA plus phenylalanine mixture provided in the same proportion and amount as that present in CAS (when supplied to provide 130, 170, 250 and 300 g RDP/kg DOM). The proportion of each amino acid in the total BCAA plus phenylalanine mixture was leucine (33.6%), isoleucine (22.1%), valine (26.2%) and phenylalanine (18.0%). All animals were offered Mitchell grass hay (*Astrebla* spp. 7.2 g N/kg DM, 799 g NDF/kg DM) *ad libitum*. MCP production (urinary purine derivative excretion), EMCP, feed intake and the concentration of ammonia-N, volatile fatty acids (VFA) and BCFA in the rumen were determined. Data were analysed using GenStat by fitting a sequence of linear mixed models to determine an appropriate low order polynomial model to describe the responses to treatment RDP (or BCAA) for each variable. Responses between supplements were then compared and when differences were not significant ($P>0.05$) a common function was fitted.

Results There was no change in MCP production in response to increasing RDP intake from the US supplement (141±12.4 g/d). In contrast, there was a linear increase in MCP production (g/d) in response to increasing RDP intake from the CAS and the USAA supplements ($Y=122+0.24X$; $P<0.001$), with no difference in the response between CAS and USAA. There was no difference in EMCP between CAS, USAA and US and no response in EMCP to increasing RDP intake from US, CAS and increasing BCAA from USAA (102±5.7 g MCP/kg DOM). Intake of Mitchell grass hay (g DM/kg liveweight per d) increased quadratically in response to increasing RDP intake from CAS ($Y=12+0.037X-0.00009X^2$; $P=0.025$). In contrast, there was no increase in intake in response to US and USAA supplements (14±0.5 g DM/kg liveweight per d). Rumen ammonia concentration was 37 mg NH_3-N/L for control animals and increased in a quadratic fashion (Ln NH_3-N mg/L) in response to increasing RDP and BCAA intake ($Y=1.8+0.023X-0.00004X^2$; $P<0.001$) with no difference between CAS, USAA and US. The total concentration of VFA (mM) decreased linearly in response to increasing RDP and BCAA intake from US and USAA, respectively ($Y=67.7-0.031X$; $P=0.006$) but there was no response in VFA to CAS (65.3±1.2). Branch chain fatty acids, as a proportion of total VFA (%), were unchanged in response to increased US intake (1.4±0.2), increased quadratically in response to increasing BCAA intake in the USAA supplement ($Y=10-0.162X+0.0007X^2$; $P<0.001$) and increased linearly in response to increasing CAS intake ($Y=-0.2+0.02X$; $P<0.001$). Retention time of Cr-EDTA in the rumen of steers fed Mitchell grass alone was 26 h and decreased linearly to approximately 20 h in response to increasing RDP intake from CAS ($Y = 26 - 0.016X$; $P<0.001$) but the other supplements had no effect.

Conclusions The supply of BCAA, in combination with adequate RDP for microbial growth, to steers fed forages low in crude protein stimulated MCP production above that of an increase in RDP intake from US alone. The increase in MCP production was similar for USAA and true protein (CAS), suggesting that BCAA and phenylalanine may be first limiting protein degradation products. The EMCP was similar for US, CAS and USAA supplements, suggesting that differences in MCP production arose through changes in intake and that factors other than RDP and BCAA, regardless of source, are required to enhance the EMCP of cattle consuming forages low in crude protein.

Acknowledgements The skilled technical assistance of P. Isherwood, A. Gibbon, L. Gardiner, M. Haliday and J. Kidd is greatly appreciated. This work was funded by Meat and Livestock Australia and the Australian Centre for International Agricultural Research.

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Compared effects of two tannin rich resources on the experimental infections of lambs with *Haemonchus contortus* and *Trichostrongylus colubriformis*

S Sotiraki[1], C Arroyo Lopez[2], F Manolaraki[2], A Stefanakis[1], V Skampardonis[1], N Voutzourakis[1], H Hoste[2]
[1]NAGREF VRI, Thessaloniki, Greece, [2]UMR 1225 INRA/DGER. Ecole Nationale Vétérinaire de Toulouse, Toulouse, France
Email: smaro_sotiraki@yahoo.gr

Introduction Parasitic nematodes of the gastrointestinal tract remain major infectious diseases which affect livestock's health, welfare and production because of the major economic losses, clinical signs and possible deaths that they provoke. Up to now, the main mode of control of these parasitic diseases relied on chemical anthelmintics (AHs). However, AH resistance within worm populations is now a worldwide phenomenon. Therefore, there is now a strong impetus for research on alternative approaches to AH drugs. Amongst those, some results underlined the efficacy of some bioactive tannin-rich (TR) plants used as nutraceuticals (Hoste *et al*, 2006; Manolaraki *et al*, 2010). The aim of the current study was to compare the AH effects of feeding experimentally infected lambs with two TR resources on different biological traits of both an abomasal and an intestinal nematode species.

Materials and methods Sixty four lambs composed 4 experimental groups (8 male and 8 female per group) depending on their feeding or AH treatments. On day-7, one week prior the experimental infection with nematodes, each group of lambs received the specialized diet *ad libitum*. The feed regimes were isoenergetic and isoproteic and included lucerne hay for the negative control (C group) and treated control group (T) and either crushed carob fruits (CAR) or sainfoin hay (S) for the two other groups. On day 0, all the animals began to be trickle infected with a mixture of 1000 infective larvae (L3) of *Haemonchus contortus* and 700 L3 of *Trichostrongylus colubriformis* larvae per week for 6 weeks. The AH treated group received an albendazole drench on Day -7. Body condition scores were recorded (weekly) throughout the trial and bodyweights measured on D0 and D63. Moreover, blood (every fortnight) and faecal (weekly) samples were performed to measure respectively packed cell volume (PCV) and faecal egg counts (FECs). The male lambs were slaughtered on D63 and the gastro intestinal tracts were taken to measure the worm burdens and their fertility in the abomasums and intestines. The statistical comparisons were performed by use of either analysis of variance on repeated measurements (for FECs and PCVs) or relying on a one way analysis of variance completed by the post hoc Bonferroni test for the worm number and fertility values. The values of FEC and worm number were (log +1) transformed before analysis

Results The statistical analyses of the parasitological data indicated significant difference ($P < 0.05$) in the egg excretion in the CAR and S groups compared to the C and T groups (See figure 1).

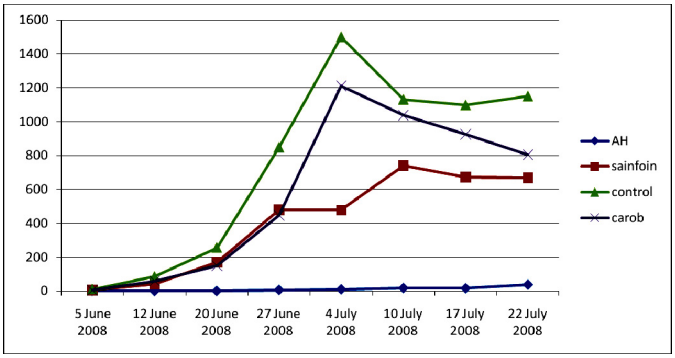

Figure 1 Faecal egg excretion in the 4 experimental groups depending on the feeding regimes

The total numbers of worms (*Haemonchus* + *Trichostrongylus*) found at necropsy per group were respectively 72 (Hc: 26; Tc: 46) in group T; 295 (Hc: 185 ; Tc:110) in group S; 442 (Hc: 257 ; Tc: 185) in group CAR; 556 (Hc: 335; Tc: 221) in the control group. The number of worms in the treated group differed from the 3 others. A trend ($P< 0.11$) was found for a reduced total number in the S group compared to the Controls. No significant difference were found in the number of *H. contortus* between the 3 non treated group but a significant reduction ($P < 0.05$) was measured in the number of *T. colubriformis* in the S vs the C group. In contrast, no significant differences were observed in the worm fertility of both nematode species depending on the feeding regime.

Conclusions These results tend to confirm 1) an effect of the distribution of both tannin rich resources on the egg excretion and subsequent pasture contamination and 2) an effect of sainfoin on the number of worms, particularly on *T. colubriformis.* This might be explained by a reduced establishment of larvae or an increased expulsion of adult worm. Last the lack of difference in worm fertility is surprising but might be due to a transient effect which is restored after the end of distribution of tannins in the diet.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Megasphaera elsdenii addition to *in vitro* cultures of equine caecal microorganisms fed oligofructose or starch

H Leventhal, T Slough, S Uwituze, J Drouillard
Kansas State University, Manhattan, KS, USA
Email: hrlvet@ksu.edu

Introduction Laminitis is a debilitating disease of the foot that affects thousands of equines each year (USDA, 2000). Excessive or rapid ingestion of starches or fructans can lead to rapid proliferation of opportunistic $Gram^+$ bacteria in the hindgut. Some $Gram^+$ bacteria produce large quantities of lactic acid, the accumulation of which can induce a cascade of inflammatory events that manifest as laminitis (Bailey, 2004). *Megasphaera elsdenii* (ME) is a bacterium that metabolizes lactic acid into volatile fatty acids (VFAs), and thus may be useful as a probiotic to prevent laminitis. The goal of this study was to determine if inoculating *in vitro* cultures of mixed caecal organisms with ME could influence fermentation profiles, substrate disappearance, and VFA and lactate accumulation.

Materials and methods Study 1 was a randomized complete block experiment with a 2 x 2 factorial treatment arrangement. Factor 1 was carbohydrate source, consisting of oligofructose (OF) or corn starch (CS). Factor 2 consisted of the level of added ME (strain KS249), which was isolated from a horse and propagated in a semi-defined lactate medium at pH 5.5 to a final density of 1 X 10^8 CFU/mL. Four mature American Quarter Horses (529 $\pm$70 kg BW; 6 yr old) fed Smooth Bromegrass (*Bromus inermis*) hay were used as donors of caecal inoculum for *in vitro* cultivations. Digesta was collected via the caecal cannulae, strained through 4 layers of cheesecloth, and allowed to stratify in separatory funnels under a stream of N_2. The particulate-free fluid layer was mixed with buffer (Russell and Martin, 1984) in a 1:2 ratio, and adjusted to pH 7.0. Buffered caecal contents (140 mL) were placed into Ankom RFS gas production vessels containing one of 4 treatments: CS; CS+ME; OF; OF+ME. For ME treatments, 1 mL of fresh culture was added to each vessel with 2.5 g of the respective substrate. The process was repeated for each horse, providing 4 observations, which were repeated for 2 days. Vessels were incubated in a 39º C water bath for 48 h and gas production was monitored. After 48 h, composition of gas within each vessel was analyzed by gas chromatography. Four mL of liquid were combined with 1.0 mL of m-phosphoric acid for VFA and lactate analysis, and the remaining contents were dried at 105ºC for 24 h to determine *in vitro* dry matter disappearance (IVDMD). Data were analysed using the Mixed procedure of SAS (2004) with fixed effects of carbohydrate source, ME level, time, and all 2- and 3-way interactions. Random effects included day and animal. A second *in vitro* experiment was conducted using the same treatments, but in 50- mL culture tubes. Incubation tubes containing 0.5 g substrate and 20 mL buffered caecal contents were terminated after 0, 3, 6, 9, 12, 15, 18, 21, 24, 30, 36, 42, and 48 h, after which final pH, concentrations of lactate and VFA, and IVDMD were determined.

Results In study 1, adding ME to cultures of mixed caecal organisms fed OF or CS increased total gas production ($P < 0.01$). Gas production ceased after only 8 hours of fermentation in cultures fed OF without ME, whereas other cultures remained active for 48 hours. Total gas production was greater for cultures fed CS compared to OF ($P < 0.01$), and addition of ME to cultures increased rate and total amount of fermentative gasses with either substrate ($P < 0.01$). No interaction was evident between carbohydrate and ME ($P > 0.05$) for the amount of gas produced. There was no evidence for an interactive effect between substrate and ME for final concentration of H_2S or CO_2 ($P > 0.05$). Cultures fed CS yielded 9-fold greater concentrations of H_2S compared to those fed OF ($P < 0.01$), but ME had no effect on H_2S production ($P > 0.7$). Differences in IVDMD were consistent with effects of treatment on gas production in that there was a greater disappearance amongst cultures fed CS as compared to cultures fed OF (substrate effect, $P < 0.01$; ME effect, $P < 0.05$). Cultures fed OF yielded greater concentrations of lactate compared to CS (55 vs 3 mM for OF and CS, respectively; $P < 0.01$). Adding ME to cultures decreased lactate concentration compared to cultures without (37 vs 21 mM for OF and CS, respectively; $P < 0.05$). Final pH of cultures were 4.5, 5.3, 5.2, and 5.3 for OF, OF+ME, CS, and CS+ME, respectively, suggesting the buffer system was overwhelmed with OF in the absence of ME. In study 2, cultures of OF yielded greater lactate, less total VFA, and greater DM disappearance compared to cultures fed CS ($P < 0.05$). Adding ME did not influence lactate, VFA, or IVDMD ($P > 0.05$). Culture pH remained higher for CS than OF ($P < 0.01$), and was not influenced by ME ($P > 0.05$).

Conclusions Adding *Megasphaera elsdenii* to mixed cultures of caecal microorganisms was helpful in attenuating severe pH depression of cultures fed OF, and improved overall fermentative activity *in vitro*. We feel these experiments provide evidence to support further evaluation *in vivo* to determine if ME has utility as a preventative for lactic acidosis in horses consuming lush pastures or appreciable quantities of starch-based feeds.

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Ingestive behaviour changes associated to grain or hay supplementation to Hereford steers grazing a Ryegrass pasture during winter

V Beretta[1], A Simeone[1], J Elizalde[2], O Bentancur[1], A Contatore[1], D Rodríguez[1], M Vago[1]
[1]Universidad de la Republica, Paysandu, Uruguay, [2]Consultant, Rosario, Argentina
Email: beretta@fagro.edu.uy

Introduction Cattle grazing temperate winter lush pastures experience low liveweight gains. This response has been attributed to low pasture dry matter (DM) content which would reduce effective DM intake (Simeone *et al.* 2002), mainly through a reduction in bite mass (Gibb *et al.* 1998). Supplementing with roughage or grains have been proposed as alternatives to overcome these constraints. The aim of this experiment was to characterize the ingestive behaviour of Hereford steer grazing a Ryegrass pasture under different supplementation strategies to help understand animal response and design improved managements.

Material and methods The study was conducted at the Experimental Station "M. A Cassinoni" in Uruguay, (32º S, 58º W) during winter 2006 (June 28 to August 31), on 23 hectares of *Lolium multiflorum* pasture naturally re-seeded. Thirty-six Hereford steers (371±37.0 kg liveweight, LW) were randomly allocated to 12 groups to receive one of four treatments (T, n= 3/T; 4 steers/replicate): control without supplementation (C); restricted (HR; 0.25 kg DM/100 kg LW) or *ad libitum* (HAD) supplementation with grass hay (*Setaria italica*); or supplementation with ground sorghum grain (SG; 1 kg DM/ 100 kg LW). Each replicate rotative grazed independent paddocks, 7-days strips with a mean daily forage allowance of 5 kg DM/ 100 kg LW. Supplements were offered daily at 8:00 am in the grazing paddock. Pre-grazing pasture biomass (kg DM/ha) and height were registered weekly to characterized grazing conditions. Random pasture samples were cut, oven dried and analyzed for DM, crude protein (CP), NDF and ADF content. On weeks 2, 4, 6 and 8, during days 2, 4 and 6 of each week, grazing activity, visits to supplement troughs, rumination and idling were recorded by visual appraisal, every 10 min during day time (8:00 am to 6:30 pm) on one animal per replicate. Bite rate was registered every two hours, during the same period, as number of bites in one minute. On same dates, to characterize pasture utilization and defoliation pattern, sward height was measured pre-grazing and every 24 hours after cattle entered a new 7-day grazing strip. Records were taken with a ruler at 50 random points per pasture replicate. Behaviour data was submitted to LOGIT transformation assuming binomial **distribution and a**nalysed through a generalised linear model as follows: $Ln(p/(1-p)) = \beta_0 + T_i + W_j + (TW)_{ij} + D(W)_j$; where Ln(p/1-p) is the probability to find an animal doing a specific activity, and D is day effect within week (W). Bite rate and pasture data were analysed through a generalised model for repeated measurements: $Y_{ijk}=\mu + T_i + \varepsilon_{ij} + W_k + (TW)_{ik} + \varepsilon_{ijk}$.

Results Average pre-grazing pasture biomass was 2854±65.7 kg DM/ha and 20.9±0.61 cm height, with 30.3% dead forage. As fed forageDM was 21%. On a DM basis, offered pasture had 10.3% CP, 50.3% NDF and 20.0% ADF. Results on ingestive behaviour are presented in Table 1.

Table 1 Probability of occurrence of different behaviour activities under different treatments

	Treatments					Effects			
	C	HR	HAD	SG	SE	T	W	T*W	D(W)
Grazing	0.47 a	0.44 ab	0.41 ab	0.39 b	0.015	*	ns	*	***
Ruminating	0.17	0.18	0.20	0.14	0.012	ns	*	ns	***
Idling	0.36a	0.31b	0.31b	0.39a	0.015	*	ns	ns	***
Trough visits	---	0.07	0.08	0.08	0.007	ns	*	ns	*
Bite rate (bites/min)	47 b	51 ab	52 a	48 ab	0.97	*	ns	ns	***

* (P<0.05) ** (P<0.01) *** (P<0.001), ns (P>0.05). SE: standard error

Supplementing with SG did not affect bite rate but it reduced grazing activity compared to C treatment. This response depended on W; however, no clear association was observed between changes in pasture condition with W and animal behaviour. Hay supplementation did not modify grazing activity compared to C, independent of hay level, but it reduced idling and increased grazing bite rate in HAD steers. Sward utilization did not vary between treatments (P>0.05); residual sward height, after steers were moved to a new strip, and mean daily defoliation rates were only affected by W (P<0.01) and pre-grazing sward height (P<0.01). Results show that, while HR just reduced idling in substitution of time spent at hay trough, steers with *ad libitum* access to hay needed to increase bite rate to keep similar sward utilization. On the other hand, grain supplemented steers reduced grazing activity without affecting bite rate, so it is probable that similar pasture utilization compared to C was due to heavier bite mass.

Conclusions Steers supplemented with roughage or sorghum grain during first grazing of a Ryegrass pasture modify ingestive behaviour without affecting pasture utilization. Adaptation of foraging strategy varies depending on type and quantity of supplement.

Acknowledgments To the Comision Sectorial de Investigacion Cientifica, University of the Republic, for financial support.

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Crude *vs.* refined glycerol supplementation to dairy cows in early lactation – effects on dry matter intake, lactation performance and metabolism

A Werner Omazic[1], J Bertilsson[1], M Tråvén[2], K Holtenius[1]
[1]Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management, Uppsala, Sweden, [2]Swedish University of Agricultural Sciences, Department of Clinical Sciences, Uppsala, Sweden
Email: anna.omazic@slu.se

Introduction The increasing production of biodiesel has improved the availability of glycerol, a high energetic and glucogenic by-product, to farmers at a low cost as an option feed for dairy cow. Glycerol supplementation to dairy cows has mainly been studied in early lactating cows. However, results are contradictive since both positive and negative responses on feed intake, milk yield and rumen metabolism have been observed (Carvalho *et al*. 2011, De Frain *et al*. 2004 and Donkin *et al*. 2009). Both crude and refined glycerol has been used in previous studies but there is a lack of comparative studies including both products. The aim of the present experiment was to evaluate possible differences between crude and refined glycerol as a feed supplement for dairy cows in early lactation and to investigate how glycerol supplementation affects energy balance and milk yield. Furthermore, the aim was to investigate if the bad taste and foul smell of crude glycerol affected feed intake.

Materials and methods The study was performed on 32 dairy cows of the Swedish Red Breed during two six months periods in spring 2008 and 2010. The experiment started two days after calving and ended four weeks *post partum*. The cows were allocated according to expected day of calving, and randomly assigned to one of three different dietary treatments, consisted of 0.5 kg crude glycerol (88.1%)/d, 0.5 kg refined glycerol (99.5%)/d and a control group without addition of extra energy supplementation. Silage and concentrates were fed separately and distributed four times/d. Glycerol was fed in equal amounts twice/d poured on top of the concentrate. Feed refusals were recorded and the amount of silage was adjusted daily to ensure *ad libitum* consumption with approximately 5 to 10% feed refusals. Silage and concentrate samples were collected, once weekly and once every two weeks, respectively. Calculation of energy balance was performed as the difference between the measured metabolized energy (ME) intake and the ME requirement for maintenance and milk production. Milk yield was recorded and samples for milk composition analysis were obtained at four consecutive milkings per week. Blood samples were drawn from the tail vein once a week. Body weight (BW) and body condition score (BCS) were registered, at start and end of the experiment. The data were analyzed using the MIXED procedure of SAS (SAS 9.2, SAS Institute 2008) and first order autoregressive covariance structure was used for measurements within cow. The fixed effects included in the models were treatment group, year, week and parity group, and all 2-way interactions.

Results Glycerol intake did not affect total dry matter (DM) intake. Average silage intake was 10.6 kg DM/d and average concentrate intake was 6.8 kg/d for all treatments. Feeding glycerol did not increase milk yield, kg ECM/d (P=0.24) relative to control group (Table 1). Milk composition, e.g. fat, protein and lactose, were not affected by treatment. A tendency (P=0.06) existed for enhanced energy balance in cows fed crude glycerol, compared to refined glycerol and control group. BW and BCS did not differ among treatments (Table 1). There was a tendency (P=0.08) for increased concentration of glucose in plasma from cows fed crude glycerol, compared to refined glycerol and control group. There were no differences due to treatments in concentration of glycerol, insulin and NEFA.

Table 1 Effect of glycerol on milk yield, feed intake, energy balance, BW change and glucose concentrations, least squares means ± standard error of the mean

	Treatment[1]		
Item	Refined	Crude	Control
Silage, kg DM/d	10.2±0.47	10.6±0.47	9.8±0.50
Milk yield, kg ECM/d	35.4±1.5	32.1±1.5	32.9±1.6
Energy balance, MJ/d	-55.5±7.6	-29.5±8.3	-52.0±8.3
BW change, kg	-46.8±15.7	2.2±17.2	-14.6±17.2
Glucose, mmol/L	2.41±0.13	2.79±0.14	2.45±0.15

[1]Treatments: Refined=0.95% glycerol; Crude=88.1% glycerol, 9.3% water, 0.9% ash and 0.8% methanol; Control=without addition of extra energy supplementation to the diet.

Conclusions Glycerol intake had no effect on total DM intake during the first four weeks of lactation. Obviously the bad taste and foul smell of raw glycerol did not affect the feed intake. Furthermore, energy balance tended to be improved and glucose concentration tended to increase in cows fed crude glycerol. This result creates favourable conditions for using crude glycerol as an energy supplementation since the process to get refined glycerol is more expensive. Milk yield did not differ among treatments, although the result indicate that intake of refined glycerol may result in increased milk yield.

Acknowledgments Financial support from the AarhusKarlshamn, Sweden, is gratefully acknowledged.

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Effect of two different types of tannins on the biohydrogenation steps of polyunsaturated fatty acids in the rumen: an *in vitro* study

M Antongiovanni, A Buccioni, S Rapaccini, S Minieri, M Mele
University of Florence, Firenze, Italy
Email: manto_1939@libero.it

Introduction Tannin containing feeds are known to have an influence on the composition of rumen microbial population and, as a consequence, on the overall rumen metabolism. Several studies have been dealing with condensed tannins (Vasta *et al.,* 2008), widely distributed in some shrubs, legume seeds and pods and agricultural by-products, but little is known about the nutritional characteristics of hydrolysable tannins in ruminants. The aim of the present study was to compare the condensed tannin from quebracho (*Schinopsis lorentzii*) with the hydrolysable tannin extracted from chestnut wood (*Castanea sativa Miller*) in terms of their influence on the biohydrogenation steps of linoleic (LA) and □-linolenic (LNA) acids in the rumen, by means of an *in vitro* approach.

Materials and methods The mixed feed, representing a previously tested sheep diet, consisted of wheat straw (300 g/kg DM), maize gluten feed (300 g/kg DM), maize meal (152 g/kg DM), soybean meal (132 g/kg DM), barley meal (96 g/kg DM) and a mineral-vitamin premix (20 g/kg DM). Samples of this feed have been referred to as the control ones. The treated ones were the same, but with tannins added: one from quebracho (*Schinopsis lorentzii*), containing 456 g/kg DM tannic acid and the second from chestnut (*Castanea sativa Miller*), containing 750 g/kg DM tannic acid. The added tannins were adjusted in order to introduce into the feed samples the same amounts of tannic acid at two levels: 49 and 82 g/kg DM. The feed samples (2 g DM), in triplicate, were fermented in glass vessels fitted with pressure valves, using an inoculum prepared from the rumen content of 4 donor ewes, after Cone *et al.* (1996). Solid associated bacteria (SAB) and liquid associated bacteria (LAB) were separated (Martin *et al.,* 1994) and analysed for fatty acids by gas-chromatography (Buccioni *et al.,* 2010). The samples to be analysed were collected at 3 fermentation times: 6, 12 and 18 hours. Data were statistically processed by GLM of SAS with diet and fermentation time as the fixed factors of the linear model.

Results Since odd branched chain fatty acids are a product of bacterial metabolism in the rumen (Vlaemink *et al.,* 2006), branched C15:0 have been observed as an evidence of bacterial activity due to the presence of tannins. Table 1 clearly demonstrates that both tannins increased significantly ($P<0.05$) the branched C15:0 production, confirming that the activity of bacteria was enhanced, even at the lower concentration level. Table 2 depicts the ongoing of the two most important steps of the rumen biohydrogenation of LA and LNA: the CLA rumenic acid (C18:2 cis9 trans 11) and vaccenic acid (C18:1 trans11), both greatly beneficial to the consumers' health when transferred into milk. Again, the presence of both tannins exerted a significant influence on the intermediate steps, in particular on the yield of vaccenic acid, possibly convertible back into rumenic acid by the □9 desaturase of the host animal.

Table 1 C15:0 iso and C15:0 anteiso acid in SAB at increasing fermentation times

	6 h	12 h	18 h	SEM		6 h	12 h	18 h	SEM
C15:0 iso					C15:0 anteiso				
control	0.26	0.19a	0.20a	0.02	control	0.34a	0.44a	0.37a	0.03
Chestnut 49	0.24	0.33b	0.33b		Chestnut 49	0.31a	0.50a	0.55c	
Chestnut 82	0.27	0.37b	0.37b		Chestnut 82	0.40	0.85b	0.66b	
Quebracho 49	0.23	0.32b	0.34b		Quebracho 49	0.44b	0.55d	0.65b	
Quebracho 82	0.22	0.37b	0.37b		Quebracho 82	0.39	0.68c	0.49c	

Table 2 Rumenic acid and vaccenic acid in SAB at increasing fermentation times

	6 h	12 h	18 h	SEM		6 h	12 h	18 h	SEM
C18:2 cis9 trans11 (rumenic acid)					C18:1 trans 11 (vaccenic acid)				
control	0.56a	0.64a	0.27a	0.01	control	2.02a	3.03a	3.45a	0.02
Chestnut 49	0.57a	0.90b	0.88b		Chestnut 49	2.14e	5.62b	5.58b	
Chestnut 82	0.51b	0.73c	0.70c		Chestnut 82	3.94b	4.13d	4.14c	
Quebracho 49	0.41c	0.41e	0.56d		Quebracho 49	2.53d	4.21c	4.19c	
Quebracho 82	0.44c	0.45d	0.92b		Quebracho 82	2.62c	4.14d	4.16c	

Conclusion As a feed additive to the diets of ruminants, the hydrolysable chestnut tannin appeared preferable to the condensed tannin from quebracho because capable of inducing higher yields of vaccenic acid, even at the lower concentration level.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

The potential of grass silages with contrasting polyphenol oxidase activity versus red clover to protect C18 polyunsaturated fatty acid across the rumen of beef steers

M R F Lee, V Theobald, N Gordon, J K S Tweed, R Fychan, N D Scollan
Aberystwyth University, IBERS, Aberystwyth, UK *Email: michael.lee@aber.ac.uk*

Introduction Polyphenol oxidase (PPO) in red clover has been shown to reduce lipolysis in silo and the rumen (Van Ranst *et al*. 2011). Lee *et al*. (2006) showed *in vitro* that grass PPO resulted in a reduction in plant mediated lipolysis similar to that observed in red clover. An *in vitro* rumen simulation (Lee *et al*. 2011) indicated that cocksfoot PPO resulted in a lower degradation of N and a trend towards lower levels of lipolysis *in vitro*. However, it is yet to be determined whether grass PPO has any effect on lipolysis *in vivo*. Therefore this study will investigate the potential of two grass silages with contrasting levels of polyphenol oxidase (cocksfoot (*Dactylis glomerata*; high PPO and perennial ryegrass (*Lolium perenne*; Intermediate PPO) and red clover (*Trifolium pratenses*) to protect polyunsaturated fatty acids across the rumen.

Materials and methods Six Hereford × Friesian steers (*c.* 450 kg), prepared with rumen and duodenal cannulae were allocated at random to one of three big bale silages: cocksfoot (CF); perennial ryegrass (PRG) or red clover (RC). All diets were offered at 16 g DM/kg live weight with the experiment consisting of two 3 × 3 Latin Squares. Each period was 21 d consisting of 14d adaptation to the diet, 4 d faecal collection, 2 d duodenal sampling and 1 d for rumen sampling. Digesta flow at the duodenum was estimated using a dual phase marker system with $Yb(CH_3COO)_3$ and Cr EDTA as particulate and liquid phase markers, respectively**.** Lipid extraction and GC analysis was as described by Lee *et al.* (2011). Statistical analysis was undertaken using ANOVA, blocking according to period + animal (Genstat 11.1).

Results All silages were well preserved with mean dry matter (DM) of 34.4, 55.3 and 45.4% for CF, PRG and RC, respectively. PPO activity in the silages was low due to deactivation but was higher (P<0.05) in CF than either PRG or RC, 0.15, 0.05 and 0.08 ukatal/g DM, respectively. Protein bound phenol (mg/g DM) as a measure of the degree of oxidation and therefore PPO protection was as expected: highest on RC (15.9), lowest on PRG (10.1) with CF intermediate (12.2). DM and fatty acid intake along with fatty acid flow and biohydrogenation are reported in Table 1. As animals did not consume all offered forage on the grass silage diets there were differences in intake with subsequent differences in fatty acid flow to the duodenum. Flow of C18:0 was comparable across treatments with highest flows of C18:1 trans, CLA and C18 PUFA on RC with PRG intermediate and CF the lowest. Biohydrogenation of C18 PUFA was significantly lower on RC compared to the two grass silages with CF higher than PRG.

Table 1 Effect of grass silage with contrasting PPO levels versus red clover silage on intake and ruminal fatty acid metabolism

	CF	PRG	RC	s.e.d	P value
DM Intake (kg/d)	5.87^{a}	6.58^{b}	7.55^{c}	0.197	***
Fatty acid intake (g/d)					
C18:0 stearic	1.52^{a}	2.62^{b}	4.04^{c}	0.150	***
C18:2*n-6* linoleic	16.6^{a}	21.0^{b}	32.5^{c}	0.84	***
C18:3*n-3* linolenic	43.5^{a}	55.1^{b}	59.6^{b}	2.21	***
Total fatty acids	93.4^{a}	119^{b}	144^{c}	4.71	***
Duodenal flow (g/d)					
C18:0 stearic	71.1	75.7	77.1	4.48	NS
C18:1 trans	8.97^{a}	12.6^{ab}	13.5^{b}	0.73	***
C18:2 CLA	0.22^{a}	0.26^{a}	0.79^{b}	0.027	***
C18:2*n-6* linoleic	1.55^{a}	2.54^{b}	7.22^{c}	0.290	***
C18:3*n-3* linolenic	2.43^{a}	4.44^{b}	13.5^{c}	0.527	***
Total fatty acids	148^{a}	160^{a}	187^{b}	8.1	**
Biohydrogenation (%)					
C18:2*n-6* linoleic	90.6^{c}	87.9^{b}	77.9^{a}	0.72	***
C18:3*n-3* linolenic	94.4^{c}	92.1^{b}	77.6^{a}	0.43	***

Values with different superscripts (^{abc}) differ significantly (P<0.01).

Conclusions As previously reported RC resulted in a lower biohydrogenation of C18 PUFA than grass silages. CF with its higher levels of grass PPO did not result in elevated levels of C18 PUFA escaping the rumen over the control PRG with lower levels of PPO. This may suggest that grass PPO has limited potential in improving lipid profiles within ruminant products, although other factors between the grasses can not be ignored within the present study, which may have contributed to the observed response.

Acknowledgement The work was funded by DEFRA, EBLEX, QMS and HCC under the project ProBeef

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The influence of grass silages with contrasting polyphenol oxidase activity versus red clover on rumen ammonia and the flow of amino acids to the duodenum in beef steers

M R F Lee, V Theobald, N Gordon, J K S Tweed, R Fychan, N D Scollan
Aberystwyth University, IBERS, Aberystwyth, UK *Email: michael.lee@aber.ac.uk*

Introduction Polyphenol oxidase (PPO) in red clover has been shown to reduce proteolysis in silo and the rumen (Lee *et al*. 2007). Lee *et al*. (2006) showed *in vitro* that grass PPO resulted in a reduction in plant mediated proteolysis similar to that observed in red clover. An *in vitro* rumen simulation (Lee *et al*. 2011) indicated that cocksfoot PPO resulted in a lower degradation of protein as indicated by reduced ammonia-N. However, it is yet to be determined whether grass PPO has any effect *in vivo*. Therefore this study investigated the potential of two grass silages with contrasting levels of PPO (cocksfoot (*Dactylis glomerata*; high PPO and perennial ryegrass (*Lolium perenne*; Intermediate PPO) and red clover (*Trifolium pratenses*) to influence N metabolism.

Materials and methods Six Hereford × Friesian steers (*c.* 450 kg), prepared with rumen and duodenal cannulae were allocated at random to one of three big bale silages: cocksfoot (CF); perennial ryegrass (PRG) or red clover (RC). All diets were offered at 16 g DM/kg live weight with the experiment consisting of two 3 × 3 Latin Squares. Each period was 21 d consisting of 14d adaptation to the diet, 4 d faecal collection, 2 d duodenal sampling and 1 d for rumen sampling. Digesta flow at the duodenum was estimated using a dual phase marker system with $Yb(CH_3COO)_3$ and Cr EDTA as particulate and liquid phase markers, respectively. Statistical analysis was undertaken using ANOVA, blocking according to period + animal (Genstat 11.1).

Results Difference in intake were due to animals not eating their whole ration. All silages were well preserved with mean dry matter (DM) of 34.4, 55.3 and 45.4% for CF, PRG and RC, respectively. Protein bound phenol (mg/g DM) as a measure of the degree of oxidation and therefore PPO protection was: highest on RC (15.9), lowest on PRG (10.1) with CF intermediate (12.2). Rumen ammonia concentration was highest on RC with no difference between the grasses: 124, 103 (PRG) and 98 (CF) mg NH_3-N/L. Intake of DM, N and amino acids with the exception of Met followed the same pattern RC>PRG>CF. Likewise duodenal flow followed the same pattern with the exception of Gly and Tyr which were not different between the grasses (Table 1).

Table 1 Effect of grass silage with contrasting PPO levels versus red clover silage on intake and duodenal flow of amino acids

	CF		PRG		RC		s.e.d		P value	
	Intake	Duodenal Flow	Intake	Duodenal Flow	Intake	Duodenal Flow	I	DF	I	DF
Dry Matter (kg/d)	5.87^{a}	4.47^{A}	6.58^{b}	5.11^{B}	6.58^{c}	5.80^{C}	0.196	0.219	***	***
Total Nitrogen (g/d)	112^{a}	121^{A}	133^{b}	161^{B}	198^{c}	205^{C}	5.0	10.3	***	***
Ala	30.5^{a}	27.1^{A}	36.8^{b}	38.2^{B}	47.6^{c}	50.1^{C}	1.18	3.62	***	***
Arg	10.1^{a}	20.0^{A}	24.3^{b}	28.0^{B}	40.9^{c}	44.6^{C}	1.41	2.97	***	***
Asp	27.9^{a}	46.6^{A}	50.6^{b}	65.5^{B}	105^{c}	91.1^{C}	2.90	6.52	***	***
Glu	24.6^{a}	47.8^{A}	38.1^{b}	67.4^{B}	75.1^{c}	95.6^{C}	2.05	6.68	***	***
Gly	19.4^{a}	28.5^{A}	26.8^{b}	37.7^{A}	38.4^{c}	57.4^{B}	0.99	4.95	***	***
His	7.08^{a}	8.88^{A}	11.0^{b}	11.9^{B}	18.6^{c}	19.9^{C}	0.523	1.27	***	***
Ile	19.6^{a}	23.2^{A}	26.3^{b}	32.7^{B}	42.6^{c}	47.3^{C}	1.04	3.20	***	***
Leu	33.1^{a}	33.6^{A}	45.3^{b}	47.2^{B}	70.2^{c}	72.9^{C}	1.80	4.58	***	***
Lys	16.6^{a}	30.2^{A}	30.3^{b}	42.2^{B}	50.2^{c}	59.9^{C}	1.56	4.20	***	***
Met	3.51^{a}	9.45^{A}	8.85^{b}	13.8^{B}	4.10^{a}	16.6^{C}	0.565	1.28	***	**
Phe	20.8^{a}	25.2^{A}	30.0^{b}	35.5^{B}	45.5^{c}	52.2^{C}	1.31	3.81	***	***
Pro	24.3^{a}	19.9^{A}	34.0^{b}	26.7^{B}	58.2^{c}	40.8^{C}	1.32	2.80	***	***
Ser	10.7^{a}	18.4^{A}	22.7^{b}	25.1^{B}	38.5^{c}	37.9^{C}	1.14	2.38	***	***
Thr	16.1^{a}	22.8^{A}	25.9^{b}	31.2^{B}	39.4^{c}	43.1^{C}	1.04	2.98	***	***
Tyr	7.28^{a}	18.7^{A}	15.5^{b}	25.4^{A}	27.0^{c}	36.8^{B}	0.841	3.16	***	***
Val	24.2^{a}	24.1^{A}	32.4^{b}	33.8^{B}	50.5^{c}	50.4^{C}	1.25	3.37	***	***
ΣAA DF / ΣAA I (g/g)	1.32^{a}		1.23^{a}		1.08^{b}		0.102		†	

Values with different superscripts (^{abc} for intake (I) and ^{ABC} for duodenal flow (DF)) differ significantly.

Conclusions Rumen ammonia-N was comparable between CF and PRG despite the greater intake of N on PRG suggesting little protective effect of grass PPO on protein. The ratio of DF:I for amino acids was also comparable for CF and PRG. RC resulted in a higher rumen ammonia-N concentration as a result of a greater N intake and a trend for lower DF:I amino acid ratio.

Acknowledgement The work was funded by DEFRA, EBLEX, QMS and HCC under the project ProBeef

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Correlations between phenolic fractions in tropical forages and ruminal C18 fatty acid metabolites *in vitro*

A Jayanegara, M Kreuzer, F Leiber
ETH Zurich, Institute of Agricultural Sciences, Zurich, Switzerland
Email: anuraga.jayanegara@inw.agrl.ethz.ch

Introduction Increasing the contents of α-linolenic acid (C18:3 *n*-3) and conjugated linoleic acids (CLA) in ruminant products is desirable due to their beneficial effects on human health. Quite a large proportion of dietary linoleic acid and α-linolenic acid undergo biohydrogenation processes in the rumen by the action of ruminal microorganisms which results in various intermediate fatty acids (FA) including CLA (mainly *c*9,*t*11-C18:2; Chilliard *et al*. 2007). Several feeding strategies have been proposed in order to increase α-linolenic acid such as feeding forage-based diets, adding various PUFA sources, using protected lipids and supplementing essential oils. In this respect, the role of plant phenolic compounds is also of increasing interest (Khiaosa-ard *et al*., 2009, Cabiddu *et al*., 2010). The present study aimed at establishing relationships between phenolic compounds in tropical forages, where these frequently occur in particular high concentrations (Jayanegara *et al*., 2011), and proportions of different C18 fatty acids after *in vitro* incubation with rumen fluid.

Materials and methods Samples of 27 tropical plant species were obtained from two sampling sites near Bogor, Indonesia, consisting of one grass, four herbs, nine shrubs and 13 tree species. For each species, approximately 3 kg fresh weight of the leafy part was collected from several individuals each. The samples were oven-dried at 50°C and ground to pass a 1-mm sieve. Determination of chemical composition of the plants included proximate analysis (crude protein, ether extract), detergent analysis (neutral detergent fibre, acid detergent fibre, acid detergent lignin), phenolic fractions (total phenols, non-tannin phenols, total tannins, condensed tannins, hydrolysable tannins) and FA profiles. About 200 mg dry matter (DM) of plant samples were incubated for 24 h at 39°C with 30 ml of buffered-rumen fluid (in four replicates) using the Hohenheim gas test method (Menke and Steingass, 1988). The incubation was done by adding 50 mg linseed oil per g plant DM, emulsified in 1:99 v/v aqueous solution of Tween® 80. After incubation, the fermentation fluid was subjected to FA analysis through extraction, transesterification into fatty acid methyl esters (FAME) and separation using a gas chromatograph (Khiaosa-ard *et al*. 2009). The individual FA data were treated as mg/g of total FAME. Correlation analysis was performed between chemical composition and FA metabolites in fermentation fluid using SPSS Software version 17.0.

Results Concentrations of total phenols in the plants ranged from 14 to 235 g/kg DM. There was a clear positive relationship between total phenols in the plants and concentrations in fermentation fluid of C18:3 *n*-3 (correlation coefficient r=0.67), C18:2 *n*-6 (r=0.69) and C18:1 *n*-9 (r=0.75 (all P<0.001) after 24 h of incubation. The relationship was of a type that increasing concentrations of total phenols decreased the degree of biohydrogenation of these fatty acids. Total phenols in plants were also positively correlated with the occurrence of *c*9,*t*11-C18:2 after incubation (r=0.54; P<0.01), but negatively correlated with C18:0 (r=–0.46; P<0.05). Similar correlations as with total phenols were found with total tannins. However, no significant relationships were detected between non-tannin phenols and any of the C18 FA. Condensed tannins revealed a different pattern compared to hydrolysable tannins; the former were positively correlated with C18:1 *n*-9 (r=0.75; P<0.001) and *c*9,*t*11-C18:2 (r=0.59; P<0.01) and negatively correlated with C18:0 (r=–0.49; P<0.01), while the latter were positively correlated with C18:3 *n*-3 (r=0.61; P<0.01) and C18:2 *n*-6 (r=0.55; P<0.01). Therefore, hydrolysable tannins turned out protecting these fatty acids from the first step of biohydrogenation.

Conclusions The significant correlations found between total phenols and several ruminal C18 FA metabolites after ruminal incubation suggest the potential of phenols to modify FA biohydrogenation and to reduce FA biohydrogenation right from the first step, at least in case of tropical forages with basically high phenol concentrations. Within the phenolic fractions, total tannins exhibited a much stronger influence on lowering biohydrogenation than the non-tannin phenols. The relationship between condensed tannins and *c*9,*t*11-C18:2 suggests that these phenolic compounds could have a role in increasing this CLA isomer in ruminant-source foods. The relatively simple determination of total phenols appears to be useful for a preliminary screening of plants with respect to their inhibitory potential in terms of ruminal ALA biohydrogenation before testing these plants in more depth.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Comparison of finishing performance and carcass characteristics of growing bulls of five different cattle breeds in China

X Xie, Q Meng, L Ren
China Agricultural University, Beijing, China
Email: xhzyzu@sina.com

Introduction It is established that breeds influence growth performance and carcass characteristics (Cozzi *et al*., 2009; Nancy and Nelson, 2009). Since 1970, China has imported some high producing cattle breeds such as Limousin and Simmental from other countries to improve growth performance and carcass traits. Chinese farmers typically raise cattle in family farms and slaughter at a common age. Little information is available about the comparison of growth performance and carcass characteristics of the imported breeds and local breeds at the same age in China. The aim of this study was to investigate the growth performance and carcass traits of five cattle breeds at the same age offered the same diet in China.

Materials and methods Fifteen male calves of Limousin (LIM), Simmental (SIM), Qinchuan (QC) and thirteen male calves of Luxi (LX) and Jinnan (JN) born around December 2007 were selected. All bulls received a similar diet on family farms until they were 6 months old. After arriving at the experimental base, all bulls accessed maize stalk silage, by-products and concentrate *ad libitum* until about 9 months. After that, the bulls' initial body weights were 397.8±56.40kg, 297.8±44.86kg, 245.3±21.69kg, 250.5±32.87kg, 255.7±43.09kg for LIM, SIM, LX, JN, QC respectively. Then all bulls were divided randomly within five breed groups and housed individually in stalls and accessed the same total mixed ration *ad libitum* for 105-day fattening period. The finishing diet consisted of (%DM) 44% maize, 3% cotton seed meal, 8.8% soybean pomace, 11% brewers dried grain, 30% maize stalk silage and 3.2% compound premix. Amounts of feed offered to the calves were determined according to live weights obtained at 35 days intervals through the trial. Eight bulls from each breed were randomly selected for slaughter at18.5 months. Hot carcass weight was individually recorded to calculate hot dressing percentage. The longissimus dorsi of the left side was cut between the 12th and 13th rib to determine backfat thickness, ribeye areas after aging at 1 - 4□ for 24h. Marbling was scored from 1 (devoid) to 5 (abundant) according to the China beef carcass grading system. The effects of breed on growth performance and carcass characteristics were subjected to one-way analysis of variance using Generalized Linear Models procedures of SAS (2000). The significance of differences between group means were compared using the PDIFF test of SAS (2000).

Results The values of FBW, ADG, DMI and G/F were highest for LIM followed by SIM (Table 1). The three local breeds had lighter carcass weight than the two imported breeds (P<0.05). LIM had higher hot dressing percentage than the other four breeds (P<0.05). Bone weight and net meat weight of the two imported breeds were heavier than the local breeds (P<0.05), while there was no breed effect on bone percentage and ratio of meat to bone. Local breeds showed the higher marbling scores and JN cattle had the highest backfat thickness. Ribeye area of the two imported breeds was larger than the three local breeds (P<0.05).

Table 1 Comparison of growth performance and carcass quality of the imported and local cattle breeds

	Breed						
Item	LIM	SIM	LX	JN	QC	SEM	P
Final body weight, kg, FBW	560.25^{a}	422.00^{b}	329.96^{c}	338.75^{c}	334.20^{c}	12.56	<0.001
Average daily gain, kg, ADG	1.50^{a}	1.20^{b}	0.82^{c}	0.82^{c}	0.78^{c}	0.05	<0.001
Dry matter intake, kg/d, DMI	8.47^{a}	7.51^{b}	6.12^{c}	6.30^{c}	6.02^{c}	0.14	<0.001
Gain efficiency,G/F	0.181^{a}	0.159^{b}	0.136^{c}	0.131^{c}	0.130^{c}	0.01	<0.001
Slaughter weight, kg	558.50^{a}	461.00^{b}	333.25^{c}	349.88^{c}	342.00^{c}	13.75	<0.001
Hot carcass weight, kg	329.50^{a}	261.88^{b}	184.75^{c}	196.50^{c}	190.13^{c}	8.62	<0.001
Hot dressing percentage, %	59.0^{a}	56.7^{b}	55.5^{b}	56.1^{b}	55.5^{b}	0.6	0.001
Bone percentage, %	12.7^{a}	12.5^{a}	11.9^{a}	11.6^{a}	12.3^{a}	0.4	0.455
Net meat weight ^{a}, kg	285.44^{a}	225.38^{b}	159.13^{c}	171.44^{c}	163.81^{c}	7.88	<0.001
Net meat weight /Bone weight	6.98^{a}	7.04^{a}	7.54^{a}	7.70^{a}	7.14^{a}	0.32	0.406
Backfat thickness, mm	2.88^{abc}	2.63^{abc}	1.69^{c}	3.75^{a}	2.94^{ab}	0.43	0.024
Marbling score ^{b}	1.50^{b}	1.75^{ab}	2.19^{ab}	2.25^{a}	2.13^{ab}	0.24	0.152
Ribeye area, cm^{2}	101.96^{a}	72.88^{b}	57.52^{c}	60.64^{bc}	56.72^{c}	3.95	<0.001

Means in the same row with different superscripts are significantly different (P <0.05)

Conclusions In this study, the two imported cattle breeds had better growth performance, efficiency of gain and some carcass traits than the local breeds at the same feeding management in China. The local breeds had a relatively higher marbling score, which is the important index for good beef quality.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Impact of climate change on the conduct of steppe's sheep breeding: case of Hadj Mechri's commune Laghouat , in Algeria

M Azeddine, H Fethi Rachid
[1]Faculté Sciences Agronomiques et Sciences Biologiques, Université Mouloud Mammeri, Tizi-Ouzou, Algeria, [2]Faculté Sciences Biologiques Environnementales, Université Houari Boumediene, Alger, Algeria
Email: mouhousazeddine@yahoo.fr

Introduction In steppe's society, sheep breeding is exploited extensively, which is based on grasslands exploitation. According to Boukhabza (1982), sheep's breeding represents an important income for households in those areas (Because of recurrent droughts, grasslands fodder production has been highly reduced; therefore, complementing animals ration, especially with barley, become necessary. During dry periods, the sedentary process increases the vulnerability of the agropastoralists, which makes them very dependent of the complement feed (Mouhous, 2005). In dry year, the sheep's reproduction performances are often weak because of insufficiency of feed. The present work aimed to answer to the following questions: i) The feed is it sufficient to permit a reproduction based on the traditional mode? ii) How is conducted the animal reproduction? iii) What are the performances obtained?

Materials and methods An exhaustive survey was conducted in steppe area of Hadj Mechri, in 2004. It concerned a community of 57 households living in scattered zone. The mains informations are collected through a structured Questionnaire. In order to complete those informations, discussions are released with agropastoralists. According to the agropastoralists, the year during which the investigation is realised is considered as a dry year.

Results The dry period has known an increase of 61.74% during the period of 1953 to 2003 (figure 1). The results show that the forage free energy which comes from grasslands covers 77% of the dietary animal's requirements and the cultivated barley cover only 22% (table 1). The reproduction mode follows traditional way, which means free mating. , Most of the breeders estimate fertility rate less than 100%, while only 9% of respondents estimate it to 200%. The average litter size was estimated to be 100%. In addition, the mortality rate remains very low, it is about 2%. These results suggest that the animal performances are strongly related to the animal's management and the availability of pastoral resources in dry periods.

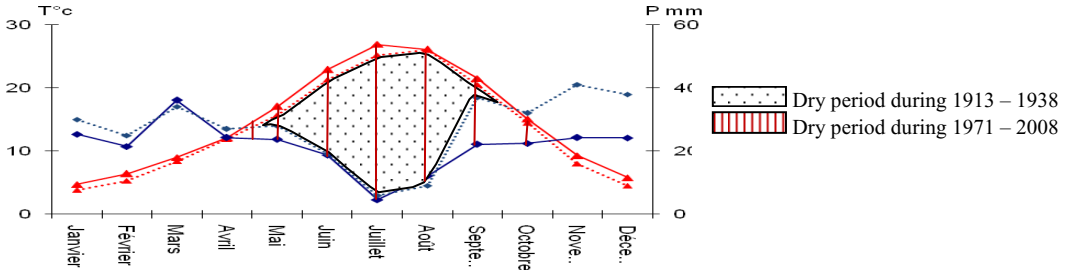

Figure1 Ombrothermic diagram of El Bayadh station.

Table 1. Fodder balance of the survey zone

Animal requirements (UF)	Feed availabilities (UF)	Balance UF)	Rate of cover
436 530	370 950	- 65 580	85 %

Conclusion In steppe area, the sheep breeding remain at extensive level. In dry period, the distribution of feed complement is daily. The weak reproduction performances are justified by the reduction of the fodder offer by the grasslands and by the high prices of the feed complement. Therefore, the economic profitability of breeding is in progressive reduction until the total loss of the livestock. The climatic changes increase the vulnerability of the steppe's area breeding and reduce the socio-economic resilience of breeder's population. This investigation will serve to think better conception of the development projects facing the climatic risk, especially concerning sheep breeding on grasslands.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Carryover effects of crude glycerine fed during the growing phase on finishing cattle performance and carcass characteristics

C Aperce, C Schneider, L Litton, K Miller, C Van Bibber, S Uwituze, C Alvarado, K Blaine, J Higgins, J Drouillard
Kansas State University, Manhattan, Kansas, USA
Email: celin@ksu.edu

Introduction Crude glycerine is a by-product of biodiesel production, and its use as a feedstuff for cattle has expanded in the last decade due to increased availability and favourable pricing compared to other energy concentrates, such as cereal grains. Effects of glycerine incorporation into cereal-based finishing diets has been studied (Parsons *et al*., 2009; Mach, *et al*., 2009), but less is known of its value as an energy source for growing animals and its possible carryover effects on finishing performance and carcass characteristics. The aim of this experiment was to feed glycerine for 90 days to growing heifers, and examine the carryover effects on performance and carcass characteristics of cattle during the subsequent fattening period.

Material and methods Three hundred sixty eight crossbred heifers (234 ± 3.2 kg BW) were randomly allocated to 48 concrete-surfaced pens (7 to 8 animals/pen). Animals were fed once daily and had *ad libitum* access to water. During the growing phase (days 0 to 90), the diet consisted of 60% roughage (maize silage) and 40% concentrate (predominantly wet maize gluten feed). Crude glycerine (derived from soya bean) was added at 0, 4 or 8% to growing diets, substituting for a portion of the maize gluten feed. During the finishing phase (days 91 to 210), all animals received 90% concentrate and 10% roughage. Heifers were weighed at the beginning and end of each period, and feed refusals were collected throughout experiment to assess dry matter intake (DMI), average daily gain (ADG), and gain to feed (G:F). Carcass data were collected at harvest. Data were analyzed using the Mixed procedure of the Statistical Analysis System (SAS, 2004). Pen was the experimental unit. Glycerine level was included in the model as a fixed effect. Pen, the random effect, was nested within glycerine level. Orthogonal contrasts were used to evaluate linear and quadratic effects of prior exposure to glycerine.

Results During the growing period, ADG was not affected by the different levels of glycerine, although cattle were more efficient (P<0.011) when fed glycerine. With respect to carryover effects, both ADG and G:F during the finishing period were enhanced as a result of prior exposure to glycerine (4 and 8%) during the growing phase. Heifers previously fed glycerine had greater DMI (P<0.025) through the finishing phase, heavier final body weights (P<0.009), improved marbling scores (P<0.009), and heavier hot carcass weights (P<0.028) compared to animals fed growing diets without glycerine.

Table 1 Carryover effects of glycerine on growth performance and carcass characteristics of fattening heifers.

	Glycerine, % of Dry Matter				*P*-values		
	0%	4%	8%	SEM	F-test	Linear	Quadratic
Dry matter intake from day 0 to 90, kg	8.88	8.62	8.53	0.133	0.166	0.069	0.166
Average daily gain from day 0 to 90, kg	1.48	1.51	1.50	0.025	0.767	0.753	0.518
Gain:feed from day 0 to 90	0.165	0.172	0.174	0.002	0.011	0.005	0.271
Dry matter intake from day 91 to 210, kg	9.64	9.87	10.06	0.274	0.076	0.025	0.919
Average daily gain from day 91 to 210, kg	1.16	1.25	1.29	0.054	0.001	0.0003	0.367
Gain:feed from day 91 to 210	0.118	0.125	0.127	0.002	0.024	0.010	0.384
Harvest weight, kg	506	518	523	9.6	0.025	0.009	0.466
Dressing percentage	61.8	61.6	61.6	0.002	0.719	0.454	0.763
Hot carcass weight, kg	312	319	322	2.9	0.074	0.028	0.554
Longissimus muscle area, cm^2	79.0	81.5	81.8	0.97	0.103	0.053	0.364
12^{th} rib subcutaneous fat thickness, cm	1.58	1.52	1.60	0.049	0.510	0.738	0.270
Marbling score$^{\phi}$	466	480	499	8.4	0.031	0.009	0.845

$^{\phi}$Marbling score: Higher numbers are desirable, indicating greater intramuscular fat deposition.

Conclusions Glycerine added to growing diets fed for 90 days improved average daily gain and efficiency in the subsequent 120-day fattening period. Marbling score, carcass weight, and final body weight also increased in response to feeding glycerine in the previous growing period. These findings suggest there is a carryover effect of glycerine feeding that influences growth performance and carcass characteristics subsequent to its removal from the diet.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effects of different additives on the aflatoxin content of alfalfa silage

F Yang, G Ma, Y Zhang
[1]Grassland Institute,China Agricultural University, Beijing, China, [2]Chinese Rural Technology Development Center,Ministry of Science and Technology, Beijing, China
Email: yfuyu@126.com

Introduction Alfalfa silage could be contaminated by aflatoxin in the process of fermentation and storage, which is harmful to animal and human above the concentration level of 15ug/kg. Experiments were conducted to investigate aflatoxin content and silage safety of alfalfa during ensiling by adding different silage additives..

Materials and methods Lactic acid bacterial(0.5%), acetic acid(0.5%), propionic acid(0.5%), urea(0.0017%)and cellulose(0.0005%) were applied to six varieties of alfalfa（Algonquin、Derby、Sandity、God Empress、Aohan and Zhongmu 1） in plastic bags for 45days during ensiling. Samples were collected at 0d and the 7th d, and dried at 65□ for 48h. Aflatoxin were measured by VICAM 4 Mycotoxin Determinator.

Results The results (figure1 and figure2) showed that the aflatoxin were detected in all alfalfa silage, except in fresh alfalfa. All additives can decrease the aflatoxin content effectively during aerobic process, and they were under the safety level (≤10 ppb) in all silage. At 0 day, the highest aflatoxin concentration was detected in control(1.63ppb), while the lowest one was found in urea treatment (0.72ppb). At the 7th day, the aflatoxin concentration increasing quickly in all silage. The highest and lowest one was control(8.21ppb) and lactic acid bacterial treatment(5.51ppb). High aflatoxin concentration in control was due to low lactic acid bacterial content and high pH value, which inhibited activities of aerobic microorganism. Aohan alfalfa silage was found higher aflatoxin concentration than other varieties because its pH value increased rapidly, which resulted lower lactic acid bacterial content and acidity.

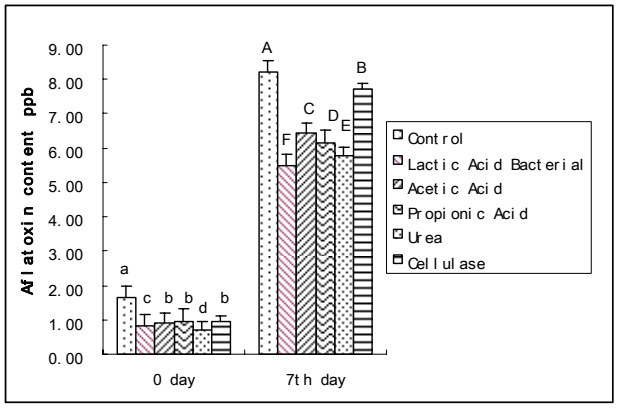

Figure 1 Aflatoxin content in alfalfa silage with different addictives

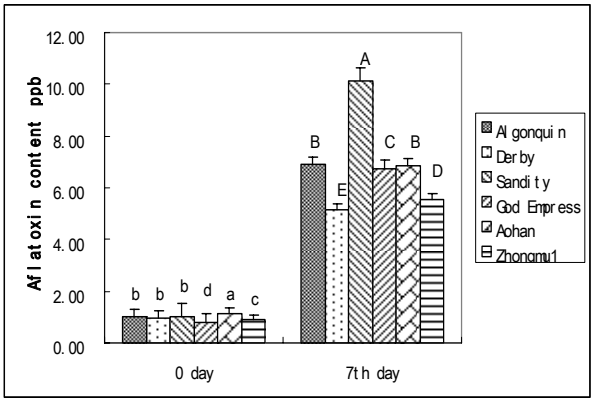

Figure 2 Aflatoxin content in alfalfa silage with different varieties

Conclusions Under aerobic condition, urea (at 0 day) and lactic acid bacterial (at 7 day) additive can decrease the aflatoxin production effectively in alfalfa silage. Varieties have effect on aflatoxin production in alfalfa silage.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Brewer's grains as protein source for growing rabbit under Algerian context: effects on growth and slaughter traits

G Lounaouci-Ouyed[1], M Berchiche[1], T Gidenne[2]
[1]Université de Tizi-ouzou Faculté des Sciences Biologiques, Tizi-ouzou, Algeria, [2]UMR 1289 TANDEM INRA de Toulouse, Toulouse, France
Email: lgouyed@yahoo.fr

Introduction Data about the utilization of brewer's grains in rabbit's diets are scarcely reported (Carabano et Fraga, 1992., Maertens and Salifou, 1997., Berchiche *et al.*, 1999). This by-product, containing a significant amount of proteins and fibres, is very suitable for rabbit feeding. In addition, the brewer's grains are abundantly available in Algeria and for a low price. In a previous study, Lounaouci *et al*., (2008) showed that the inclusion of 30% of brewer's grains in the diet seems to reduce the growth performances of rabbits, but not affect the slaughter rate. The aim of the present experiment was to study the effects of incorporation of brewer's grains (up to 25%) as main and alternative source of proteins of those of soya bean meal, in balanced and practical diet, on health, growth and slaughter performances of growing rabbits.

Materials and methods Eighty eight mixed-sex rabbits, belonging to the local Algerian population (Lounaouci *et al*., (2008), weaned at 35 days of age and weighing 598g on average, were assigned to the two experimental groups (44 rabbits/diet) and received, *ad libitum,* two isonitrogenous (crude proteins: 16.1% as fed), isoenergetic (12 MJ/Kg as fed) and isofibrous (NDF: 32% as fed) diets : a control diet based on 10% of soya meal (SM10 diet) and an experimental diet (BG25 diet) based on 25% of sun dehydrated brewer's grains, in complement of maize (27 and 15%), alfalfa (30 and 19%) and hard wheat bran (30 and 40%), respectively for SM10 diet and BG25 diet). Rabbits were placed into collective cages (4 rabbits / cage). At the end of the trial (at 84 days of age), 10 rabbits/diet were slaughtered in order to measure hot and cold carcass weights. The chemical analyses were performed at research unit (UMR 1289 TANDEM) of INRA of Toulouse according to ISO methods and considering the recommendations proposed by the EGRAN group. Data were analyzed as a completely randomized design with the Statistical Analysis System (SAS Version 6.1, SAS Institute Inc., Cary, USA; 1987) by using the general linear model (GLM procedure). The variance analysis was performed with type of diet as the main source of variation.

Results The incorporation of sun dehydrated brewer's grains in the diet, up to a level of 25%, permit for rabbits of BG25 group to reach the same level of animal performance as rabbits fed with SM10 diet. The daily feed intake, the daily weight gain (Table 1) and the slaughter performances (Table 2) of rabbits fed the brewers' grains (BG25) diet were not significantly (P>0.05) different compared to those rabbits on the control. In addition, the mortality rate of rabbits received BG25 diet is significantly (P<0.05) lower comparatively of that of SM10 group (respectively 15.9 *vs* 34 %).

Table 1 Growth rate and feed intake during the fattening period

Growth traits	Diets		SEM[1]	P[2]
	SM10	BG25		
Initial weight (g)	595	601	119	ns
Final weight (g)	2001	2144	172	ns
Daily weight gain (g/d)	32.2	33.7	4.4	ns
Daily feed intake (g/d)	103	111	11	ns
Feed conversion ratio	3.95	3.65	0.2	ns

1SEM: standard error of mean
2ns: no significant (P>0.05)

Table 2 Slaughter performances of the two groups of rabbits

Killing-out %	Diets		SEM[1]	P[2]
	SM10	BG25		
Rabbits / treatment	10	10	-	-
Slaughter weight (SW) g	2189	2112	68	ns
Hot carcass g (HC) g	1489	1466	52	ns
Cold carcass (CC) g	1427	1432	49	ns
Slaughter rate HC/SW %	68.8	69.5	1.4	ns
Slaughter rate CC/SW %	65.9	67.8	1.2	ns
Skin % SW	9.6	9.0	1.1	ns
Digestive tract % SW	19.0	19.1	1.5	ns
Abdominal fat % CC	1.10	1.33	0.3	ns
Muscle / Bone ratio	6.14	6.10	0.5	ns

1SEM (standard error of mean)
2ns: no significant (P>0.05)

Conclusions The results of this study indicate that brewer's grains, in complement of hard wheat bran, can be included at a level of 25% in the diet, without any adverse effect on growth traits and slaughter performances of growing rabbits. The brewer's grains would constitute interesting alternative and locally source of proteins for rabbit feeding, which is especially important for reduced the importation of soya bean meal proteins. In addition, the low cost of this by products makes it of interest to reduce the feeding cost, which is the most discouraging problems for rabbit breeders in Algeria

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effects of forage to concentrate ratio in the diet and pH on ruminal fermentation in Rusitec fermenters

J E Vargas[1,2], S Andrés[1], D R Yañez-Ruiz[3], L López-Ferreras[4], S López[1]
[1]Instituto de Ganadería de Montaña (CSIC-Universidad de Leon), Leon, Spain, [2]Universidad de Caldas, Manizales, Caldas, Colombia, [3]Estación Experimental del Zaidín (CSIC), Granada, Spain, [4]Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Madrid, Spain
Email: s.lopez@unileon.es

Introduction Diet and rumen pH are the main factors affecting rumen fermentation (Calsamiglia *et al*., 2008; Martínez *et al*., 2010). The Rusitec system simulates rumen fermentation under controlled conditions allowing for examining separately the effects of both factors. The objective of this work was to study the effects of forage to concentrate ratio in the diet and of pH on ruminal fermentation patterns, methane production and digesta fatty acid composition in Rusitec fermenters.

Materials and methods Four treatments were randomly allocated to 16 Rusitec vessels in a completely random design with a 2 x 2 factorial arrangement. High forage (**HF**, 127 g of CP and 502 g of NDF/kg of DM) diet contained 800 g/kg of hay, whereas high concentrate (**HC**, 186 g of CP and 284 g of NDF/kg of DM). Both diets were supplemented with 30 g of sunflower oil/kg of diet. To vary ruminal pH and culture medium buffering capacity, composition of the artificial saliva infused into the vessels was modified changing the concentration of bicarbonate, so that rumen pH was 6.82 with the high bicarbonate buffer and 6.44 with the low bicarbonate buffer. The experiment consisted of a 7-day adaptation period followed by a 14-day sample collection period. On collection days (1, 2, 3, 8, 9 and 10 of the collection period) total gas and liquid effluent outputs were recorded and sampled in order to determine CH_4 and VFA, L-lactate and NH_3-N. Organic matter and NDF disappearance from the bags was measured after 48 h of incubation in the vessels. On day 6 of the collection period, microbial protein synthesis was estimated using ^{15}N as microbial marker, measuring its concentration in samples of digesta and microbial pellets after an infusion of the marker into the vessels.

Results The interaction diet x rumen pH was not significant for any of the studied parameters. There was no difference (P>0.05) between diets in rumen pH. Substrate disappearance from the bags was greater for the more digestible HC diet than for the HF diet (0.661 vs. 0.491, SEM 0.035), and was not affected by rumen pH, although tended (P=0.088) to be greater at a higher pH (0.592 vs. 0.560). Total gas production was greater for HC than for HF diet (2.41 vs. 1.89 l/day, P<0.001), and for high than for low rumen pH (2.31 vs. 1.99 l/day; P=0.019). Methane daily output, expressed per g of fermented OM was not affected by diet (0.720 vs. 0.743 mmol/g of fermented OM for HC and HF, respectively, P=0.833) or rumen pH (0.790 vs. 0.674 mmol/g of fermented OM for high and low pH, respectively, P=0.303). Total VFA production was higher for HC than for HF diet (43.4 vs. 38.2 mmol/d, P=0.006). There were no significant differences between diets in the production of acetic or propionic acids (P>0.05), whereas iso-butyric, butyric, valeric, isovaleric and caproic outputs and acetic to propionic ratio (2.35 vs. 2.02, P<0.001) were greater for the HC diet. L-lactate was higher for HC diet (12.7 vs. 7.02 mg/day, P<0.001). Total VFA production and acetic to propionic ratio tended to be higher at higher pH (42.5 vs. 39.1 mmol/day, P=0.052 and 2.25 vs. 2.12, P=0.083). Ammonia output was greater with HC diet (133.5 vs. 80.6 mg/day, P<0.001) and at higher pH (114.0 vs. 100.1 mg/day, P=0.004). The efficiency of microbial protein synthesis was not affected by diet or buffer composition (P>0.05); however daily microbial protein synthesis was higher with HC diet (0.568 vs. 0.464 g/day, P=0.015). Rumen pH had little effects on fatty acid composition of digesta (Table 1) with high pH giving rise to greater stearic and vaccenic acids proportions. Diet affected fatty acid composition of digesta to a greater extent (Table 1) so that stearic and CLA percentages were lower and oleic and linoleic percentages were greater with HC compared with HF diet.

Table 1 Profile of medium and long chain fatty acids in rumen digesta (% of total fatty acids measured)

	Rumen pH			Diet			SEM
	6.82	6.44	P =	High Concentrate	High Forage	P =	
Palmitic acid	23.5	19.8	0.080	21.4	21.9	0.801	14.83
Stearic acid	12.3	9.9	0.012	10.0	12.2	0.017	2.70
Vaccenic acid	2.34	1.53	0.030	1.89	2.00	0.745	0.379
Oleic acid	21.6	20.8	0.743	23.3	19.5	0.050	10.66
Linoleic acid	10.2	13.6	0.114	14.3	9.5	0.033	15.94
Linolenic acid	1.68	2.06	0.359	1.59	2.15	0.179	0.632
Conjugated linoleic acid (CLA)	0.204	0.218	0.690	0.167	0.241	0.032	0.0023

Conclusions Diet (forage to concentrate ratio) and, to a lesser extent, rumen pH had significant effects on rumen fermentation, in particular on substrate degradation, VFA output and fatty acid profile in the digesta. Methane output was not affected by diet or rumen pH. Fatty acid would be hydrogenated to a greater extent with a high forage diet. Although Rusitec system mimics rumen fermentation, simulation of *in vivo* fermentation may be affected by the type of diet (Martínez *et al*., 2010).

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Substitution of soybean meal for inactive dry yeast in diets of beef cattle: intake and total and partial apparent digestibilities of nutrients

A Campos, O Pereira, S Valadares Filho, K Ribeiro, L Rosa
Brazilian Society of Animal Science, Vicosa, Minas Gerais, Brazil
Email: odilon@ufv.br

Introduction The feedlot of beef cattle is an attractive alternative in the dry season by allowing higher weight gain in times of shortage of forage in pastures, reducing the age of slaughter of animals, providing uniform carcasses and increasing the rate of enjoyment of property. But, in this system the diet is one among the factors more expensive in the production costs. Soybean meal is classified as a protein concentrate with excellent nutritional characteristics for the diets, but it is expensive. Thus, the co-products generated by Brazilian industries have potential to substitute conventional ingredients, due to their protein composition. Inactive dry yeast is a co-product obtained during the process of sugarcane alcoholic fermentation that is used as high protein ingredient for animal feed and has potential of substitution of soybean meal, because the crude protein content ranges from 30 to 45% (Valadares Filho *et al*. 2006) and it has high concentrations of vitamins and the aminoacids lysine, threonine and methionine (Ezequiel *et al*., 2000). The objective of this study was to evaluate the intake and total and partial apparent digestibilities of nutrients of beef cattle fed diets containing different levels of inactive dry yeast in substitution of soybean meal.

Material and methods Five Nellore cattle, castrated, with initial live weight of 320±39 kg, fistulated in rumen and abomasum, were allotted in a 5x5 Latin Square design. The animals were kept in individual pens of approximately 10 m^2, with protected feeders and wateries. Diets consisted of 60% corn silage and 40% concentrate, containing different levels of inactive dry yeast in substitution of soybean meal (0, 25, 50, 75 and 100%, dry matter basis). Daily intake was measured by determining the difference between the weight of supplied feed and refusals. The chromic oxide (15 g daily) was used as an external marker to estimate fecal and abomasal flows. Each experimental period lasted 16 days, being 10 for diet adaptation and six for sample harvest. The samples of supplied feed, refusals, abomasal digesta and feces were analysed for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber corrected for ash and protein (apNDF) and determination of non-fiber carbohydrates (NFC) and total digestible nutrients (TDN). The DM, OM, apNDF and NFC ruminal and intestinal apparent digestibilities were calculated relative to total digestible, while the CP and EE ruminal and intestinal digestibility were calculated in relation to quantities of nutrients present in each study site. The data were evaluated by regression analysis using SAS software (1999).

Results There was a linear negative effect ($P<0.10$) of inactive dry yeast levels on DM, OM, CP, EE, NDF and TDN intakes. The decrease in DM intake as yeast levels increased in the diets may have been influenced by the physical characteristics of this ingredient, especially for fine grind and sticky feature for the animals. With a reduction of DM intake, the intake of other ingredients also declined, with exception of the intake of NFC, which was not affected ($P> 0.10$) by levels of yeast in the diet, due to higher concentration of this nutrient in the diets with highest levels of yeast. No effect of yeast levels were observed on total, ruminal and intestinal apparent digestibility of all nutrients, except for the NFC, in which there was a quadratic effect ($P<0.10$) of yeast for both ruminal and intestinal digestibility, with minimum and maximum values of 50.51 and 49.49% at 48.1% of inactive dry yeast, respectively.

Conclusion Soybean meal might be replaced by inactive dry yeast until 30% of concentrate.

Acknowledgements Authors thank the National Council for Scientific and Technological Development – CNPq - INCT-CA, for the financial support.

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Intestinal digestibility of rumen undegraded protein of co-products from new generation of bioethanol processing plant using three-step *in vitro* method

I Hettiarachchi Gamage, P Yu
University of Saskatchewan, Saskatoon, Canada
Email: peiqiang.yu@usask.ca

Introduction Accurate intestinal digestibility of rumen undegraded protein (%dRUP) of the co-products from bioethanol processing is key information for dairy and beef diet formulation. The newly developed bioethanol plants differ from the old generation plants in many way. The processing will affect nutrient availability. The objectives of this study were to (1) determine the intestinal digestibility of rumen undegraded protein of the co-products (wheat based DDGS) from a new generation of bioethanol processing plant in western Canada using the three-step *in vitro* method, and (2) detect the batch effects within original feedstock and within wheat DDGS; and (3) compare between original feedstock and wheat DDGS.

Material and methods Total 30 samples included original 15 parental feedstock samples and 15 wheat DDGS samples were collected from newly built bioethanol processing plant in western Canada. Intestinal digestibility of rumen undegraded protein of wheat DDGS from new generation of bioethanol processing plant was used three-step *in vitro* method. The samples were incubated in rumen for 16 h, then rumen residue were estimated for its digestibility in the intestine using an *in vitro* enzyme method.
The N was determined using standard lab AOAC method.
All data were analyzed using the MIXED procedure of SAS software (SAS Institute, Inc.) with CRD model. When a significant difference was detected ($P < 0.05$), means were separated using the Tukey-Kramer post test.

Results The results showed that the %dRUP in wheat DDGS was lower (P<0.05) than that in wheat (67 vs. 79% of RUP). There was a significant effect (P<0.05) between batches within original feedstock with digestibility ranged from 76 to 83% of RUP. The results also showed a significant batch effect with wheat DDGS on %dRUP with a range from 64 to 71% of RUP).

Conclusions This study suggest that it is not a good approach to use a fixed intestinal digestibility data of the wheat DDGS in a diet formulation even within a plant. It is necessary to regularly analyse %dRUP.

Acknowledgements Funding provided by ABIP-FOBI, Science Cluster AAFC-BCRC-Beef Cattle Research Council. The authors wish to express their gratitude to Z. Niu (Department of Animal and Poultry Science, University of Saskatchewan).

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Chemical profiling: new strategies to more efficiently utilize cereal grains (oat, barley, corn) and bioethanol co-products for beef cattle

D Damiran, P Yu
University of Saskatchewan, Saskatoon, Canada
Email: peiqiang.yu@usask.ca

Introduction The goals of this research program were to develop new strategies to more efficiently utilize grain barley, oat and corn by integration with bioethanol co-products (high protein, RUP and DVE values) in sustainable beef production for improving animal production and health and to assist the beef industry to develop low-cost feeding strategies by utilizing alternative feed resources.
The hypothesis of the research program was that the bioethanol co-product (DDGS) as a natural feed additive can slow down the degradation rate and extent of degradability of cereal grains, improve the effective degradation ratio between nitrogen and energy, increase nutrient availability and nutrient supply for beef cattle.
In this specific chemical study, the objective was to determine chemical characterization and nutrient profiles: COMPARISON among various combinations of barley, oat and corn with natural feed additives - bioethanol co-products.

Material and methods Five feed combinations for each grain (Oat, barely or corn to co-products ratio) were 4:0 ratio; 3:1 ratio; 2:2 ratio; 1:4 ratio; 0:4 ratio. The detailed chemical compositions were determined using standard lab analysis methods, including (1) basic chemical profiles: DM, ash, OM, crude fat; (2) structural carbohydrate profile: NDF, ADF, ADL, Hemicellulose, Cellulose; (3) Non-structural carbohydrate profile: starch and sugars; (4) Protein profile: CP, soluble CP, NPN, NDICP and ADICP.
All data were analyzed using the MIXED procedure of SAS software (SAS Institute, Inc.) with factorial treatment design: $Y_{ijr} = \alpha_i + \beta j + e_{ijr}$; where Y_{ijr} is the variable studied, α_i is the cereal grain type effect, βj is the DDGS level effect, and e_{ijr} is the residual standard deviation used as the error term.
When a significant difference was detected ($P < 0.05$), means were separated using the Tukey-Kramer post test. Grain, DDGS level (DDGS), and Grain×DDGS were set as fixed effects and DDGS plant set as a random effect.
Orthogonal contrasts were used to compare linear and quadratic and cubic effects of including 0, 25, 50, 75 or 100% DDGS. Each sample was considered the experimental unit.

Results The results showed there were differences ($P < 0.05$) in chemical profiles among the combinations. Within each grain (corn, barley, or oat), the chemical profiles in five combinations were also different ($P < 0.05$). Sugars ranged from 26 to 49, 12 to 63, 17 to 59 g/kg DM for barley, corn and oat combinations respectively. Soluble CP ranged from 245 to 356, 306 to 385, 407 to 393 g/kg CP for barley, corn and oat combinations respectively. Starch ranged from 609 to 161, 751 to 187, 435 to 120 g/kg DM for barley, corn and oat combinations respectively. CP ranged from 138 to 340, 89 to 332, 139 to 341 g/kg DM for barley, corn and oat combinations respectively. Crude fat ranged from 17 to 44, 33 to 46, 41 to 50 g/kg DM for barley, corn and oat combinations respectively. NDF ranged from 172 to 406, 162 to 445, 358 to 444 g/kg DM for barley, corn and oat combinations, respectively. ADL ranged from 7 to 39, 9 to 43, 25 to 44 g/kg DM for barley, corn and oat combinations, respectively. NPN ranged from 636 to 842, 592 to 802, 557 to 784 g/kg SCP for barley, corn and oat combinations, respectively. NDIN and ADIN ranged from 164 to 445, 11-145; 272 to 489, 92-177; 99 to 456, 16-163 g/kg DM for barley, corn and oat combinations, respectively. There were non-linear responses to adding co-products at different ratios in chemical profiles ($P < 0.05$).

Conclusions Overall, the study data suggested that through varying inclusion rate of wheat DDGS in feed mixtures, chemical profile of grain based animal diets can be manipulated.

Acknowledgements Funding provided by the Science Cluster AAFC and BCRC-Beef Cattle Research Council (Project # FED.02.09). The authors wish to express their gratitude to Z. Niu (Department of Animal and Poultry Science, University of Saskatchewan).

The comparison of CNCPS and *in situ* assessment of protein and NDF in lucerne and maize silages

M Chrenková[1], Z Ceresnaková[1], M R Weisbjerg[2], Z Formelová[1], M Polácikova[1]
[1]Animal Production Research Centre, Nitra, Slovakia, [2]Faculty of Agricultural Science, Aarhus University, Tjele, Denmark
Email: chrenkova@cvzv.sk

Introduction Silages are the dominant part of dairy rations and so they are one of the key determinants of overall profitability. Traditionally, modern European systems have used *in situ* methods to describe protein and NDF availability in the rumen and in the small intestine of ruminants, whereas the Cornell Net Carbohydrate and Protein System (CNCPS) mainly rely on chemical fractionation of nitrogen (Sniffen *et al*., 1992) and cell walls (Weisbjerg, 2010). The aim of our experiment was to compare the *in situ* and CNCPS description for lucerne silage (LS) and maize silages (MS).

Material and methods According to Licitra *et al*. (1996) soluble and insoluble subfractions (in buffer, neutral detergent (ND) and acid detergent (AD) were determined in six samples of maize silage (MS) and seven samples of lucerne silage (LS). For effective CP and NDF degradability was used *in situ* method. The data were analysed One-Way Analysis of Variance and linea. The significant differences were declared at $P<0.01$ and $P<0.05$ using T-test. The correlation (Pearson) between two CNCPS and *in situ* characteristics was measured.

Results Both LS and MS samples varied highly, with concentrations (in $g.kg^{-1}$DM): CP 90.6 – 196.0 and 56.0 – 82.5; NDF 366.3 – 690.2 and 362.8 – 482.2; ADF 305.7 – 592.9 and 201.7 – 243.2; ADL 43.7 – 77.2 and 23.8 – 29.2, respectively. Mean concentrations in LS and MS were significantly different for CP ($P<0.05$), ADF ($P<0.01$), and lignin ($P<0.01$). Buffer insoluble protein minus the protein insoluble in ND is fraction B2. Concentration of B2 was significantly higher in LS than in MS ($P<0.05$), but in % of total N the difference was non-significant (32.1 and 39.9, resp.). The fraction C (unavailable protein – NIAD) varied considerably in both LS and MS (for LS from 6.2 to 30.9 % and for MS 6.8 to 42.6 % of total N) but the means were similar (12.3 % and 17.4 %, resp). *In situ* effective CP degradability (ECPD) of LS and MS were Table 1). Effective NDF degradability was higher for than for LS but the rate of NDF degradability was higher ($P<0.05$) for LS than for MS

Table 1 Characteristics of protein and NDF assessed by the *in situ* or CNCPS methods

Item	Method	Feedstuffs				P-value
		Maize silage (n=6)		Lucerne silage (n=7)		
		x	v %	x	v %	
CP (g/kg DM)		70.6	15.6	157.0	22.5	0.0108
NDF (g/kg DM)		457.0	13.9	458.7	24.8	0.9747
ADF (g/kg DM)		237.8	13.6	389.4	24.6	0.0037
ADL (g/kg DM)		29.8	19.5	95.2	48.5	0.0057
NDIN (g/kg DM)	CNCPS	16.9	46.6	24.9	71.0	0.3347
% of total N	CNCPS	24.0	47.6	16.5	79.9	0.2987
ADIN (g/kg DM)	CNCPS	12.2	79.8	18.3	57.3	0.3010
% of total N	CNCPS	17.4	77.5	12.3	68.8	0.4269
B_2 (g/kg DM)	CNCPS	27.2	28.3	48.0	32.7	0.0134
% of total N	CNCPS	39.9	37.7	32.1	39.7	0.3314
EDCP (%)	*In situ*	75.6	6.7	76.3	12.1	0.8735
cEDCP (%/h)	*In situ*	0.0423	50.1	0.0628	43.0	0.1619
EDNDF(%)	*In situ*	36.5	32.0	33.2	21.9	0.4563
cEDNDF(%/h)	*In situ*	0.020	29.8	0.0379	37.2	0.0150
CPID (%)	*In situ*	40.4	36.4	46.5	15.9	0.3599

Conclusion There was found very weak correlation between selected parameters of quality. It could be affected by the large variability of observed parameters in both groups of silages.

Acknowledgements The work has been carried out with financial support from the Commission of the European Communities, FP7, KBB-2007-1 and Ministry of Agriculture

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Performance of Santa Inês lambs supplemented with whole cottonseed and its co products

P de Mello Tavares Lima[1], P Batelli de Oliveira[1], A Campeche Lopes[2], P Galvão Viana[1], H Louvandini[2], C Concepta Margaret McManus Pimentel[3], V Alves Gonçalves da Silva[1], F Amorim de Sousa[1]
[1]Universidade de Brasília - UnB, Brasília, Distrito Federal, Brazil, [2]Centro de Energia Nuclear na Agricultura - CENA, Piracicaba, São Paulo, Brazil, [3]Universidade Federal do Rio Grande do Sul - UFRGS, Porto Alegre, Rio Grande do Sul, Brazil
Email: paulotavares29@gmail.com

Introduction Sheep production is a globally distributed economic activity with its products, such as wool, meat and milk being highly valued in several places all over the world (Viana, 2008). It is known that one of the major sources of expenditure on livestock production can be attributed to animal feeding, and animal feeding is very closely related to the final product quality. Taking that into consideration, the whole cottonseed and its co products are being used to obtain a better quality product without raising production costs. This is a cheap alternative protein source, showing great potential to reduce costs in the production system. The aim of this study was to evaluate the performance of Santa Inês lambs supplemented with whole cotton seed and its co products from birth to weaning.

Materials and methods Twenty two single lambs (11 males; 11 females), born in the same week with live weight average 3.54±0.79 kg were used. The animals were assigned in four collective pens. Each pen had 5 dams and their respective lambs. The lambs had full access to their dams, received water, mineral supplementation and *Cynodon dactylon* cv. Coast Cross hay *ad libitum* and they had access to a creep-feeding system with four different concentrate isoenergectic (TDN = 76.29%) and isoproteic (CP = 23.88%) mixture according to the treatment group: (1) CON - Ground corn and soybean meal; (2) CAR - Ground corn, soybean meal and whole cotton seed; (3) FAR - Ground corn, soybean meal and cotton seed meal; (4) TOR - Ground corn, soybean meal and cotton seed cake. The gossypol content of the concentrate mixtures was CAR- 0.102%; FAR- 0.035% and TOR- 0.043%. Lambs were weaned at 90 days of age. At weaning they were weighed and three biometric measurements were taken: withers height - WH; chest perimeter - CP; and carcass length - CL. Statistical analysis was performed taking in account the treatment as a fixed effect and weight at birth as a co variate using the Statistical Analysis System Software (SAS) (the significance was declared at $p<0.05$).

Results The average body weight at weaning (BWW) was (1) 15.45 kg, SED: 1.94; (2) 12.03 kg, SED: 2.17; (3) 16.42 kg, SED: 2.61; and (4) 13.96 kg, SED: 2.66. The BWW had shown no significant statistical difference between the four groups ($p>0.05$). The average biometric measurements were: WH – (1) 54.41 cm, SED: 1.56; (2) 53.95 cm, SED: 1.74; (3) 55.58 cm, SED: 2.11 and (4) 52.6 cm, SED: 2.21; CP – (1) 58.74 cm, SED: 1.91; (2) 52.28 cm, SED: 2.14; (3) 56.75 cm, SED: 2.59 and (4) 54.39 cm, SED: 2.72; CL – (1) 51.57 cm, SED: 3.91; (2) 41.03 cm, SED: 4.37; (3) 52.54 cm, SED: 5.28 and (4) 50.91 cm, SED: 5.94. There was no statistical difference between the four groups in any of the biometric measurements ($p>0.05$). However, the group (2) CAR, compared to the group (1) CON, had statistical tendency for a difference in the biometric measurements for CP and CL ($p<0.1$).

Table 1 The average body weight at weaning (BWW) and the average biometric measurements: withers height (WH), chest perimeter (CP) and carcass length (CL) for all treatments ($p>0.05$).

Treatments	CON	CAR	FAR	TOR
BWW (kg)	15.45, SED: 1.94	12.03, SED: 2.17	16.42, SED: 2.61	13.96, SED: 2.66
WH (cm)	54.41, SED: 1.56	53.95, SED: 1.74	55.58, SED: 2.11	52.6, SED: 2.21
CP (cm)	58.74, SED: 1.91	52.28, SED: 2.14	56.75, SED: 2.59	54.39, SED: 2.72
CL (cm)	51.57, SED: 3.91	41.03, SED: 4.37	52.54, SED: 5.28	50.91, SED: 5.94

Conclusions Compared to the control group of the experiment (1) CON, group (3) FAR and group (4) TOR, had very similar results. However group (2) CAR had poorer results compared to the other three treatments. This suggests that the cottonseed co products (cottonseed meal and cottonseed cake) have the potential for being an efficient alternative supplement for young lambs, with satisfactory performance and reducing production costs.

Acknowledgements Ministério da Agricultura Pecuária e Abastecimento – MAPA and Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (Process: 578541-2008/4).

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Nutritional evaluation of sorghum grain (*Sorghum bicolor L. Moench*) as silage or reconstituted using the in *vitro* gas production technique

U A Gonzalez G[1], M Gonzalez-Ronquillo[1], L Corona Gochi[2], J G Estrada F[1]
[1]Universidad Autonoma del Estado de Mexico, Toluca, Mexico, Mexico, [2]Universidad Nacional Autonoma de Mexico, Mexico, DF, Mexico
Email: mrg@uaemex.mx

Introduction Grain sorghum (*Sorghum bicolor L. Moench*) and other cereals, are rich in starch. The grain sorghum has a potentially attractive energy source for industry in ruminant feeding and used as a main grain in feedlots diets. The main problem was its low nutritional value compared with corn grain (Ezeogu *et al*., 2005). The aim was to determine the extent of dry matter fermentation using the *in vitro* gas production technique to evaluate four processing methods of sorghum with the cracked corn. Total gas production was used to measure the extent of dry matter fermentation of different grain processing (dry or reconstituted).

Material and methods Five treatments were used: Dry cracked corn (DCC), Reconstituted sorghum cracked (RSC), Dried whole sorghum (DWS), Reconstituted whole sorghum (RWS), Dry cracked sorghum (DCS), ground to 3 mm diameter. The reconstitution of the grain was carried out by adding water to the entire grain, to raise the humidity of 35% during the first 42 days ensiled of RWS and the 21 days of RSC, while the DCC was used as control. To determine the kinetics of ruminal degradation using the *in vitro* gas production technique (Menke *et al*., 1988) and modified by Theodorou *et al*. (1994), 800 mg DM per sample were incubated in 125 ml serum bottles during 3,6,9,12,18 and 24 h, to measure gas production (ml gas / g DM) by pressure transducer (Delta OHM, Manometer, 8804), At the end of incubation, dry matter digestibility (DMd, mg/100 mg) was determined as well as the relative gas production (RGP, ml gas / g DMd). Gas production data was fitted in the model of proposed by France *et al*. (1993) (Table 1 and Figure 1)), $GP = A\ \{1\text{-}e^{-b^{(t-T)} - c^{(\sqrt{t}-\sqrt{T})}}\}$, where: GP, Gas production (ml gas / g DM), A, asymptote of the curve (total gas production, ml) b (h^{-1}) c ($h^{-1/2}$) constant gas production and the lag time before the fermentation starts (T, h). An analysis of variance was performed using a completely randomized design, which includes cereal (n = 5) and replication (3 rounds of incubation).

Results Gas production (ml gas / g DM) until 24h (Figure 1) during incubation of the cereals, showed that the RWS and SCC shown a lowest (P <0.001) gas production at 3 h compared to other treatments, while at 6 hours RWS shows the lowest (P <0.001) gas production. However, there were no differences (P> 0.05) at 9 h, whereas at 12h RWS increased gas production *versus* to RCS (P <0.05) *versus* DCS that had the lowest production. RCS was higher (P <0.001) at 18 h, whereas DCC and RWS showed the same gas produced (10.23 ± 0.14) while DWS and DCS had the lowest production (7.74 ± 0.16). The highest gas production (P <0.001) was at 24h in RCS, whereas RWS and DCC showed a similar gas production (9.27 ± 0.44). DMd was greater in DSC and DWS (78.61 ± 1.55), and lower (P> 0.05) in SCC (61.3). DMd at 24 hours was higher (P< 0.001) in dry sorghum *versus* reconstituted grain (Table 1).

Table 1 *In vitro* gas production (ml gas/g DM) of the cereals, A: gas total production (ml gas/g DM incubated), b: fermentation rate (h^{-1}), c: fermentation rate ($h^{-1/2}$), lag time (h), DMd_{24h}: DM disappeared at 24h (mg/100 mg), RGP: (ml gas 24h/g DMd_{24h}). ^{defg} Different letters in the same row ** P< 0.01; *** P<0.001

Item	DCC	RCS	DWS	RWS	DCS	SEM	*P* <
A	89.02^{d}	81.8^{e}	106.5^{f}	88.79^{d}	94.94^{g}	0.3	***
b	0.021^{d}	0.015^{f}	0.015^{f}	0.021^{d}	0.018^{e}	0.1	***
c	-0.037^{d}	-0.023^{e}	-0.022^{e}	-0.038^{d}	-0.031^{f}	0.3	***
Lag time	1.20^{de}	0.74^{e}	1.69^{d}	1.42^{d}	0.74^{e}	0.01	**
DMd	68.16^{d}	61.3^{d}	77.51^{f}	68.43^{d}	79.72^{f}	0.4	***
RGP	130.6^{d}	133.4^{e}	137.4^{f}	179.7^{d}	119.1^{g}	0.7	***

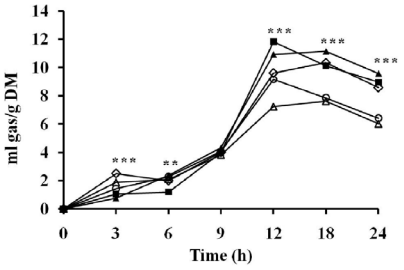

Figure 1 Gas production volume (ml gas/ g DM per h) produced during in vitro fermentation of DCC(□), RWS (■),RCS (▲), DWS (○),DCS (Δ) *** P<0.01; *** P<0.001*

Conclusion Gas production was increased at 12 h in reconstituted sorghums, but when the data was fit mathematically, it was increased in the sorghum dry. However, our recommendations of feeding animals is depending on the actual increased in gas production not after mathematical adjustment

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effect of *Saponaria officinalis* L. on *in vitro* gas production kinetics in cattle

P Zmora[1], M Szumacher-Strabel[1], E Pers-Kamczyc[1], J Szczechowiak[1], B Szajwaj[2], A Stochmal[2], A Cieslak[1]
[1]Poznan University of Life Sciences, Department of Animal Nutrition and Feed Management, Poznan, Poland, [2]Institute of Soil Science and Plant Cultivation, Department of Biochemistry and Crop Quality, Pulawy, Poland
Email: pzmora@jay.up.poznan.pl

Introduction The antimicrobial properties of saponins were confirmed by many authors (Holtshausen *et al*., 2009; Saraf *et al*., 2011). On the basis of earlier studies, the further research are conducted to come to know the mechanisms which are responsible for the effect of saponins on the rumen fermentation and as the consequence for the possibility of their practical usage in ruminants nutrition. The saponins are present in plants, e.g. peppermint or common soapwort (*Saponaria officinalis* L.), which can be used in the animal nutrition. The aim of carried research was to evaluate the effect of powdered *Saponaria officinalis* L. root on rumen gas kinetic and methanogenesis *in vitro*.

Materials and methods The experiment was conducted in an *in vitro* conditions, using Hohenheim Gas Test. The experimental factor was powdered root of *Saponaria officinalis* L. in amount of 0, 4.0, 8.0, 12.0 mg per 40 ml of rumen fluid, supplemented to feed ratio, differing in the level of structural feed (corn silage, high dry matter grass silage or alfalfa silage). The gas kinetics was checked at 2, 6, 12, 24 and 48 hours of incubation. Methane concentration was determined in afterfermentation gases in 24th hour of incubation by gas chromatography in a SRI 310 (SIR Instruments, USA) equipped with a thermal conductivity detector (TCD) and Carboxen – 1000 column (mesh side 60/80, 15 FT x 1,8 INS.S, SUPELCO). The fluorescence *in situ* hybridization (FISH), according to Soliva *et al*. (2004) was used to quantify methanogens in the population in 24th of incubation. The specific oligonucleotide probe was performed for all methanogens (*Archeae*) S-S-GTGCTCCCCCGCCAATTCCT-a-A-20 (Lin *et al*. 1997), which was complementary to 16S rRNA. The dyeing 4′,6-diamidino-2-phenilindol (DAPI, Vectashield® Mounting Medium, Vector) was used to corroborate that the observed fluorescence with the FISH technique corresponded to bacteria cells. Samples were viewed with a fluorescence microscope (Axiovert 200, Zeiss). The number of protozoan population in 24th hour of incubation, with the division into *Entodiniomorph* and *Holotrich*, was determined using the light microscope, according to Michalowski *et al.* (1986). To demonstrate protein fermentation processes, the profile of protein subunits was determined, using the SDS-PAGE technique. The obtained data were subjected to variance analysis using SAS general linear model (GLM) procedure (version 9.1). Differences between the means were tested using the Duncan test.

Results In the following experiment, no significant differences were observed. However, addition of *Saponaria officinalis* to the diet, caused some numerical changes in gas production and methane concentration (Tab. 1). Moreover, in the experimental group the protozoa and bacteria population increased. Ammonia concentration and pH were on similar levels in all groups (8.0 mmol/L and 5.4, respectively).

Table 1 The effect of Saponaria officinalis on the *in vitro* gas production in 24th hour of incubation.

	The addition of *Saponaria officinalis* L. (mg)			
	0.0	4.0	8.0	12.0
100% corn silage				
IVGP (ml)	84.00	85.00	83.67	84.67
Methane (mmol)	5.14	4.90	4.83	5.45
70% corn silage : 30% alfalfa silage				
IVGP (ml)	86.67	87.00	87.00	87.00
Methane (mmol)	4.92	4.78	4.52	5.10
50% corn silage : 50% alfalfa silage				
IVGP (ml)	88.00	87.67	86.3	85.57
Methane (mmol)	5.36	5.00	4.90	5.49

Conclusions The present *in vitro* study suggests that supplementation of ruminant diet with *Saponaria officinalis*, should be higher than 3% of dietary dry matter. Probably the amount of 12 mg per 40 ml of rumen fluid, applied to this experiment was not enough to affect the microbial metabolism, especially the gas production and methanogenesis.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Dose- and time-dependent changes of rumen fatty acids in goat on diets supplemented with algae

H Zhu, S Mao, W Zhu
Nanjing Agricultural University, Nanjing,Jiangsu, China
Email: zhlxiao@126.com

Introduction Previous studies have shown that marine products, such as fish oil and algae, were effective in inhibiting rumen biohydrogenation of unsaturated fatty acids. The long-chain polyunsaturated fatty acid (PUFA) eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA) were active compounds resulting in a wide range of intermediates, predominantly $C_{18:1}$ *t*11 and $C_{18:1}$ *t*10, and finally the end product C18:0 (Boeckaert *et al*., 2008) accumulating. The study aimed to investigate the dose- and time-dependent shifts in rumen fatty acid composition in the rumen of goats when the diet was supplemented with algae.

Materials and methods Six crossbred wethers (18.40±0.95kg), fitted with a ruminal cannula, in a replicated 3×3 Latin square design were offered Chinese wildrye hay plus 1 of 3 concentrates (A0, A1, and A3, referring to the amounts of algae: 0, 10, and 30g.kg $^{-1}$dry matter (DM), respectively. The forage:concentrate ratio was 60:40 (DM).The experimental period lasted for 21 d with 14 d interval between experimental periods. Ruminal digesta were collected from each animal on d 0, 3, 7, 14 and 20 and sampled before (0 h) and 2, 4, 6, and 9 h after morning feeding. Equal amounts of ruminal digesta for each animal and sampling day were pooled and freeze-dried, and stored at -20°C until FA determined. Data were analysed as repeated measures using the mixed procedure of SAS (version 9.1, SAS Institute Inc., Cary, NC, USA).The model included the fixed effect of period, treatment, days of sampling and the interaction term treatment×day and the random effect of goat.

Results Selected data only for d 0, 7, and 20 are given in Table 1. Inclusion of algae in the diet reduced saturated fatty acids proportion (P=0.04), while monounsaturated fatty acid were not affected (Table1). PUFA *n*-3 proportion (P < 0.001) increased upon algal feeding, whereas the proportion of PUFA *n*-6 decreased, this was mainly due to the accumulation of C22:6*n*-3 (P<0.0001) and the reduction of C18:2*n*-6 in the rumen fluid. The high level of algae addition (30 g.kg^{-1}DM) increased the total *trans*C18:1fatty acids (P< 0.001) by 2.5 times relative to control, mainly C18:1*t*10 and C18:1*t*11, whereas it reduced the proportion of *cis*C18:1fatty acids due to the reduction of C18:1*c*9(P<0.001). The level of C18:1 *t*11 (P<0.001) in the treatment with algae addition at 30 g.kg^{-1}DM showed maximum proportion on d7, after which it declined, whereas the maximal level of C18:1 *t*10 was observed on d14. Proportions of CLA *c*9*t*11and CLA *t*10*c*12 on all sampling days did not differ from the values before algae feeding.

Table 1 Dose- and time-dependent effect of inclusion of algae in the diet on the rumen fatty acid composition (g/100 g total fatty acid)

Fatty acid	A_0			A_1			A_3				Significance		
	d 0	d 7	d 20	d 0	d 7	d 20	d 0	d 7	d 20	SEM	Dose	Time	Dose×Time
EPA	0.72	0.72	0.72	0.69	0.72	0.78	0.72	0.52	0.71	0.03	0.44	0.35	0.47
DHA	0.04	0.05	0.04	0.02	3.17	3.32	0.05	8.78	7.00	0.01	<0.0001	<0.0001	<0.0001
Total CLA	0.47	0.41	0.58	0.44	0.37	0.47	0.43	0.28	0.32	0.05	0.04	0.18	0.75
Trans C18:1	4.86	4.87	5.01	4.42	6.30	4.92	4.44	10.5	11.4	0.15	0.01	0.01	0.09
Cis C18:1	7.50	7.48	7.78	6.63	5.56	5.45	6.94	6.18	4.88	0.22	<0.0001	0.16	0.18
SFA	64.0	65.0	64.0	62.4	61.8	64.9	63.8	54.4	56.6	0.44	0.04	0.03	0.14
MUFA	14.4	14.2	14.5	13.1	13.8	12.1	13.7	18.4	18.1	0.32	0.14	0.12	0.60
PUFA *n-6*	6.02	6.23	7.28	5.88	5.35	4.89	6.06	5.86	4.87	0.22	0.003	0.95	0.17
PUFA *n-3*	2.41	2.26	2.16	2.32	5.44	5.41	2.43	10.27	8.84	0.07	<0.0001	<0.0001	<0.0001

[1] *Trans* C18:1∑(C18:1 *t*4; C18:1 *t*5; C18:1 *t*6 to *t*8; C18:1*t*9; C18:1 *t*10; C18:1 *t*11; C18:1 *t*12; C18:1 *t*13 to *t*14)
[2] *Cis* C18:1 ∑(C18:1 *c*9; C18:1 *c*11; C18:1 *c*12; C18:1 *c*13; C18:1 *c*15)
[3] SFA, saturated fatty acids. ∑(C4:0;C6:0;C8:0;C10:0;C12:0; C14:0; C16:0; C17:0; C18:0; C20:0).
[4] MUFA, monounsaturated fatty acids. ∑(C14:1 *c*9; C16:1 *c*9; C18:1 *t*4; C18:1 *t*5; C18:1 *t*6 to *t*8; C18:1*t*9; C18:1 *t*10; C18:1 *t*11; C18:1 *t*12; C18:1 *t*13 to *t*14; C18:1 *c*9; C18:1 *c*11; C18:1 *c*12; C18:1 *c*13; C18:1 *c*14 +*t*16; C18:1 *c*15).
[5] Total CLA ∑(C18:2 *c*9*t*11; C18:2 *t*10*c*12; C18:2*c*9*c*11+*c*10*c*12;C18:2*t*9*t*11+*t*10*t*12).
[6] PUFA *n-6* ∑(C18:2*n-6*;C22:4*n-6*).
[7] PUFA *n-3* ∑(C18:3 n-3; C20:5*n-3*; C22:5*n-3*;C22:6 *n*-3).

Conclusion The results suggest that supplementation of the diet with algae inhibited rumen C18 biohydrogenation, resulting in an increase of C18:1 *t*11 and C18:1 *t*10 proportions and decrease of C18:0 proportion and that this effect was dose- and time-dependent.

Acknowledgments This work was supported by the Natural Science Foundation of Jiangsu Province(Q200710).

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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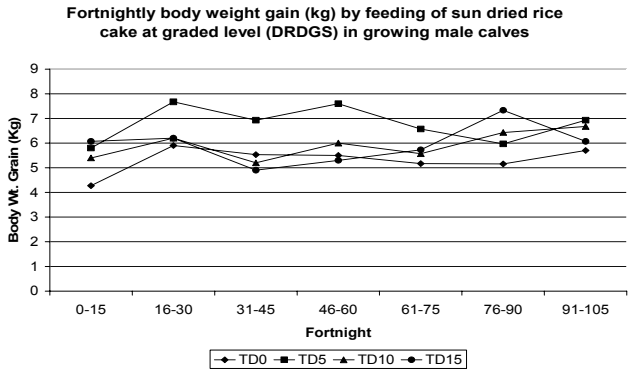
Evaluation of rice distillers grain and soluble as an alternative feed resources for livestock

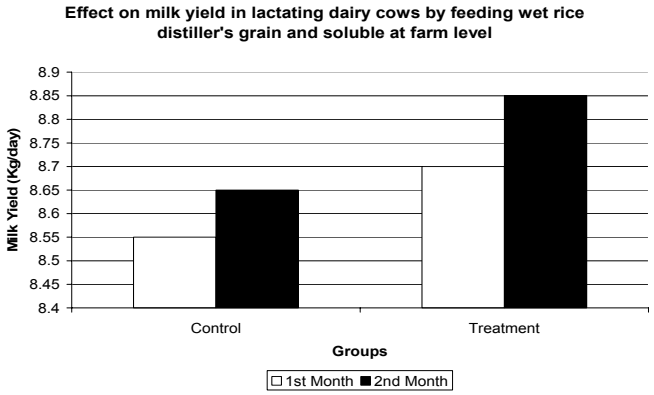
M C Pakhira, P Biswas, L Mondal
West Bengal University of Animal & Fishery Sciences, Kolkata, West Bengal, India
Email: drpbiswas56@yahoo.com

Introduction Rice distiller's grain & soluble (RDGS), an important by-product of the distillery industries in Asian Countries is a good source of protein for dairy cattle (Warner, 1970), high fat and low fiber that stimulate cellulose digestion in the rumen (Hatch, 1993). Use of corn distillers grain and soluble in dried form in livestock and poultry feed is very common. The form of DG with soluble, meaning wet DG with soluble (WDGS) or dried DG with soluble (DDGS) may affect animal performance when fed to lactating dairy cows because there is possibility of heat damage during drying of DDGS, and this may have effects on digestibility and use of nutrients (Powers et. al., 1995). When WDGS is fed, the greater concentration of water in diets may decrease DMI (Lahr et. al. 1983; Hippen et. al., 2003). Wet DGS was well utilized at 31% of diet DM (Schingoethe et. al., 1999) with a slight decrease in DMI. Accordingly, digestibility of rice distillers grain & soluble was determined and fed to calves and lactating cows to find out the effect on growth and milk production in addition to analysis for its nutrient content.

Materials and method Six cross bred male calves were fed measured quantity of sun dried RDGS and paddy straw. Faeces output was recorded and sample kept for chemical estimation daily to find out the digestibility co-efficient. Sixteen cross bred calves were randomly distributed into control(without RDGS) and three test groups(RDGS @ 5, 10 & 15 percent respectively).Sun dried RDGS was included in the in the test feed @ 5, 10 & 15 percent respectively on iso nitrogenous & iso caloric basis as per NRC (2001). Actual feed intake was recorded daily for 90 days of growth trial. The feeding experiment with wet RDGS was conducted with twenty lactating Jersey cows allocated into two dietary treatment groups for 60 days. Control group was fed 4Kg straw + 6 Kg concentrate and test group was fed 5 Kg straw + 4Kg concentrate + 4 Kg wet RDGS (75% moisture).

Results Proximate principles of sun dried RDGS: DM:90.57± 0.35; CP: 60.92 ± 0.27; CF: 0.90 ± 0.03; EE: 4.68 ± 0.07; NFE: 15.22 ± 0.18; Ash: 8.85 ± 0.06 & AIA : 3.12 ± 0.06 percent. No detectable quantity of aflatoxin B1 was found in sun dried rice cake. Average DCP & TDN value of sun dried RDGS was determined as 47.16 and 74.77% respectively. Precisely feed intake by the growing calves (roughage & concentrate) was not significantly affected by dietary supplementation of sun dried RDGS. The overall ADG (g/day) on 105 days of growth period were 354.95, 452.09, 394.95 and 396.19 respectively for control, TD_5, TD_{10} & TD_{15} respectively. The TD_{10} & TD_{15} groups showed almost similar result and TD5 showed better result. The highest ADG was found in TD_5 group & the lowest was in control (TDO) group. All the above values did not bring forth any significant difference (P>0.05) among treatment groups as well as with control group.





Conclusion Rice distillers grain & soluble is a potent source of high quality protein feed for livestock and sun dried rice distillers grain & soluble can be included up to 15% level in the concentrate mixture to support growth of calves and wet rice distillers grain & soluble can replace 33% of concentrate mixture to support milk production.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Improving the nutrition and health of cattle in Cambodia by successful introduction of forages to small-holder farmers

R Bush[1], S Sothoeun[2], J Young[1], L Rast[1], P Windsor[1]
[1]University of Sydney, Camden, NSW, Australia, [2]Department of Animal Health & Production, Ministry of Agriculture, Forestry and Fisheries, Phnom Penh, Cambodia *Email: russell.bush@sydney.edu.au*

Introduction Lack of knowledge and effective implementation of interventions to improve the nutrition and health of cattle in smallholder village-level enterprises in Cambodia is a major deficiency that limits the potential expansion of cattle production from subsistence inactivity to a productivity focus. Most Cambodian farmers raise cattle extensively in the traditional manner where feed is based on the natural grasses available along the road sides, surrounding households, in Chamkars or in the paddy rice fields after the rice harvest. The introduction of forages for Cambodian farmers has been attempted since early 1960 with minimal success. This paper focuses on the recent successful introduction, adoption and utilisation of forages by intensive farmer training, to address year round feed deficits and improve cattle weight gains and health. Associated social implications will also be discussed.

Materials and methods This project trialled the integration of interventions to improve cattle productivity in 3 villages (High Intervention) located in the 3 provinces of Kampong Cham, Kandal and Takeo, comparing productivity and health parameters to measures obtained from 3 closely matched villages (Low Intervention) where the interventions were limited to vaccination only. Initially 5-6 farmers were selected in each of the three intervention villages to grow, maintain and harvest recommended forage species using supplied seed, seedlings or rooted tillers. This number increased each year based on farmer interest and demand. Farmer selection was based on commitment, having in excess of one animal plus the potential capability to fence and irrigate at least 300m^2 of land. For the period 24 March 2008 until 06 September 2010 (30 months), data on weight gain of 319 cattle in the control and intervention groups was obtained from six weighings at approximately 5 months intervals.

Results In 2008, the project planned to recruit 5 to 6 households in each of the three project locations. However due to the enthusiasm of participants the project established 52 forage plantation sites among 52 households on 26,520m^2, including 34 households in Takeo province, 12 in Kampong Cham and 6 in Kandal. It was noted that many farmers in nearby villages commenced planting forages by themselves, having visited the project forage plantations where they learned techniques from farmers and from information provided on TV and radio. In 2009, 347 project households had established forage plantations on 130,950m^2, including 212 households in Takeo province, 65 in Kampong Cham and 70 in Kandal. By November 2010, 456 project households had established forage plantations on 195,938m^2, including 263 households were located in Takeo province, 148 in Kampong Cham, 15 in Kandal, and when forage plantations adjacent to the project sites were included, a total of 854 plantations had been established on 355,056 m^2 with spread from Takeo to the adjacent province of Kampot involving 30 households.

Cattle weight gains were recorded across six age groups with the 2 year old age group presented in Table 1. Note the difference in weight gain between the low intervention villages (0.044-0.068 kg/hd/day) and the high intervention villages (0.108-0.166 kg/hd/day). This trend was typical for all age groups.

Table 1 Comparison of weight gain in cattle (1-2 years of age in high and low intervention villages

Village Intervention	n	Initial weight (kg)	Final weight (kg)	Weight Gain (kg)	Ave. Daily Gain (kg/hd/day)
Low	14	155.6	211.8	56.2	0.062
Low	7	120	181	61	0.068
Low	7	239.1	278.4	39.3	0.044
High	14	123.3	220.6	97.4	0.108
High	14	110.4	208.6	98.2	0.109
High	8	156.3	305.3	149	0.166

Conclusions The project has successfully demonstrated that despite initial resistance from farmers that complained that growing grass for cattle was unnecessary, when farmers are trained to understand the year round energy deficiency that exists in the rice-based subsistence system of cattle raising, forages technology can be rapidly adopted in project sites and surrounding villages. Within 30months and despite the limited amount of forage available, we recorded improved weight gains in excess of 0.1 kg/hd/day in young cattle compared to less than 0.07 kg/hd/day in animals relying on traditional sources of nutrition such as native grasses, rice straw and rice stubble. The social implications of forage utilisation include labour savings due to major reductions in time taken to supervise grazing animals and cut-and-carry grasses for feeding (10mins instead of 60mins per animal per day) plus the benefits of readily collected manure from forage-fed cattle for production of gas for lighting and cooking through biodigestion. Although these average daily weight gains are small when compared to more advanced intensive feeding systems, it should be noted that initially, the forages grown are distributed between all the animals owned by a farmer. The introduction of targeted feeding in order to fatten identified animals will result in vastly improved average daily weight gains and additional training is required to enable progressive farmers to better utilise their new forage resources for fattening. Importantly, increased interest by farmers to invest in disease risk management is emerging, with demonstration of the absence of infectious diseases (such as FMD and HS) in trial sites despite nearby epidemics. Our studies suggest that integration of nutrition and health is an important strategy for potentially alleviating poverty of rural smallholder farmers and for addressing regional transboundary disease control initiatives and local food security management (Windsor, 2011).

Acknowledgements The Australian Centre for International Agricultural Research is acknowledged for funding this work.

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Supplementary effect of thermotolerant probiotic yeast (*Saccharomyces cerevisiae*) on growth and carcass traits in Nellore lambs

C Harikrishna, M Mahender, Y Ramana Reddy, M Gnana Prakash, K Sudhakar, M Pavani
Sri Venkateswara Veterinary University, Hyderabad, India
Email: drhkvet@gmail.com

Introduction A number of studies reported beneficial effect of yeast supplementation on the meat performance traits including body weight, daily gains and growth rate (Milewski, 2009). But generally the yeast used as probiotic in livestock rations may not exert more beneficial action due to the harsh environmental (temperature, variation in pH and bile concentration) conditions under which they have to survive. Lankaputra and Shah (1995) reported that, high temperature in the gut of animals makes the limitation of using yeast as probiotic. Hence, an attempt was made to know the effect of supplementation of thermo, acid, bile and osmo tolerant strain of *Saccharomyces cerevisiae* (OBV-9), isolated by Bhima *et al*. (2008), in comparison to mesophilic strain of *Saccharomyces cerevisiae* on intake, growth, feed conversion and carcass traits in Nellore ram lambs.

Materials and methods Eighteen Nellore ram lambs (12.33 ± 0.08 kg initial body weight (BW), aged 3 months) randomly divided into three groups comprising of six lambs each were allocated to one of three sorghum straw based complete rations (50R:50C, 12% CP) in the 180 days experiment; without yeast (CON; n=6), supplementation with 1 g/kg mesophilic yeast-MTCC-1813 (MPY_1; n=6) and with 1 g/kg thermotolerant yeast-OBV-9 (TPY_1; n=6) at the rate of $5x10^8$ cfu/g. Ram lamb groups were kept separately under hygienic conditions in well ventilated pens and test diets were offered twice daily. The ram lambs were weighed fortnightly for two consecutive days and the mean was taken to represent the BW. Average daily gain (ADG, g) was calculated as the (final BW (g) – initial BW (g))/number of days on trial. Feed conversion ratio (FCR) was calculated as the amount of feed consumed (kg DM) per BW gain (kg). At the end of the trial, three lambs from each group were slaughtered following standard procedures described by Gerrand (1964), to evaluate the carcass traits. Data were analysed using the GLM procedures of SAS (2001). In all analyses, when least squares means were different at $P<0.05$, they were separated by the PDIFF option of SAS.

Results Dry matter intake (g/day or g/kg $w^{0.75}$/day was higher ($P<0.01$) in lambs fed TPY_1 ration than those fed CON and MPY_1 rations (Table 1), while intake of OM and CP was similar among all the groups. Intake of NDF and ADF was higher ($P<0.01$) in lambs fed TPY_1 diet compared to those fed CON and MPY_1 diets. The final BW, total gain and ADG were higher ($P<0.01$) in lambs fed TPY_1 diet followed by MPY_1 compared to CON diet. Lambs receiving TPY_1 and MPY_1 diets had superior ($P<0.05$) FCR and cost/kg gain (£) than those fed CON diet. The dietary treatments did not affect the dressing percentage, though there was a higher ($P<0.01$) carcass weight on MPY_1 and TPY_1 diets. There was no effect of test diets on per cent proportions of all carcass cuts, except proportions of leg, which is higher ($P<0.05$) in TPY_1 group. There was also no effect of test diets on proportions of lean, bone and fat and bone to meat ratios. Significantly ($P<0.01$) higher per cent weights of edible components and lower ($P<0.01$) per cent weights of non edible components were recorded on TPY_1 and MPY_1 diets than CON diet.

Table 1 Intake, FCR and growth performance of Nellore lambs fed diets supplemented with thermotolerant probiotics yeast

Parameter	Test diets			
	CON	MPY_1	TPY_1	SEM
DMI (g/day)	908.27[a]	938.78[a]	994.06[b]	12.29
DMI/kg $w^{0.75}$ (g/day)	86.47[a]	88.30[a]	90.01[b]	0.53
Initial body weight (kg)	2.27	2.33	2.40	08
Final body weight (kg)	5.33[a]	7.13[b]	9.70[c]	78
Total gain (kg)	3.07[a]	4.80[b]	7.30[c]	75
ADG (g)	72.59[a]	82.22[b]	96.11[c]	4.18
FCR[*]	12.51[c]	11.42[b]	10.34[a]	2.56
Cost/kg gain (£)[*]	0.94[c]	0.87[b]	0.83[a]	1.08

[a, b, c] : Means with different superscripts row wise differ significantly (P<0.01); [*](P<0.05).

Conclusions Dietary supplementation of thermotolerant probiotics yeast (1 g/kg diet) improved the growth performance, feed conversion ability and certain carcass characteristics of Nellore ram lambs economically, when compared to mesophilic yeast.

Acknowledgements The authors are thankful for the financial assistance from Department of Biotechnology, Government of India, New Delhi, India.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Fattening and slaughter performance of Simmental bulls fed isoenergetic diets with varying levels of grass silage

T Ettle, A Obermaier, H Spiekers, H Meiser, S Weinfurtner
Bavarian State Research Center for Agriculture, Institute for Animal Nutrition and Feed Management, Poing, Germany
Email: thomas.ettle@lfl.bayern.de

Introduction Increased need of maize silage for biogas production or occurrence of pests as *Diabrotica virgifera virgifera* may result in a shortage of availability of maize silage for animal feeding. Studies on use of grass silage, which may be considered as an alternative roughage source in fattening bulls, mainly resulted in depressed performance due to inclusion of grass silage in diets. In those studies, diets were corrected for low protein concentration of maize silage, but not for lower energy concentration of grass silage (e.g. Juniper *et al*. 2005). For this reason, the present study aimed to investigate effects of partial replacement of maize silage by grass silage in diets for fattening bulls on an isoenergetic basis. The study was part of the "Diabrotica programme" set up by the federal government of Germany and the federal state of Bavaria.

Material and methods 72 Simmental bulls (250 ± 18 kg, 197±7 days old) were equally assigned to three feeding groups according to body weight and ancestry. Group 1 was fed a Total Mixed Ration (TMR) based on maize silage, straw and concentrates. In diets for groups 2 and 3, 300 and 600 g/kg of maize silage and straw (based on DM) were substituted by grass silage. Extracted soybean meal in concentrates of group 1 was replaced by corn and rape cake in concentrates of groups 2 and 3 to obtain similar CP and ME concentrations of TMR. Portion of concentrates in TMR was comparable for feeding groups in fattening period 1 (260 to 270 g/kg of DM) and 2 (190 to 210 g/kg of DM), but increased from 210 and 230 to 280 g/kg of DM in groups 1, 2 and 3 in fattening period 3. TMR and water were provided *ad libitum*. Individual feed intake was automatically recorded daily, live weight was recorded every 4 weeks and back fat depth every 12 weeks. Data was evaluated by a one-factorial ANOVA and SNK-test using SAS. Data is presented as means ± SD. Because of animal losses not associated to feeding regime, for groups 1, 2 and 3 data of 21, 19 and 21 animals were considered.

Results Feed, CP and energy intake in group 2 was slightly higher than in other groups (Tab 1). Time to reach end weight of 750 kg was 316, 308, and 321 days for groups 1, 2 and 3, respectively. Average daily gain was not affected by treatment. Moreover, there was no influence on hot carcass weight, dressing or carcass conformation. Carcass fatness classification was slightly higher in animals fed diets with grass silage, and back fat depth in the middle of the fattening period and at slaughter was significantly ($P<0.05$) increased. Intramuscular fat content and pH value, share force, drip losses and grilling time of meat were not influenced by treatment. ω-6/ω-3 – ratio of meat of m. l. dorsi decreased ($P<0.05$) when higher proportions of grass silage were fed. There was no influence on meat colour, but subcutaneous fat was more yellow ($P<0.05$) in grass silage fed bulls (Tab. 1). There was no effect on serum GLDH-activity and concentration of urea, total protein and glucose.

Table 1 Feed intake, nutrient and energy intake, growth and slaughter performance and selected meat characteristics (mean±SD)

	group 1		group 2		group 3	
Feed intake (kg DM/day)	9.3	± 1.1	9.6	± 0.8	9.3	± 1.1
CP intake, g/day	1215	± 151	1322	± 125	1285	± 153
ME intake, MJ/day	110	± 13	113	± 10	109	± 13
Initial weight, kg	248	± 19	252	± 15	252	± 17
End weight, kg	748	± 13	747	± 12	744	± 28
Average daily gain, g	1595	± 158	1615	± 122	1550	± 196
Dressing proportion, %	58.7	± 1.1	58.8	± 1.4	59.0	± 1.1
Carcass classification (EUROP)*	2.48	± 0.51	2.63	± 0.50	2.57	± 0.51
Fat classification**	2.57	± 0.51	2.84	± 0.50	2.76	± 0.54
Back fat depth at slaughter (cm)	1.74	± 0.26^{c}	1.90	± 0.37^{b}	2.12	± 0.31^{a}
Intramuscular fat (%)	2.49	± 0.72	2.59	± 0.5	2.54	± 0.72
Fat colour (b*; haunch)	5.29	± 1.81^{b}	7.07	± 2.16^{a}	8.12	± 2.59^{a}
ω-6/ω-3 – ratio of m. l. dorsi	11.96	± 1.39^{a}	6.81	± 0.74^{b}	5.14	± 0.84^{c}

^{a,b} Values differ at $P< 0.05$; *E=1,…, P=5;** 1=lean, …, 5=fat

Conclusions Inclusion of grass silage in diets for fattening bulls had only minor influence on growth performance or carcass characteristics of fattening bulls. Therefore, partial replacement of maize silage with grass silage may be a valid alternative in situations where availability of maize products is limited, but lower energy concentration of grass silage has to be accounted for diet formulation.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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

M A Shahzad, M Sarwar, M U Nisa
Institute of Animal Nutrition and Feed Technology, University of Agriculture, Faisalabad, Pakistan
Email: aasifshah9@hotmail.com

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.

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.

Table 1 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

Conclusions 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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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Fattening performance and meat characteristics of crossbred cattle (*Bos indicus*) and crossbred water buffalo (*Bubalus bubalis*) in the Philippines

M Kondo[1], T Ban[1], R M Lapitan[2], A N del Barrio[2], J R V Herrera[2], T S Saludes[2], A Tandang[2], M Fujii[1], M Yamaguchi[1], T Nade[3], H Matsui[1], T Fujihara[1]
[1]Mie University, Tsu, Mie, Japan, [2]Philippine Carabao Center, Los Banos, Laguna, The Philippines, [3]Nippon Veterinary and Life Science University, Musashino, Tokyo, Japan
Email: makok@bio.mie-u.ac.jp

Introduction In the Philippines, the demand for milk and meat from water buffalo has been increasing. However, limited information is available on fattening performance of water buffalo at a younger age. Recent studies have shown that crossbred cattle and crossbred water buffalo at the same age (18-24months) and fed with high grain-based diets were comparable in terms of meat production, carcass and meat quality (Lapitan *et al*. 2004).Fattening at 18-24 months resulted to high growth rate and better organoleptic quality of meat. However, there is still a higher cost of grain feed for smallholder farmers. In tropical countries, napier grass is the major forage because it is perennial and high yielding. As alternative feed resources to grain, agro-industrial byproducts such as copra meal and rice bran are also readily available for use. Regarding the meat of water buffalo, it has been reported that nutritional compositions (cholesterol, fatty acids, etc.) are different or similar to cattle (Spanghero *et al*. 2004, Lapitan *et al*. 2007), but they are could be affected by the type of feeds. Therefore, this study aimed to compare the fattening performance and meat characteristics of crossbred cattle and crossbred water buffalo based on napier grass and locally available agro-industrial byproducts.

Materials and methods Five crossbred male cattle (Philippine native cattle x Brahman) and five crossbred male water buffalo (Philippine native water buffalo x Murrah), with an average age of 22 months were housed in individual pens. The feeds were offered *ad libitum* and composed of fresh napier grass and concentrate mixture, at ratios of 50% napier grass and 50% concentrate mixture on a dry matter (DM) basis. The concentrate mixture consisted of 40% copra meal, 20% rice bran, 20% wheat bran, 10% of molasses, 5% of wheat, and 5% of minerals and vitamins. The feeding trial lasted for 16 weeks and dry matter intakes (DMI) were recorded daily. The digestion trial was done in the 8th week. The total faecal output for 24 h was collected for 6 consecutive days to determine the nutrient digestibility. After finishing the feeding trial, animals were slaughtered and the carcass weights were measured. After chilling the carcass for 48 hours, the ninth to 11th rib cuts were taken for the determination of proximate compositions of the meat. All data were analyzed using two-tailed Student's t-test.

Results The feed intake of napier grass and concentrate mixture among crossbred cattle and crossbred water buffalo was not significantly different. During the feeding trial, average daily gains of both species were almost same at 0.74 kg per day and feed conversion ratios were not statistically different (9.9 vs 10.7, $P>0.05$, for crossbred cattle and crossbred water buffalo, respectively). The apparent nutrient digestibility (organic matter, crude protein, non-fibre carbohydrate and NDF) between species were not significantly different (Table 1). In terms of slaughter traits, the percentage of dressing yield based on the chilled carcass weight were significantly higher in crossbred cattle than crossbred water buffalo (52.3 vs 48.6%, $P<0.05$), but the percentage of wholesale cut yields based on chilled carcass (chuck, rib, round loin, etc.) were comparable between species. There was no significant species difference in crude protein content in *longissimus dorsi* muscle, but crude fat and cholesterol contents were significantly lower in crossbred water buffalo than crossbred cattle (Table 2). The weight percentage of unsaturated fatty acid in *longissimus dorsi* was higher in crossbred water buffalo than in crossbred cattle.

Table 1 Apparent nutrient digestibility of crossbred cattle and crossbred water buffalo fed napier grass and concentrate mixture

	Cattle	Buffalo
Organic matter	0.62±0.005	0.65±0.025
Crude protein	0.61±0.010	0.65±0.023
Non-fibre carbohydrate	0.80±0.004	0.79±0.016
NDF	0.49±0.007	0.54±0.032

Table 2 Proximate composition of longissimus dorsi muscle of crossbred cattle and crossbred water buffalo

	Cattle	Buffalo
Moisture (g $100g^{-1}$)	$74.3±0.26^{a}$	$76.0±0.35^{b}$
Crude protein (g $100g^{-1}$)	20.9±0.25	20.7±0.47
Crude fat (g $100g^{-1}$)	$2.7±0.37^{a}$	$1.0±0.12^{b}$
Cholesterol (mg $100g^{-1}$)	$53.5±2.65^{a}$	$43.5±1.15^{b}$

ConclusionsBased on the locally available feedstuffs (napier grass and concentrate mixture from agro-industrial byproducts) in the Philippines, crossbred water buffalo showed comparable fattening performance in terms of weight gain, feed conversion ratio and meat production. The meat quality of *longissimus dorsi* in crossbred water buffalo is slightly different from crossbred cattle in terms of fat, cholesterol and fatty acid composition in this study. Further research is necessary to investigate meat production based on local feedstuffs, as well as characterise the meat in crossbred water buffalo in the Philippines.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effect of different level of soybean oil on methane emission and celluloytic bacteria *in vitro*

W Hongrong, W Rina, Y Lihuai
Yangzhou University, Jiangsu Province,, China
Email: hrwang@yzu.edu.cn

Introduction Plant oils are considered as a natural source of unsaturated fatty acids (UFA). Soybean oil mainly consists of linoleic acid (18:2, 64%). Added to ruminant diets plant oils can modify the metabolic processes occurring in the rumen, e.g. methane formation, fibre degradation, bacteria' activity (Cies'lak *et al*., 2003; Giger-Reverdin *et al*., 2003). The aim of the present study was to ensure diets with suitable level of soybean oil affect the methane formation and celluloytic bacteria community in ruminants.

Materials and methods 50mL injector tubes (with a tree -way valve) contained 200mg synthetic diet and 200ml conical flask (with stopple, with two holes for venting and colleting samples) contained 2g synthetic diet was prepared in gas production experiment and incubation trials respectively. The synthetic diet contained 675.4 g, cellulose (packed by nylon and weighed for detecting Cellulose degradation), 346.6 g starch, 178 g casein, and 0 g fat per kg dry matter (DM). Fat from the supplements represented 0%(group A), 2%(group B), 4%(group C),6%(group D) ,8%(group E) of the substrate DM in each sample tube. Inoculums was obtained via rumen cannula from two goats before they received their morning aliquot of a total mixed ration (70% forage and 30% concentrate). Rumen contents were obtained from four sites within the rumen and the associated liquid squeezed through two layers of cheesecloth. The following parameters were measured: pH was measured immediately and the volatile fatty acids were measured with a gas chromatograph. Fermentation gases were collected daily and analyzed for gas volume, and then methane was quantified with a gas chromatograph. Celluloytic bacteria' enzyme activity was detected through absorbency on spectrometer and relative quantity were calculated through Bandscan system on specific primer PCR products (Kobayashi et al., 2001). All data were analyzed using LSD procedure of SPSS15.0 (User's Guide, 2006).

	Adding level					
	Gro A	Gro B	Gro C	Gro D	Gro E	S.E.M
48h Gas production (ml)	15.0^{b}	12.2^{ab}	12.5^{ab}	11.0^{a}	10.7^{a}	0.75
48h Methane(mMol/L)	0.20^{c}	0.13^{ab}	0.13^{ab}	0.09^{a}	0.08^{a}	0.02
TVFA (mMol/L)	41.6	37.41	39.86	40.43	38.54	5.2
pH Average value	6.22^{a}	6.50^{ab}	6.54^{ab}	6.61^{bc}	6.72^{bc}	0.06
Filterpaper-enzymes(IU)	0.85	0.87	0.84	0.87	0.74	0.05
CMC-enzymes(IU)	0.93	0.99	0.95	0.93	0.8	0.1
Cellulose powder disappearance (%)	68.32^{a}	67.72^{a}	67.58^{a}	65.26^{b}	64.07^{c}	0.07

Results With the increase level of soybean oil supplement, gas production, methane production decreased gradually; The activity of the filter paper-enzymes fluctuate with the incubation time ,but no obviously difference among groups ; There were no significantly difference on CMC-enzymes among groups at different time except 10h the E group lower than A、B ($P<0.05$) . Treatment with different level of plant oil had different effect on relative quantity of celluloytic bacteria: ⑴: After 24 hours incubation , the relative quantity of *Fibrobacter succinogenines* for group E was highly significantly lower than group A ($P<0.01$) and lower than other groups significantly ($P<0.05$) ; ⑵For *Ruminococcus flavefaciens,* ,there were similar trend , the relative quantity in group E was lower than control group significantly ($P<0.05$) , although there was no obviously difference among treatments until 12h or 24h incubation. ⑶For *Ruminococcus albus, a*fter 24h incubation, the relative quantity in group E was significantly lower than group A ($P<0.01$) and other groups ($P<0.05$) respectively. ⑷For *Butyrivibrio fibrisolvens,* after 24h incubation, the relative quantity in group D and E ware significantly lower than group A ($P<0.01$) and other groups ($P<0.05$) respectively. The suitable supplement level of oil was 2% and 4% group in this experiment through several indexes including gas production、pH level、VFA concentration and fibre degradation rate. It has to be underlined that counting total bacteria reveal possible fluctuations in the population of individual microbial species. Therefore, some microbial species may be replaced by others, methane release was significantly reduced by all plant oils giving evidence that UFA inhibited methane formation by directly affecting the ruminal methanogens in their metabolic activity.

Conclusions It was concluded that with the increase level of soybean oil supplement, gas production, methane production decreased gradually. The activity of the filter paper-enzymes varies with the incubation time and treatments although no obviously difference among groups. Different level of plant oil had different effect on relative quantity of celluloytic bacteria.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Serum mineral concentration in dairy heifers fed with different protein sources diets

R C Canesin[1], D A Mota[2], T T Berchielli[1], G Fiorentini[1], A F Ribeiro[1], B L Rosa[1], J D Messana[1]
[1]São Paulo State University, Jaboticabal, São Paulo, Brazil, [2]Federal University of Amazonas, Parintins, Amazonas, Brazil
Email: betacanesin@hotmail.com

Introduction The protein sources addition in the diet to animals fed with sugar cane is essential to a correct nutritional balance, which can be determined by the some metabolites blood concentration. Mineral deficiencies are very common, and most of those deficiencies or unbalances are not clinically manifest, but they can cause losses in production indexes even at subclinical levels. The study aimed evaluate the effect of different protein sources in serum mineral concentration, total calcium, phosphorus, and magnesium, as well ionized sodium, potassium, and calcium in dairy heifers fed with diets based on sugar cane.

Materials and methods Eight Holstein x Zebu dairy heifers crossbred, averaging 202.12 ± 11.54 kg body weight and 18 months of age, were used. The sugar cane was used as exclusive forage, it was hand-picked and chopped daily to provide to the animals particle size of about 2.0 cm; and 60:40 diet ratio (forage:supplement) having 13% CP. The diets were composed by different protein sources: soybean meal (60% sugar cane, 15% soybean meal, 23.5% corn grain, 1% mineral and 0.5% urea); cottonseed meal (60% sugar cane, 14.2% cottonseed meal, 23.8% corn grain, 1% mineral and 1% urea); peanut meal (60% sugar cane, 13% peanut meal, 25.3% corn grain, 1% mineral and 0.7% urea); and sunflower meal (60% sugar cane, 16.2% peanut meal, 21.75% corn grain, 1% mineral and 0.85% urea). The experimental design used was double 4x4 latin square with four treatments, eight replications, and four experimental periods. The experimental period was twenty-eight days, being eight days to adaptation and twenty to data collection, within these, one day was used to blood sampling, which was performed before (0 hours) and after (4 hours later) feeding. The blood sampling was performed after prior disinfection with *vacutainer* tubes without anticoagulant by jugular vein puncture with needles. The *vacutainer* tubes were conditioned in an isothermal box, at 8-15°C. Subsequently, blood was centrifuged at 1367 *x g* for 15 minutes to separate the serum. The serum was retained and stored in *eppendorfs* (l.5 mL) and frozen at -20°C. To determine total serum calcium, phosphorus and magnesium, and ionized sodium, potassium and calcium commercial kits were used and the analysis were performed in a semi-automatic biochemical analyzer model LabQuest®. Data were subjected to analysis of variance by PROC GLM of SAS (2004) and adjusted means were compared using the Tukey test (5% probability).

Results There was no interaction among diets and sampling time to total minerals serum concentration (P>0.05). Significant differences in total phosphorus serum concentrations to the sunflower meal and peanut meal (P=0.01), and total magnesium to the peanut meal and cottonseed meal protein sources (P=0.03) were presented. To total calcium, ionized sodium, potassium, and calcium the difference was no significant (P>0.05) between protein sources though.

Table 1 Total calcium (Ca), phosphorus and magnesium (Mg) serum, and ionized sodium (iNa), potassium (iK), and calcium (iCa) of heifers fed with different proteic sources.

	Protein sources*				*P*
	SBM[1]	SFM[2]	PNM[3]	CTM[4]	
Ca (mg dL^{-1})	8.00	8.03	8.21	8.08	0.94
P (mg dL^{-1})	6.26 ab	7.25 a	5.87 b	6.93 ab	0.01
Mg (mg dL^{-1})	2.19 ab	2.26 ab	2.18 b	2.38 a	0.03
iNa (mmol L^{-1})	137.81	138.81	136.21	138.19	0.84
iK (mmol L^{-1})	3.66	3.63	3.69	3.53	0.94
iCa (mmol L^{-1})	0.96	0.94	1.00	0.95	0.77

*The diet means were obtained using the sampling time averages (n= 8 dairy heifers $diets^{-1}$); [1]Soybean meal; [2]Sunflower meal; [3]Peanut meal; [4]Cottonseed meal. P= significance. Means followed by different letters are significantly different at P<0.05.

Conclusions The protein sources influence total serum concentration of minerals. However, the values are in the normal reference level, 8-12 mg dL^{-1} to total calcium, 2-3 mg dL^{-1} to magnesium and higher 3 mg dL^{-1} to phosphorus (González, 2000). The ionized sodium, potassium, and calcium concentration was not affected by different protein sources.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Molecular diversity of Methanogens in the hindgut of Bactrian camels (*Bactrianus camelus*) from two zoos

K L Turnbull, R P Smith, A-D G Wright
University of Vermont, Burlington, VT, USA
Email: rachel.p.smith@uvm.edu

Introduction In Asia and the Middle East, camelid husbandry represents an increasingly popular market. The role of camels in the more arid regions of the world is becoming more prominent as they are discovered to be an economic alternative to traditionally raised livestock, and they are well suited to the climate. Camels are pseudoruminants, possessing a three-chambered stomach, and like ruminants, they rely heavily upon their gut microbial consortium to digest their food and to allow them to obtain adequate nutrients (Mackie *et al*., 1997). They are also host to methanogens, which are able to produce methane from hydrogen and carbon dioxide in a process called enteric fermentation. The objectives of the present preliminary study were to identify and compare methanogenic archaea from fresh fecal samples from Bactrian camels.

Materials and methods Fresh faecal samples were obtained from two captive Bactrian camels at Southwick Zoo (Massachusetts, USA) and two captive Bactrian camels at Potter Park Zoo (Michigan, USA). Samples were sent frozen to the University of Vermont (USA). The camels at Southwick Zoo were offered a diet of grass hay supplemented with Canadian alfalfa and trace minerals, while the camels at Potter Park Zoo received grass hay supplemented with alfalfa along with a high starch pelleted feed. DNA extraction and purification was performed as previously described (Yu & Morrison, 2004), and methanogenic 16S rRNA genes were PCR amplified. Amplicons from camels from the same zoo were pooled and used to construct separate 16S rRNA gene clone libraries. Based upon a species-level sequence identity criterion of 98%, MOTHUR (Schloss *et al*., 2009) was used to assign sequences across the two libraries to operational taxonomic units (OTUs).

Results In total, 108 clones were examined from the two 16S rRNA gene clone libraries, revealing 75 phylotypes assigned to 11 OTUs (Figure 1). OTUs 1 and 3 were the only OTUs found in both libraries (Figure 1) and accounted for 96% and 79% of the clones from Southwick Zoo and Potter Park Zoo, respectively. Interestingly, phylotypes belonging to OTU 1 were nearly 4 times higher in the Southwick library than the Potter Park library, whereas phylotypes belonging to OTU 3 were nearly 3 times higher in the Potter Park Library than the Southwick library (Figure 1). Furthermore, 98% of the clones from the Southwick library belonged to the genus *Methanobrevibacter,* as did 96% of the clones in the Potter Park library.

Figure 1. Pie-chart comparison of the distribution of clones assigned to OTUs from the Southwick Zoo and the Potter Park Zoo, based upon a species-level 98% sequence identity criterion

Conclusions Findings from this study are consistent with previous reports that *Methanobrevibacter* species are the dominant methanogen phylotypes found in herbivores worldwide. While none of the 16S rRNA gene sequences generated from the present study branched within the clade consisting of uncharacterized archaea (Wright *et al*., 2006), we also identified methanogen sequences related to *Methanocorpusculum* and *Methanosphaera*. *Methanocorpusculum* species are not usually found in the foregut, but they have been identified in a variety of hindgut fermenters (A-DG Wright, unpublished data). Since our results are primarily consistent with similar studies conducted on ruminants, it is likely that archaea involved in hindgut fermentation were also identified because faecal samples were used. The differences between the two libraries may be due to either subtle differences in diet composition between zoos, or sampling density. Therefore, additional studies utilizing alternative techniques, such as next generation sequencing, are warranted in order to further investigate the microbial diversity in Bactrian camels.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Differences in the rumen methanogen population of lactating Jersey and Holstein dairy cows under the same diet regimen

E E King, R P Smith, B St-Pierre, A-D G Wright
University of Vermont, Burlington, VT, USA
Email: rachel.p.smith@uvm.edu

Introduction Holstein and Jersey breeds account for the vast majority of animals used in the dairy cattle industry within the US and world-wide. Methane emission is emerging as an important efficiency trait, due to its negative impact on animal production and its contribution to climate change (Hook *et al.*, 2010). Since the continuous growth of the human population is expected to result in an increase in the number of domesticated ruminants, reducing livestock's methane contribution has become a priority and an integral part of climate control policies (Thorpe, 2008). The specific objectives of our study were: 1) to identify methanogens that reside in the rumen of Jersey and Holstein cattle and to determine their phylogeny; 2) to investigate whether methanogen population structures vary significantly between breeds; and 3) to compare our findings with previously published reports.

Materials and methods Nine Holsteins and 10 Jerseys were randomly selected from a lactating herd from Burlington, Vermont (USA) for rumen sampling by stomach tubing. The herd was maintained under the same environmental conditions and fed the same diet. Microbial DNA from rumen samples was isolated as described by Yu and Morrison (2004). Methanogen 16S rRNA gene sequences were amplified from purified rumen microbial DNA by PCR as previously described (Wright & Pimm, 2003). Amplicons produced from animals of the same breed were pooled, cloned, and sequenced to generate breed-specific methanogen 16S rRNA gene clone libraries. All non-chimeric sequences from both libraries were pooled *in silico*, then assigned to operational taxonomic units (OTU) by the open-source program MOTHUR (Schloss, *et al*., 2009) using distance data according to the Kimura two-parameter model based on a 98% sequence identity cut-off. PHYLIP was used to construct a neighbour-joining tree which was bootstrap resampled 1,000 times.

Results A total of 365 non-chimeric sequences were obtained, with 180 and 185 clones isolated from the Holstein and Jersey clone libraries, respectively. Sequences from both libraries were assigned to 55 OTUs based on a 98% sequence identity criterion. Twenty OTUs, representing 85% of combined library sequences, were common to both breeds, while 23 OTUs (36 sequences) were only found in the Holstein library and 12 OTUs (18 sequences) were only found in the Jersey library, highlighting increased diversity in the Holstein library. Other differences included that sequences in OTUs with species-like sequence identity to *Methanobrevibacter millerae* were more highly represented in the Jersey breed, while *Methanosphaera*-related sequences and novel uncultured methanogen clones were more frequent in the Holstein library. In contrast, sequences in OTUs with species-level sequence identity to *Methanobrevibacter ruminantium* were similarly represented in both libraries.

Conclusions Our study is the first to compare Holstein and Jersey breeds under the same diet and environmental conditions, allowing confirmation of suggested differences between breeds. We found that clones with species-like identity to *Methanobrevibacter ruminantium* or *Methanobrevibacter millerae* were present in similar frequencies in lactating Jersey cows. In contrast, methanogen 16S rRNA gene clones with 95 – 97.9% identity to *Methanobrevibacter* species were found to be the most abundant (47.8%) in Holsteins. We have recently proposed elsewhere to divide *Methanobrevibacter*-related environmental clones into two categories based on their phylogenetic distribution and representation. When all sequences with at least genus-level sequence identity are included and divided between the two phylogenetic subgroupings of *Methanobrevibacter* (i.e. the *Methanobrevibacter smithii* - *Methanobrevibacter gottschalkii* - *Methanobrevibacter millerae* – *Methanobrevibacter thaurei* (or SGMT) clade and the *Methanobrevibacter ruminantium* -*Methanobrevibacter olleyae* (or RO) clade), each breed exhibited a distinct population structure (Figure 1). Since SGMT and RO groups sequences tend to show an opposite distribution pattern, each group perhaps represents methanogens that thrive in conditions that are not optimal for members of the opposite group. While this model still remains to be validated, it may potentially provide a means of estimating the methane synthesis potential of a methanogen community.

Figure 1 Pie-chart representations of methanogen 16S rRNA gene clone distribution in lactating Holstein and Jersey cows.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Integrative predictions of free water intake, urine volume and fecal water excretion in dairy cows under thermoneutral condition

H Khelil Arfa, A Boudon, G Maxin, P Faverdin
INRA,Agrocampus Rennes, UMR 1080, Production du Lait, Saint-Gilles, Rennes, France
Email: hajer.khelil@rennes.inra.fr

At a world scale, the concomitant increase of livestock production and risks of global warming will reinforce the issue of the use of water for livestock (Chapagain and Hoekstra, 2003). Even though the requirements for animal watering are small compared to the water requirements for irrigation for feed production, it is difficult to reduce it without directly affecting the integrity of the herds. In temperate climates, concurrences in the uses of water can occur during drought periods and in this context, it will be essential to predict accurately the water requirement of the herds, particularly dairy herds. Moreover, at the farm level, the volume of water excreted by livestock partly determines the size of the manure storage facilities, which can affect the dynamics of land applications. The aim of this work was to establish a set of predictive equations of the main daily water flows at the level of a dairy cow (free water intake, urine and feces excretion), according to the diet characteristics and the animal performance, from a dataset of cows water balances collected during the last 30 years at the experimental farm of INRA at Méjussaume (France).

Materials and methods The dataset used to build predictive equations included 342 individual measurements of daily water balance in dairy cows (free water intake FWI, water ingested with the feed, excreted in urine, in feces, or in milk), collected from 18 energy and N balance conducted between 1983 and 2005. 281 measurements were obtained from lactating cows and 61 from dry cows. Considering the diets offered to the cows, 92 measurements were obtained on diets based on freshly cut grass, 94 measurements on diets based on corn silage based diets and 156 measurements on diets based on dried forage. Explicative parameters included in the dataset were milk yield (MY), dry matter intake (DMI), body weight (BW), percentage of dry matter in diet (DM), percentage of concentrate in diet CONC, content of CP ingested with forage and concentrate qtCPf and qtCPc. Predictive equations were built by multiple regressions using the REG procedure and the stepwise statement to select the significant regressors. An external validation of the equation was performed from a dataset including 197 results of water flows collected from 43 studies gathered from the literature. Selected studies were obtained from bibliographic research in the CAB and from the bibliographic list of retained references. In this latter dataset ambient temperature was also collected.

Results The dataset used to build the predictive equations in the present study is unique because it included a large variability of FWI (varied from 2.3 to 140 kg/d) which is likely due to the large variability of % DM of diets (varied from 11.5 to 91.4%). The variable % DM was the first predictor of FWI and explained most of the variability with a partial R^2 of 0.57. The variability of urine volume was explained mostly by the qtCPf associated with DMI and that of fecal water was explained by CONC and DMI. Ambient temperature affected FWI, the mean prediction error (MPE) of FWI decreased when 26 data obtained at ambient temperature >25°C were eliminated (13.61vs 23.92). Fecal water was predicted from the product of fecal DM and the percentage of DM in feces (fDM %) to avoid negative prediction of low producing cows (Table 1).

Table 1 Prediction equations of FWI, urine volume and fecal water excretion

Prediction equations	Model adjustment			External validation	
	n	R^{adj}	rsd	n	MPE
FWI (kg/d)= 0.83 (±0.03) × DM + 3.22 (±0.23) × DMI + 0.92 (±0.07) × MY – 0.28 (±0.03) × CONC + 0.04 (±0.007) × BW –77.61(±6.08)	232	0.92	9.35	120	23.92
Urine water (kg/d)= – $2.2.10^{-4}$(±$8.4.10^{-5}$)× qtCPf ^{2}+ 0.88 (±0.11) × DMI + 0.26 (±0.03) × qtCPf + $9.3.10^{-4}$ (±$10.5.10^{-5}$) × $qtCPc^2$ – 19.77 (±3.20)	227	0.75	5.31	99	10.21
Faecal water (kg/d)= Fecal DM x fDM %				84	7.28
Fecal DM (kg DM)= 0.43 (±0.009)× DMI – $1.98.10^{-5}$ (±$3.09.10^{-6}$) × qtCPf ^{2} – 2.30 (±0.17)	261	0.91	0.49		
fDM %= 0.04 (±0.004) × CONC – 0.03 (±0.005) × MY – 0.14 (±0.03) × DMI – $4.5.10^{-5}$ (±$1.03.10^{-5}$) × $qtCPf^2$ + 16.28 (±0.53)	261	0.52	1.34		

Conclusions The main strength of the predictive equations proposed in the present study is that they were build from a dataset with a large variability of diet DM. Our predictive equation of urine volume was less precise than equation that included mineral intakes but it is simpler, as mineral intakes are difficult to assess in most farms. Also, it appears form the external validation that ambient temperature clearly affects water flows. This illustrates the necessity to integrate climatic parameters in the prediction equation .Further work will be necessary to integrate these parameters in such a way that they can be used across geographic localization.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effect of a constant high ambient temperature on the intake and excretion of water in dry and lactating Holstein cows

H Khelil-Arfa, P Faverdin, A Boudon
INRA,Agrocampus Rennes,UMR 1080,Production du Lait,Saint-Gilles, Rennes, France
Email: hajer.khelil@rennes.inra.fr

Introduction At a world scale, the concomitant increase of livestock production and risks of global warming reinforce the issue of the use of water for livestock. In this context, it will be important to predict accurately the water requirement of the herds, particularly dairy herds. An issue for theses predictions is to include climatic parameters in predictive equations in such a way that they could be used across geographic contexts. Even though mechanistic models of the impact of the climate on animal water evaporation have been developed, their use assumes to quantify the compensations between evaporation and water consumption by cows when ambient temperature increases. The aim of the present study was to determine the effect of two ambient temperatures (under or above upper critical temperature for cow) on all water flows on dairy cows either dry or lactating. An increase of Na and K supplementation is recommended when ambient temperature is high. Given that supplementation could also affect the partition between flows, the interaction of temperature with sodium supplementation was also tested.

Materiel and methods The effects of two constant ambient temperature thermoneutrality TN (15°C) and High Temperature HT(28°C) in interaction with two mineral complementation Na^-(0.15%DM) and Na^+(0.55% DM) were compared according to 2 Latin square design, on 4 dry and 4 middle lactation Holstein cows (98.5±3.32 day in milk, 42 kg/d milk yield). Cows were housed in two climate chambers (2 dry and 2 lactating cows) during 4 periods of 2 weeks each. Feed intake, milk yield, water drunk and vaginal temperature were daily recorded. Urine and faeces were collected during the last 3 days of each period. The volume of evaporated water was calculated as the difference between water drunk, ingested with feed, urine, faeces and milk, retained or produced by metabolic reactions. Cow diets consisted on 76% maize silage, 10% dehydrated alfalfa and 13.8% soybean meal. Dry cows were restricted to their protein and energy requirement. Animal's data were analyzed using PROC MIXED of SAS Institute (1990).

Results The daily average of temperature-humidity index (THI) was 59 for TN and 73 for HT (Mader *et al*.2006). Daily average vaginal temperature increased from TN to HT by 0.12°C for dry cows and 0.91°C for lactating cows. Dry matter intake (DMI) of lactating cows decreased significantly from 21.1kg/d at TN to 18.8 kg/d at HT (P<0.01) and remained constant at 13.9 kg/d for dry cows. Both dry and lactating cows increased significantly the volume of water evaporated at HT compared to TN (P<0,001), with no significant interaction with the physiological stage. For dry cows, the increase of water drunk between HT and TN was very similar to the increase of evaporation and most of the other flows of water were not affected by the temperature. For lactating cows, the increase of water drunk was lower to that of evaporation, likely because of a decrease of the amount of water excreted in faeces. When all water flows were expressed as a proportion of DMI, the decrease of faecal water and increase of urine water remained significant in lactating cows but the range of variation were low compared to those of evaporated and drunk water. Mineral complementation only increased urine and water drunk with no interaction with physiologic stage.

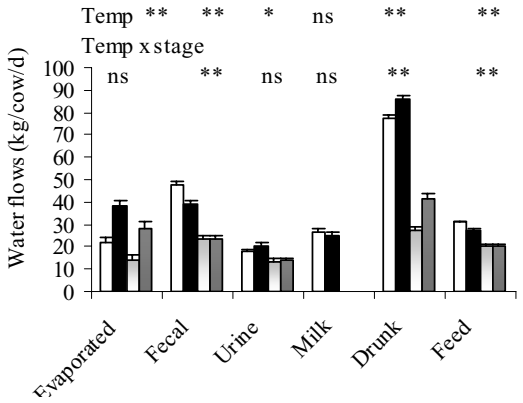

Figure 1 Water flows of dry and lactating cows (kg/cow/d)
** :P<.01,*:P<.05,NS: Not significant

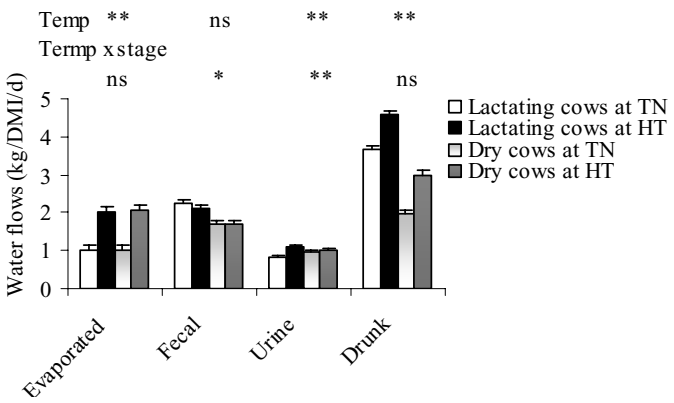

Figure 2 Water flows of dry and lactating cows (kg/DMI/d)

Conclusion These results shows that when expressed as a proportion of intake, the increase of evaporation with increased temperature in dairy cows is mainly compensated by increasing volumes of water drunk whatever the physiological stage of the cows, at least in conditions of moderate heat stress. This means that the development of predictive models of water intake including climatic parameters could be based on mechanistic models of evaporation.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Greenhouse gas emissions from alternative dairy calf-to-beef production systems

P Crosson[1], PA Foley[2], P O'Kiely[1]
[1]Teagasc, Animal & Grassland Research and Innovation Centre, Grange, Dunsany, Co. Meath., Ireland, [2]University College Dublin, School of Agriculture, Food Science and Veterinary Medicine, Ireland
Email: paul.crosson@teagasc.ie

Introduction The greenhouse gas (GHG) emissions associated with beef production have been quantified by many authors (e.g. Beauchemin *et al*., 2010). However, to date the focus of these studies has been on suckler beef cow production systems. Fewer studies have investigated dairy calf-to-beef production systems and, where they have, the analysis has been on quantifying emissions at the national level (e.g. Verge *et al*., 2008). The objective of this present study was to quantify the GHG emissions and associated profitability of alternative dairy calf-to-beef systems.

Materials and methods The BEEF Greenhouse gas Emissions Model (BEEFGEM; Foley *et al*., 2011) was used to conduct the analysis. This is a single-year, static model developed in Microsoft Excel which integrates the beef enterprise production profile with various GHG emission factors. Direct GHG emissions associated with farm activities and indirect GHG emissions associated with inputs brought onto the farm, nitrate leaching and ammonia (NH_3) volatilization are simulated. No land use or land use change emissions were assumed to occur and emissions associated with gestation of the calf are allocated to the dairy production system. A bioeconomic model of beef production systems, the Grange Beef Systems Model was used to conduct economic analysis and to develop the production profile which was used as input data for BEEFGEM (e.g. animal inventories, feed budgets, nitrogen application rate, etc.). Four alternative scenarios representing dairy calf-to-beef production systems using the male progeny of February-calving Holstein-Friesian dairy cows and purchasing supplementary concentrate requirements were evaluated. The first system (Steer 30) was based on national survey data (Connolly *et al*., 2008) and was chosen to represent average levels of management for dairy farmers in Ireland finishing male calves as steers at pasture at 30 months of age. The three alternative systems were based on research farm conditions (Teagasc, Grange) and thus were representative of better management and more intensive production. The first two systems (Bull 16 and Bull 20) involved finishing males as bulls at 16 and 20 months of age, respectively, whereas the final system (Steer 24) was a steer system finishing cattle at 24 months of age.

Results Stocking rate was higher for the research farm systems (Bull 16, Bull 20 and Steer 24) at 2.6 LU/ha relative to average farm conditions (Steer 30; Table 1). Although individual carcass weight was greater for Steer 30, total carcass output per ha was much higher for the other scenarios as a result of much higher stocking rates. Net farm margin was lowest for Steer 30 and highest for Steer 24 and Bull 20. Greenhouse gas emissions were highest for Steer 30 at 16.5 kg CO_2e/kg beef carcass and lowest for Bull 16 at 10.7 kg CO_2e/kg beef carcass (Figure 1). In general, research farm systems had lower emissions than the system representing average farm conditions, and bull systems had lower emissions than steer systems. Methane represented the largest share of total emissions being responsible for 58% of total emissions across all scenarios. The main source of methane emissions was enteric fermentation which was the source of 49% of all GHG emissions on average across the four systems. Sensitivity analysis indicated that bull systems were most sensitive to emissions associated with concentrate feeding.

Table 1. Annual technical and financial performance of four alternative dairy calf-to-beef production systems

	Steer 30	Bull 16	Bull 20	Steer 24
Stocking rate (LU/ha)	1.8	2.6	2.6	2.6
Animal units per ha[1]	1.2	5.2	3.3	2.5
Fertiliser nitrogen (kg/ha)	106	139	142	142
Conc. fed (kg per animal unit)	846	1328	1054	924
Carcass weight (kg)	370	272	321	323
Carcass output (kg/ha)	431	1407	1042	806
Financial performance (€/ha)[2]				
Gross output	1155	3991	2639	2217
Gross margin	367	899	861	848
Net farm margin	-208	182	201	198

[1]Animal Unit = calf to slaughter. [2]Beef price = €3.10/kg beef carcass; Concentrate price = €250/t DM; Fertiliser price (CAN) = €275/t.

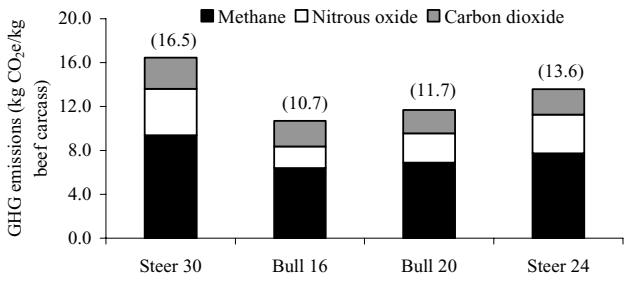

Figure 1. Total greenhouse gas emissions and sources from four alternative dairy calf-to-beef production system (total emissions in parentheses)

Conclusion Adopting research farm levels of efficiency has the potential to reduce GHG emissions from dairy calf-to-beef systems by 35%. Within the research farm systems explored in this analysis, bull systems had 18% lower emissions than steer beef systems. However, systems finishing steers at 24 months of age had a higher net farm margin than bull beef systems finishing cattle at 16 months of age and were less sensitive to emissions associated with concentrate feeding.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effects of enzyme supplementation in oil palm fronds either or not pre-inoculated with white rot fungi to improve rumen degradability *in vitro*

H Abu Hassim[1,2], M Lourenco[1], Y M Goh[2], V Fievez[1]
[1]University of Ghent, Ghent, Belgium, [2]Universiti Putra Malaysia, Serdang, Selangor, Malaysia
Email: haslizahassim@gmail.com

Introduction Oil palm fronds (OPF), a by-product of Malaysian oil palm industry, are used in ruminant diets. However, the high fiber content of OPF limits its rumen degradability. Due to the lignocellulosic bonds in OPF, there is a limitation for ruminal microbial population to access the digestible fiber. Therefore, there is a need to breakdown this lignocellulosic bond as shown by Rahman *et al*. (2010) using white rot fungi (WRF). In addition, enzyme supplementation to fungi inoculated OPF could enhance the cellulose degradation and improve the digestibility of OPF.

Materials and methods Two *in vitro* incubations (Experiment 1and 2), each consisting of 2 runs, with 2 replicates/treatment/run were performed (Hassim *et al*., 2010). Mixed rumen fluid of 2 fistulated sheep (8 ml), bicarbonate+phosphate buffer (32 ml) and OPF non-inoculated or inoculated with fungi (0.560 g) were added to each syringe. *Ceriporiopsis subvermispora* inoculated for 3 weeks (CSW3) and *Lentinula edodes* inoculated for 9 weeks (LEW9) were selected based on the results of Rahman *et al*. (2010). Enzymes were supplemented according to *in vivo* dose advised by the companies (Alltech; Synergen® and Vitamex N.V.; Hemicell®), both in active or inactive forms. The dosage was converted to *in vitro* on substrate basis assuming 20 kg DMI/d (0.110 ml/kg and 0.2 g/kg of feed for Hemicell® and Synergen®, respectively; Exp. 1) or on rumen volume basis (2.2 ml/100L RF and 4 g/100L RF for Hemicell® and Synergen®, respectively; Exp. 2). Syringes were placed in the incubator at 39°C for 96 h. Total gas production was recorded at different time points up to 96 h. Rumen fluid was collected at 0 h and total net SCFA production was assessed after 96 h of incubation. In addition, SCFA production curves were constructed based on total gas production and fitted to the model of Schofield *et al*. (1994). Data were analyzed using General Linear Model according to $Y_{ij}=\mu+A_i+B_j+\xi_{ij}$, and $Y_{ij}=\mu+C_i+B_j+CB_{ij}+\xi_{ij}$ where Y_{ij} is the response; μ the overall mean; A_i the effect of WRF (fixed factor); B_j the effect of incubation runs (random factor); C_i the effect of enzyme supplementation (fixed factor); CB_{ij} the interaction between enzymes and incubation runs; ξ_{ij} the residual error and Dunnet as post-hoc test. Effects with $P<0.05$ were considered statistically significant.

Results Inoculation of OPF with CSW3 and LEW9 resulted in increased total SCFA production by 10 – 15 % compared to the non-inoculated OPF (Table 1). However, it did not affect the rate of SCFA production. The addition of enzymes to the fungi inoculated OPF did not further increase the total SCFA production or the rate of SCFA production (Kf) compared to the respective controls.

Table 1 Effects [mean (SD)]of enzyme supplementation to OPF non-inoculated and inoculated with WRF on total SCFA production (µmol/incubation) and rate of SCFA production (Kf; %/h) after 96 h of *in vitro* incubation (N=4, two replicates per run)

OPF		Experiment 1					Experiment 2				
		Hemicell®		Synergen®		Control	Hemicell®		Synergen®		Control
		Active	Inactive	Active	Inactive		Active	Inactive	Active	Inactive	
CSW3	SCFA	1880	1798^{K}	1816	1862	1902^{aa}	2412^{K}	2670	2468	2621	2741^{aa}
		(181)	(209)	(225)	(175)	(249)	(296)	(328)	(411)	(267)	(317)
	Kf	3.53	3.78	3.55	2.98^{k}	4.33	3.83	3.15	2.83	2.53	3.13^{aa}
		(0.359)	(0.457)	(0.370)	(0.685)	(0.826)	(1.190)	(0.881)	(1.056)	(1.153)	(0.833)
LEW9	SCFA	1769	1828^{K}	1787	1698^{K}	1766^{a}	2577^{k}	2657	2768	2733	2782 ^{aaa}
		(121.1)	(155.0)	(157.2)	(91.9)	(136.4)	(236.0)	(266.0)	(92.9)	(109.3)	(148.7)
	Kf	3.30	3.45	4.10	3.78	4.08	5.55	5.13	5.43	4.95	5.43
		(0.860)	(0.755)	(1.023)	(0.877)	(0.465)	(0.943)	(2.155)	(1.353)	(1.060)	(1.394)
Non-inoculated	SCFA	1621	1721^{k}	1665	1583	1597	2561	2455	2436	2416	2462
		(172)	(289)	(251)	(185)	(188)	(208.8)	(68.9)	(57.5)	(103.5)	(24.1)
	Kf	4.40	4.63	4.45	4.43	4.95	5.40	5.98	5.35	5.13	5.33
		(1.219)	(1.034)	(0.854)	(1.015)	(1.300)	(1.208)	(1.263)	(0.238)	(0.512)	(0.619)

a $0.05<P<0.01$, aa $0.01<P<0.001$, aaa $P<0.001$; differences reported between control of OPF inoculated and non-inoculated with fungi
K = trend ($0.1<P<0.05$), k $0.05<P<0.01$; differences reported between feed additives (active and inactive form) and control

Conclusion Enzyme supplementation did not further enhance rumen degradability of fungi inoculated OPF.

Acknowledgement This research was funded by the EU-community (Project TH/Asia-Link/014-141-176).

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Biennial canola for forage and ecosystem improvement in dryland cropping systems

R Kincaid, K Johnson, J Michal, S Hulbert, W Pan, J Barbano, A Huisman
Washington State University, Pullman, WA, USA
Email: rkincaid@wsu.edu

Introduction Biodiesel production has increased demand for oilseeds such as canola, which can be grown in crop rotation with wheat and barley. Winter canola, when seeded in spring or early summer will grow as a biennial crop that allows for a forage crop to be harvested in year one (Kirkegaard *et al*. 2008). The aim of this experiment was to evaluate biennial canola as a dryland forage for silage production and to determine the feeding value of the silage for cattle.

Materials and methods A mixture of peas (113 kg/ha) and canola (5 kg/ha) were planted and 70-d later the standing forage was ensiled into an air-tight plastic silo bag. The harvested forage contained 31% DM and yielded about 4940 kg DM/ha. Samples of canola/pea ensilage were obtained 40-d post-ensiling for chemical analysis. Digestibility 48-h *in vitro* of the ensilage was determined using a Daisy™ ruminal incubation system. A feeding study was done using 36 multiparous Holstein cows in which cows were group-fed either a control total mixed ration (TMR) or a TMR that contained 9% canola/pea silage (DM basis). In the TMR, the canola/pea silage replaced a portion of the lucerne hay and maize silage such that CP and NDF percentages were similar between treatments. Samples of the TMRs were taken daily and composited by week for chemical analysis. After 21-d the canola/pea silage was increased to 15% (DM basis) of the TMR. Individual milk yields were recorded daily and milk composition determined on composite samples collected every 21-d. Data were statistically analyzed using Proc GLM of SAS.

Results The ensiled canola/pea forage contained 13.3% CP, 29.8% NDF, 24.1% ADF, 38.6% NFC, 0.31% S, a pH of 4.3, a desirable concentration of lactic acid (6.92%), and an acetic acid concentration of 1.97% with no detectable concentration of butyric acid, which is an indicator of undesirable secondary fermentation. The nitrate-N concentration in the pre-ensiled forage (139 ppm nitrate-N) was reduced about 80% (27.7 ppm nitrate-N) in the ensilage as the nitrate presumably was converted to nitrous oxide and emitted. The high percent ash (17.5%) resulted from soil contamination during harvest. The estimated NE_L value of the canola/pea silage was 1.56 Mcal/kg, DM. Digestibilities *in vitro* of the DM, NDF and ADF were 55.4, 39.9 and 41.1%, respectively, compared to digestibilities of a lucerne hay standard that were 59.5, 40.7 and 40.5%, respectively.
The control TMR had 16% CP, 36% NDF, and 25% ADF with an estimated NE_L value of 1.61 Mcal/kg DM. With 9% canola/pea silage incorporated into the TMR, the estimated NE_L was 1.60 Mcal/kg DM, and with 15% canola/pea silage in the TMR, the estimated NE_L was 1.58 Mcal/kg DM. Average dry matter intake (25.4 kg) was not affected by treatment. Likewise, neither average daily milk yield (35 kg) nor milk composition were affected ($P>0.05$) by the partial substitution of canola/pea silage for lucerne hay and maize silage.

Conclusions Creating sustainable systems for agriculture in dryland regions should include economical options for producers to diversify. The intercropping of canola and peas yielded an ensilage that was palatable and contained high feeding value as indicated by the chemical composition, *in vitro* digestibility, and feeding study with lactating cows. Thus, biennial forage canola appears to be a viable option in crop rotation systems in dryland areas to diversity crop production and obtain forage for ruminants.

Acknowledgement
This study was supported by the College of Agriculture, Human and Natural Resources at Washington State University and by a grant from the BioAg Program, WSU Center for Sustaining Agriculture and Natural Resources.

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Molasses-based n-alkane tablets to estimate feed intake and digestibility in cattle consuming low quality roughage diets

M Bezabih[1,2], W F Pellikaan[1], W H Hendriks[1,3]
[1]Wageningen University, Wageningen, The Netherlands, [2]Hawassa University, Hawassa, Ethiopia, [3]Utrecht University, Utrecht, The Netherlands
Email: melkamu.derseh@wur.nl

Introduction Ruminant animal production systems in the tropics are mainly based on extensive grazing of natural grasslands. Assessment of the nutritional status of animals grazing on such lands requires adequate knowledge of the level of feed intake, diet composition and organic matter digestibility of the feed ingested. As direct measurement of these parameters on free-ranging animals is difficult, indirect approaches are commonly employed. Animal-based indirect estimation of feed intake and diet quality using plant wax components as markers has got wider acceptance. The current experiment was conducted to evaluate the suitability of using molasses based n-alkane tablets to indirectly measure the feed intake and digestibility of grazing cattle in the rangelands of Ethiopia.

Materials and methods Doses of C32 and C36 alkanes were prepared using molasses, linseed powder, and hydrated calcium sulphate as carrier materials.. Four experimental diets were prepared by mixing different roughage feeds. The diets varied in their species richness and composition. The experiment was conducted indoors in a Latin square design, with the four diet mixes and four feeding rounds (runs). Eight bulls were used, two bulls randomly assigned to each diet in each run, the duration of which was 21 days. Samples of diets consumed were taken and pooled over the experimental period. The daily dose contained 380 mg of each alkane, administered orally in two equal amounts, with aid of a balling gun. In the last 5 days of each run, total daily faecal output was collected and after thoroughly mixing, 10% of the faeces was sampled. Faecal spot samples were taken two times a day in the morning and in the afternoon during the total faecal collection period. The gross chemical compositions as well as the n-alkane profile of the diets and faecal samples were analyzed following standard procedures. Feed intake was estimated using the double n-alkane method according to Mayes *et. al.* (1986). Faecal output was estimated from C36 concentration in the faeces. Comparison of measured and estimated values of intake and digestibility was performed.

Results The concentration of dosed n-alkanes in faeces increased linearly in the first three days of marker dosing and a steady state of concentration was achieved after the fourth day onwards (graph not presented). This showed that estimation of intake and digestibility can be done from the fifth day of marker dosing onwards. Comparison of actual and alkane-estimated feed intake of experimental animals showed that intake was under estimated by about 13% when the ratio of C31/C32 or C33/C32 was used in the calculation. On the other hand intake was over estimated only by about 1.2% when the ratio of C35/C36 was used and the difference was statistically insignificant. Generally, total faecal collection samples and spot samples produced similar results, although the former appeared to be on the better side. DMD and OMD were accurately estimated using C36 as external marker to estimate faecal output.

Table 1 Actual versus alkane-estimated dry matter intake (DMI, kg/day), dry matter digestibility (DMD, %) and organic matter digestibility (OMD, %) in cattle fed low quality diets

	Diet 1	Diet 2	Diet 3	Diet 4	Mean	P-value
Actual intake	2.78	2.69	2.80	2.81	2.78	
Estimated intake C31/C32						
Total collection samples	2.27	2.38	2.53	2.59	2.47	.0001
Spot samples	2.33	2.45	2.45	2.52	2.44	.0001
Estimated intake C33/C32						
Total collection samples	2.22	2.34	2.46	2.50	2.47	.0001
Spot samples	2.29	2.42	2.42	2.44	2.39	.0001
Estimated intake C35/C36						
Total collection samples	2.64	2.56	2.74	2.85	2.75	.4227
Spot samples	2.56	2.75	2.78	2.81	2.76	.5607
Actual DMD	50.00	49.95	49.35	51.99	50.32	
Estimated DMD	48.36	49.31	50.13	53.01	50.57	.7489
Actual OMD	55.35	55.14	54.69	57.16	55.48	
Estimated OMD	51.35	52.12	53.97	55.81	53.49	.0839

Conclusion Five days of dosing of molasses based boluses would be adequate to conduct measurement of faecal output and intake in cattle feeding poor quality tropical roughages. Unlike previous reports where C31 and C33 were priority choices for intake estimation, in the present experiment C35 appeared to produce better results in combination with dosed C36. The result generally highlighted that molasses-based alkane tablets can be used to estimate intake and digestibility with adequate precision.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effects of polyethylene glycol on *in vitro* rumen fermentation characteristics of six Mediterranean tree and shrub leaves

A Boubaker[3,1], M R Mosquera-Losada[2], M Cherif[1], B Rekik[1], A Rigueiro Rodriguez[2]
[1]Ecole Supérieure d'Agriculture de Mateur, Mateur, Tunisia, [2]Ecole Polytechnique, Université Santiago de Compostela, Lugo, Spain, [3]Institut National Agronomique de Tunisie, Tunis, Tunisia
Email: azizaboubaker@yahoo.com

Introduction Tree and shrub leaves play an important role in nutrition of goats grazing Mediterranean woodland (Boubaker *et al*., 2005). In recent years, research has focused on using browse species that contain tannins as feed supplements for ruminants to reduce CH_4 production. The aim of the present study was to evaluate inhibitory effects of condensed tannin (CT) on methane (CH_4) and gas production (Experiment 1), *in vitro* organic matter digestibility (IVOMD) and ammoniacal nitrogen (N NH_3-N) concentration of rumen liquid incubated with some Mediterranean tree and shrub leaves with or without polyethylene glycol 6000 (PEG), a tannin complexing agent.

Materials and methods Leaves from trees of *Quercus suber (Q. suber)* and *Olea europea (O. europea)* and shrubs of *Erica arborea (E. arborea), Phyllerea angustifolia (P. angustifolia.), Myrtus communis (M. communis), Pistacia lentiscus (P. lentiscus)* were collected in northern Tunisia during March 2009, where the climate is Mediterranean. Samples collected from 8 plants of each plant species were used for chemical analyses and *in vitro* gas production (Menke and Steingass; 1988). Effects of various tannin containing samples on *in vitro* gas production, methanogenesis (Experiment 1), IVOMD and NH_3-N concentration at 72 h (Experiment 2) was assessed by incubating samples with and without PEG at a dose of 1 g/g DM. Total gas and CH_4 production were recorded at 48 and 72 h of incubation. The IVOMD was calculated as the difference between the OM weight of the sample at the start of incubation and the weight of OM remaining at the end of incubation after correcting for blank. Results of Experiment 1 were subjected to analysis of variance in a 6 x 2 x 2 factorial arrangement to assess the effect of plant species, PEG and time of incubation and their interaction. Results of experiment 2 were subjected to a 6 x 2 factorial analysis. Means were compared by the Student-t test at the 5% level.

Results There were wide variations in the chemical composition of the plant species. Total phenols ranged from 160 (*Q. suber*) to 270 g/kg DM (*E. arborea and P. lentiscus*). The highest CT content was in *E. arborea* (253 g/kg DM) while the lowest were in *O. europea* (20 g/kg DM) and *P. angustifolia* (10 g/kg DM*)*. The ranking of species based on their CT content was *E. arborea* > *Q. suber* > *M. communis* > *P. lentiscus* > *O. europea*> *P. angustifolia*. An interaction was found between plant species and PEG addition for gas production and CH_4 proportion . Gas production was lower ($P < 0.05$) in incubations containing samples of *E. arborea*, *M. communis*, *P. lentiscus*, *Q. suber* without PEG than in incubations containing PEG, and differed ($P < 0.05$) at 48 and 72 h of incubation. Addition of PEG resulted in higher ($P < 0.05$) proportions of CH_4 after 48 h incubation in *O. europea,* and after 72 h in *P. angustifolia* (Table 1). There was an interaction between plant species and PEG addition for IVOMD and NH_3-N concentration after 72 h incubation. The addition of PEG increased the IVOMD in *E. arborea* and *M. communis,* while NH_3-N concentration increased in most of plant species, except *O. europea* and *P. angustifolia* (no change).

Table 1 Effects of PEG on *in vitro* gas production and CH_4 proportion at 48 and 72 h incubation of plant species leaves (* Standard error of overall mean)

	Gas production (ml gas /200 mg DM)				CH_4 (% of gas production)			
	48 h		72h		48 h		72h	
Plant species	- PEG	+ PEG	- PEG	+ PEG	- PEG	+ PEG	- PEG	+ PEG
E. arborea	50 [a]	62 [b]	54 [a]	65 [b]	15.1 [a]	20.2 [a]	9.5 [a]	3.8 [a]
P. angustifolia	65 [a]	70 [a]	75 [a]	77 [a]	20.4 [a]	18.8 [a]	9.2 [b]	14.5 [a]
M. communis	29 [a]	74 [b]	36 [a]	87 [b]	9.7 [a]	6.5 [a]	12.2 [a]	12.0 [a]
P. lentiscus	49 [a]	80 [b]	54 [a]	84 [b]	10.8 [a]	15.2 [a]	30.4 [a]	26.8 [a]
Q. suber	53 [a]	72 [b]	60 [a]	77 [b]	20.2 [a]	21.0 [a]	31.8 [a]	26.9 [a]
O. europea	77 [a]	79 [a]	83 [a]	85 [a]	15.3 [b]	18.6 [a]	29.0 [a]	25.5 [a]
SEM*	18.0	9.2	18.3	9.7	5.75	6.80	10.37	16.30

[a,b]Within species and within incubation time, means with different superscripts differ ($P < 0.05$);

Conclusions The improvement in gas production and IVOMD with PEG demonstrates the negative effect of tannins on digestibility of the studied plant species. To confirm the potential of *O. europea,* and *P. angustifolia* as antimethanogenic feed for ruminants, more experiments are needed.

Acknowledgements This study was supported by the "Laboratoire d' Economie Agro-alimentaire" and AECID of Spain (Project code A/024952/09).

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Use of nutritional blocks as supplement for sheep in dry tropical highlands of Central Mexico

F Avilés Nova[1], O Castelán Ortega[2], A García Martínez[1], J Estrada Flores[3], P Vázquez Mendoza[1], B Albarrán Portillo[1]
[1]Universidad Autónoma del Estado de México/Centro Universitario Temascaltepec, Temascaltepec Estado de México, Mexico, [2]Universidad Autónoma del Estado de México/Facultad de Ciencias Agrícolas, Toluca Estado de México, Mexico, [3]Instituto de Investigación en Ciencias Agropecuarias y rurales-Universidad Autónoma del Estado de México, toluca Estado de México, Mexico
Email: franavilesnova@yahoo.com.mx

Introduction In Mexican tropical regions, sheep feeding is based on the intake of native and introduced grasses as well as crop residues like corn stover. During the dry season, forages are scarce and of low quality, limiting dry matter intake and affecting sheep performance, forcing farmers to confine and supplement their sheep with grounded corn stover and grains, increasing feeding costs. It is therefore necessary to implement feeding strategies based on available feed resources in the area. The aim of this study was to evaluate sheep performance in confinement, when supplemented with nutritional blocks.

Materials and methods Fifteen sheep (Dorper x Pelibuey) were assigned to individual pens receiving three treatments (five sheep per treatment). The base diet (BD) contained 30% wheat bran, 35% corn stover, 29% ground maize, 5% soybean and 1% of a mixture of minerals and vitamins, providing 130g kg^{-1}/DM of crude protein (CP) and 10.4 MJ/kg^{-1}/DM of metabolizable energy (ME). Sheep had 2 weeks of adaptation to treatments and 8 experimental weeks; the treatments were T1= Base Diet (BD) + nutritional block (BN) with 30% *Leucaena leucocephala* foliage (NB1), T2= BD + Nutritional Block with 30% wheat bran (NB2), and T3= BD (control treatment). The response variables were: daily weight gain (DWG), nutritional block intake (NBI), base diet intake (BDI), *in vitro* apparent dry matter digestibility of nutritional block (IDNB), and *in vitro* apparent dry matter digestibility of base diet (IDBD). Chemical composition and nutritive value of the blocks were assessed in terms of ashes, CP, NDF and ADF. *In vitro* digestibility of organic matter (IVDOM) was determined with the gas production technique (Theodorou *et al.,* 1994), and ME was estimated by multiplying the IVDOM x 0.15 according to AFRC (1993). The results were analyzed using a completely randomized design, and for comparison of means, the Tukey test (P<0.05) was used.

Results Significant differences (P<0.05) were observed in DWG. T1 and T2 showed the highest DWG with 92 and 102 $g/day^{-1}/sheep^{-1}$, respectively. T3 showed the lowest DWG, with 64 g $day^{-1}/sheep^{-1}$. Significant differences (P<0.05) were observed for NBI, showing that NB1 had higher intake (135 $gr/animal^{-1}/day^{-1}$) than BN2. BDI had the lowest intake in T3 (P<0.05) (Table 1). Chemical composition and nutritional value of blocks did not have significant differences (P>0.05) for ashes and CP. The content of NDF was lower in NB2 (P>0.05), and had the highest organic matter digestibility of NDF and ME (Table 2).

Table 1 Averages of response variables of sheep supplemented with nutritional blocks

VARIABLES	T1	T2	T3	P	EEM
NBI (g day^{-1})	135[a]	89[b]	-	0.008	7.7
BDI (g day^{-1})	916[a]	932[a]	887[b]	0.05	15.80
TDMI (g day^{-1})	1051[a]	1021[a]	887[b]	0.05	21.02
DWG (g day^{-1})	92[a]	102[a]	64[b]	0.009	6.98
IDNB (g kg^{-1} MS)*	568	647	-	0.052	20.65
IDBD (kg^{-1} MS)	780	768	742	0.207	13.61

T1= Base diet + NB1; T2= Base diet + NB2; T3= Base diet
[a] and [b] Different letters in rows represent significant differences P <0.05, NBI= Nutritional Block Intake, BDI= Base diet intake, TDMI= Total dry matter intake, DWG= Daily weight gain, IDNB= *In vitro* Apparent Digestibility of nutritional block, IDBD= *In vitro* Apparent Digestibility of Base Diet.

Table 2 Chemical composition and nutritional value of blocks used in supplementation to sheep in confinement

	Ashes	CP	NDF	ADF	IVDOM	ME*
NB1	228.3[a]	294.1	115.78	72.36[a]	462.51[a]	7.26[a]
NB2	181.4[b]	293.3	114.53	37.26[b]	651.31[b]	10.23[b]
P	0.035	0.677	0.534	0.015	0.004	0.004
EEM	2.05	1.21	1.18	3.06	8.73	0.13

CP=Crude Protein, NDF=Neutral detergent fiber, ADF=Acid detergent fiber,
IVDOM=*In vitro* digestibility of organic matter, ME= Metabolizable energy,
[a] and [b] = Different letters in columns represent significant differences P < 0.05.

Conclusions Sheep supplemented with nutritional blocks had improved productive performance. Using nutritional blocks with *L. leucocephala* foliage and wheat bran blocks can be a recommended feeding strategy for sheep during the dry season, due to their good acceptability. In addition, the foliage is a forage resource available in the study area in the South Region of the State of Mexico.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Botanical composition of sheep diet and nutritive value of consumed forages in highlands of Mexico

F Avilés Nova[1], O Castelán Ortega[2], R Rojo Rubio[1], J Estrada Flores[3], C Milpa Cruz[1]
[1]Universidad Autónoma del Estado de México/Centro Universitario Temascaltepec, Temascaltepec Estado de Mexico, Mexico, [2]Universidad Autónoma del Estado de México/Facultad de Ciencias Agrícolas, Toluca, Estado de México, Mexico, [3]Instituto de Investigación en Ciencias agropecuarias y rurales-Universidad Atónoma del Estado de México, Toluca, Estado de México, Mexico *Email: franavilesnova@yahoo.com.mx*

Introduction Native grasslands in tropical regions of Mexico have significant importance for farming, as they are the most important source of forage for domestic animals and wildlife, mainly because of their availability and floristic diversity. In the southeastern part of the State of Mexico, tropical areas represent 80% of the land surface, serving as the base of sheep production systems, especially in the rainy season. Determining the botanical composition of the herbivores diet makes it possible to identify the fodder species preferred by sheep, and to establish the effect of botanical variation on selectivity intake, and variation in the diet's nutritional value. The aim of this study was to determinate the botanical composition of the diet of grazing sheep, and the nutritional value of species consumed while grazing native pastures during the rainy season.

Materials and methods In a 1.5 ha plot of native pastures continuously grazed by 25 hair sheep (Dorper X Pelibey), with a liveweight of 41.0 kg during the rainy season. Three time periods were measured: P1 (start of rainy season, June), P2 (mid rainy season, July-August) and P3 (end of rainy season, September-October). In each period the botanical composition of the diet was determined according to the selectivity index (SI), selection rate (SR), relative frequency (RF) and relative density (RD) (Coates and Penning, 2000), and diversity index (DI) (Shanoon, 1948). Micro-histological analysis from feces (n= 3 sheep) (Holechek *et al*., 2001) was used. In each species, crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) were measured. In-vitro organic matter digestibility (IVDOM) was determined with the gas production technique (Theodorou *et al.,* 1994). Nutritional results were analyzed using a completely randomized design. For comparison of means, the Tukey test ($P<0.05$) was used.

Results The *Poaceae* family in P1had the highest selectivity index. *C. dactylon*, *A. compressus* and *P. nonatum* showed the highest selectivity indexes, followed by *C. dactylon* and *P. notatum* in P2, and *A. compressus* had a negative value, indicating it was rejected by the sheep during this period. However, in P3 this species had higher SIs (Table 1). The highest SRs in P1 were obtained by *A. compressus* and *C. dactylon.* In P2 the highest SR was *E. alsinoides,* and in P3, *A. compressus* (Table 1). Nutritional value of *P. notatum* showed significant differences ($P<0.05$) in its contribution of CP during the three periods, P1>P2>P3, with 85.0, 76.4 and 59.7 g, respectively. The IVDOM in P1 and P2 had higher values, with a lower value in P3 ($P<0.05$).

Table 1 Species' selectivity indexes and selection rates based on the frequency distribution of intercepted numbered fragments from micro-histological analysis during assessment periods in the rainy season

FAMILY	SPECIES	Selectivity index			Selection rate		
		P1	P2	P3	P1	P2	P3
POACEAE	*Brachiaria brizantha*	0,741	-1,542	0,241	1,16	0,73	1,05
POACEAE	*Setaria pallide*	-	3,245	-	-	1,96	-
POACEAE	*Paspalum lividum*	-	-0,208	-	-	0,49	-
POACEAE	*Axonopus compressus*	5,293	-3,651	7,981	3,25	0,47	8,90
POACEAE	*Paspalum notatum*	3,699	0,169	-3,111	2,17	1,03	0,53
POACEAE	*Cynodon dactylon*	6,075	2,859	3,245	4,10	1,80	1,96
POACEAE	*Eragrostis pilosa*	-	-1,150	-5,731	-	0,79	0,27
POACEAE	*Andropogon gayanus*	-	-7,191	-2,500	-	0,16	0,6
CONVOLVULACEA	*Evolvulus alsinoides*	2,789	7,146	-	1,77	6,01	-
ASTERACEAE	*Conyza sophiifolia*	1,227	-	-	1,28	-	-
FABACEAE	*Senna hirsuta*	-4,540	0,909	0,769	0,38	1,2	1,17
MALVACEA	*Sida rhombifolia*	-0,660	-	-	0,88	-	-
FABACEAE	*Lysiloma acapulcensis*	-0,424	-	-	0,92	1	-

P= Period; P1: Start of rains; P2: middle of rains; P3: end of rains, - without value.

Conclusions Poaceae family species were the main components in the sheep diet during the rainy season. Species had different SIs, depending on availability and their nutritional value and animal preference, which was related to the growth stage of the plants.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Estimation of feed ingredients and intake in cattle using stable isotope analyses

Y Yanagi, H Kumagai, K Oishi, H Hirooka, M Kitagawa
Graduate School of Agriculture, Kyoto, Japan
Email: hkuma@kais.kyoto-u.ac.jp

Introduction It is important to measure feed ingredients and intake individually for cattle management. However, in group feeding, such as grazing, the measurement is difficult. Stable isotope techniques have been used to estimate the dietary histories of wild animals (Mizukami *et al.* 2005). Only a few studies have examined the utilization of this technique to measure dietary history in cattle. The present study was conducted to monitor C and N isotope values in cattle hair after feed ingredient are changed, to calculate the values of isotopic enrichment and to estimate the proportions of feed ingredients ingested using a mathematical model.

Materials and methods Five Japanese Black heifers (16.8±1.6 months of age) were used for the experiment. The heifers were individually fed Italian ryegrass silage (IR) at 7 kg/day for 4 weeks (C_3 period) and Sudan grass hay (SG) at 5 kg/day for 18 weeks (C_4 period) thereafter. A 1-week transient phase was set between the C_3 and C_4 periods to replace IR with SG gradually. Two kilograms of concentrate mixture (CM) was supplemented daily throughout the experiment. Feed samples were taken 4-9 times and tail hair samples were taken from the heifers every 1 or 2 weeks. Samples of hair at 7 mm from the roots (temporal samples) were subjected to isotope analyses to calculate isotopic enrichment and to estimate the feed ingredients ingested. The hair taken on the last sampling day of the experiment was cut at 7-mm intervals, and 20 segmental samples (Nos. 1-20: from the top direction to the root edge) were also analysed to estimate feed ingredients ingested. The C and N isotope ratios ($\delta^{13}C$ and $\delta^{15}N$(‰)) of the feed and hair samples were measured using a mass spectrometer coupled with an elemental analyzer. The isotope ratios of temporal samples were analyzed by general linear model (GLM) procedure of Statistical Analyses Systems (SAS), and the fixed effect of the period (C_3 vs. C_4) was examined. The proportions of feed ingredients ingested were estimated by the following stable isotope mixing model of Phillips and Koch (2002) using the contents of C and N in the feed, the isotope ratios in the feed and hair, and the values of isotopic enrichment:

$$\delta_{13}C_{mix} = f_{XC}\delta_{13}C_{x'} + f_{YC}\delta_{13}C_{Y'} + f_{ZC}\delta_{13}C_{Z'}$$
$$\delta_{15}N_{mix} = f_{XN}\delta_{15}N_{x'} + f_{YN}\delta_{13}N_{Y'} + f_{ZN}\delta_{15}N_{Z'}$$

where $\delta_{13}C_{mix}$ and $\delta_{15}N_{mix}$ represent isotopic ratios in the hair. $\delta_{13}C_x$ and $\delta_{15}N_x$ represent the C and N isotopic ratios for source X (CM), and similarly for sources Y (IR) and Z (SG). This isotopic ratios for the dietary sources have been corrected for trophic enrichment, as designated by the prime (') symbol. The dietary source fractional contributions for biomass, C and N are constrained to sum to 1:

$$1 = f_{XB} + f_{YB} + f_{ZB}$$
$$1 = f_{XC} + f_{YC} + f_{ZC}$$
$$1 = f_{XN} + f_{YN} + f_{ZN}$$

where f_{XB}, f_{YB} and f_{ZB} represent the fractions of assimilated biomass of X, Y and Z, respectively, in the hair; f_{XC}, f_{YC}, f_{ZC}, f_{XN}, f_{YN} and f_{ZN} similarly represent the fractions of assimilated C and N of the individual dietary sources in the hair.

Results The dietary $\delta^{13}C$ values were -29.80±0.18, -12.34±0.61 and -26.02±0.35‰, and the dietary $\delta^{15}N$ values were 6.06±0.94, 3.00±1.55 and 2.89±0.51‰ for IR, SG and CM, respectively. In the whole feed, the$\delta^{13}C$ values were -28.67 and -16.25‰, and the$\delta^{15}N$ values were 5.11 and 2.97‰ for C_3 and C_4 periods, respectively, on average. The $\delta^{13}C$ values were -23.73±0.22 and -15.72±1.35‰, and the$\delta^{15}N$ values were 8.08±0.38 and 6.54±0.42‰ for the C_3 and C_4 periods, respectively, in the temporal samples. The isotope ratios of the temporal samples changed abruptly after the transient phase, and significant differences were observed between the C_3 and C_4 periods ($P<0.01$). The values of isotopic enrichment of$\delta^{13}C$ were 5.05 and 1.00‰, and those of $\delta^{15}N$ were 3.68 and 3.62‰ for the C_3 and C_4 periods, respectively. The estimated proportion of feed ingredient ingested was 65-75% for SG in the last week of the experiment on a DM basis. This was similar to that of the actual proportion: 70%. The $\delta^{13}C$ values were -22.38±1.49 and -15.22±1.22‰, and the$\delta^{15}N$ values were 8.01±0.34 and 6.47±0.44‰ for Nos. 1-7 and 10-20, respectively, in the segmental samples. The changes in isotope ratios in the segmental samples from numbers 7 to 20 were similar to those of the temporal samples after 5 weeks. When the segmental samples were used, the estimated proportion of IR ingested was drastically replaced by SG from numbers 7 to 10. The estimated proportions of feed ingredients were 65-80% for SG in no. 20 on a DM basis.

Conclusion The isotope ratios, $\delta^{13}C$ and $\delta^{15}N$, in tail hair clearly reflected the changes in feed composition. The changes in temporal samples were similar to those in segmental samples in the mid-to-late period of the experiment, and the estimated proportions of feed ingredients ingested were similar to those of the actual proportions at the end. The results suggest that the stable isotope technique might be utilized to estimate feed ingredients that cattle ingest.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Effect of castration on performance of Sudan Nubian kids

A Nasr, H Ali
[1]Sudan University of Science and Technology, Khartoum North, Sudan, [2]University of Juba, Khartoum North, Sudan
Email: hafizabdo@hotmail.com

Introduction Nubian goats comprise 70% of goats in Sudan. They are mainly kept for dairy production (M.A.R, 2006). However, male kids are not being kept as future flock sires and are usually castrated and culled as a meat source (Stanton, 1999; Ensminger and Parker, 1986). This study was conducted to investigate the effect of castration on dry matter intake (DMI), body weight gain (BWG) and feed conversion ratio (FCR) of Sudan Nubian male kids.

Materials and methods The experiment was conducted at the Animal Production Research Center, Helat Kuku, Khartoum North-Sudan for 3 months (November 2006 to January 2007). Ten male Nubian kids (9-14kg body weight) at sexual maturity were used. Animals were divided randomly into two groups (A & B) of 5 animals each. Animals of group B were castrated using a Burdizzo castrator, whereas those of group A remained intact. Animals were treated against endo- and ecto-parasites prior to the experiment. Animals were fed *ad libitum* on a diet of chopped *Sorghum vulgare* (Abu70) and concentrates which included: crushed sorghum feterita grains 50%, wheat bran 22%, ground nut cake 25%, lime stone 2% and salt 1%. Lick stone and clean water were available all the time. Live body weight was taken a week before castration for all kids as a control and then weekly throughout the experimental period in the morning before feeding. Data were analyzed using the Statistical Package for Social Science (SPSS). Differences between means were assessed by the independent student t-test. The correlations among the traits were tested using the same statistical package. For the significant correlations, linear regressions were done to quantify the relationships (Gomez and Gomez, 1984).

Results Table 1 shows the DMI, BWG and FCR of intact and castrated kids. It revealed that BWG was significantly ($P < 0.05$) higher in the intact kids. There were no significant ($P > 0.05$) differences in the DMI and FCR between the two groups.

Table 1 Feedlot performance of the intact and castrated kids.

Item	Intact Mean ±SD	Castrated Mean ±SD	T	Probability and level of significance
No. of animals	5	5	-	-
Dry matter intake (g/d)	751±318.4	586±49.7	1.205	0.274 NS
Initial weight (kg)	14.6±3.41	14.8±3.36	-2.96	0.999 NS
Body weight gain (g/d)	103.5±14.09	76.2±14.4	2.62	0.04 *
Feed conversion ratio (g feed/g gain)	7.3±2.83	7.8±.88	-0.40	0.701 NS

SD: standard deviation. T: calculated (t). NS: not significant. *: significant at $P < 0.05$.

Conclusion Castrated kids had lower body weight gains compared to uncastrated ones and hence castration is not recommended for Nubian male kids at this age.

Acknowledgments The authors are indebted to the staff and administration of Animal Production Research Centre, for financial support and sample collection.

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Effect of castration on some blood metabolites of Sudan Nubian male kids

A Nasr, H Ali
[1]Sudan University of Science and Technology, Khartoum North, Sudan, [2]University of Juba, Khartoum North, Sudan
Email: hafizabdo@hotmail.com

Introduction In Sudan, most dairy goats male kids are not being kept as future flock sires. They are usually castrated and culled as a meat source (Stanton, 1999). Blood metabolites for domestic animals have been investigated as indicator for metabolism by various workers (Zubcic, 2001). This study was initiated in order to investigate the effect of castration on some blood metabolites of Sudan Nubian male kids.

Materials and methods This experiment was conducted at the Animal Production Research Center, Helat Kuku, Khartoum North-Sudan for 3 months (November 2006 to January 2007). Ten male Nubian kids (9-14 kg average body weight) at sexual maturity were used. Animals were divided randomly into two groups (A & B) of 5 animals each. Group B was randomly selected to be castrated using a Burdizzo castrator; whereas those of group A remained intact. Animals were treated against endo- and ecto-parasites. Animals were fed *ad libitum* on a diet of chopped *Sorghum vulgare* (Abu70) and concentrates which included: crushed sorghum feterita grains 50%, wheat bran 22%, groundnut cake 25%, lime stone 2% and salt 1%. Lick stone and clean water were available all the time. Three ml of blood were collected from the jugular vein one week before castration and weekly throughout the experimental period. Analysis of the blood serum was conducted spectrophotometrically using commercially available diagnostic kits (Crescent Test kits, KSA) for metabolites which included: glucose, total cholesterol, total protein, albumin, globulin, urea and uric acid. Using the Statistical Package for Social Science (SPSS) computer software, the data obtained were analyzed by independent t-test.

Results Table 1 shows the concentrations of blood metabolites of the intact and castrated kids. It was observed that there were no significant ($P > 0.05$) differences between the two groups in total cholesterol, total protein, albumin, globulin and uric acid concentrations. The glucose blood level in intact kids was significantly ($P < 0.05$) lower. For urea concentrations, the intact group were significantly ($P < 0.05$) higher.

Table 1 The blood metabolites concentrations of the intact and castrated kids.

Item	Intact Mean ± SD	Castrated Mean ± SD	Calculated T	Probability and level of significance
No. of observations	40	40	-	-
Glucose (mg/dl)	55.3 ± 9.4	61.4 ± 15.8	-2.2	0.04*
Total Cholesterol (mg/dl)	70.8 ± 20.4	58.5 ± 18.1	3.02	0.613 NS
Total protein (g/dl)	6.03 ± 0.8	6.4 ± 1.3	-1.6	0.281 NS
Albumin (g/dl)	4.2 ± 0.5	4.2 ± 0.5	-0.34	0.516 NS
Globulin (g/dl)	1.9 ± 1.0	2.2 ± 1.3	-1.3	0.723 NS
Urea (mg/dl)	57.2 ± 19.7	51.5 ± 11.2	1.7	0.05*
Uric acid (mg/dl)	0.3 ± 0.4	0.5 ± 0.5	-1.3	0.190 NS

SD: standard deviation. T: calculated t. NS: not significant. *: significant at P<0.05.

Conclusion Castration had no effect on most blood metabolite concentrations. This study documented the examined parameters and made them available for further studies on this aspect.

Acknowledgment The authors are indebted to the staff and administration of Animal Production Research Centre, for financial support and sample collection

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Mathematical model to calculate the energy and protein requirement for sheep

R Burlacu, C Nitu
the Agricultural University and Veterinary Medicine, Bucharest, Bucharest, Romania
Email: radu.burlacu@fifim.ro

Introduction Feeding norms assessment for animals is a dynamic process related both to the continuous process of improving animal capacity to ingest and use the forage, and to the exogenous factors other than the type of forage, the technology of forage processing and preserving, the exploitation technology in general. A major element concerns diet balancing in energy and nitrogen. Each forage will therefore be characterized by two potential values as digestible protein, which ensures diet balancing with non-protein matter if fermentable organic matter predominates in one of the forages. This manner of expressing the potential dietary protein level avoids the waste of energy, when the dietary nitrogen level is not high enough, and the waste of nitrogen, when dietary energy level is too low. These novel elements outlined by the most recent investigations in the physiology of nutrition are presented by mathematical modelling of energy and protein metabolism with the purpose to optimise diet formulations according to production requirements, providing thus the possibility to develop strategies for an efficient animal production under specific soil, climacteric and socio-economic conditions.

Materials and methods The class of models that we consider aims mainly to describe the processes connected to protein metabolism and energy metabolism. The existence of estimates of the daily rate of protein, lipid, water and ash retention, leads naturally to a dynamic discrete model that determines the evolution over time of the variables of state, as soon as the initial states and the feeding strategy ("command") represented by the daily values of the digestible protein and energy are known. We shall now choose a certain set of relations among the most widely accepted and verified experimentally, referring to metabolism processes of sheep.

Results

Energy norms (Net Energy for meat and milk using maintenance energy and climatic stress energy)

For growing lambs younger than one year, wethers reared in stables

$$ENC(M+Q") = Kmp/Km[1.15(0.25(G/1.08)^{o'75})+(0.13+5.00+1.56)G/1000] \quad [MJ/day]$$

For growing lambs younger than one year, wethers reared on the pasture:

$$ENC(M+Q") = Kmp/Km\ [1.15(0.25(G/1.08)^{0.75}) + 13.0+ 2.8 +5.0+3.1)G/1000][MJ/day]$$

For growing lambs younger than one year, ewes or wethers reared in stables:

$$ENL(M+Q") = [1.0(0.25(G/1.08)^{o.75}) + (0.13+5.0 + 1.56)G/1000]K1/Km \quad [MJ/day]$$

For growing lambs younger than one year, ewes or wethers reared on the pasture:

$$ENL(m+Q") = [1.0(0.25(G/1.08)^{0.75})+ (13.0+2.8+5.0+3.1)G/1000]KI/Km \quad [MJ/day]$$

For lambs older than one year, wethers reared in stables:

$$ENC(m+Q") =1.15(0.23(G/1.08)^{o.75})+(0.13+5.83+3.64)G/1000]KJ/Km \quad [MJ/day] \quad (12)$$

calculating 50 m of movement (0.13), 14 hours of standing (5.83) and 14 changes of position

For lambs older than one year, wethers reared on the pasture:

$$ENC(M+Q") = [1.15(0.23(G/1.09)^{o.75}) + (13.0+2.8+5.0+3.1)G/1000]KI/Km \quad [MJ/day]$$

For the ewes or wethers older than one year, reared in stables:

$$ENL(m+Q") = [1.0\{0.23(G/1.08)^{0.75}) + (0.13+5.83+3.64)G/1000]KI/Km \quad [MJ/day]$$

For the ewes or wethers older than one year, reared on the pasture:

$$ENL(m+Q") = [1.0(0.23(G/1.08)^{0.75}) + (13.0+2.8+5.0+3.1)G/1000]KI/Km \quad [MJ/day]$$

Conclusions The method of modelling, as a synthetically method, shows the unknown or partially known links of the studied system and makes evident at the same time those aspects which at a certain moment are well enough known. From this point of view the modelling activity is a very efficient instrument for orienting the research efforts towards the priority aspects. What is more these models are effective instruments of decision orienting on both biologic and economic levels

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effect of dietary supplementation with Hainanmycin on ruminal methane production and protozoa and fungi numbers relative to bacteria *in vitro*

H Xin, Y Zhang, W Liu
Northeast Agricultural University, Harbin, China
Email: xinhangshu@163.com

Introduction It is well known that methane is a potent greenhouse gas and its release into the atmosphere is directly linked with animal agriculture, particularly ruminant production. It has been reported that the supplementation of ionophores, such as monensin, alters rumen fermentation pattern and inhibits the methane production in cattle (Guan, *et al*., 2006; Weimer, *et al*., 2008). Hainanmycin is a new type of ionophore antibiotics which was developed by Chinese researchers and has already shown its effect on gas production and VFA concentrations *in vitro* (Li, *et al*., 2006). Therefore, the aim of the present experiment was to investigate the effect of dietary supplementation with Hainanmycin on ruminal methane production and microorganisms *in vitro*.

Materials and methods An *in vitro* experiment was conducted according to Menke *et al*. (1979). All the diets were firstly formulated with corn (32%), soybean (8%) and Chinese wild rye grass hay (60%) and then supplemented with four different levels of Hainanmycin i.e. i) no additive, ii) 2.5 mg/kg (DM basis), iii) 5.0 mg/kg and iv) 10 mg/kg. Each of the four diets was added to 100-mL glass syringe for 24 h ruminal fermentation *in vitro*. Ruminal fluid was obtained from three ruminally-fistulated Holstein cows fed a similar diet without any additive. Three syringes for each treatment diet were incubated. Gas was recorded for volume and then subsampled for methane determination at 0, 2, 4, 6, 8, 12, 16, 20 and 24 h time points by gas chromatography. Real-time PCR was employed with primers designed to detect the 16S rDNA of *Ruminococcus flavefaciens, Ruminococcd. albus, Methanogens, fungi* and *protozoa* relative to total bacterial 16S rDNA in samples withdrawn after 24h.

Results Increasing the level of Hananmycin quadratically inhibited methane production by 37.6%, 45.6% and 47.3%, respectively, as compared to the control at 24 h incubation (Table 1). And it could reach the lowest level theoretically when the supplementation level of Hainanmycin was 7.2mg/kg by curve fitting and mathematical derivation. The percentage of total bacterial 16SrDNA of *Ruminococcu. albus* and *Methanogens* did not differ among treatments while the quantities of *Ruminococcu. flavefaciens, Fungi* and *Protozoa* differed significantly. Quadratically lower *R. flavefaciens* and *Fungi* contents were observed when Hainanmycin was added in the diet. And the quantity of *Protozoa* linearly decreased with the level of Hainanmycin addition increasing. However, no significant differences were found in these microorganisms among the different levels of Hainanmycin addition.

Table 1 Effects of different levels of dietary supplementation with Hainanmycin on methane production and quantities of microorganisms after 24h incubation *in vitro*

Item	Level of Hainanmycin addition (mg/kg)				SEM	P	Contrast, P	
	0	2.5	5.0	10			Linear	Quadratic
Methane production (mL)	6.2^{a}	3.9^{b}	3.4^{c}	3.3^{c}	0.07	<0.0001	<0.0001	<0.0001
16SrDNA (% total bacteria)								
R. flavefaciens	0.018^{a}	0.002^{b}	0.002^{b}	0.002^{b}	0.001	<0.0001	<0.0001	0.001
R. albus	6.80	6.79	6.64	6.52	0.13	0.44	0.13	0.68
Methanogens	0.23	0.23	0.23	0.22	0.004	0.32	0.13	0.54
Fungi	0.034^{a}	0.021^{b}	0.017^{b}	0.016^{b}	0.002	0.0002	<0.0001	0.01
Protozoa	0.70^{a}	0.32^{b}	0.41^{ab}	0.26^{b}	0.09	0.04	0.02	0.28

Conclusions Supplementation with Hainanmycin in the diet reduced ruminal methane production, and affected the micro flora significantly *in vitro*. Methane production would reach the lowest level theoretically at 24h incubation when the supplementation level of Hainanmycin was 7.2mg/kg.Hainanmycin addition could inhibit the growth of *Ruminococcu. flavefaciens, Fungi* and *Protozoa*, but there were no effect on *Ruminococcu. albus* and *Methanogens.*

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Yield, chemical composition and *in vitro* gas production of maize hybrids cropped in high valleys of Mexico in fresh or hay

J A Ruiz-Perez, M Gonzalez-Ronquillo, A Morales O, M G Gutierrez M, N Pescador S
Universidad Autonoma del Estado de Mexico, Toluca, Mexico, Mexico
Email: mrg@uaemex.mx

Introduction Forage maize is the main source of animal feed in the center of Mexico, the increase in demand for feed and the low availability of land for cultivation has led to implement programs for the choice of maize varieties with higher production And the search for new varieties of hybrid maize (Johnson *et al*., 2003; Ivan *et al*., 2005) that heterosis by increasing their nutritional value and is the forage or grain and digestibility. Yellow corn is the main type produced internationally destined for animal feed. The aim of this study was to evaluate and compare the performance, chemical composition and gas production *in vitro* both fresh hay of eight varieties of maize grown in the high valleys of the state of Mexico (2600 m above sea level) during the spring summer 2009 cycle.

Materials and methods Eight varieties of maize hybrids (3 white and 3 yellow) and two local Native white and yellow were used for this study, which were sown on May 12, 2009. Once the dough stage of grain (180 d) varieties were collected, was determined yield production, chemical composition and *in vitro* gas production of fresh and hay, gas production was determined by the method proposed by Theodorou *et al*. (1994), It was used 125 ml flasks for each sample preservation methods in triplicate, in three series of incubation were introduced 0.8 g DM of each sample, and proceeded to record gas production at 0, 3 , 6, 9, 12, 18, 24 and 30 h using a pressure transducer (DELTA OHM, Manometer, 8804). To estimate the degradation and fermentation of forage was used the equation proposed by Krishnamoorthy *et al*. (1991). The gas production data were analyzed using a completely randomized design with 19 x 2 factorial arrangement considering the varieties and two treatments (fresh and hay) with three replicates. The data were analyzed using an analysis of variance using the SAS software (1999). The averages of each variable ($P < 0.05$) were compared with the Tukey test.

Results

Table 1 Forage yield (ton DM/ ha), chemical composition (g/ kg DM) and *in vitro* gas production (GP, ml gas / g DM) as fresh and hay.^{defg} Diferent letters in the same row $P<0.5$

	Native yellow	Cobre	CML 460	Pioner	Native white	H47	H66	H51	sem	P<
Yield	14.1^{e}	21.7^{de}	21.3^{de}	18.2^{e}	17.8^{e}	21.4^{de}	19.4^{e}	29.8^{d}	1.902	0.001
CP	73.7^{d}	55.6^{de}	63.3^{de}	68.5^{de}	57.8^{de}	49.3^{e}	62.7^{de}	64.0^{de}	0.04	0.055
NDF	596.5^{f}	686.6^{d}	672.9^{de}	541.6^{g}	599.0^{f}	629.9^{ef}	594.5^{f}	588.8^{fg}	10.94	0.001
In vitro GP fresh										
B	344.3^{d}	375.8^{d}	342.3^{d}	334.9^{d}	347.1^{d}	389.1^{d}	361.3^{d}	344.9^{d}	18.19	0.431
C	0.047^{d}	0.039^{d}	0.043^{d}	0.052^{d}	0.045^{d}	0.036^{d}	0.048^{d}	0.048^{d}	0.032	0.058
Lag time	1.40^{def}	1.17^{fg}	1.59^{df}	1.76^{d}	1.02^{g}	1.17^{fg}	1.24^{efg}	1.30^{efg}	0.024	0.001
In vitro GP hay										
B	328.1^{d}	382.4^{d}	539.2^{d}	450.4^{d}	282.4^{d}	328.8^{d}	343.2^{d}	405.4^{d}	73.13	0.337
C	0.037^{d}	0.034^{d}	0.032^{d}	0.033^{d}	0.048^{d}	0.042^{d}	0.037^{d}	0.038^{d}	0.127	0.922
Lag time	1.58^{e}	3.85^{d}	1.89^{e}	1.92^{e}	1.50^{e}	1.75^{e}	1.81^{e}	1.95^{e}	0.367	0.011

Conclusions The local Native yellow variety despite having a lower yield compared to maize hybrid breeding, has a higher protein content, with increased gas production in the first 6 hours of incubation and intermediate from 9 to 30 h, H51 variety is an alternative to show the highest yield (ton DM / ha) and intermediate crude protein content (64 g / kg DM) and an intermediate gas production after 12 hours. The highest yields both fresh hay under white varieties, but higher protein concentrations correspond to the yellow varieties.

Acknowledgement This project was funded by Fundación PRODUCE 2009 estado de Mexico and UAEMEX 2750/2009E

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Performance and ruminal parameters of lambs fed maize silage inoculated with *Lactobacillus buchneri* and the combination of *L. buchneri* and *L. plantarum*

F Carvalho Basso, R Andrade Reis, C H Silveira Rabelo, J V Yamagushi, P A Ribeiro Salvo, E C Lara
Animal Science Departament, Agricultural and Veterinary Sciences College, São Paulo State University., Jaboticabal, São Paulo, Brazil
Email: rareis@fcav.unesp.br

Introduction Microbial inoculants in maize silages have been used to inhibit undesirable organisms during the fermentative process. Inoculants are added to improve the fermentation, and to increase the dry matter recovery of conserved forage, resulting in final products that stimulate the animal intake and performance (Kung Jr. *et al*., 2003). However, the mechanisms of action of the inoculants are not totally clear, and so it necessary for more studies. The aim of this research was to evaluate the performance and ruminal parameters of lambs fed maize silages inoculated with *L. buchneri* and the association of *L. buchneri* and *L. plantarum*.

Materials and methods Three stack silos containing 10 t of maize were prepared. The treatments were maize silage untreated (control - MS); maize silage inoculated with *Lactobacillus buchneri* NCIMB 40788 ($1x10^5$ cfu/g of fresh forage - MSB) and maize silage inoculated with of *L. buchneri* and *L. plantarum* MA18/5U ($1x10^5$ cfu/g– MSBP). Two trials were developed simultaneously. The first experiment was to evaluate the lambs' performance (Experiment I). The second was to evaluate the ruminal parameter of rams (Experiment II). *Experiment I*: thirty lambs Santa Inês and Dorper (20 kg) crossbred males were used. The animals were adapted to environment and diets during 15 days. Experiment had three experimental periods. The relation roughage: concentrate was 80:20. The silages were MS, MSB and MSBP. The concentrate presented: 160 g/kg of ground maize, 575 g/kg of soybean meal, 50 g/kg of cottonseed meal, 70 g/kg of wheat meal, 125 g/kg of citric pulp and 20 g/kg of mineral salt. The diet was offered twice for day, permitting *ad libitum* intake (refusal of 10%). Average daily dry matter intake (DMI) was measured subtracting the orts from the offered. To determine the average daily gain (ADG), the lambs were weighed after a fast of 16 hours in the initial and final of experiment (45days). The lambs were slaughtered at 30kg. The experiment was conducted using a randomized blocks design with three treatments and ten replicates. *Experiment II*: six ram Santa Inês and Dorper crossbred (40kg), 10 months old and rumen cannulated were used. The animals were fed with the same diet of the Experiment I. Rumen fluid samples were collected 0, 3, 6, 9, 12 hours after feeding to measure pH values and ammonia levels. The experimental design was Latin square (3x3) twice repeated. The data of experiments were submitted to variance analysis and the averages were compared by Tukey test ($P<0.05$).

Results The average of dry matter intake (DMI), average daily gain (ADG), fed conversion ratio (FCR), and pH and rumen ammonia were not affected by microbial inoculants ($P>0.05$) (Table 1). Dry matter intake is an important factor on animal performance, considering the nutrients required to attendance the maintenance and gain (Sniffen *et al*., 1993). The volatile fatty acids (VFA) produced from inoculated silage fermentation could affect the DMI (Kung *et al*., 2003). However, this did not occur in this study. The average DMI was 1.018 g/day and the ADG was 207 g/day. Although a possible increase in the VFA could happened in this silage, the ruminal parameters (pH and ammonia) are according to the recommendation to a normal rumen metabolic condition.

Table 1 Dry matter intake (DMI), average daily gain (ADG), fed conversion ration (FC), pH and ammonia ruminal

Item	Treatments[1]			
	MS	MSB	MSBP	VC (%)[2]
DMI (g/day)	994	1.025	1.035	7.68
ADG (g/day)	202	200	220	14.98
FCR (g of DMI/WGD)	4.99	5.21	4.75	15.09
ruminal pH	6.06	6.12	6.07	1.28
ruminal ammonia (mg/dL)	17.09	13.87	15.11	12.57

[1]Treatments: MS - maize silage untreated; MSB - silage inoculated with *Lactobacillus buchneri*; MSBP - silage inoculated with *L. buchneri* and *L. plantarum*. [2]VC: variation coefficient.

Conclusions The maize silage with microbial inoculants did not affect the performance and ruminal parameters.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Intervention of the insulino/somatotropic axes in the development of digestive function in pre-weaning calves fed a sodium (Na)-butyrate supplemented diet

K Katoh[1], S Kato[1], S-G Roh[1], S Owada[1], P Guilloteau[2]
[1]Graduate School of Agricultural Science, Sendai, Miyagi, Japan, [2]INRA, Saint-Gilles, France
Email: kato@bios.tohoku.ac.jp

Introduction Many countries in the world limit the prophylactic use of antibiotics in animal agriculture because of the emergence of antimicrobial resistance and in the interests of public health. A group from INRA and Warsaw University has published a series of experiments on the beneficial effect of supplementing calf and pig diets with Na-butyrate, and this research has indicated this chemical is a promising substitute for antibiotics (see Guilloteau *et al*. 2010). However, the endocrine background involved in the actions of Na-butyrate on animal production remains to be clarified. In the present study, pre-weaning calves were given milk replacer (MR) supplemented with Na-butyrate for 6 weeks in order to investigate its effects on the postprandial changes in plasma concentrations of metabolic hormones, including growth hormone (GH) and insulin, and on the weight of various organs and rumen papilla development. Postprandial endocrine features in response to MR ingestion are interesting because MR feeding caused a concomitant increase in plasma concentrations in GH and insulin in young calves (Katoh *et al*. 2004). The insulino/somatotropic axes involving these hormones are indispensable for growth and nutrient deposition during animal development.

Materials and methods Twenty four Holstein milk-fed male calves were held in individual calf hutches from the age of 3 d (D1). They were divided into 2 groups (n=12, Control group (C-group) and Na-butyrate-supplemented group (B-group)), and were used in the experiment up to D42 (6 weeks). The mean body weight (BW) at D12 was similar in the C- and B-groups (43.9 ± 0.4 and 43.7 ± 0.4 kg, respectively, NS). The two groups received the same amount of MR twice daily at 0830 and 1630 h (2 L x 2 times/day) through the whole experiment. From D7, calves also received a starter diet *ad lib*. The MR and the calf starter consisted of 24.5% and 20.0% crude protein and 21.9% and 2.0% crude fat, respectively (Yonekura *et al.* 2002). For the B-group, MR was supplemented with increasing amounts of Na-butyrate (3, 5 and 7 g/day from D1 to D3, from D4 to D7, and from D8 to D42, respectively). On D42 for each group (B and C), 4 calves for each group and treatment received a meal based on the MR described previously (2 L), or a lactose solution (100 g/2 L water, LS); MR and LS were given with a "milk bottle". All the 16 calves that received MR or LS on D42 morning were carried out for blood sampling from jugular vein, from 30 min before to 120 min after the ingestion of MR or LS, via a jugular catheter inserted on D41. Postprandial changes in plasma hormones (GH, insulin and IGF-I) and metabolites (glucose and non-esterified fatty acids, NEFA) were measured. Plasma concentrations of GH, insulin and IGF-I were analyzed by radioimmunoassay as described previously (Katoh *et al*. 2004). Glucose and NEFA concentrations were determined using commercially available kits (Wako Pure Chem., Osaka, Japan). On the other hand, all the 16 calves (n = 8 for each group) which were fed MR during the whole experiment, were killed at a slaughterhouse at the age of D43/44 by stunning, and were subjected to the studies for performance (growth, feed intake, etc.) and morphological analyses of several organs and tissues (liver, spleen, kidney, forestomachs and peri-renal fat). For statistical analyses, one-way ANOVA followed by Duncan's multiple range test, and unpaired t-test were used. The value of $P < 0.05$ was taken as the level of significance, and $P < 0.10$ was considered to indicate a tendency.

Results. The Na-butyrate supplementation did not change the growth rate and feed intake during the whole experiment, as well as the weight of the different parts of the stomachs measured at slaughter. The weight of the peri-renal fat tended to be increased (+43.8%, P=0.07) as well as the length of rumen papilla (+13.4%, P = 0.08). Ingestion of meal based on MR concomitantly and significantly increased plasma concentrations of GH and insulin as well as that of glucose, but decreased plasma NEFA concentrations. However, in the B-group, the postprandial increase in plasma GH and insulin concentrations induced by the MR ingestion showed a tendency to be reduced (-49.3%, P=0.07 and -73.4%, P=0.06, respectively) when compared with the calves of the C-group. Plasma IGF-I levels were not changed by the addition of Na-butyrate. On the other hand, the postprandial increase in plasma insulin concentration induced by the lactose ingestion was smaller (-71.5%, P<0.02) in the calves of the B-group than the C-group.

Conclusions In pre-weaning Holstein calves, feeding milk replacer or lactose supplemented with Na-butyrate caused a significant reduction in plasma concentrations of insulin and moderate changes in glucose concentrations. The supplementation also caused a tendency to increase the length of rumen papilla and the weight of peri-renal fat weight. From these findings, we conclude that the accelerated growth performance of Na-butyrate as feed additive (as it was previously reported in milk-fed calves), could be partly linked to the hormonal regulation by intervention of the insulino/somatotropic axes on the development of the metabolic and digestive functions.

Acknowledgements This study was partly supported by the financial grant from YP tech, Tokyo, Japan.

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Effect of peppermint (*Mentha piperita* L.) and sage (*Salvia officinalis* L.) as a source of phytofactors on ruminal methanogenesis

M Szumacher-Strabel[1], P Zmora[1], E Pers-Kamczyc[1], A Nowak[1], L Pecio[2], A Stochmal[2], A Cieslak[1]
[1]Poznan University of Life Sciences, Department of Animal Nutrition and Feed Management, Poznan, Poland, [2]Institute of Soil Science and Plant Cultivation, Department of Biochemistry and Crop Quality, Pulawy, Poland
Email: pzmora@jay.up.poznan.pl

Introduction The quest of effective plant extracts (phytofactors) for inclusion in ruminal diets to enhance animal performance and efficiency has been subject of much research (Benchaar and Greathead, 2011). The investigations were focused on the evaluation of the effect of phytofactors on rumen microorganisms and practical implications. Few experiments have been completed to evaluate the possibilities of using the native plant species rich in biologically active components in animal nutrition. The present experiments were conducted to explore the effects of Polish native plants rich in flavonoids and rosmarinic acid: peppermint (*Mentha piperita* L.) and sage (*Salvia officinalis* L.) on rumen fermentation parameters *in vitro*.

Materials and methods Experiments were carried out using rumen simulation technique RUSITEC (Czerkawski and Breckenridge, 1977). Rumen fluid inoculum was obtained from a ruminally fistulated Polish Holstein-Friesian cows. Experiments were repeated three times for each source of phytofactors. The diet was formulated to meet the feeding requirements of dairy cow of 600 kg, yielding 25 kg milk per day, containing 3.2% protein and 4.0% fat. Diets of experimental groups were supplemented with 200, 400 and 600 mg of dried peppermint or sage in dietary dry matter. The concentration of flavonoids and rosmarinic acid in dried peppermint and sage was determined. The following parameters were analyzed in rumen fluid samples: pH, redox, ammonia and fermentation gases concentration. Changes in microbial profile, including the number of protozoa and bacteria were tested using the light microscopy. The fluorescence *in situ* hybridization (FISH), according to Soliva *et al*. (2004), was used to quantify methanogens in the microbial population of the rumen fluid. The obtained data were subjected to variance analysis using SAS general linear model (GLM) procedure (version 9.1). Differences between the means were tested using the Duncan test.

Results Dried peppermint used in the experiment contained 5.6 mg/g of rosmarinic acid and 8.2 mg/g of flavonoids, whereas sage 7.9 mg/g and 6.6 mg/g, respectively. There were no significant effects of peppermint or sage on the rumen microorganisms, nor the methane concentration. However, some numerical differences were observed. The supplementation of peppermint decreased the total number of protozoa and bacteria. Also the methane concentration was reduced in the groups with *Mentha piperita* L. Changes were dose-dependent and the biggest effect was showed in the group with the highest dose (600 mg) of peppermint. The supplementation of peppermint caused the significant ($P<0.05$) changes in methanogen population in the last day of incubation. The addition of 200 mg increased the number of *Archaea* by 43.24%, whereas 600 mg decreased by 51.16%, in comparison to the control. The addition of sage affected protozoa and bacteria population and methane concentration, but significant differences were not observed. The effect of sage was day-dependent. Namely, in the 1st day of analysis, in the group with 600 mg, the methanogen population was higher by 78.74% ($P<0.05$) than in the control. However, in the last day of incubation, the number of *Archaea* was lower in all experimental groups in comparison to the control, respectively by 41.78%, 49.82% and 71.23%.

Table1 The effect of peppermint on the rumen fermentation

	The addition of peppermint			
	0 mg	200 mg	400 mg	600 mg
Protozoa (cells/ml)	4544	4062	3767	3498
Methanogens (%)	21.30	25.29	26.67	20.47
Methane (mmol)	3.01	2.77	2.68	2.36

Table 2 The effect of sage on the rumen fermentation

	The addition of sage			
	0 mg	200 mg	400 mg	600 mg
Protozoa (cells/ml)	1988	1953	1689	1701
Methanogens (%)	23.57	22.55	26.06	23.39
Methane (mmol)	2.51	2.23	2.12	2.21

The values are shown as a mean of analysis from 1st, 3rd and 5th day of experimental period

Conclusions These *in vitro* studies suggest that supplementation of ruminant diet with peppermint and sage may alter the rumen microbial population. However, the methane was not decreased despite changes in methanogens. More research is needed to explain the effect of the native plants on the rumen fermentation using standard material with known concentration of bioactive components that could be applicable in dairy cow rations.

Acknowledgement This study was supported by the Ministry for Science and Higher Education, Grant No. N 311 239638

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A novel approach: Relationship of protein molecular structures to degraded protein balance, protein intestinal digestibility and metabolisable protein

P Yu
University of Saskatchewan, Saskatoon, SK, Canada
Email: peiqiang.yu@usask.ca

Introduction There have been no studies of the effect of bioethanol processing on changes to the molecular structure of proteins in cereal grains. Additionally, there have been no studies on how bioethanol processing-induced changes on molecular structure changes the availability of nutrients to animals. The objective of this study was to investigate protein molecular structures affected by bioethanol processing in relation to levels of metabolizable protein of the new co-products of dried distillers grains with solubles using a novel approach.

Material and methods Various bioethanol co-products and cereal grain feeds were collected from bioetheanol plants. The degraded protein balance and metabolisable protein value were predicted using the Dutch DVE/OEB protein evaluation system and NRC-2001 model.
The protein molecular structures were identified using synchrotron radiation-based FTIRM, DRIFT or ATR-FT/IR infrared (micro)spectroscopy. The protein secondary structure profiles were analyzed using multi-peak modelling procedure in amide I area with OMNIC and Origin Software. Multivariate molecular spectral analyses were done using Statistica statistical software, including cluster analysis and principal component analysis.

Results The results showed that the changes of the protein molecular structure α-helix to β-sheet ratio and the amide I to II ratio during bioethanol processing (either due to fermentation processing or due to heat drying) were highly associated with estimated protein intestinal digestibility and degraded protein balance, but were not associated with total intestinally absorbed protein supply from the DDGS to dairy cattle.

Conclusions This study indicates that a potential novel method could be developed based on the protein molecular structure parameters to improve estimating protein value after validation on a large scale is done.

Acknowledgements Funding was provided by the NSERC, ADF and Ministry of Agriculture Strategic Research Program. Synchrotron facilities NSLS, CLS and Saskatchewan Structure Science Center (SSSC,) University of Saskatchewan.

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Lactating dairy cows fed indoors with either cut pasture or Lucerne cubes.

K Macdonald, G Waghorn, R Weintjes
DairyNZ, Hamilton, New Zealand *Email: kevin.macdonald@dairynz.co.nz*

Introduction A trial was undertaken to compare milk production, eating behaviour and rumen parameters in 30 multiparous cows fed either freshly cut perennial ryegrass pasture (*Lolium perenne* L.) or Lucerne (*Medicago sativa* L.) cubes. The measurements were made to assist with the interpretation of a larger trial designed to identify individuals with divergence for residual feed intake (RFI). The RFI trial evaluated over 1000 weaned calves (aged 5-9 mo) and required accurate measurement of intake, as well as daily gain. These measurements required a dry forage diet to be fed because of the logistical problems associated with harvesting pasture and accurate measurement of intake by large numbers of calves. In addition, a later evaluation is planned with lactating cows, selected from calves with divergent RFI, when fed either cubes or pasture in separate trials.

Grazed pasture is the predominant diet (90% of intake) for cows in New Zealand and results from the RFI evaluation and feeding trials based on cubes need to be interpreted in a way that applies to pastoral feeding. This trial will define effects of fresh pasture and Lucerne cube diets on eating behaviour, intakes, rumen parameters and production of lactating cows.

Materials and methods Thirty, lactating mixed-age Holstein-Friesian cows (age 3-9 yrs, days in milk 66 standard deviation (SD) 16 days, balanced for age and milk production) were assigned to one of 2 groups in a cross-over design. Each group included 4 animals fitted with a permanent rumen fistula. The cows were housed indoors, and fed either cut pasture or Lucerne cubes for 15 days, comprising a 4 day acclimatisation and 11 day experimental period. Prior to entering the feeding facilities, the cows were introduced to the Lucerne cubes over a 5 day period at pasture. There was a transition period of 10 days between the cross-over when the cows fed cubes were reassigned to pasture and those to be fed Lucerne were introduced to them. Cows accessed their feed through an electronic identification operated Calan gate system. All cows were offered approximately 10% more than their daily intake. The fresh cut pasture was placed in bins at 0830, 1530 and 2100 h. The Lucerne cubes were fed to the cows daily at 0830 h. The pasture offered (average 3346 kg dry matter (DM)/ha) was mowed twice daily (0700 & 1430 h) and was, 21.7 SD 2.40 cm prior to mowing, with a 9.1 SD 0.93 cm residual height. Pasture averaged 16.4% DM, and 20.1 SD 0.50 crude protein; 45.6 SD 2.09 neutral detergent fibre (NDF); and predicted organic matter (OM) digestibility 85.4 SD 1.60 (g/100g DM from near infra red spectroscopy (NIR) analysis). The Lucerne cubes had been imported from Canada and contained 88.5 DM with 17.2 SD 1.70 crude protein; 48.6 SD 2.47 NDF; and predicted OM digestibility of 60.6 SD 1.06 (g/100g DM).
In addition, measurements were recorded of time spent eating, ruminating and resting over 2 x 24 h periods for each cow. Rumen digesta was measured in the fistulated cows by removal and return of contents before morning and afternoon feeding (0630 and 1430h) for each animal on each diet. Samples were taken to determine the particle size of digesta DM. Rumen contents were sampled at 6 h intervals over 24 h for measurement of pH, ammonia and volatile fatty acid (VFA) concentrations.

Results Although the intakes (Table 1) of Lucerne cubes were higher than for pasture DM, the milk, milk fat and protein concentrations were lower for the Lucerne fed cows. This resulted in 0.26 kg less milk fat and 0.19 kg less protein/cow/day (P <0.001), for cows fed Lucerne, compared with pasture.

Table 1 Intake and milk yield and its components for cows fed cut pasture or Lucerne cubes

	Pasture	Lucerne	SED[#]	P value
Intake (kg DM/c/d)	19.9	20.7	0.26	0.006
Daily milk yield (kg/d)	27.3	23.1	0.26	<0.001
Milk fat (%)	4.32	3.95	0.050	<0.001
Protein (%)	3.38	3.19	0.017	<0.001

Molar proportions of VFA suggested a lower propionate and butyrate production from the cube diet. The concentration of ammonia (mmol/L) was 14.0 when [#]SED Standard error of the difference pasture was fed, compared with 5.5 with the cube diet (P<0.001). The weight of rumen contents was similar for both diets, but the high DM% of the cube diet (15.7 vs 10.9) resulted in a much larger rumen DM pool in cows fed Lucerne cubes (16.6 SD 3.01 kg vs 11.1 kg SD 0.94 kg). The percentage of rumen DM retained on a sieve with a 2 mm aperture was 27.2 with pasture and 7.9 when cubes were fed, and chewing (eating + rumination) occupied 68.1% and 54.7% of the day for respective treatments.

Discussion Although DM intakes were similar, production and digestion differed substantially between diets, as indicated by the differences in rumen digesta composition and ammonia concentration. However the lower production by cows fed cubes was probably a consequence of lower energy availability, with 26% less digestible OM available to cows fed cubes compared with ryegrass. These animals spent 55 % of their time chewing, compared with 68% for those fed grass, and in combination with the short particle length of the Lucerne cubes resulted in a high DM% in the rumen.

Conclusion Lucerne cubes resulted in similar DM intakes to cows fed fresh pasture, but rumen digesta characteristics and products of digestion differed substantially, despite similar NDF concentrations of the two feeds. The lower predicted digestibility of Lucerne cubes was matched by a lower milk production. The results indicate that selection of divergent animals for RFI based on cubes may underestimate both animal production and the calculated divergence between extremes. This has identified a need for further evaluations of divergent selections using pasture.
This trial was funded by Foundation for Research, Science and Technology

Effects of supplemental copper and Linpro on performance and carcass traits of finishing heifers

C Alvarado[1], C Aperce[1], K Miller[1], C Van Bibber[1], S Uwituze[1], J Drouillard[1], J Higgins[2]
[1]Kansas State University, Department of Animal Science & Industry, Manhattan, Kansas, USA, [2]Kansas State University, Department of Statistics, Manhattan, Kansas, USA
Email: chrialva@ksu.edu

Introduction Omega-3, essential fatty acids for human nutrition, have been demonstrated to have a positive effect on health (Calder, 2001). Human diets often are low in these fatty acids, and frequently contain high levels of saturated fatty acids which are considered to have negative consequences for human health. Feeding cattle with flax-based feeds can increase concentrations of omega-3 fatty acids in beef (Drouillard *et al*., 2004), despite the fact that omega-3 fats from flax are extensively hydrogenated into saturated fats by ruminal microbes (Montgomery *et al*., 2008). Engle (2011) has reported that elevated levels of dietary copper can inhibit biohydrogenation. Consequently, our objective was to evaluate whether feeding elevated copper concentrations in conjunction with Linpro, a co-extruded blend of field peas and flaxseed, affected performance and plasma lipid profiles of fattening beef heifers.

Materials and methods The study was conducted as a randomized complete block experiment with a 2 x 2 factorial treatment arrangement. Supplementation consisted of dietary copper (10 or 100 mg/kg added copper) and Linpro (0 or 10% of diet, dry basis). Linpro is an extruded blend of flaxseed and field peas containing 12% linolenic acid, added vitamins and minerals (22% CP; 23% fat). Crossbred yearling heifers (n = 261; 351 ± 23 kg initial body weight) were blocked by weight into heavy and light groups and assigned randomly to experimental pens containing 10 or 11 heifers each. Pens (n=24) were assigned randomly to each of the 4 treatments. Cattle were fed once daily and had *ad libitum* access to feed and water. Basal diets included (DM basis) 35% wet maize gluten feed, 35% cracked maize, 15.8% pelleted soya bean hulls, 10% maize silage, vitamins, and minerals, and provided 14% crude protein, 300 mg/d monensin, 90 mg/d tylosin, 2200 IU/kg vitamin A, 0.7% calcium, and 0.7% potassium. For Linpro diets, the extrudate was added at 10% of DM, replacing soya bean hulls. Heifers were implanted (estradiol/trenbolone acetate), dewormed (Safe Guard®), and vaccinated against common viral and clostridial diseases (Vista® 3, Vision® 7). Starting 23 d before harvest, zilpaterol hydrochloride was added to the diet for 20 days. At day 64, blood samples were drawn from the jugular vein for analysis of copper and long chain fatty acid (LCFA) concentrations. Heavy and light blocks were harvested on day 117 and 136, respectively. Data were statistically analyzed using the MIXED procedure of SAS (Version 9.1) with Linpro, copper, the interaction between Linpro and copper, and block as fixed effects, pen nested within Linpro, copper, and block as the random effect, and pen as the experimental unit.

Results There were no significant interactions between levels of Linpro and supplemental copper. Added copper did not affect performance (P>0.15). Final body weights were similar for cattle fed 0 and 10% Linpro (581 vs 588 kg; SEM: 5.18; P>0.05), but cattle fed diets with Linpro consumed less feed (14.08 vs 13.59 kg/d; SEM: 0.21; P<0.05), and therefore were more efficient (0.131 vs 0.141, for 0 and 10% Linpro, respectively; SEM: 0.0017P<0.01). Carcass traits were not affected by treatment. Feeding elevated levels of copper did not appreciably alter proportions of polyunsaturated fatty acids in plasma (Table 1). Plasma concentrations of omega 3 fatty acids, including C18:3, C20:5, and C22:5, were greater for heifers fed Linpro (P<0.05).

Table 1 Effects of Linpro and copper levels on carcass traits and blood parameters of finishing heifers.

	Control		Linpro			P values		
	10 Cu	100 Cu	10 Cu	100 Cu	SEM	Linpro	Cu	Linpro*Cu
Carcass weight, kg	371	367	377	370	3.32	0.195	0.133	0.571
Plasma fatty acids, μg ml^{-1}								
C18:0	356	401	414	411	20.21	0.114	0.318	0.248
C18:1	132	158	152	153	9.18	0.444	0.157	0.205
C18:2	1227	1416	1267	1324	81.83	0.755	0.151	0.434
C18:3n6	6.74	9.44	4.67	5.57	1.42	0.053	0.224	0.536
C18:3n3	39.22	61.32	209.83	206.63	9.73	<0.001	0.346	0.212
C20:3n6	26.13	43.11	22.92	23.71	4.17	0.015	0.049	0.070
C20:4n6	34.17	44.04	27.15	30.04	3.45	0.008	0.083	0.327
C20:5n3	4.28	5.55	8.92	10.86	0.93	<0.001	0.103	0.722
C22:5n3	14.20	18.45	19.17	20.86	1.62	0.036	0.085	0.438

Conclusions Linpro can be used effectively as an energy source in finishing cattle diets, and to modify tissue concentrations of polyunsaturated fatty acids. However, increasing dietary concentration of copper does not appear to be an effective strategy for decreasing ruminal biohydrogenation and promoting subsequent assimilation of polyunsaturated fatty acids into tissues.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Response of pen-fed weaner cashmere goats to lucerne hay harvested at different times

F Hou[1], X Chen[1], L Xu[1], Y Kang[1], Z Nan[1], S Pain[2], L Bell[3], M Robertson[3], W Bellotti[4]
[1]Lanzhou University, Lanzhou, China, [2]Massey University, Palmerston North, New Zealand, [3]CSIRO Ecosystems Sciences, Canberra, Australia, [4]University of Western Sydney, Penrith, Australia
Email: cyhoufj@lzu.edu.cn

Introduction In Loess Plateau region of China, there is a trend to increasing animal numbers and production (Brown *et al*. 2009). Cashmere goats for meat and wool production are the most common livestock enterprise and pen feeding of forages is dominant especially with grazing now prohibited on the native grasslands in this region (Hou *et al*. 2008). The predominant feed sources are lucerne (*Medicago sativa* L.) and residues from maize grain crops. Often higher quality lucerne leaf is lost from stored material or lucerne is harvested later than optimal and hence has reduced it nutritional value for stock. We aimed to quantify the effects of feeding lucerne/ maize straw-based diets of different qualities to weaner Cashmere goats in northern China, to understand how much improvement in animal productivity might be obtained by better feeding management of their lucerne and other feed resources.

Materials and methods To compare animal growth response to feeding 2 rations comprising 40% g/g of good quality lucerne hay with a high leaf proportion (leafy lucerne), compared with poor quality lucerne hay with a low leaf proportion (stemy lucerne), study was conducted in Huanxian County, Gansu province, on the Loess Plateau of Northwestern China from 5 December 2010 to 15 January 2011. To maintain a common lucerne source for the experiment, leaf was removed from the good quality hay material to produce the lower quality treatment. The ration also consisted of 40% g/g corn straw and 20% g/g FW cracked corn grain. Forty-eight 3 month old male cashmere goats were selected for this experiment and were ranked randomly to 8 pens by weight. Four replicates of the two ration treatments were fed to pens (space, 6.25 m^2) of 6 animals arranged in a randomised block design. All animals were drenched for worms prior to their induction in the experiment. The 3 feed components and the 2 rations sampled were dried in an oven at 80 ^oC to determine DM content and then dried samples were ground for determination of: N content using the Kjeldhal method; Acid Detergent Fibre (ADF) and Neutral Detergent Fibre (NDF) using the ANKOM apparatus (ANKOM 200 Fiber analyser, ANKOM Technology, Macedon, New York, USA); and ash content by ignition in a muffle furnace at 550 ^oC for 4 hours. Crude protein concentration was determined as Kjeldahl N × 6.25. The live-weight of individual animals was measured every week. The feed and water intake of each pen was recorded daily. During two consecutive 6 day periods during the first 2 weeks, second 2 weeks and third 2 weeks of the experiment, the residual feed was retained and faeces were collected for analysis of fibre, crude protein and ash content. Also during these periods, rectal faecal samples (approximately 30 to 40 grams per animals) to assess worm egg counts and louse counts were conducted on 2 animals per pen. Growth rates were calculated using a linear regression of individual animal weights over the experimental period (n =7) and the slope of these were then compared in ANOVA. Because groups of animals were separated according to their live-weight, ANOVA was conducted with these groups as a block term. The 6 day periods when residue feed and faeces were collected during the first, second and third fortnight of both studies were discrete and hence these 3 periods were treated as a factor in the analysis of measurements using this data.

Results No significant differences were found on feed intake (Table1). Leafy lucerne caused a significant (P=0.009) enhancement of liveweight gain by about 35% (71.9 vs. 53.2 g/hd/d) but had no significant effect on feed conversion ratio (P=0.138). Goats fed with leafy lucerne had a higher CP, ADF and NDF intake than that fed with stemy lucerne. The differences in OM intake (451 vs. 465 g/hd/d) were statistically significant between the goats fed with leafy and stemy lucerne but differences in lice count and faecal egg counts for the 2 meal rations were numerically.

Table 1 Liveweight gain per day (LWG), faeces egg count, lice count, water intake, forage DM intake, CP intake, OM intake, feed conversion ratio (FCR) vs. ration (mean±SE)

	Ration 1 (leafy Lucerne)	Ration 2 (stemy Lucerne)	P
LWG (g/hd/d)	71.9±5.9	53.2±4.6	0.009
Feed intake (g DM/hd/d)	493.2±22	498.3±21	0.247
Water intake (L/hd/d)	0.60±0.04	0.58±0.04	0.044
FCR (g LW/g DM intake)	0.15±0.01	0.11±0.02	0.144
CP intake(g/hd/d)	76.7±3.0	48.3±2.0	<0.001
ADF intake (g/hd/d)	167±9.3	238±10.0	<0.001
NDF intake (g/hd/d)	258±16	348±17	<0.001
OM intake (g/hd/d)	451±22	465±22	0.019
Lice count (louse/goat)	11.9±0.52	11.2±0.67	0.186
Faecal egg count (eggs/g)	906±116	911±113	0.930

Conclusions In the Loess Plateau, cashmere goat performance could be improved by reducing higher quality lucerne leaf lost during the storage and harvesting practices of lucerne that is fed.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Dairy ewes fed with fresh citrus by-product feedstuffs: the effect on Vastedda cheese characteristics

M Todaro, M L Scatassa
[1]University of Palermo - Department DEMETRA, Palermo, Sicily, Italy, [2]Istituto Zooprofilattico Sperimentale, Palermo, Sicily, Italy
Email: zootmax@unipa.it

Introduction Citrus by-product feedstuffs (BPF) are important components of ruminant feeding systems in many areas of the world, and are commonly used as sources of dietary energy. The main citrus BPF fed to ruminants are fresh citrus pulp, citrus silage, dried citrus pulp, citrus meal and fines, citrus molasses, citrus peel liquor, and citrus activated sludge. Other minor BPF include cull or excess fruit. Citrus silage and pulp are generally very rapidly accepted by most classes of ruminants. Citrus BPF have similar digestibility among ruminant species. Supplementation of forages with citrus BPF that are rich in pectin or highly degradable NDF usually has a less negative effect on the rumen ecosystem, and thus on cellulolysis, than supplementation with starch- or sugar-rich feeds. Citrus BPF contain a variety of energy substrates for ruminal microbes, including both soluble carbohydrates and rapidly digested NDF. Citrus BPF improve the utilization of other dietary NDF, possibly due to positive effects on rumen microflora. Citrus BPF can be used as a high energy feed in rations that support growth and lactation in ruminants (Bampidis & Robinson, 2006).
In Sicily the utilisation of fresh lemon pulp (FLP), namely "Pastazzo", consisting of a mixture of peels, inside portions, seeds and culled fruit, in sheep fed during the dry season (from May to August) is frequent. The FLP contain particular flavors, as limonene, that can transfer to milk and cheese (Chiofalo *et al*., 2004). During the dry season the Valle del Belice ewes are in milking, so their milk is utilized to produce a particular fresh *pasta filata* cheese, named PDO Vastedda della valle del Belice. The aim of this study was to evaluate the effects of FLP feeding in milk composition, in Vastedda cheese making process and in cheese characteristics.

Materials and methods Sixty-eight multiparous Valle del Belice ewes at the end of lactation (122 ± 68 days) were allocated homogeneously, based on days in milking and age at lambing, to 2 groups and differentiated only for supplementation of 2 kg/head/day of FLP (DM 12.30%; CP 11.24%, EE 4.55%, NDF 35.13%, cellulose 22.76%, ash 5.56%) after the evening milking. From the 8.00 am to the 4.00 pm the ewes of both groups were fed at pasture together. The adaptation period for animals to the experimental diet was 3 weeks. Weekly, milk tank for each group (Pasture group and FLP group) was analyzed and the cheese making process was monitored. Additionally, the Vastedda della valle del Belice cheeses were analyzed for chemical and microbiological composition. Fatty acids composition of Vastedda cheeses was analyzed too. To statistical analysis a two-factors ANOVA model was employing, considering feeding group (Pasture and FLP) and control data (1..5) as fixed factors.

Results The milk pH was influenced by FLP supplementation, (6.68 *vs* 6.71; P<0.01), this lower pH value was also observed in the curd (6.41 *vs* 6.43; P<0.01), in the pasta at the end of acidification process (5.02 *vs* 5.16; P<0.01) and in the Vastedda cheese (5.21 *vs* 5.28; P<0.05). The lower pH values observed in milk and cheese produced by FLP ewes could be due to higher citric acid concentration present in the milk instead of the activity of lactic microflora. In fact the total bacterial count (7.75 *vs* 8.03 log; P<0.05) and mesophilic lactobacilli (7.95 *vs* 8.32 log; P<0.01) was significantly lower in the cheese from the FLP group.
Overall, the Vastedda cheeses presented high lactic microflora concentration, around 10^8 ufc/g. Cheese chemical composition (table 1) did not differ between groups with the exception of a_w (available water) that resulted higher in the FLP cheeses. On the another hand, the fatty acid composition was not influenced by FLP supplementation.

Table 1 Chemical composition of PDO Vastedda della valle del Belice cheese

Chemical Composition	Pasture group	FLP group	Standard error
Water (%)	28.26	27.84	0.73
Crude protein (% of DM)	38.65	38.49	0.30
Ether Extract (% of DM)	50.59	50.64	0.21
Ash (% of DM)	5.18	5.20	0.14
Chlorides (g/100g)	0.33	0.35	0.02
pH of Vastedda cheese	5.28 a	5.21 b	0.022
AW	0.95 A	0.97 B	0.00

A, B, in the same row, significant at P<0.01; a, b, in the same row, significant at P<0.05

Conclusions The PDO Vastedda della valle del Belice is a particular *pasta filata* ovine cheese, that could present different flavour and taste with the variation of pH. The supplementation of FLP to milking ewes could present some problems due to the high presence of citric acid in the milk and in the curd that reduce pH values without increasing the lactic microflora. In conclusion, if the FLP, such as the BPF, are considered the good by-products, their use in lactating ewes should be evaluate in function of milk destination and in particular if milk is used to PDO Vastedda della valle del Belice cheese production.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Can frequent and long-term consumption of beef with elevated concentrations of n-3 fatty acids improve the health of people with metabolic syndrome?

S Kronberg
EcoSun Prairie Farm, Inc., Brookings, South Dakota, USA
Email: scott.kronberg@ars.usda.gov

Introduction Intake of beef from cattle that are produced while consuming feedstuffs that have substantial concentrations of n-3 fatty acids (e.g. immature grass, linseed) can raise the n-3 concentrations in blood plasma (Sinclair *et al*. 1994; McAfee *et al*. 2010), but evidence is needed to indicate that consuming this beef improves the health of people who do not have good health. Metabolic syndrome is a growing health problem world-wide because it is associated with type-2 diabetes and cardiovascular disease, and in the USA it is frequently observed in young and older adults and in men and women. Abdominal obesity, elevated blood triglycerides, low blood levels of high-density lipoprotein cholesterol (HDL-C), elevated blood pressure, and elevated fasting glucose are associated with the condition. Lastly, many medical doctors in the USA recommend that their metabolic syndrome patients consume more fatty fish that contain high amounts of n-3 fatty acids per serving, but these patients often do not follow their doctor's advice because they either do not like to eat fatty fish or can not afford it. Therefore, although beef can not compete with foods such as wild-caught salmon or tuna in respect to amounts of n-3 fatty acids per serving, it can compete with these fish in respect to a commonly consumed source of these fatty acids to many people who eat little if any fatty fish.

Evidence Compared to a diet that is relatively high in carbohydrate (65% of energy as carbohydrate, 13% as protein, and 22% as fat with 17% of this unsaturated fat), a diet that is relatively rich in protein and monounsaturated fat and low in carbohydrate (48% of energy as carbohydrate, 19% as protein, and 33% as fat with 24% of this as unsaturated fat) has been shown to improve components of metabolic syndrome (except for HDL-C) for obese patients (Muzio *et al*. 2007). Also, intake of lean red meat is not associated with coronary heart disease (Li *et al*. 2005), but it is not clear if intake of n-3 fatty acids in the muscle cell membranes of lean beef contributes to the reduced risk of coronary heart disease since lean beef also has a low concentration of saturated fatty acids. Elevated levels of inflammatory cytokines such as C-reactive protein (CRP) in the blood are also associated with metabolic syndrome, and intakes of the n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are associated with reduced levels of blood triglycerides and CRP in the blood. Increased intake of alpha-linolenic acid (ALA) can also reduce CRP levels in the blood, and this relationship may exist because ALA is converted in the body to EPA. Prolonged intake of ALA by people raises levels of EPA and the n-3 fatty acid docosapentaenoic acid (DPA) in their red blood cells (Barceló-Coblijn *et al*. 2008). Seal oil, which contains high levels of DPA along with EPA and DHA, appears to be as effective as fish oil, which contains low levels of DPA along with similar levels of EPA and high levels of DHA, in lowering blood plasma triglycerides and blood pressure (Meyer *et al*. 2009). Therefore, high intake of DPA may help lower blood triglycerides and blood pressure, which is important because beef is a good source of DPA (Noci *et al*. 2005; Kronberg *et al*. 2011).

Conclusion It is reasonable to assume that consumption of several servings per week (for at least several months) of lean beef that has elevated concentrations of n-3 fatty acids will improve the health of people who have metabolic syndrome. However, this hypothesis needs to be evaluated.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Performance and carcass traits of finishing Nellore steers fed crude glycerin

E van Cleef, J Ezequiel, J Sancanari, A D`Aurea, V Fávaro, D Silva, F Scarpino
São Paulo State University, Jaboticabal, São Paulo, Brazil
Email: ericvancleef@gmail.com

Introduction The introduction of by-products from agribusiness, such as cakes and meals, in animal nutrition is becoming increasingly important as they can replace conventional ingredients such as soybean and corn, without impairing the performance of animals. With the advent of biodiesel new by-products are being generated, such as glycerin. Approximately 10% of any oil or fat used in making biodiesel, becomes glycerin (Dasari *et al*., 2005), so with the increase in world production of this product, the surplus amount must have an ecologically appropriate and economically viable destination. In this context, the objective of this study was to evaluate the inclusion of crude glycerin in diets for beef steers Nellore and its effect on carcass traits of these animals.

Materials and methods Thirty Nellore steers (277.7 ± 23.8 kg BW) were blocked by initial weight and assigned randomly to one of the five treatments for 82 d. Experimental diets consisted of 30% corn silage and 70% concentrate (corn grain, soybean hulls, sunflower meal, glycerin) and were labeled as: 1) diet with no added glycerin (CON), 2) 7.5% glycerin on diet dry matter basis (7.5GLY), 3) 15% glycerin on diet dry matter basis (15GLY), 4) 22.5% glycerin on diet dry matter basis (22.5GLY), and 5) 30% glycerin on diet dry matter basis (30GLY). Cattle were housed on concrete surfaced individual pens (16 m^2) with roofs covering half the pen and the entire feed bunk. On the arrival, the animals received *ad libitum* access to fresh water and corn silage before processing. For the first 21 days, animals were offered 4 step-up diets with increasing levels of concentrate (10, 20, 30, 50%). Cattle were fed twice daily *ad libitum* and the feed and refused feed weights were recorded to determine dry matter intake (DMI). After 82 days on feed, cattle were weighed and slaughtered in a commercial abattoir, when the carcass data were collected. Longissimus muscle area and 12th-rib fat thickness were collected after a 24-h chill. Data were analyzed as a randomized complete block design by using the MIXED procedure (SAS Inst. Inc., Cary, NC). Animal was the experimental unit, and model effects included block and treatment. Orthogonal contrasts were used to determine the linear, quadratic, and cubic effects of glycerin, and 0% glycerin vs. glycerin treatment.

Results There was a cubic effect of inclusion of glycerin on steers DMI (P = 0.020) with the lowest values observed on 7.5 and 30% glycerin. Parsons, *et al*. (2009), working with beef heifers, reported that increasing glycerin to 4, 8, 12, and 16% reduced DMI to 8.66, 8.61, 8.40, and 7.80 kg, respectively. Similarly, Pyatt *et al*. (2007) observed a 10.1% reduction in DMI when glycerin was added at 10% to finishing steers diets. These authors also reported an improvement in feed efficiency, a fact not observed in the present study. Carcass and meat characteristics did not change (P > 0.05) with the addition of crude glycerin in this study. Mach, *et al*. (2009) also observed no differences on carcass and meat quality of Longissimus muscle from Holstein bulls fed high-concentrate diets containing different glycerin content (0, 4, 8, or 12%).

Table 1. Carcass characteristics of steers fed 0, 7.5, 15, 22.5, or 30% crude glycerin

Item	Crude Glycerin, (%)					Contrast, P-value				EPM
	0	7.5	15	22.5	30	L[1]	Q[2]	C[3]	0 vs. Gli[4]	
Dry matter intake kg	8.96	7.81	8.49	8.75	7.79	0.26	0.98	0.02	0.09	0.38
Average daily gain, kg/day	1.54	1.69	1.75	1.70	1.44	0.68	0.12	0.80	0.56	0.15
Gain:feed, kg/kg	0.17	0.22	0.21	0.20	0.19	0.97	0.14	0.33	0.21	0.02
Hot carcass weight, kg	219.50	225.17	227.50	231.92	214.00	0.86	0.13	0.44	0.55	7.59
Dressed yield, %	53.05	52.70	53.87	54.32	53.03	0.37	0.24	0.08	0.50	0.56
Longissimus muscle area, cm^2	65.17	63.33	64.83	63.67	64.50	0.92	0.83	0.90	0.77	8.14
Subcutaneous fat, cm	0.36	0.45	0.48	0.37	0.22	0.25	0.12	0.92	0.88	1.97

[1]Linear, [2]Quadratic, [3]Cubic, [4]Effects of 0% glycerin with the combined glycerin treatment.

Conclusions Feeding up to 30% crude glycerin in by-product diets do not alter the gain, efficiency and carcass characteristics of Nellore finishing steers. Further studies must be conduct to determine the real feed value of glycerin, such as digestibility and ruminal parameters trials.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effect of pre-grazing herbage and sward specie richness on forage nutritive value, botanical composition, dry matter intake and milk solids production of dairy cows during the mid-lactation

D Collucci, S Greenwood, R Bryant, G Edwards
Lincoln University, Lincoln,Canterbury, New Zealand
Email: dcollucc@gmail.com

Introduction Forage nutritive value (FNV) in diverse swards can be associated with botanical composition (BC) richness; as sward dry matter (DM) inherently comprises both different chemical composition and distinct stages of maturity among plant species compared with simple swards (Deak *et al*., 2007). Pre-grazing forage mass (FM) and post-grazing FM are two essential factors when considering required daily DM intake (DDMI). Sustaining high mass (HM) grass clover swards can reduce forage quality (FQ) and therefore milk yield (MY) and milk solids (MS) production relative to low mass (LM) grass clover swards (Holmes *et al*., 1992). However, HM complex swards could maintain FNV because of the unique chemical composition. Therefore, the objective of this study was to evaluate the effects of sward diversity (poor or complex) managed at 2 levels of FM (low and high) on BC, FNV, DDMI, MY and MS production of dairy cows feed on pastures.

Materials and methods Twenty-four multiparous lactating Holstein-Friesian cows were allocated in 2 replicates in a 2X2 factorial design (3 cows per treatment replicate), according to days in milk, live weight and (MS) production. Two levels of FM, low (2500 kg DM ha^{-1}) and high (3300 kg DM ha^{-1}) were randomly assigned to 2 pasture mixtures: a poor mixture (HS) of high sugar perennial ryegrass (*Lolium perenne* L.) + white clover (*Trifolium repens* L.), and a diverse mixture (HSD) of high sugar perennial ryegrass + white clover + chicory (*Cichorium intybus* L) + plantain (*Plantago Lanceolata* L.). Cows feed on treatments for 9 days, were milked twice a day (7 am and 3pm) and offered fresh daily allowance (15 kg DM cow^{-1} day^{-1}) after pm milking. Fresh break area (strip-grazing management) for each cow group, was calculated based on target DDMI and on a daily assessment of pre- and post- grazing FM (1500 kg DM ha^{-1}) using a rising plate meter calibrated by double sampling of standing FM. Treatment replicate group daily intakes were estimated by the difference between pre- and post-grazing FM (McEvoy *et al*., 2007). Milk yield was recorded daily and milk samples were collected from each cow on days 0, 5 and 8. Milk samples were analyzed for fat and protein content. On days 4 and 7, for 3 hours, a sward stick was used to record the decline in height as cows progressive grazed down the fresh strip. The sward layers removed over this period were sampled, freeze-dried, ground and analysed for FNV using near infrared spectroscopy. Pre-grazing forage snips were analyzed for BC. Within each treatment, group of cows were the experimental units. Treatment effects were analyzed using analysis of variance.

Results There was no effect of sward richness on daily MS (1.69 vs 1.67 kg MS, SEM: 0.02, P>0.10, for the HS and HSD respectively). However, the effect of FM on MS was significantly different between the LM and HM swards (1.72 vs. 1.63kg MS, SEM: 0.02, P<0.10). The effects of sward diversity (SEM: 0.13, P<0.001) and sward FM (SEM: 0.13, P<0.05) on DDMI were significant. Greatest DDMI was observed for HSD (16.48 kg DM) compared with HS (12.56 kg DM). This was related with the lower residuals on HSD compared with HS (1089 vs. 1344 kg DM ha^{-1}, SEM: 49.6, P<0.05, for the HSD and HS respectively). In addition, HM swards had slightly greater DDMI (14.78 kg DM) compared with LM (14.28 kg DM). There was an interaction between HSD and FM for DDMI (P<0.10). That is, HSD at HM had slightly lower DDMI compared with HSD at LM (Table 1). FM did not impact the sward BC (P>0.10), nevertheless, HS had greater ryegrass and white clover percentage compared with HSD (Table 2). FM did not affect FNV (P>0.10); however, HS had greater dry matter digestibility (DMD; 856 vs. 837 g kg DM^{-1}, SEM: 5, P<0.05) and higher neutral detergent fiber (NDF; 337 vs. 194 g kg DM^{-1}, SEM: 8, P<0.001) compared with HSD. Crude protein (CP) was not affected by FM or sward specie richness.

Table 1 The effect of sward richness and forage mass and on dry matter intake, milk yield and milk solids

Treatment	DMI (kg day^{-1})	MY (Litres day^{-1})	MS (kg day^{-1})
HS (HM)	13.07	15.54	1.63
HS (LM)	12.05	19.25	1.75
HSD (HM)	16.44	17.06	1.64
HSD (LM)	16.51	17.65	1.70
SEM	0.19	0.09	0.27

Table 2 Botanical composition (%), forage mass (kg DM ha^{-1}) forage nutritive value (g kg DM^{-1}) of four pasture mixtures

Treatment	FM	Rye	Clover	Chicory	Plantain	Other	NDF	CP	DMD
HS (HM)	3754	85	12	0	0	3	346	212	855
HS (LM)	2735	76	20	0	0	4	326	242	857
HSD (HM)	3209	35	7	33	22	3	193	214	841
HSD (LM)	2622	43	8	26	19	4	195	212	832
SEM	51	3	4	9	6	1	11	11	5

Conclusions Sward species diversity did not impact MS production, nevertheless, species richness impacted NDF and DMD. HSD swards had lower NDF compared with HS due to herbs species and low grass percentage.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effect of incremental amounts of fish oil in the diet on milk fatty acid composition in lactating dairy cows

P Kairenius[1], A Ärölä[1], H Leskinen[1], S Ahvenjärvi[1], A Vanhatalo[2], V Toivonen[1], M Griinari[3], K J Shingfield[1]
[1]MTT Agrifood Research Finland, Animal Production Research, Jokioinen, FI-31600, Finland, [2]University of Helsinki, Department of Agricultural Sciences, Helsinki, FI-00014, Finland, [3]The Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management, Uppsala, SE-75323, Sweden
Email: piia.kairenius@mtt.fi

Introduction Based on the potential benefits for human health there is interest in enhancing the concentrations of *cis*-9, *trans*-11 conjugated linoleic acid (CLA) and the long chain n-3 polyunsaturated fatty acids (PUFA), 20:5n-3 (EPA) and 22:6n-3 (DHA) in ruminant derived-foods. Inclusion of fish oil (FO) in the diet has been shown to be an effective strategy to increase *cis*-9, *trans*-11 CLA, and to a lesser extent EPA and DHA, in ruminant milk but these changes are also accompanied by higher *trans* fatty acid (TFA) concentrations (Chilliard *et al*., 2007). The current experiment examined the effects of increasing levels of FO in the diet on milk fat composition in lactating cows, with specific emphasis on TFA.

Materials and methods Four lactating dairy cows (2 Friesian and 2 Finnish Ayrshire; 159 ± 28 days in lactation) fed a grass silage and cereal based-concentrate (forage:concentrate ratio 58:42 on a DM basis) were used in a 4 x 4 Latin square with 28d experimental periods to examine the effects of increasing levels of FO (0, 75, 150 or 300 g/d) on milk fat composition. Milk samples were collected on d 21 of each period. Fatty acid composition was determined by GC and HPLC analysis of fatty acid methyl esters and GC-MS analysis of 4,4-dimethyloxazoline derivatives (Kairenius *et al*., 2011). Data were analysed by ANOVA using a statistical model that included the random effects of cow and fixed effects of period and treatment. Sum of squares were further divided to the orthogonal polynomial contrasts to examine the linear, quadratic and cubic components of the response to incremental amounts of FO in the diet.

Results Incremental amounts of FO in the diet resulted in linear decreases (P<0.01) in DM intake (18.7, 18.8, 17.8 and 15.6 kg/d, SEM 0.45 for 0, 75, 150 and 300 g FO/d, respectively), milk fat concentration (38.0, 41.2, 32.9 and 31.5 g/kg, SEM 1.94) and milk fat yield (883, 966, 783 and 578 g/d, SEM 67.9). Inclusion of FO in the diet altered milk FA composition, resulting in decrease (P≤0.001) in milk fat saturates (68.8, 66.4, 61.3 and 57.0 g/100g FA, SEM 0.67) and an increase (P≤0.001) in PUFA (3.73, 4.70, 7.23 and 11.2 g/100g FA, SEM 0.37), EPA and DHA concentrations (Table 1). Milk fat *cis*-9, *trans*-11 CLA content increased in a quadratic manner (P=0.040) from 0 to 150 g FO/d (Table 1). Incremental amounts of FO in the diet also elevated (P<0.001) the concentration of 16-, 18-, 20- and 22-carbon TFA in milk (Table 1). Most of the increase in total TFA content in milk was due to enrichment of total *trans* 18:1 (P<0.001; 3.9, 6.1, 11.1 and 13.5 g/100g FA, SEM 0.45).

Table 1 Effect of fish oil in the diet on milk fatty acid composition (g/100g total fatty acids).

	Fish oil (g/d)				SEM	Significance[1]		
	0	75	150	300		L	Q	C
Σ4:0-16:0	58.7	57.2	54.4	51.8	0.947	0.001	0.671	0.502
18:0	10.1	8.90	6.06	4.25	0.370	<0.001	<0.001	0.127
cis-9 18:1	17.5	16.2	12.2	8.76	1.052	0.001	0.762	0.307
trans-10 18:1	0.34	0.46	1.06	4.20	0.231	<0.001	0.004	0.852
trans-11 18:1	1.37	2.43	5.31	5.46	0.453	<0.001	0.035	0.059
cis-9, *trans*-11 CLA	0.61	1.03	2.15	2.07	0.206	0.002	0.040	0.085
18:2n-6	1.23	1.18	1.21	1.17	0.047	0.496	0.999	0.515
18:3n-3	0.41	0.38	0.39	0.48	0.047	0.274	0.302	0.962
20:5n-3	0.09	0.09	0.10	0.20	0.011	<0.001	0.014	0.501
22:6n-3	0.03	0.03	0.05	0.10	0.009	0.001	0.258	0.802
Σ*trans* 16-carbon FA	0.45	0.71	1.21	1.54	0.056	<0.001	0.061	0.055
Σ*trans* 18-carbon FA	4.89	7.52	13.2	17.6	0.573	<0.001	0.106	0.036
Σ*trans* 20-carbon FA	0.13	0.34	0.65	1.07	0.041	<0.001	0.626	0.223
Σ*trans* 22-carbon FA	0.01	0.02	0.06	0.19	0.009	<0.001	0.004	0.857
Σtrans FA	5.68	8.74	15.2	20.4	0.600	<0.001	<0.001	0.089

[1] Significance of linear (L), quadratic (Q) and cubic (C) effects of fish oil in the diet.

Conclusions Fish oil in the diet elevated milk fat CLA, EPA and DHA concentrations in a dose dependent manner. However, *cis*-9, *trans*-11 CLA content was not increased in response to FO above 150 g/d due to a shift in ruminal biohydrogenation towards the formation of *trans*-10 18:1 at the expense of *trans*-11 18:1. Enrichment of potentially beneficial fatty acids were accompanied by substantial increases in milk fat 16-, 18-, 20- and 22-carbon TFA containing at least one double bond and decreases in nutrient intake, milk yield and milk fat content.

Acknowledgments Financial support from the Finnish Ministry of Agriculture and Forestry is gratefully acknowledged.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Factors affecting biohydrogenation of 22:6*n*-3 during batch *in vitro* incubations

B Vlaeminck, V Fievez
LANUPRO, Ghent University, Melle, Belgium *Email: bruno.vlaeminck@ugent.be*

Introduction Previous *in vitro* experiments showed a decrease in ruminal biohydrogenation (BH) of 22:6*n*-3 with increasing amounts of 22:6*n*-3 (Fievez *et al*., 2007). However, in addition to *in vitro* 22:6*n*-3 concentration, other factors were suggested to contribute to the observed variation in the disappearance of 22:6*n*-3. Variable composition of the 22:6*n*-3 supplements as well as a lack of information on the rate of lipolysis of 22:6*n*-3 might contribute to inter-experimental differences (Fievez *et al*., 2007). In addition, preparation of rumen inoculum and the type of fermentation substrate might contribute to the variation on the disappearance of 22:6*n*-3 (Vlaeminck and Fievez, unpublished results). The aim of the current experiment was to evaluate the effect of the rumen fluid:buffer ratio, substrate and time of addition of 22:6*n*-3 on the disappearance of 22:6*n*-3 during batch *in vitro* incubations.

Materials and methods A 2×2×2 factorial design was used to examine the effect of rumen fluid:buffer ratio (RF, 5/20 or 10/15), fermentable substrate (200 mg; cellulose/casein (C/C, 85/15, w/w) or cellulose/glucose/casein (C/G/C, 45/40/15, w/w/w)) and time of 22:6*n*-3 supplementation (2 mg in 100 µL of ethanol added at the start or after 6h of incubation). Cultures were run in triplicate at 39°C as described by Van Ranst *et al*. (2010). After 48 h, flasks were removed from the incubator and placed immediately in an ice bath. In a second experiment, kinetics of 22:6*n*-3 disappearance and rumen fermentation were evaluated using RF 10/15. 22:6*n*-3 (2 mg in 100 µL of ethanol) was added at the start of the incubation. Fermentable substrates were as before and cultures were removed after 6, 24 and 48h of incubation. A 2 ml sample was taken for VFA analysis (Van Ranst *et al*., 2010). Fatty acids were determined in 5 ml freeze dried culture solution. Toluene (2 ml) containing the internal standard (13:0, 0.4 mg) and methanolic NaOH (2 ml) was added and the mixture was incubated at 70°C (60 min.) followed by 30 min at 50°C after addition of methanolic HCl (3 ml), prepared by dissolving 10 ml acetyl chloride in 50 ml methanol. Fatty acid methyl esters were extracted with hexane and analyzed by gas chromatography. Kinetic parameters were estimated using the exponential model $b \times (1 - e^{-k \times (\text{incubation time} - \text{lag time})})$. When lag time was not different from 0, it was removed from the model.

Results Biohydrogenation of 22:6*n*-3 was mainly affected by the rumen fluid:buffer ratio and to a lesser extent by time of addition of 22:6*n*-3 and substrate after 48 h of incubation (Table 1). Net production of acetate and butyrate was greater with RF 10/15 and C/G/C as substrate whereas propionate production was greater with RF 5/20 and C/C as substrate (Table 1). The effect of substrate on kinetic parameters of net production of VFA and BH of 22:6*n*-3 are shown in Table 2. C/G/C as substrate increased the rate of acetate and butyrate production and BH of 22:6*n*-3 and a greater asymptotic value for BH of 22:6*n*-3.

Table 1 Effect or rumen fluid:buffer ratio, fermentable substrate (C/C, cellulose/casein and C/G/C, cellulose/glucose/casein) and time of 22:6n-3 addition (after 0h or 6h of incubation) on net production of VFA (µmol/incubation) and biohydrogenation of 22:6n-3 (g/g)

	rumen fluid/buffer ratio			substrate			time of 22:6*n*-3 addition			SEM
P	10/15	5/20		C/C	C/G/C	P	0h	6h	P	
Acetate	1043	687	<0.001	796	934	<0.001	855	874	0.217	10.7
Propionate	672	824	<0.001	790	706	<0.001	768	728	0.034	12.1
Butyrate	140	96	<0.001	88	149	<0.001	121	115	0.016	1.5
22:6*n*-3	0.933	0.379	<0.001	0.643	0.669	0.043	0.680	0.633	0.001	0.008

Table 2 Effect of fermentable substrate (C/C, cellulose/casein and C/G/C, cellulose/glucose/casein) on kinetic parameters of net production of VFA (µmol/incubation) and biohydrogenation of 22:6n-3 (g/g)

	asymptotic value (b)		fractional rate (k)		lag time	
	C/C	C/G/C	C/C	C/G/C	C/C	C/G/C
Acetate	1210 (78.9)	1160 (36.9)	0.037 (0.006)	0.067 (0.006)*	1.34 (0.752)	-
Propionate	744 (19.8)	642 (8.9)*	0.097 (0.013)	0.111 (0.006)	4.24 (0.337)	-
Butyrate	171 (30.8)	171 (5.9)	0.022 (0.006)	0.086 (0.010)*	-	-
22:6*n*-3	0.942 (0.009)	0.978 (0.006)*	0.118 (0.004)	0.155 (0.004)*	-	-

* parameters differ significant ($P < 0.05$)

Conclusions Disappearance of 22:6*n*-3 during batch *in vitro* incubations was largely affected by the amount of rumen fluid. In addition, a mixture of cellulose and glucose as substrate increased the rate of BH of 22:6*n*-3.

Acknowledgements This work was financially supported by the Fund for Scientific Research–Flanders and the Special Research Fund of the University of Ghent (Belgium). Bruno Vlaeminck is a Postdoctoral Fellow of FWO-Vlaanderen.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Effect of lasalocid supplementation on performance of lambs grazing natural pastures.

L Piaggio[1], M L del Pino[1], M de J Marichal[2]
[1]Secretariado Uruguayo de la Lana, Montevideo, Uruguay, [2]Facultad de Agronomía, Montevideo, Uruguay
Email: mariadejesus.marichal@gmail.com

Introduction In 1993, the Uruguayan Wool Secretariat began a research program to generate new products that would complement wool production. The goal was to produce a "heavy weight lamb" in production systems where animal continuously grazed pastures. Animals should reach, in spring (October), a final weight of 32 to 45 kg, a body score of 3.5, and 10 to 30 mm of wool length (Azzarini *et al*, 1996). The main limitation to reach that goal in animals grazing natural pastures is the fluctuation in quantity and quality of available forage during the winter. Piaggio *et al* (2009) reported that supplementation with concentrates in winter will result in satisfactory lamb growth rates, and desirable final weights could be achieved in spring. The inclusion of lasalocid in rations fed to sheep could shift rumen VFA toward less acetate and more propionate, reduced methane production, and increase the efficiency of energy utilization (Ricke *et al*., 1984). There is limited information about the effect of ionophores supplementation on performance of fattening lambs grazing natural pastures. The objective of this study was to examine the effect of the inclusion of lasalocid in concentrates offered to lambs grazing natural pastures.

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 May 18 to October 14 2010 (29 days of adaptation period plus 120 days of measurements), seventy two (36 ewes and 36 wethers) 7 months old Corriedale lambs were divided into twelve homogenous groups according to sex, live weight, and body score. Animals were allocated into six paddocks (twelve lambs paddock-^{1}, 14 lambs/ha) of natural pastures (2636 kg DM ha^{-1}; 80, 660 and 322 g kg DM^{-1} of CP, NDF,and ADF, respectively). Lambs in three paddocks were supplemented with a concentrate containing (70:30, respectively) of whole sorghum grain (85 g kg DM-^{1} CP), and soybean meal (509 g kg DM-^{1} CP) . In each paddock, animals were assigned (3 replicates treatment-^{1}) to one of the following treatments: continuous grazing + 500 g concentrate lamb-^{1} (Control), or continous grazing + 500 g concentrate lamb-^{1} + 1 mg kg BW-^{1} of Lasalocid [Bovatec, Sinervia, Uruguay). Lambs were weighed first day of adaptation period, one month later (June 16), and every 15 days thereafter, body score was determined on the first and last day of the experimental period. Because lambs were sheared on August 31, average daily gain was calculated for the pre, and post shear periods, and the weighted average daily gain was estimated. Results of initial body weight, final body weight, average daily gain pre and pos shear, whole period average daily gain , wool production, and difference between initial and final body score (DIFBS), were analyzed (PROC GLM, SAS) in a completely randomized design (Tukey test), considering the group of twelve lambs by paddock as the experimental unit.

Results In both treatments, lambs achieved the final target weight (32 to 45 kg) in Spring . Although the inclusion of lasalocid could have modified rumen metabolism, no differences ($P > 0.13$) in any of the variables studied were observed when the additive was included in the concentrate fed to lambs.

Table 1 Effect of inclusion of lasolacid in a concentrate on the performance of lambs grazing natural pastures .

	Control	Control + Lasalocid	Pooled SEM	P =
N	36	36		
Initial live weight, kg	25.3	26.2	0.91	0.29
Pre-sheared live weight, kg	33.3	36.0	3.73	0.43
Final live weight, kg	36.0	38.7	4.54	0.51
Pre-sheared average daily gain, g lamb-1 day-1	85	85	0.09	0.26
Post-sheared average daily gain, g lamb-1 day-1	122	122	0.08	0.93
Weighted average daily gain, g lamb^{-1} day^{-1}	96	96	0.02	0.71
Initial body score (IBS)	3.5	3.4		
Final body score (FBS)	3.5	3.6		
Difference between IBS and FBS	0.0	0.2	0.02	0.34
Wool Production	2.6	2.7	0.30	0.13

Conclusion Performance of fattening lambs grazing natural pastures was not improved when animals were supplemented with a concentrate containing lasalocid.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effect of diet on yield of meat cuts from two indigenous breeds of cattle from Tanzania

A Zakaria, A Kimambo, L Mtenga, G Laswai, A Mwilawa, D Mgheni
Sokoine University of Agriculture, Morogoro, Tanzania
Email: abiliza2010@hotmail.com

Introduction Meat produced and consumed in Tanzania comes mainly from low weight indigenous cattle that are grazed and finished on poor quality natural pastures (MLDF 2009). Carcasses produced fall short of requirements desired by the domestic upper end consumers (Mapunda 2007). This study determined the effects of high molasses based concentrate on yield of prime cuts from the indigenous breeds

Materials and methods Thirty six Tanzania Shorthorn Zebu (TSHZ) steers (mean age 3.5yrs; 177±9 kg) and 36 Boran steers (mean age 2.5yrs; 225±12kg) were randomly allocated to three treatments for 90 days during the dry season of 2008. Where: Hay+C100, animals were stall fed on hay (mixture of *Cenchrus ciliaris* & *Chloris gayana*) plus ad lib concentrates, Graz+C50, free grazing for 9 h plus 50% ad lib concentrate and Graz +C00 grazing only. Concentrate used had 135g CP /kg DM and 12MJME/kg DM formulated from maize meal, cotton seed cake, molasses, mineral mix, salt and urea. Animals had free access to clean water. Initial live-weight of the animals was taken as mean of 3 consecutive weighing at the start of experiment. At the end of the experimental feeding all steers were weighed after 12hr of fast to get slaughter weights. The steers were slaughtered, the carcasses were split into halves and the hot carcass weights recorded. The carcasses were held for 10h at room temperature thereafter chilled at 4^0C for further 38h. On the 48h post-mortem, the right halves were jointed into 18 retail cuts as per Tanzania Bureau of Standards meat cuts (NARCO 2004 Manual) and weighed. The carcass components data were analysed by two way analysis of variance for complete randomised design where the main effects were the breed and feeding regimes. Initial weights were used as covariates within breeds in the model.

Results Boran steers had higher (P<0.05) slaughter weight, but their carcass weights and meat cuts yield were not different (P>0.05) from those of TSHZ (Table1) due to higher dressing % for TSHZ. Slaughter and carcass weights were higher (P<0.05) for steers on Hay+C100 than those for Graz+C50 and for Graz+C00 respectively. Similarly animals on Hay+C100 produced more kg of prime cuts than those on grazing only. Cuts from Gaz+C50 animals weighed higher than those from grazing only (Graz+C00).

Table 1 Ls Means effect of breed and dietary treatments on the weight of half carcasses and of prime cuts (kg)

	Breed effect				Dietary effect				
Parameters	Boran	TSHZ	SEM	P	HayC100	GrazC50	GrazC00	SEM	P
Slaughter weights	248	219	5.010	***	278	231	192	5.263	***
Dressing %	49.0	53.4	1.59	ns	54.2	50.9	48.5	1.670	*
Half carcass (kg)	57.8	55.3	1.61	ns	67.4^{a}	58.0^{b}	44.2^{c}	1.700	***
Saleable cuts (kg)	48.5	46.7	1.56	ns	56.3^{a}	49.2^{b}	37.3^{c}	1.640	***
Prime cuts (kg)	34.7	32.0	0.99	ns	39.0^{a}	34.9^{b}	26.1^{c}	1.040	***
Un-primed cuts (kg)	13.6	14.6	0.61	ns	17.0^{a}	14.4^{b}	11.0^{c}	0.630	***
Prime cuts portions (kg)									
Shoulder	6.17	5.32	0.21	*	6.68^{a}	6.06^{a}	4.50^{b}	0.260	***
Chuck	6.62	5.25	0.22	*	7.61^{a}	6.46^{b}	4.64^{c}	0.226	***
Rib	5.59	5.25	0.29	ns	6.97^{a}	5.59^{b}	3.73^{c}	0.303	***
Thick flank	2.05	1.90	0.11	ns	2.17^{a}	2.02^{ab}	1.73^{b}	0.117	**
Loin	4.25	3.82	0.17	ns	4.73^{a}	4.13^{b}	3.24^{c}	0.176	***
Rump	2.07	2.18	0.13	ns	2.39^{a}	2.33^{a}	1.66^{b}	0.133	***
Top side	3.45	3.45	0.15	ns	3.69^{a}	3.74^{a}	2.92^{b}	0.153	***
Silver side	3.32	3.07	0.11	ns	3.50^{a}	3.40^{a}	2.69^{b}	0.120	***
Shin beef	0.68	0.75	0.07	ns	0.75	0.75	0.65	0.070	ns
Fillet	0.51	0.40	0.05	ns	0.55^{a}	0.46^{ab}	0.36^{b}	0.050	***
T-bone	0.33	0.29	0.02	ns	0.35^{a}	0.33^{a}	0.24^{b}	0.020	***

Conclusions Local breeds finished on Hay+C100 yield carcasses with desired prime cuts for domestic upper end markets.

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Financial support from ENRECA IGMAFU-Meat Project and Belgian Technical Cooperation is acknowledged

Study of the effect of reducing methane production on milk composition and fatty acid profile in dairy goats

L Abecia[1], P G Toral[2], A I Martin-Garcia[1], G Martinez[1], E Molina-Alcaide[1], N W Tomkins[4], C J Newbold[3], D R Yañez-Ruiz[1]
[1]Estación Experimental del Zaidín (CSIC), Granada, Spain, [2]Instituo Ganadería de Montaña (CSIC-ULE), León, Spain, [3]IBERS, Aberystwyth University, Aberystwyth, Spain, [4]CSIRO Livestock Industries, Townsville, Australia
Email: david.yanez@eez.csic.es

Introduction The synthesis of methane in the rumen represents a loss of between 5 and 12% of the gross energy intake and significantly contributes to worldwide greenhouse gas emissions (Gill *et al.*, 2003). Several technologies have been tested *in vitro* to reduce methane production in the rumen, but very few have been successfully used in practical conditions in producing animals. Methane reduction may be based on diverting H_2 away from CH_4 formation in the benefit of the propionate production metabolic pathway in the rumen (Newbold and Rode, 2006). However, the side effects that a shift in rumen fermentation might have on fatty acid (FA) metabolism are still poorly understood. Very recently we have observed that dairy goats treated with bromochloromethane (BCM), an antimethanogenic compound, emit 38% less methane and produce 28% more milk after 9 weeks of treatment (Abecia *et al.*, 2011). This work aimed to study whether the increase in milk production in dairy goats treated with BCM and its putative effect on rumen microbiota had an impact on milk composition and its FA profile.

Material and methods Eighteen goats housed in individual tie stalls were fed *ad libitum* alfalfa hay and a concentrate. After giving birth, goats were divided in 2 groups of 9 animals each: treated (BCM+) or not (BCM-) with 0.60 g of BCM /100 kg LW and day. The BCM was administered *per os* in 2 equal doses for a 9-week period from parturition to 1 week post-weaning. Animals were weighed at the beginning and at the end of the trial. One week after weaning, daily milk production was recorded and samples were collected for determination of fat and protein contents (Person, 1976) and FA composition by gas chromatography (Shingfield *et al.*, 2003). Data were evaluated by one-way ANOVA using the MIXED procedure of SAS (Version 9.1).

Results The increase in milk production with BCM administration had no effect (P>0.10) on the concentration or daily production of milk fat and protein (Table 1). Animals body weight did not change (P>0.10) throughout the lactation period (on average –6.3 kg/goat). Furthermore, besides a small increase (5%) in total saturated FA content and a concomitant reduction in monounsaturated (–13%) and polyunsaturated FA (–17%), changes in milk FA profile were fairly small (Table 2). These were characterized by a slight decrease in some FA that are products of the enzyme Δ9-desaturase in the mammary gland (i.e., *c*9-14:1 and *c*9-16:1), but there was no significant increase in its potential substrates. The concentration of FA derived from *de novo* mammary synthesis (<16 carbons) or from the uptake of circulating plasma lipids (18 or more carbons) was not significantly modified with BCM administration either (on average 39.6 and 30.8 g/100 g total FA, respectively).

Table 1 Milk content (g/100 raw milk) and production (g/d) of protein and fat in goats treated (BCM+) or not (BCM-) with BCM

		BCM-	BCM+	SED	P
Content	Protein	3.22	3.80	0.317	0.66
	Fat	5.15	5.11	0.596	0.95

		BCM-	BCM+	SED	P
Production	Protein	37.0	41.9	7.31	0.52
	Fat	53.1	62.5	10.31	0.38

Table 2 Partial milk FA profile (g/100 g total FA) in goats treated (BCM+) or not (BCM-) with BCM

	BCM-	BCM+	SED	P		BCM-	BCM+	SED	P
14:0	10.82	11.78	0.605	0.14	*t*11-18:1	0.82	0.56	0.156	0.12
*c*9-14:1	0.19	0.14	0.026	0.05	*c*9*c*12-18:2	2.32	2.05	0.259	0.31
16:0	27.92	28.53	1.345	0.66	*c*9*t*11-18:2	0.56	0.36	0.109	0.08
*c*9-16:1	0.78	0.58	0.085	0.03	Σ saturated FA	73.00	76.75	1.599	0.03
18:0	6.59	6.91	0.860	0.71	Σ monounsaturated FA	20.92	18.16	1.278	0.05
*c*9-18:1	15.49	14.01	0.993	0.16	Σ polyunsaturated FA	3.89	3.24	0.422	0.15
*t*10-18:1	0.60	0.32	0.160	0.11	Σ odd- and branched-chain FA	3.43	3.30	0.137	0.34

Conclusions The improvement in milk production with BCM administration had no effect on milk fat and protein content and had a relatively minor impact on its FA profile. Changes in milk FA profile could have been a consequence of the increase in milk fat synthesis in goats that were treated with BCM rather than shifts in ruminal biohydrogenation pathways.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effect of yeast supplementation on nutrients utilization and milk production in dairy cows

S M A Sallam[1], A N M Nour El-Din[1], S A Mahmoud[2]
[1]Alexandria University, Alexandria, Egypt, [2]Suez Canal University, Al-Ismalia, Egypt
Email: s_sallam@yahoo.com

Introduction Microbial additives such as *Saccharomyces cerevisiae* products have been widely used in ruminant nutrition to manipulate rumen fermentation and improve animal performance. Yeast or yeasts products are widely utilized as feed ingredient or feed additive in animal nutrition. Various *S. cerevisiae* based yeast products have been shown to impact dry matter intake (DMI), rumen pH and nutrient digestibility (Dann *et al*., 2000; Lehloenya *et al*., 2008). The objective of this study was to determine effects of two types of yeast on ruminal fermentation, nutrients utilization and milk production of multiparous Holstein cows.

Materials and methods Eighteen lactating multiparous Holstein cows (500±12.34 kg LW) with similar milk production at early lactation were randomly assigned into three groups (6 cows each) and fed concentrate mixture and green forage without (CTRL) or with addition of Tonilisat (YT, 3.75 g/cow/d of Tonilisat yeast powder; China Way Corp., Taiwan) or Grow (YG, 15 g/cow/d of yeast grow powder; Vet Green Co, Egypt) for three months. Cows were randomly assigned to the experimental groups after being sorted by parity. The daily amount of yeast was mixed individually for each cow with the concentrate mixture at 0600 h. cows were milked three times daily and milk yield was recorded weekly. Biweekly milk samples were analysed immediately by milk scan (Eko-milk of milk analyzer, Milkana KAM 98-2A, USA) based on infrared technology for fat, crude protein, density and solid not fat and preserved with potassium dichromate, stored at 4°C total solids and ash analysis. Lactose was calculated by difference. Twelve Barki sheep are allocated into three groups (live weight = 45.7 ± 2.16 kg) for the digestibility and nitrogen balance trail. The control group (CTRL) received only the basal diet (berseem, *Trifolum alexandrinum*, hay plus concentrate mixture), the second group received the basal diet plus 0.225 g of Tonilisat /head/day while, the third group received the basal diet plus 0.90 g of Grow/head/day. Feed (offered and refusal) and faeces samples were then composited for seven days period and analyzed for contents of DM (AOAC, 2000, Method # 925.40), ash (AOAC, 2000; method 923.03), N and fiber. Neutral detergent fiber was assayed utilizing a heat stable amylase according to Van Soest *et al*. (1991) and with sodium sulfite, and it is expressed including the residual ash. Composed urine samples were analyzed for N content. Means were compared by Tukey test

Results The results at Table 1 showed that inclusion of Grow or Tonilisat yeast increased (P<0.05) milk yield by 7.7 or 13.8%, respectively compared to the untreated group, while the inclusion of the two types of yeasts had no significant effects on dry matter intake or milk composition in dairy cows. The results of digestibility trail and nitrogen balance (Table 2) indicated that Grow yeast supplementation increased (P<0.05) the dry matter intake (DMI), while Tonilisat yeast had no significant effect on DMI compared to the control group. Digestion coefficients of crude protein (CP) and neutral detergent fiber (NDF) were improved (P<0.05) by inclusion of Tonilisat yeast but the yeast grow did not effect on nutrients digestion coefficients compared to untreated group. Moreover, the nitrogen balance (NB) was enhanced (P<0.05) when Tonilisat or Grow yeast was supplemented compared to untreated group.

Table 1 Effect of yeast supplementation on dry matter intake, milk yield and composition in dairy cows

Items	CNTRL	Tonilisat yeast	Grow yeast
DMI, kg/d	18.77	18.72	19.01
Milk yield, kg/d	24.6^{b}	28.0^{a}	26.5^{ab}
Milk composition, %			
Total solid	14.4	13.20	13.90
Fat	3.84	3.64	3.66
CP	3.45	3.32	3.42
SNF	9.07	8.90	9.07

a, b means with different superscripts, within column, are different (Tukey test; P<0.05).

Table 2 Effect of yeast supplementation on nutrients utilization in Barki sheep

Items	CNTRL	Tonilisat yeast	Grow yeast
DMI, g/d	889^{b}	910^{ab}	934^{a}
Digestion coefficients			
OM	75.3	75.6	73.4
CP	77.5^{b}	79.5^{a}	75.8^{b}
NDF	74.4^{b}	76.1^{a}	73.6^{b}
ADF	63.4	63.8	61.0
N balance, g/d			
N Intake	18.9^{b}	19.4^{a}	19.9^{a}
Faeces N	4.9	4.6	5.4
Urinary N	5.9	6.0	5.8
NB	8.1^{b}	8.8^{a}	8.7^{a}

Conclusions It is concluded that yeasts supplementation improved significantly both milk yield in dairy cows and nutrients utilization in ruminants.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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The relationship of temperature-humidity index with milk components and energy output of dairy cows

U Serbester[1], E Yazan[2], Z Sahan[3], M Gorgulu[3], L Celik[3]
[1]Nigde University, Bor Vocational School, Nigde, Turkey, [2]Cukurova Agriculture Research Institute, Adana, Turkey, [3]Cukurova University, Agriculture Faculty, Department of Animal Science, Adana, Turkey
Email: ugurserbester@gmail.com

Introduction Temperature-humidity index (THI) incorporates the effects of both ambient temperature and relative humidity in an index. Heat stress occurs when the THI exceeds 72, which is when the cow's body is unable to cool itself adequately. Relationship among heat stress and milk production have been well documented (Ominkski *et al.* 2002; West *et al*. 2003). On the otherhand, limited number of studies have focused on the effect of heat stress on milk components. Therefore, the objective of the present study was to determine the relationship of THI with milk fat, protein, lactose, urea nitrogen(MUN), citric acid, and milk energy output of dairy cows.

Materials and methods Twenty-six Holstein-Friesian cows (14 primiparous and 12 second lactation), averaging 37.5±6.2 days in milk and 26.0±2.97 kg/d, were observed for a total period of 17 weeks between May and September 2008. All cows were housed in a free-stall barn and fed ad libutum the same total mixed ration (TMR) which consisted of corn silage, alfalfa hay, wheat straw, and home blend concentrate. The calculated composition of TMR was 638 g of DM/kg, 7.03 MJ of NE/kg of DM, 180 g of CP/kg of DM. The cows were milked twice daily at 0500 and 1700, individual milk yields were recorded. Individual milk samples were collected at morning milkings weekly. Milk fat, protein, lactose, MUN, citric acid contents were determined by infrared analysis (Milkoscan FT 120, Foss Electric, Hillerød, Denmark). Milk energy output was calculated as: Milk energy output (Mcal/d) = 2.2046 × (milk yield, kg/d) × {[31.63 × (milk fat, %)] + [24.13 × (milk protein, %)] + [21.6 × (milk lactose, %)] − 11.72} / 1000 (Khorasani *et al*. 2001). Meteorological data were recorded hourly by weather stations (Vantage Pro2, Davis Instruments, USA) near the free stall barn. The temperature-humidity index (THI) was calculated as described by Bianca (1962). The effect of parity on milk yield was determined not significant and therefore was removed from the statistical model. The relationship between THI and milk components were estimated by Pearson correlations. Finally, linear regression coefficients of THI on the variables were derived. All analyses were conducted using SAS statistics program.

Results Average temperature, relative humudity, and THI were 26.8°C, 68.1%, 76.9 respectively during the study. Milk fat and protein were positive but weakly (r= 0.240 and r= 0.164, P< 0.001) related to THI (Table 1). Lactose and citric acid were inversely (r= -0.210 and r= -0.294, P< 0.001) related to THI. Also, milk energy output was inversely (r= -0.601, P< 0.001) but more stronger related to THI. No association between MUN and THI was observed (P> 0.05). Linear regression analysis revealed that 35% of the variation in the milk energy output was accounted for by THI (Table 2).

Table 1 Pearson correlation coefficients for relationships between THI and milk components and milk energy output

Milk Components	THI
Fat	0.240***
Protein	0.164***
Lactose	-0.210***
Urea-nitrogen	-0.07
Citric acid	-0.294***
Milk energy output	-0.601***

*** P< 0.001

Table 2 The linear regression coefficients of THI on milk components and milk energy output

Milk Components	R^2
Fat	4%
Protein	1.8%
Lactose	3.6%
Urea-nitrogen	2.2%
Citric acid	13.4%
Milk energy output	34.9%

Conclusions The results of the current study indicated that milk energy output was found to be the parameter most closely related to THI. Therefore, it can be suggested that the milk energy output is suitable measurement to observe effects of heat stress.

Acknowledgements The authors are grateful to farm and technical staff for data collection.

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Analysis of total methanogens in rumen fluid using quantitative real-time PCR and gas chromatography/mass spectrometry

C McCartney[1,2], I Bull[2], R J Dewhurst[1]
[1]Animal & Grassland Research and Innovation Centre, Teagasc, Dunsany, Co. Meath, Ireland, [2]School of Chemistry, Univeristy of Bristol, Bristol, UK
Email: christine.mccartney@teagasc.ie

Introduction Archaeol is a lipid that is ubiquitous in the membranes of Archaea, including methanogens. The use of archaeol as a proxy for methanogenesis has already been investigated by Gill *et al*. (2011), however there were concerns over the reliability of the methane measurements with which it was compared. An alternative way to evaluate archaeol as a methanogen marker is to make comparisons with estimates of methanogen numbers using quantitative real-time PCR (qPCR).

Materials and methods Twelve rumen fluid samples from 4 ruminally cannulated Rotbunde-Holstein steers on divergent diets with increasing concentrate content were selected from a previous study (McGeough *et al*. 2010). Genomic DNA was isolated from the ruminal fluid using a repeated bead beating method and a qPCR SYBR Green assay was used to quantify methanogens. Two methanogen specific PCR primers targeting DNA sequences of methyl coenzyme M reductase (*mcrA*; Denman *et al*. 2007) and methanogen specific 16S rRNA (qMet; Hook *et al*. 2009) were used, along with a prokaryote 16S rRNA as a reference gene (Muyzer *et al*. 1993). To estimate the proportion of methanogen within total prokaryotes, the formula $\Delta Ct = 2^{-(Ct\ methanogen - Ct\ reference)}$ was used where Ct represents threshold cycle. ΔCt values were normalised, related to total DNA concentrations (measured using a ND-1000 photospectrometer, NanoDrop Technologies, Wilmington) and expressed per g of original sample DM. For archaeol analysis, the rumen fluid samples were lyophilized. The total lipid extract (TLE) was then obtained using a modified Bligh-Dyer method, and subjected to acid methanolysis. The TLE was then separated into apolar and alcohol fractions, the alcohol fraction trimethylsilylated, dissolved in ethyl acetate, and then analysed by gas chromatography/mass spectrometry. Archaeol was identified, and then quantified by comparison to an internal standard (1,2-di-*O*-hexadecyl-*rac*-glycerol). The relationship between ΔCt values for the methanogen primers, and between archaeol (μg/g) and the ΔCt values expressed on a DM basis were analysed by linear regression analysis.

Results There was a relatively good agreement between estimates of methanogen populations based on the qMet and *mcrA* primers (Figure 1). After removal of an outlier, highlighted in Figure 2, regression analysis showed a significant relationships between archaeol concentration and methanogen populations estimated using the qMet ($P = 0.011$) and *mcrA* primers ($P = 0.073$).

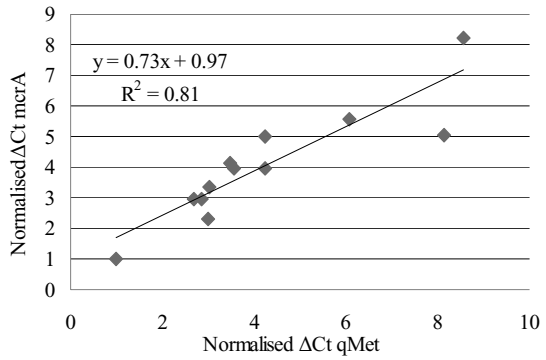

Figure 1 Relationship between estimates of methanogen populations using primers for methanogen specific16S rRNA and *mcrA* genes

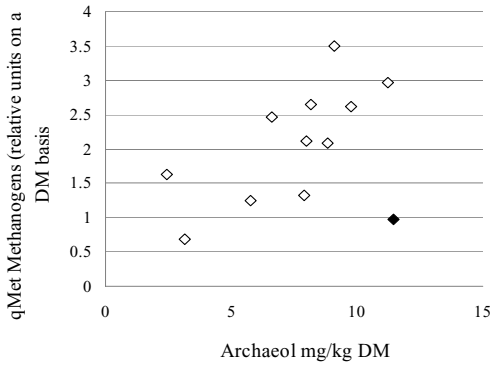

Figure 2 Relationship between methanogens quantified using qMet primers and archaeol in the rumen fluid samples, both expressed on a DM basis

Conclusions Some of the variation in the relationships between archaeol and qPCR estimates of methanogen populations may be due to technical difficulties with the analyses, as well as variation in the archaeol concentration of archaea. However, it is most likely that the weakness of the relationships is due to differences in gene copy number (Hook *et al*. 2009), genome copy number (Hildenbrand *et al*. 2011), or both. The presence of non-methanogenic archaea may lead to additional variation in the case of the *mcrA* gene, since these would contain archaeol, but not the *mcrA* gene.

Acknowledgements Technical assistance from J. Larkin, C. Carberry and M. McCabe, as well as financial support from the Teagasc Walsh Fellowship Scheme is gratefully acknowledged.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effects of season and species on *in sacco* degradability of plant species in the sub-humid subtropical savannah, South Africa

N Basha[1,3], P Scogings[2], I Nsahlai[1]
[1]University of KwaZulu-Natal, Pietermartizburg, KwaZulu-Natal, South Africa, [2]University of Zululand, Empangani, South Africa, [3]University of Khartoum, Khartoum North, Sudan *Email: nasrbashabasha@yahoo.com*

Introduction In general, smallholder farmers in subtropical savannah of Africa keep different ruminant species which survive on natural pastures (Aganga & Tshwenyane, 2003). The productivity of ruminant species depends on quantity and quality, which are affected by seasonal fluctuations (Abusuwar & Ahmed, 2010). The study objective was to determine the effects of season and plant species on *in sacco* degradation characteristics of edible forage of browse species.

Materials and methods Leaves of five plant species were sampled during the dry (DS), early wet (EWS) and late wet (LWS) seasons. The plant species were *Acacia natalitia* (Ana), *Acacia nilotica* (An), *Dichrostachys cinerea* (Dc), *Scutia myrtina* (Sm) and *Chromolaena odorata* (Co). Three rumen-fistulated cows (350 kg LW) were used following the nylon bag technique described by Meherz and Ørskov (1977). Dried samples were ground (2-mm screen) and 3 grams/bag were weighed and incubated for 0, 3, 6, 9, 12, 24, 48 and 72 h. A residual for each incubated bag was analyzed for nitrogen. The DM and N degradation data were fitted to the model of McDonald (1981). The effective degradability (ED) of dry matter (DM) or nitrogen (N) was calculated at a rumen out flow rate (r) of 0.03 h^{-1} using this equation: $ED_{(DM\ or\ N)} = a + b*c/(c+r)$, where, a is the soluble fraction, b is the slowly degraded fraction, c is the degradation rate (h^{-1}). The data were analysed using the general linear model (GLM) procedure of SAS in a 3×5 factorial design with three replicates, statistical significance being declared at P<0.05. Means were compared by least significant difference (LSD).

Results Season (Se) affected (P<0.001) a_{DM}, $(a+b)_{DM}$, ED_{DM}, lag time (lt) of DM, a_N and ED_N. Browse species (Sp) and Se x Sp affected (P<0.001) all DM and N degradability variables, except b_N. Among the three seasons, the a_{DM}, $(a+b)_{DM}$ and ED_{DM} were higher in the DS than in the wet seasons, while a_N and ED_N were higher in the EWS than in the DS and the LWS. *Chromolaena odorata* had best estimated parameters of degradation during the three seasons compared to other browse species. Based on potential and effective degradation, the plant species followed this decreasing order: *C. odorata*, *A. nilotica*, *A. natalitia*, *S. myrtina* and *D. cinerea*.

Table 1 *In sacco* degradation of plant species harvested at different seasons from sub-humid subtropical savannah, South Africa

		Dry matter degradation (g kg^{-1} DM)						Nitrogen degradation (g kg^{-1} DM)					
Se	Sp	a_{DM}	b_{DM}	c_{DM}	$(a+b)_{DM}$	ED_{DM}	lt_{DM}	a_N	b_N	c_N	$(a+b)_N$	ED_N	lt_N
Dry	Ana	309	521	0.0317	829	576	-1.26	268	646	0.0297	914	570	0.53
	An	528	360	0.0655	887	774	0.85	351	522	0.0569	873	692	1.65
	Dc	307	289	0.0257	597	430	-0.38	172	543	0.0141	715	338	0.71
	Sm	292	519	0.0223	812	493	-2.10	336	450	0.0326	786	536	-0.09
	Co ‡	353	484	0.3411	837	796	-0.06	434	481	0.2822	914	868	-0.07
Early wet	Ana	268	451	0.0354	719	511	-0.24	232	545	0.0359	776	526	-0.75
	An	500	357	0.0657	857	745	1.00	432	452	0.0538	885	722	1.69
	Dc	331	227	0.0400	559	458	1.05	276	256	0.0562	532	443	-0.89
	Sm	242	449	0.0203	690	423	-0.57	287	432	0.0262	719	464	-0.62
	Co	329	609	0.2803	938	879	0.06	407	567	0.2652	974	916	0.11
Late wet	Ana	208	391	0.0420	598	434	0.24	242	404	0.0371	646	464	0.30
	An	327	443	0.0882	771	658	-0.26	299	467	0.0771	765	632	0.84
	Dc	222	324	0.0394	546	394	0.54	261	434	0.0372	695	454	-0.76
	Sm	255	399	0.0205	654	409	0.83	279	507	0.0235	786	462	-1.00
	Co	317	632	0.2292	949	876	-0.03	388	581	0.2089	969	896	0.04
	P	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.018	0.001	0.001	0.001	0.122
	LSD	6.863	49.18	0.028	48.43	10.27	0.626	19.330	102.51	0.013	100.09	15.111	1.158

DM = dry matter; N = nitrogen; a = soluble fraction; b = slowly degradable fraction; c = degradation rate of b per hour; ED = effective degradability; lt = lag time; P = probability; LSD = least significant difference.

Conclusion Significant variations in the parameters of in sacco degradability were reported among seasons and among plant species. The results suggest that the studied plant species have potential to be used as feed supplements. *Chromolaena odorata* can be use as best supplementary protein source like high-quality leguminous forages. However, the reluctance of livestock to consume *C. odorata* (personal observation) is not related to its high potential and effective degradation must be for other reasons. Further studies would need to investigate these reasons and establish new methods for promoting intake rate of *C. odorata*.

Acknowledgement This research was funded by the National Research Foundation, South Africa.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

In vitro evaluation of some plant extracts as a methane-inhibiting agents with diets with different degradability using rumen liquor from goats

G Martinez[1], L Abecia[1], A. I Martin-Garcia[1], E Ramos Morales[1], E Molina-Alcaide[1], M J Ranilla[2], D R Yañez-Ruiz [1]
[1]Estacion Experimental d el Zaidin (CSIC), Granada, Spain, [2]IGM-Universidad de Leon, Leon, Spain
Email: david.yanez@eez.csic.es

Introduction A large number of plant secondary compounds have been identified to have potential to inhibit methane production in the rumen; although the influence of the diet is not clearly understood (Hart *et al*., 2008). Furthermore, goats have been shown to exhibit a different nutritive response than other ruminants to diets containing antimicrobial compounds which has been suggested to be, in part, due to a distinctive rumen microbial population (Kamra, 2005). The aim of the present experiment was to evaluate the effect of two doses of different antimicrobial compounds on methane emissions and rumen fermentation of diets including concentrates with different ruminal degradability using batch cultures and inoculum from goats' rumen.

Material and methods Two doses (Table 1) of propyl propane thiosulfinate (PTS), propyl propane thiosulfonate (PTSO), diallil disulfide (DDS), carvacrol (CA), cinamaldehide (CI) and, bromochloromethane (BCM) were added to 1 g of the experimental diets and incubated for 72 h in bottles with 120 ml of goats rumen inoculum. The experimental diets were a 50:50 alfalfa hay:concentrate mix, in which the concentrate was based on faba beans-barley or corn-sunflower meal with rapid (R) or slow (S) rumen degradation, respectively. The rumen content for preparing the fermentation inoculum was obtained before the morning feeding from three rumen cannulated goats fed oats and alfalfa hay (40:60). The BCM compound was used as positive control with known anti-methanogenic affect as reported by Tomkins *et al*. (2008). Three equal incubation runs were carried out, with 2 bottles per treatment and run. In each run a control with diet and no additive was included. Total gas volume in the bottle headspace was measured at 2, 4, 6, 8, 12, 24, 36, 48 and 72 hours after inoculation following the procedure described by Theodorou *et al*. (1994). After 24 hours of incubation a sample of the gas produced was collected in a graduated syringe and transferred to a 5 ml vacuum tube and kept at room temperature before methane concentration was measured by gas chromatography. Data were analyzed by randomised block analysis of variance with the measure of each two bottles as blocks using SPSS 19.0®.

Results The addition of both doses of BCM decreased (P< 0.001) methane production by 95% compared to the control (Table 1), while the asymptotic gas produced by diet S fermentation was only reduced by 20% (P< 0.001). The higher dose of CA reduced methane production while both doses of CI had any effect (P>0.05). However, 320 mg/L of both essential oils reduced A value for diet S wile only CA affect that value for diet R. Among the 3 garlic derived compounds, only PTS and DDS exhibited methane reduction activity (up to 90% and 60 %, respectively). However, only DDS significantly (P<0.001) affected the asymptotic gas production for both diets and doses used.

Table 1 Effect of additives on *in vitro* methane produced (CH_4, ml) after 24 h of incubation and on asymptotic gas production (A, ml).

CH_4 ml														
		Control	CA		CI		PTS		PTSO		DDS		BCM	
Diet	Dose, mg/L	0	160	320	160	320	160	320	40	160	80	320	10	20
R	Mean	3.74	3.31	1.85***	3.47	3.17	2.90	0.27***	3.27	3.12	1.13***	0.81***	0.10***	0.10***
S	Mean	3.17	3.07	1.62***	3.30	2.99	2.78	0.26***	2.95	2.99	1.19***	0.86***	0.13***	0.09***
	SEM		0.144		0.128		0.116		0.133		0.121		0.115	
A, ml														
R	Mean	118.8	108.2	80.7***	120.4	116.1	103.7	85	117.2	112.5	98.9***	82***	114.4	117.9
S	Mean	102.7	92.4***	64.6***	106	99.3*	92.3	88.5	105.9	98.3*	88.3***	70.4***	91.9*	95.8*
	SEM		1.14		0.82		4.54		0.80		0.88		0.85	

**P < 0.05; **P < 0.01; ***P < 0.001: significance of the differences of each dose compared to the control.*

Conclusions Results of this experiment suggest that some organosulphurous compounds at dose between 80 to 320 mg/L have the potential to reduce methane emissions without compromising the digestibility of the diet in goats and that the effect is diet-dependent.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Locally produced protein feeds for dairy bull calves

B Johansson, T Fernqvist, A Hessle, E Nadeau, A Arnesson, K-I Kumm
Swedish university of agricultural sciences, P.O. Box 234, S-532 23 Skara, Sweden
Email: birgitta.johansson@slu.se

Introduction Some cases have shown that feeds grown on the own farm contributes less to environmental problems than far-distant grown feeds. Soya bean meal is widely used in the world as a protein feed of good nutritional quality but the ethics around the cultivation of the beans are often questioned. Calves need high concentrations of protein with high protein quality in their feed for proper growth. The purpose of this study was to compare dry matter intake (DMI), live weight gain (LWG), feed efficiency (FE), rumen function and profitability in calves fed protein feeds produced in Sweden vs. soya bean meal.

Materials and methods The experiment was carried out at Götala Beef and Sheep Research Station, Swedish University of Agricultural Sciences (SLU), Skara. Dairy bull calves (84 Swedish Red and Swedish Holstein per year) were used in a completely randomized design. The protein feeds studied were rolled peas (PE) and rolled field beans (FB) in year 1, Swedish grown rolled soya beans (SSB) and dried distiller's grains with solubles (DDGS) in year 2, which were compared to imported soya bean meal (SBM) both years. The DMI, FE and faecal traits were recorded at a pen level (four pens, each with seven calves, per treatment) while LWG was recorded on the individual calves. The calves were weighed regularly and averaged 90 and 93 kg in live weight at the start of year 1 and 2, respectively, and ended at 245 and 271 kg. A total mixed ration consisting of a grass/clover silage (90/10), rolled barley and vitaminised minerals, together with either PE, FB, SSB, DDGS or SBM, was fed to the calves. Feed was offered *ad libitum* once a day. The diets were balanced to be isonitrogenic and isoenergetic. Diets were rebalanced four times according to changed nutrient requirements during growth. Cold-pressed rapeseed cake (322 g CP per kg DM) was included in the diet of calves given PE, FB, DDGS and SSB until the calves reached an average live weight of 175 kg. Nutrient composition in DM of the silage (154 g CP) and concentrates (PE 211, FB 281, DDGS 349, SSB 400, SBM 528 g CP, respectively) were analysed by conventional methods. All DDGS came from the same batch and contained 21% ADIN of total N. One fresh faecal sample was collected from each pen four times during each year. The faeces consistency was determined visually on a scale, DM and content of grains and long particles (> 10 mm) in the faecal matter were analysed. Analyses of DMI and FE were done with PROC GLM, SAS (ver. 9.1, 2003), whereas PROC MIXED, SAS, was used for analyses of faecal traits and LWG. The profitability was calculated as value of calf growth less cost of feeds consumed at 2010 price level in Southern Sweden. In sensitivity analyses different prices were used.

Results In year 1, no differences in daily DMI or LWG were found between calves with the different diets (Table 1). There was, however, a tendency for a higher intake of ME for the calves fed PE than for the other calves, but the FE did not differ between treatments. In year 2, feeding DDGS resulted in the highest LWG due to higher intakes of ME, CP and also to a strong tendency for higher DM intake than calves fed SBM, but FE was similar in all treatments (Table 1). The faecal traits differed little or not at all between the treatments except for the last sampling occasion in year 2 where a firmer consistency, a higher DM content and a higher number of grains were found in faeces from calves fed DDGS and SSB compared to calves fed SBM. Most profitable in the basic price situation was PE year 1 and DDGS year 2. However, relatively small increases in cost of PE production or decreases in SBM-price would make SBM most profitable year 1. DDGS was most profitable year 2 at any probable price situation.

Table 1 Average daily intake of dry matter (DM), neutral detergent fibre (NDF), metabolizable energy (ME) and crude protein (CP), average daily live weight gain (LWG) and feed efficiency (FE) of bull calves, means and standard error of the means (SEM)

	Year 1					Year 2				
	PE	FB	SBM1	SEM	P [1]	DDGS	SSB	SBM2	SEM	P [1]
Intake of DM (kg day-1)	4.66	4.38	4.41	0.091	NS	4.81	4.50	4.45	0.098	0.058
Intake of NDF (kg day^{-1})	1.65	1.62	1.64	0.033	NS	1.78^{a}	1.57^{b}	1.66^{b}	0.035	**
Intake of NDF (% of LW)	1.00	1.05	1.01	0.022	NS	1.01^{a}	0.91^{b}	1.00^{a}	0.013	***
Intake of ME (MJ day^{-1})	57.1	53.2	53.3	1.11	0.054	60.9^{a}	57.7^{ab}	55.5^{b}	1.24	*
Intake of CP (g day^{-1})	760	710	741	17.3	NS	811^{a}	750^{b}	725^{b}	16.8	*
LWG (kg day^{1})	1.16	1.08	1.12	0.029	NS	1.34^{a}	1.25^{b}	1.21^{b}	0.027	**
FE (g gain MJ^{-1} ME)	20.1	20.2	21.0	0.27	NS	22.2	21.7	22.0	0.21	NS

Means with different superscripts (a, b) differ significantly ($P < 0.05$).

Conclusion Swedish grown protein feeds can replace imported SBM with maintained or, regarding DDGS, even improved performance in dairy calves. All diets resulted in good rumen function and diets with PE and DDGS resulted in the best profitability. The high ADIN concentration of DDGS should decrease the availability of important amino acids needed for optimizing LWGs of the calves fed the DDGS diet. However, this was apparently not the case in this study.

Acknowledgements Funding from Swedish Farmers' Foundation for Agricultural Research, SLNFBS and SLU is greatly appreciated, as well as Agroetanol for supply of DDGS.

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Relationship between intake and excretion of nitrogen in dairy cows

C C Jobim, J S Souza, A F Branco, J C Barreto, M P Osmari, S Teixeira
Universidade Estadual de Maringa, Maringa, Brazil
Email: mileneosmari@yahoo.com.br

Introduction In farms with intensive systems of milk production is common an excessive supply of crude protein (CP) and high nitrogen excretion. Reducing N loss from the farm must begin with proper animal feeding and management to reduce N excretion. Even with good management though, large quantities of N are in the manure (Rotz, 2004). Many experiments have shown that diets with 16% CP, and balanced for rumen degradable protein (RDP) and rumen undegradable protein (RUP), may be sufficient to maintain high production in dairy cows. According to Butler *et al*. (1996), an overfeeding of CP increases N excretion in feces and urine, contributes to pollution and reduces the animal's performance in relation to fertility. Thus, the objective was to evaluate the nitrogen balance and the association between N intake and N excretion in dairy cows.

Material and methods Were used 16 lactating Holstein cows over 60 days in milk, in a farm located in Castro, Paraná State, Brazil. The experiment lasted 21 days and diet was balanced to 16% CP, 1.55 Mcal of NE_L/kg dry matter (DM), and cows yielded on average 31 kg of milk/day. Dry matter intake (DMI, kg/day) was calculated by the amount of feed (kg of DM/day) provided to the lot of cows, discounting orts (kg of DM/day), and divided by the number of cows (16). Milk samples were collected at the time of milking and were preserved using Bronopol (2-bromo-2-nitropropane-1,3-diol) under refrigeration (4ºC) until analyzes. Faecal samples were collected directly from the rectum. The faecal dry matter output (kg) was estimated from the observed DMI (kg) multiplied by the percentage of dry matter indigestible obtained from *in vitro* dry matter digestibility. Were collected two spot urine samples of each cow, on days 20 and 21 for estimation of daily urine production using urine creatinine concentration. Nitrogen intake (g/cow/day) was calculated using DMI (g/day) and % of N in the diet. The excretion of N in milk was calculated from the values of milk yield (kg/day) and milk protein (%). Faecal N (g/cow/day) was determined by faecal DM output (g/day) and faecal N (%) for each cow. Urine N (g/cow/day) was determined by urine production (L/day) and urine N (g/L) for each cow. Were collected (observed) data for N intake, N excreted in faeces and milk N for each cow. Linear regression was performed to describe relationship between total nitrogen excretion and nitrogen intake.

Results The daily intake of nitrogen was 502 g/day on average. Nitrogen excretion was 28.4% in feces (142.5 g/day), 32.3% in urine (164.6 g/day) and 30.7% in milk (149 g/day) (Table 1). Nitrogen intake did not influence (P>0.05) N excretion (Figure 1).

Table 1 Nitrogen balance

Item	Mean	SD	Minimum	Maximum
N intake (g/cow/day)	502.7	30.1	439.4	555.6
N excreted (g/cow/day)				
Feces	142.5	9.5	122.1	163.8
Urine	164.6	61.6	58.4	283.2
Milk	149.0	20.2	111.6	192.2
N excreted (% of intake)				
Feces	28.4	1.0	26.6	29.9
Urine	32.3	11.5	12.1	51.0
Milk	30.7	4.7	21.8	40.7

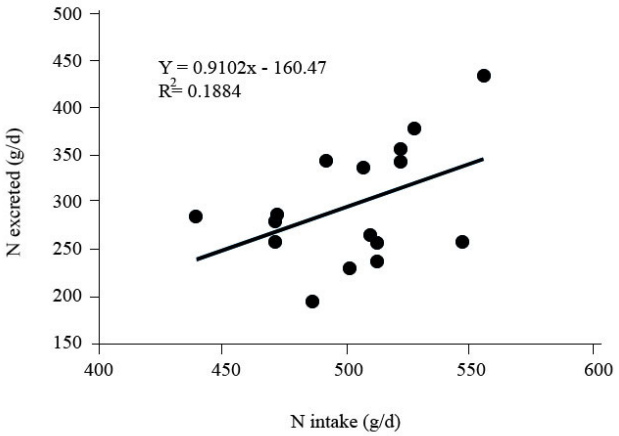

Figure 1 Relationship between N intake and N excreted

Conclusion Relationship between nitrogen intake and nitrogen excretion was low.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Milk yield and efficiency of nitrogen utilization in dairy cows

A F Branco, J S Souza, J C Barreto, M P Osmari, S Teixeira, G T Santos, B N Marsíglio
Universidade Estadual de Maringa, Maringa, Brazil
Email: mileneosmari@yahoo.com.br

Introduction In farms with intensive systems of milk production is common an excessive supply of crude protein (CP) and high nitrogen excretion. Many experiments have shown that diets with 16% CP, and balanced for rumen degradable protein (RDP) and rumen undegradable protein (RUP), may be sufficient to maintain high production in dairy cows (Dinn *et al*., 1998; Broderick, 2003). Thus, the objective was to evaluate the relationship between efficiency of nitrogen utilization and milk yield in dairy cows.

Material and methods Were used 31 lactating Holstein cows (15 on experiment 1 and 16 on experiment 2) over 60 days in milk, in a farm located in Castro, Paraná State, Brazil. The experiments (1 and 2) lasted 21 days and diet was balanced to 16% CP, 1.55 Mcal of NE_L/kg dry matter (DM), and cows yielded on average 31 kg of milk/day. Dry matter intake (DMI, kg/day) was calculated by the amount of feed (kg of DM/day) provided to the lot of cows, discounting orts (kg of DM/day), and divided by the number of cows (15). Milk samples were collected at the time of milking and were preserved using Bronopol (2-bromo-2-nitropropane-1,3-diol) under refrigeration (4ºC) until analyzes. Nitrogen intake (g/cow/day) was calculated using DMI (g/day) and % of N in the diet. The excretion of N in milk was calculated from the values of milk yield (kg/day) and milk protein (%). Data for milk yield, N intake (NI) and N excreted in milk (NEM) were collected for each cow during 21 days. The efficiency of N utilization (ENU, %) was obtained by ratio between nitrogen intake (NI) and nitrogen excreted in milk (NEM). Data were analyzed by ANOVA and a linear regression was performed to describe relationship between efficiency of N utilization (%, Y axis) and milk yield (kg/day, X axis).

Results Milk yield influenced efficiency of nitrogen use ($P<0.01$). There was a positive linear response between milk yield and efficiency of nitrogen use (Figure 1). Results show that milk production of the cows is an important factor to reduce nitrogen excreted in relation to nitrogen intake, and decrease environmental impact.

Table 1 Nitrogen intake, nitrogen excreted in milk and efficiency of N utilization

Item	Mean	SD	Minimum	Maximum
	Experiment 1			
N intake (g/cow/day)	502.7	30.1	439.4	555.6
N excreted on milk (g/cow/day)	149.0	20.2	111.6	192.2
N excreted on milk (% intake)	32.3	4.7	21.8	40.7
	Experiment 2			
N intake (g/cow/day)	585.4	32.1	500.2	628.5
N excreted on milk (g/cow/day)	172.4	25.8	141.7	222.2
N excreted on milk (% intake)	28.5	4.0	22.2	36.5

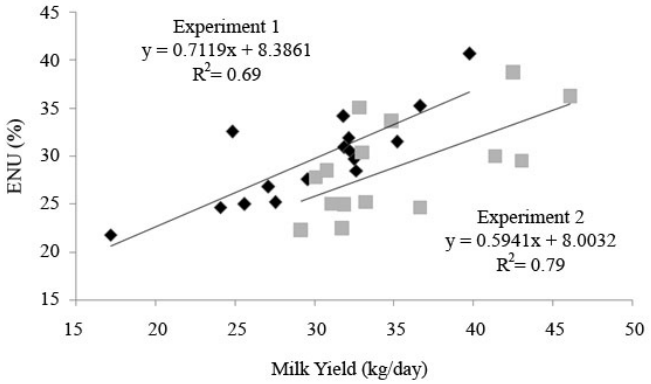

Figure 1 Relationship between milk yield and efficiency of nitrogen use

Conclusions There is a high relationship between milk production of the cow and efficiency of nitrogen use.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Relationships between milk fatty acids and methane emissions in dairy cows fed on diets which altered methane outputs

M A S Gama[1,2], D Li[1], J H Hernandez-Medrano[1], N Saunders[1], J Craigon[1], P C Garnsworthy[1]
[1]The University of Nottingham, School of Biosciences, Loughborough, UK, [2]Embrapa Dairy Cattle, Juiz de Fora, MG, Brazil
Email: phil.garnsworthy@nottingham.ac.uk

Introduction Methane emissions by dairy cows are affected by diet composition, particularly proportions and sources of forage and concentrates, and oil supplementation. There is limited evidence that milk fatty acids vary with *in vivo* methane output in dairy cows fed on diets containing different forms of lipid (Chilliard *et al*., 2009). Milk fatty acid profile might therefore provide a proxy for methane emission on farms, but a broader range of dietary conditions needs to be tested. The aim of this study was to evaluate associations between milk fatty acids and methane emissions in dairy cows fed on diets which altered methane outputs through changing forage and concentrate composition.

Materials and methods Forty-two Holstein dairy cows were used in a cross-over design with 14-d treatment periods. Each cow received both of the following dietary treatments *ad libitum*: 1) Control diet (C): a commercial TMR (31.9 maize, 13.2 grass and 12.6 whole-crop silages; 5.0 straw, 9.6 beet pulp, 13.2 rapeseed meal, 8.4 soyabean meal, 2.3 fat, 3.7 minerals; g/100g of diet DM), and 2) High methane diet (HMD): an experimental TMR (31.6 grass, 19.1 Maize and 7.8 whole-crop silages; 3.0 straw, 11.3 beet pulp, 10.7 peas, 7.8 rapeseed meal, 5.0 soyabean meal, 1.4 fat, 2.3 minerals; g/100g of diet DM) designed to increase methane output through its higher grass silage content and inclusion of peas. A dairy concentrate was also fed during milking (1.6 kg/d plus 0.16 kg/kg milk yield above 23 kg/d). Methane concentrations in exhaled air were measured at each milking and eructation data (peak area and frequency) were used to calculate individual daily means for methane emission rate during milking (MERm), as described by Garnsworthy *et al*. (2011). Milk samples were collected on the last day of each experimental period and analysed for fatty acid profile by gas chromatography (GC). Treatment effects on milk fatty acid profile were analysed by ANOVA using diet, period and diet vs. period interaction as sources of variation, with individual cows as blocks. Additionally, correlation analyses were performed between milk fatty acid concentrations and MERm expressed as mg/min (MERm) or mg/min per kg dry matter intake (MERmDM).

Results The effect of dietary treatments on methane emissions was presented by Garnsworthy *et al*. (2011) and showed that when cows were fed on treatment HMD significantly ($P<0.05$) higher values were obtained for MERm (+5%, $P=0.042$) and MERmDM (+20%, $P<0.001$). A total of 58 milk fatty acids were identified, and 40 of them differed significantly ($P<0.05$) between C and HMD. Overall, short and medium chain fatty acids (≤16C), including the odd-numbered (straight and branched) fatty acids produced by rumen microorganisms, were higher ($P<0.05$) in milk fat of cows fed HMD. On the other hand, most long chain fatty acids, including C18:0, C18:1 cis-9, trans-C18:1 isomers (trans-4, trans-5, trans-6 to 8, trans-9, trans-10, trans-11, trans-12, trans-13 to 14 and trans-16), minor cis-C18:1 isomers (cis-11 and cis-12), non-conjugated C18:2 (except C18:2 trans-11, cis-15), linoleic acid and cis-9, trans-11 CLA, were lower ($P<0.05$) with HMD. By contrast, concentrations of α-linolenic, C18:2 trans-11, cis-15, C22:0, C20:4, C20:5 and C22:0 were higher ($P<0.05$) in milk fat of cows fed HMD. Correlation analysis between individual milk fatty acids and methane output revealed positive ($P<0.05$) associations with MERm for C11:0 ($r=0.225$), C12:0 ($r=0.246$), C13:0 ($r=0.269$), C14:0 ($r=0.258$) and C15:0 ($r=0.223$), and with MERmDM for C11:0 ($r=0.228$), C12:0 ($r=0.226$), C13:0 ($r=0.269$), C14:0 ($r=0.222$) and C15:0 ($r=0.330$). Positive correlations were also observed between MERm and C10:0 ($r=0.232$, $P=0.038$) and C20:5 ($r=0.308$, $P=0.005$) as well as between MERmDM and C16:0 ($r=0.220$, $P=0.049$), C21:0 ($r=0.249$, $P=0.025$) and C22:0 ($r=0.236$, $P=0.034$). Although concentrations of odd-numbered chain fatty acids (grouped as: straight-chain C5 to C17; iso; and anteiso) were significantly increased ($P<0.01$) in milk fat of cows fed HMD, only odd straight-chain C5 to C17 was significantly correlated with MERm ($r=0.225$, $P=0.043$) and MERmDM ($r=0.319$, $P=0.004$). Of the long chain fatty acids which were reduced by HMD, none was correlated with MERm, and MERmDM was correlated only with linoleic acid ($r= -0.247$, $P=0.026$) and cis-11 C18:1 ($r= -0.257$, $P=0.021$). A few milk fatty acids that were unidentified due to the lack of appropriate standards were significantly correlated with one or both methane measurements. Because these fatty acids could also be potential indicators of *in vivo* methane emissions, GC-MS will be used to identify them.

Conclusion The results show that concentrations of certain fatty acids in milk fat were significantly correlated with methane emissions in dairy cows fed on diets which altered methane output. Based on correlation analysis, the even-numbered medium chain fatty acids (predominantly synthesized from rumen acetate) and the odd-numbered straight-chain fatty acids (mostly synthesized by rumen bacteria) appear to be the best potential predictors of methane output.

Acknowledgements The authors thank: Defra for funding the methane study; Embrapa for supporting M. Gama; Morag Hunter for technical assistance.

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Conversion of dietary N into milk N may be enhanced by the energy source in isoenergetic diets

G Cantalapiedra-Hijar[1], J-L Peyraud[2], S Lemosquet[2], P Noziere [1], I Ortigues-Marty[1]
[1]INRA, UH1213 Herbivores. Centre de recherches de Clermont-Ferrand-Theix, F-63122 Saint Genès Champanelle, France,
[2]INRA, UMR1080 Production du Lait, F-35590 Saint-Gilles, France
Email: gonzalo.cantalapiedra@clermont.inra.fr

Introduction Meta-analysis studies (Castillo *et al*., 2001) have shown that the increase of N input in dairy rations promotes, in general terms, an increase in milk protein yield. However, it has been also shown that diets with greater protein concentration result in lower milk protein conversion efficiency (Huhtanen and Hristov, 2009) and thus in higher urinary N output to the environment. Although the N input has been identified as the principal driver of N excretion other covariates may also play a role, like the energy content or even the energy type (Kebreab *et al*., 2001). The aim of this study was to determine the effect of the energy source (starch vs. fibre) on milk N conversion efficiency when animals were fed iso-energetic diets with either low or high N level.

Material and methods Four multiparous Jersey cows in late lactation, averaging 354 ± 23 kg of BW and 211 ± 13 DIM at the onset of the experiment, were used in a 4 x 4 Latin square design experiment with a 2 x 2 factorial arrangement of dietary treatments. Treatments were two energy sources (rich in starch [S] or fibre [F]) combined with two N levels (low and high). The four diets were formulated to be isoenergetic and with a 50:50 forage to concentrate ratio. Concentrates were based on either cereals (corn, oat, barley and wheat) or highly degradable rich NDF by-products (dehydrated beet pulp, citrus pulp and soybean hulls) with or without a rumen protected soybean meal. The forage part was composed by different proportion of grass silage, grass hay, dehydrated whole corn plant and molassed straw cubes. The starch and NDF content was 350 and 320 g/kg of DM for S diets and 50 and 490 g/kg of DM for F diets, respectively. The high N level (H: 16.5% CP) was formulated to meet 120% of MP cow requirements expressed in the French protein digestible in the intestine (PDI) system (INRA, 1989) with an adequate supply in ruminal degradable N, whereas the low N level (L: 12.0% CP) covered 90% of PDI requirements with a slight shortage in ruminal degradable N. Diet was distributed eight times daily in equals amounts at fixed and limited quantities throughout the study according to the expected milk yield and composition at the middle of the experiment. Each experimental period lasted 21d and consisted of 15d of adaptation to the diet and 6d of measurements. The digestibility was determined by total faeces collection. The N content of milk, feed, faeces and refusals was assessed by the Kjeldahl method. Statistical analysis was performed using GLM procedure of SAS with N level (N), energy source (E), interaction N x E, as well as animal and period as main effects.

Table 1 Milk yield and N balance in dairy cows receiving diets rich either in starch or fibre at low or high N level

	L		H		SEM	Statistical analysis		
	S	F	S	F		E	N	E x N
Dry matter intake (kg/d)	13.9	13.1	14.4	13.7	0.25	**	ns	ns
N intake (g/d)	268	234	362	370	7.2	***	ns	**
Apparent digestible OM intake (kg/d)	9.37	9.15	10.0	9.35	0.27	ns	ns	ns
Apparent digestible N intake (kg/d)	160	139	257	265	6.8	ns	***	ns
Milk yield (kg/d)	14.1	13.0	16.2	14.9	0.32	**	***	ns
Milk N yield (g/d)	86	71	104	90	2.3	***	***	ns
Milk N/ N intake	0.320	0.305	0.286	0.242	0.013	*	**	ns
Milk N/Apparent digestible N intake	0.534	0.517	0.403	0.356	0.024	*	***	ns

N: effect of N level; E: effect of energy source; ns: non significant; *: $P \leq 0.1$; **: $P < 0.05$; ***: $P < 0.01$

Results Although DMI was around 6% higher ($P < 0.05$) with S than F diets (Table 1), the apparent digestible OM intake was similar ($P > 0.05$) among dietary treatments compatible with a similar net energy intake as defined by the experimental design. Both N intake and apparent digestible N intake were similar ($P > 0.05$) regardless the energy source, although a significative ($P < 0.05$) N x E interaction was found for the former. The S diets promoted greater milk ($P < 0.05$) and milk N ($P < 0.01$) yield as well as a tendency for the efficiency of conversion of the dietary N and the apparent digestible N intake into milk to be higher ($P < 0.10$) than F diets. As expected the milk and milk N yield increased ($P < 0.01$) as the dietary CP content increased from 12.0% to 16.5%. Conversely, the efficiency of N transfer into milk decreased as the N intake ($P < 0.05$) and the apparent digestible N intake ($P < 0.01$) increased.

Conclusions The efficiency of conversion of the dietary N into milk N decreased as the CP content of dairy diets increases from 12.0% to 16.5%. However, at similar apparent digestible OM and N intake the dietary energy source may affect this efficiency.

Acknowledgment This study was granted by the Commission of the European Communities; project FP7-KBBE-2007-1 "Rednex"

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On-farm dietary nitrogen use efficiency of lactating dairy cows in North-West of Portugal

P Magalhães[1], M R G Maia[2], A C Gomes[3], A J M Fonseca[2], A R J Cabrita[1]
[1]REQUIMTE, Departamento de Geociências, Ambiente e Ordenamento do Território, Faculdade de Ciências, Universidade do Porto, Campus Agrário de Vairão, Rua Padre Armando Quintas, 4485-661 Vairão VC, Portugal, [2]REQUIMTE, ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Campus Agrário de Vairão, Rua Padre Armando Quintas, 4485-661 Vairão VC, Portugal, [3]Cooperativa Agrícola de Vila do Conde, CRL, Rua da Lapa nº 293, 4480-757 Vila do Conde, Portugal
Email: rita.cabrita@mail.icav.up.pt

Introduction Dairy industry has been pointed out as an important contributor to environmental pollution, the main sources of reactive nitrogen (N) in the environment being fertilizers and manure (van Egmond *et al*., 2002). Manure N is strongly correlated with N intake; around 70% of the N ingested being excreted in urine and faeces. Manipulation of feeding strategy could simultaneously decrease the amount of N excreted in manure and increase the efficiency of N use (Weiss *et al*., 2009), along with economical benefits. The present study aimed to better understand nutritional management practices that affect dietary N use efficiency on commercial dairy farms located in the North-West of Portugal, an important dairy region.

Materials and methods Herd data were collected from 20 commercial dairy farms (with 63 ± 21.9 lactating Holstein dairy cows) located in Vila do Conde, Portugal, during 12 consecutive months (July 2009 – June 2010). Information about milk production and composition, nutritional management, and collection of feed samples for chemical analysis were performed every month in all farms. Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Carry, NC, USA). The model included the fixed effects month, base forage (maize silage, MS; and mixtures of maize and grass silages, MS+GS), and feeding strategy (total mixed single ration, TMR; partly mixed ration – TMR plus concentrate according to milk yield, PMR; and two TMR rations according to milk yield, TTMR), and the interactions between the fixed effects, farm as a random effect, days in milk as a covariate and the random residual error. When the effect of covariate was not significant ($P > 0.15$), it was removed from the model.

Results Month significantly affected dietary crude protein (CP) content. Maize silage-based diets and TTMR presented significantly higher starch content (Table 1). Dry matter intake (DMI) did not differ between treatments. Milk yield, milk protein, and milk urea-N were not affected by month and base forage, but milk yield was significantly higher for TTMR. Efficiency of conversion of feed N into milk N was greater for PMR and TTMR, base forage not having a significant effect.

Table 1 Diet composition and animal performance.

	Feeding strategy			SEM	P-value	Base Forage		SEM	P-value
	TMR	PMR	TTMR			MS	MS+GS		
Diet, %DM									
CP	16.4	16.2	16.5	0.18	0.329	16.5	16.3	0.18	0.286
Soluble CP	4.81	5.50	5.33	0.113	<0.001	5.07	5.36	0.109	0.070
Fat	3.89	3.60	3.95	0.309	<0.001	3.85	3.77	0.057	0.280
Starch	21.7	22.4	23.2	0.37	0.032	23.3	21.5	0.32	<0.001
NDF	39.4	39.5	39.0	0.41	0.721	38.9	39.6	0.32	0.039
DMI, kg/d	19.7	20.5	21.4	0.59	0.222	20.3	20.7	0.40	0.182
Milk									
Yield, kg/d	27.8	28.9	33.5	0.93	0.001	30.2	30.0	0.60	0.458
Protein, %	3.26	3.30	3.28	0.021	0.327	3.27	3.29	0.015	0.153
Urea-N, mg/dl	14.07	13.71	14.81	0.435	0.271	14.27	14.12	0.311	0.627
Milk-N/Feed-N	0.26	0.28	0.28	0.008	0.022	0.27	0.27	0.008	0.894

Conclusions This study showed that commercial dairy farms can significantly increase efficiency of dietary N use and thus decrease their negative environmental impact through nutritional management practices. This also has economical advantages as, in this region, almost all protein sources are imported, not being produced in the farm.

Acknowledgements This work was partially financed by Projectos em Co-Promoção QREN – Sistema de Incentivos à Investigação e Desenvolvimento Tecnológico (SI I&DT); Projecto N.º 5343, LEITE SAUDÁVEL), S. Magalhães being a fellowship of the project. M.R.G. Maia gratefully acknowledges an individual scholarship from FCT, Portugal (SFRH/BDP/70176/2010).

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Quantity and quality of goat milk from a herd grazing on a cultivated pasture with tedera (*Bituminaria bituminosa)* and barley as main constituent.

P Méndez[1], S Álvarez[1], E Morales[2], L Bermejo[2]
[1]Instituto Canario de Investigaciones Agrarias, Tenerife, Spain, [2]Universidad de La Laguna, Tenerife, Spain
Email: pmendez@icia.es

Introduction The current diet of the goat population in the Canary Islands has insufficient fibre; most of the cereal straw for animal feed is imported at high prices and furthermore underestimates the potential of local forage. Moreover abandoned cropland have suffered a considerable increasing in recent years due to various socio-economic circumstances. The purpose of this experiment was to evaluate the possibility of using abandoned farmland in the mediterranean semi-arid climate as grazing areas for dairy goats by establishing an artificial prairie undemanding species with a high degree of resistance such as tedera (Bituminaria bituminosa), a forage legume used traditionally as hay with a nutritive value suitable for feeding dairy goats (Alvarez *et al*., 2007), and barley.

Material and methods Sixteen Canarian goats were divided into two randomized groups based on their lactation number, milk production and kidding number. One group (GD, Grazing Diet) was placed in a pen (15m^2) at the experimental grazing area while the other group (ID, Intensive Diet) remained in their usual place under intensive regimen. The tedera and barley from grazing plot was the fibre source in the GD group while the cereal straw was the fibre supply in ID group. The two diets were supplemented with a cereal and legume mixture for lactating goats and the rations were balanced for energy and protein intake providing the requirements of high yielding dairy goats in mid and late stage of lactation. The grazing area (3000 m^2) was subdivided into 6 subplots of 500 m^2 for rotational grazing. The daily grazing duration was 6-7 h/day on average with a stocking rate between 5.4 and 8 h. The experiment lasted three months from mid-April to mid-July, with a preliminary adaptation period to grazing. Milk yiled was obtained by daily morning milking and the milk control was based on two weekly checks. Milk quality (fat and protein) was measured in a representative sample using a MilkoScan 133b. Descriptive statistics and ANOVA tests for milk yield and quality was carried out with SPSS 15.0.

Results Significative differences (P <0,005) in the average milk yield were found only at the second month for the ID group (1.90 ± 0.06 l/day) versus GD group (1.63 ± 0.14 l/day), but although the differences were not significant during the first month milk yield was slightly higher in the GD group (Table 1). Milk production of Canarian goats can exceed 2l /day but mean values obtained in this experiment for both groups are similar to those reported by other authors for the Canarian goat breeds (Capote et. al, 1999). No differences were detected in protein content; however such levels were slightly higher for the GD group during the first two months. The fat content was more affected by feeding system, showing a slight superiority of the average fat content in the ID group (Figure 1), however these differences do not become significant.

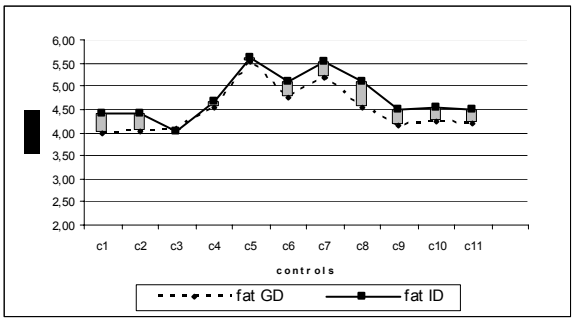

Table 1 Effect of the feeding system on daily milk yield (l/day)

	Feeding system	
	Grazing Diet (GD))	Intensive Diet (ID)
April-May	1,77 ± 0.19 (100%)	1.72 ± 0.14 (103%)
May-June	1.63 ± 0.14[a] (100%)	1.90 ± 0.06[b] (117%)
June-July	1.51 ± 0.12 (100%)	1.63 ± 0.14 (108%)

Figure 1 Fat content (%) on the GD and ID group during the experience

Previous experience has shown that voluntary intake of tedera forage was significant lower in summer than in winter (Ventura *et al*., 2009) probably due to the highest content on the secondary compounds (coumarins) in fruits and seed, that although in amounts not harmful to livestock it could be the cause of rejection in animals accustomed to its use as hay where the characteristic smell of bitumen disappear. This fact could influence a negative effect on milk production and fat content in our experience.

Conclusions: The abandoned farmland could be considered potential areas for livestock grazing. Although this study requires further research, both in livestock and forage management, preliminary results indicate that it is possible to obtain similar values of milk yield and quality from goats under intensive feeding system than those obtained from goats under semi-extensive feeding system with tedera and barley as main pasture component, on areas of mediterranean semiarid type climate.

Acknowledgements: This study was supported by the project RTA2007-0085 with FEDER funds

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Utilisation of ensiled by-products mixture in the feeding of lactating goats

S Alvarez[1], P Mendez[1], M Fresno[1], T Pérez[2]
[1]Instituto Canario de Investigaciones Agrarias, La Laguna, Tenerife, Spain, [2]Canarias Forestal SL Santa Cruz Tenerife, Spain *Email: salvarez@icia.es*

Introduction The goat milk production obtained in the Canary Islands (Spain) in 2008 exceeded 92 million litres, showing an increasing trend in the last decade, where output has increased by 30%. In this region there are three autochthonous dairy breeds: Majorera, Palmera and Tinerfeña. These breeds have a high productive potential, which often is constrained by feed consumption. The cost of raw materials used in animal feeding is one of the most important problems in the sector that has to import over 80% of the food consumed by livestock. An interesting option that can reduce the cost of diets is the use of agro-industrial by-products to produce animal feed. *Giro Ambiental*® silage made mainly from brewer and flour industry waste could be a cheaper alternative of similar quality. Ruminant feeding is considered the external factor most influential, not only in the amount of milk (Alvarez *et al.* 2005), but also in the quality (chemical composition and coagulation properties) and by derivation in the cheese (Alvarez *et al.* 2007a b). The aim of the present experiment was to evaluate the effect of using agroindustrial by-products in goat's diet.

Materials and methods Forty five lactating goats of the Canarian dairy breeds were randomly divided into three groups of 15 animals each. The first group was used as control and given a conventional diet (CD), whereas the second and third group was offered the experimental diets containing increasing amounts of *Giro Ambiental*® silage (GD1 and GD2). The ration for the control consisted of pelleted lucerne, supplemental feed (mixture of cereals and legumes)and festuca hay, whereas that for the experimental groups was pelleted lucerne, supplemental feed, festuca hay and *Giro Ambiental*® silage (GD1: 18% DM and GD2: 36% DM). The amounts of the diets fed, which were made isoenergetic and isonitrogenous, in order to meet requirements for maintenance and production of 3500 g milk/day. Goats were fed twice per day in the morning and evening. Rations were totally consumed with no leftovers. The adaptation period of animals to the experimental diet was 30 days. The experimental period when measurements were taken lasted for 5 months. Milking of the goats was carried out once daily, using a milking machine, in the morning (07:00 h). For determining milk yield, fat, protein, lactose and non-fat-solids of milk, samples were obtained from each goat and every 2 weeks until the end of the experimental period. Milk samples were analyzed for fat, protein, lactose and non-fat solids using a *Milko Scan 133B* apparatus. Milk yield and composition data were analysed by analysis of variance performed with Tukey test using the statistical package SPSS 15.0.

Results No significant differences were detected for milk yield in the controlled period (Table 1). CD goats showed higher daily milk values than those fed experimental silage, except for the first period where the GD2 group was more productive. The average daily milk yield at 60 days was 22% higher in CD group, this difference decreased quickly after 90 days, equaling GD registers as lactation progressed. In 120 and 150 days controls the differences between groups were null. The effect of diet on the protein content was moderate. GD1 and GD2 values were higher than CD values in all the controls, but significant differences appeared only at 60 and 90 days of experiment. This moderating influence of dietary factors on the variation in the percentage of milk protein has been reported by several authors (Letourneau *et al.* 2000, Morand-Fehr *et al.* 2000). Analyzing the fat content, GD1 and GD2 values were higher with significant differences ($P<0.01$) in the controlls at 60, 90 and 150 days (Table 2). After 90 days, milk fat content in the GD2 group was 20% higher than that of the goats fed the conventional diet ($P<0.01$). The present work indicates that the easily digestible fibre in experimental silage, created favourable conditions in the rumen of goats for enhancement of microbial activity, which brings about fibre degradation, with subsequent production of acetic acid which in turn promotes milk fat synthesis.

Table 1 Effect of experimental silage on daily milk yield (l/day)

Controls	Diets		
	CD	GD1	GD2
60	2.32 ± 0.77	1.95 ± 0.56	2.38 ± 0.86
90	2.42 ± 0.65	2.24 ± 0.47	2.26 ± 0.80
120	2.20 ± 0.69	2.20 ± 0.51	2.18 ± 0.83
150	2.14 ± 0.81	2.10 ± 0.54	2.13 ± 0.65

Table 2 Effect of experimental silage on fat content (%)

Controls	Diets		
	CD	GD1	GD2
60	4.56 ± 0.58^{a}	5.19 ± 0.50^{b}	5.02 ± 0.71^{ab}
90	4.21 ± 0.52^{a}	4.82 ± 0.58^{b}	5.02 ± 0.78^{b}
120	4.67±0.51	4.90±0.51	5.15±0.85
150	5.00 ± 0.53^{a}	5.22 ± 0.50^{ab}	5.47 ± 0.40^{b}

Conclusions The results of the present study show that the *Giro Ambiental*® silage used can be fed to goats without any negative effects on the performance of the animals. Results of this study indicate that experimental silage can replace part of the conventional ration of goat, thus lowering the cost of production. Since milk from the Canarian goat breeds is not used for human consumption, but all is destined for cheese production, its high fat and protein content is greatly appreciated by the cheese manufacturing industry.

Acknowledgements This study was supported by the project RTA2008-00108 with FEDER funds and Canarias Forestal contract

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Performance of Nellore steers grazing *Brachiaria brizantha* cv. xaraes supplemented with different lipid sources during the spring

A Valente, R Reis, I Carvalho, T Berchielli, A Ruggieri, N Santos, D Casagrande
Unesp, Jaboticabal, Sao Paulo, Brazil
Email: rareis@fcav.unesp.br

Introduction Due to the economical, cultural, and biological reasons, the Brazilian`s beef cattle operation is based on pasture, and forage is the main source of nutrients for animals maintained in grazing condition around the year. In this context, the xaraes grass has been an option to diversify the *Brachiaria* cultivars, offering alternatives for good quality and high productivity pasture, when submitted an appropriate gazing management. In Central Brazil region, the spring is usually characterized by a critical period for forage quantity and quality in the pastures. Thus, animal supplementation becomes an important strategic to supply energy, protein, and minerals deficient on the available forage, and resulting in a high animal performance. Energy supplementation of beef cattle in pasture, using lipid sources, can increase the diet energy density, and animal weight gain. This trial aimed to evaluate the effects of lipid supplementation on performance of beef cattle grazing *Brachiaria brizantha* cv. Xaraes pasture during the spring.

Materials and methods The experiment was conducted at Animal Science Department of the São Paulo State University, Campus Jaboticabal, during 84 days in a 19.0 ha area divided in 10 paddocks, using 90 Nellore steers in continuous stocking rate grazing system. Lipid sources supplements evaluated were: soybean oil, linseed oil, palm oil, fat-protected with calcium salts, and control. Animals were supplemented daily, 0.5 g/kg body weight, between 11:00 am to 14:00 pm to minimize the effects of supplement on grazing behavior. All supplements presented the same content of crude protein, and total digestible nutrition (TDN), 260 g/ kg DM, and 980 g/kg DM, respectively. Control treatment consisted of mineral salt, with 560 g/kg DM of crude protein (NPN). Evaluation of forage were taken every 28 days, cutting the grass near the soil surface, to determine the herbage mass, and separating into leaf blade, sheath plus stem, and dead material, to determining the characteristics of the canopy. Chemical composition of the grass was performed in samples harvested by hand-plucking technique each 28 days. The grazing animals behavior measure was taken during daytime (12 hours) for two consecutive days. The experiment was analyzed by a complete randomized design with five treatments and two replications (paddocks) with nine animals in each one. Three periods were analyzed by repeated measures.

Results The variables total herbage mass, green mass, and green/dead material relationship were not affected (P> 0.05) by the sources of supplements, these variables were affected only by the evaluation periods (P <0.05). The greatest value of total mass was observed in the first period 11,997 kg/ ha. The highest amount of leaf was observed in the last period (4,930 kg / ha), associated to the nitrogen application in the previous period. Stem and dead material present the highest values in the first period, 3.741 and 4.308 kg / ha, respectively.

Table 1 Total herbage mass and their components (leaf, stem and dead) of Xaraes-grass pasture

Variable	Experimental period			CV %
	1°	2°	3°	
Total HB	11997 a	7056 c	10815 b	7,2
Leaf DM	3948 b	3740 b	4930 a	10,6
Stem DM	3741 a	1903 b	3622 a	32,3
Dead DM	4308 a	1412 c	2262 b	24
Leaf/Stem	1,20 b	2,06 a	1,39 b	33,8
Green/Dead	1,96 b	4,38 a	3,98 a	39,4

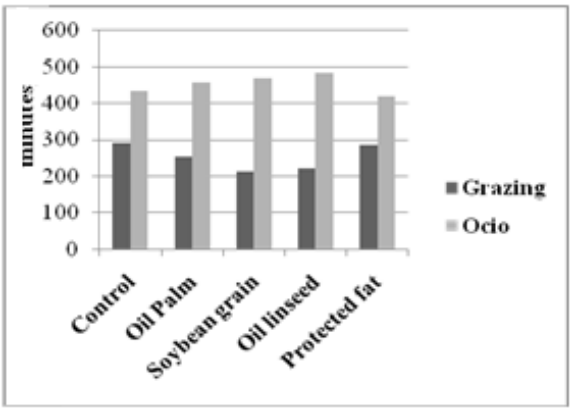

Figure 1 Grazing, ruminating and idling time of bovines supplemented with different lipid sources

The supplements used did not affect the forage chemical composition; it was observed mean values of crude protein, neutral detergent fiber and acid detergent fiber of 90.5, 615.1, and 324.1 g/kg DM, respectively. Grazing time varied due to the supplements and periods (P < 0.05). Animals in the control treatment and supplemented with protected fat spent more time in grazing (Figure 1). There was no effect of the treatments in weight gain of animals (P> 0.05). Animals of control group, and those supplemented with palm oil, soybean oil, linseed oil, and protected fat showed average daily gain of 0.631, 0.562; 0.683, 0.679, 0.709 kg / day, respectively.

Conclusions Supplementation with different lipid sources is not a viable approach to increase animal performance in tropical pasture with high herbage allowance during the spring.

https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Development of fat depots in cattle and associations with beef quality

P Greenwood[1,2], J Siddell[1,2], M McPhee[1,2], B Walmsley[1,2], G Geesink[1,3], D Pethick[1,4]
[1]Australian Cooperative Research Centre for Beef Genetic Technologies, Armidale, NSW, Australia, [2]Industry & Investment NSW, Armidale, NSW, Australia, [3]University of New England, Armidale, NSW, Australia, [4]Murdoch University, Murdoch, WA, Australia
Email: paul.greenwood@industry.nsw.gov.au

Introduction Information on development of fat depots as affected by genotype, stage of production, nutrition during the immediate post-weaning period, and their interactions is limited. The objectives of this study are to 1) determine whether a high energy, starch based supplement during the immediate post-weaning period enhances marbling; 2) determine whether nutrition and genotype interact to affect development of fat depots; 3) obtain data and samples for detailed study of fat depot development; 4) understand factors influencing tenderness of beef and provide data for the Meat Standards Australia (MSA) cuts-based palatability model.

Materials and methods Steers (n=165) within three Genotypes were studied from weaning. Targeted genotypes were high intramuscular (IMF) and high subcutaneous (SCF) fat (Angus, A), low IMF and high SCF (Hereford, H) and high IMF and lower SCF (Wagyu x Angus, WA). From weaning, steers were fed pasture (P) or pasture plus high energy pellets (12.3 MJME/kgDM, 110g CP/kgDM) at 1% live weight (LW) (Supplemented, S) within 2 replicates per treatment for 168d. Steers were then backgrounded within 2 replicates until feedlot entry at 18 months of age. Steers were then short (100d) or long (250d) feedlot fed. Live weight did not differ due to nutritional treatment at any stage of the experiment. Base-line steers (n=15, Kill 1) were slaughtered at weaning, and groups slaughtered at the end of nutritional treatments (n=30, Kill 2), prior to feedlot entry (n=30, Kill 3), and after short (n=30, Kill 4) and long (n=60, Kill 5) feedlotting. Genotype, Kill and Post-weaning nutritional effects and interactions on carcass and chiller assessment traits and on IMF percentages were assessed by analyses of variance, with initial LW as a covariate due to Angus being heavier at the start of the experiment.

Results Hereford steers had more SCF at the P8 site and less marbling than the other Genotypes (Table). Carcass weight, SCF depths, marbling and IMF percentages increased with Kill number. Among the Genotypes, Wagyu-steers had the largest eye muscle area and highest ossification score and fat colour. Post-weaning supplement depressed Rib fat depth compared with forage feeding. No interactions were evident for the chiller assessment traits apart from a Genotype x Nutrition interaction for ossification score due to Herford cattle fed supplements having a lower score (127.4) than those fed pasture only (137.7). Interactions were evident for IMF percentages, and are being assessed as part of more complete analyses of the data.

Table 1 Effects ($P < 0.05$) on carcass chiller assessment traits and intramuscular fat percentages, adjusted for LW at start of the experiment (iLW). Within columns and effects, means with different superscripts differ significantly. Numbers of animals in parentheses are for intramuscular fat percentages

	Genotype			Kill					Post-weaning nutrition	
Variable	A	WA	H	1	2	3	4	5	P	S
Age (mo.)				6	12	18	21	26		
n	55(40)	55(40)	55(40)	15	30	30	30	60(30)	75(60)	75(60)
iLW (kg)	277^{b}	216^{a}	215^{a}	234	237	237	235	236	236	236
Carcass weight (kg)	337	331	327	117^{a}	188^{b}	267^{c}	381^{d}	457^{e}	354	349
P8 rump fat (mm)	13.2^{a}	14.3^{a}	17.7^{b}	1.4^{a}	4.2^{b}	7.3^{c}	19.1^{d}	25.5^{e}	17.0	15.9
Rib fat (mm)	9.1	9.8	10.2	1.3^{a}	3.0^{b}	5.2^{c}	13.1^{d}	15.4^{e}	11.1^{b}	9.9^{a}
Loin marble score	451^{b}	435^{b}	338^{a}	154^{a}	288^{b}	330^{b}	455^{c}	541^{d}	447	418
Intramuscular fat (%)										
Loin	7.26^{b}	7.04^{a}	4.76^{b}	1.96^{a}	2.57^{ab}	4.81^{b}	7.19^{c}	13.03^{d}	6.97	6.84
Oyster blade	10.17^{b}	10.55^{b}	6.19^{a}	3.25^{a}	4.36^{a}	6.79^{b}	11.03^{c}	16.71^{d}	9.30	10.13
Outside flat	6.08^{b}	7.51^{c}	5.43^{a}	2.11^{a}	3.21^{a}	5.37^{b}	6.69^{b}	12.20^{c}	6.77	6.97
Chuck tender	5.90^{b}	6.27^{b}	3.92^{a}	2.43^{a}	2.93^{a}	5.25^{b}	6.43^{c}	8.31^{d}	5.89	5.57
Eye round	4.27	4.45	4.03	2.02^{a}	2.46^{a}	3.94^{b}	4.45^{b}	7.27^{c}	4.47	4.60
Eye muscle area (cm^2)	73.0^{ab}	77.0^{b}	70.3^{a}	37.6^{a}	51.3^{b}	65.2^{c}	83.9^{d}	91.6^{a}	78.1	75.8
Ossification score	131.5^{ab}	136.5^{b}	130.2^{a}	106.8^{a}	110.6^{a}	123.9^{b}	135.7^{c}	152.4^{d}	136.1	134.4
Fat colour score	0.57^{a}	0.90^{b}	0.74^{ab}	1.94^{e}	1.00^{c}	1.53^{d}	0.53^{b}	0.03^{a}	0.67	0.57
Ultimate pH	5.55	5.57	5.58	5.59	5.60	5.53	5.56	5.57	5.57	5.56

Conclusions and further research The post-weaning supplement did not enhance marbling, and had a somewhat suppressive effect on SC fat. The genotypes had predicted marbling characteristics, although SC fat did not differ overall between A and WA. Data is currently being generated from CT-scans and depot weights obtained at slaughter to quantify amounts of total body and carcass fat and of all major fat depots. Phenotypic data presented in the Table above, and data for the weights of fat depots and consumer assessments of eating quality currently being generated, will be used to inform detailed studies of fat depot development and meat quality. The data will also be used to refine the MSA model.

https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Effect of wheat DDGS to grain ratio on changes of protein subfraction profiles in grain-based feed (oats, barley and corn)

D Damiran, P Yu
University of Saskatchewan, Saskatoon, SK, Canada
Email: peiqiang.yu@usask.ca

Introduction The goals of this research program were to develop new strategies to more efficiently utilize grain barley, oat and corn by integration with bioethanol co-products (high protein, RUP and DVE values) in sustainable beef production for improving animal production and health and to assist the beef industry to develop low-cost feeding strategies by utilizing alternative feed resources.
The objective of this specific study was to investigate the effects of wheat dry distiller grains with solubles (DDGS) to cereal grain ratio on changes of true protein and protein (CP) subfractions.

Material and methods The CP fractions were partitioned according to the Cornell Net Carbohydrate and Protein System and included True protein (TP), PA, PB1, PB2, PB3 and PC for protein subfractions. Each subfraction has different degradation behaviour (degradation rate and extend) which are highly related to component nutrient availability. Three type of grains with wheat DDGS samples from two bioethanol plants were mixed manually to combine in ratios of 4:0, 3:1, 2:2 and 1:3. This has resulted in total of 24 (3 × 2 × 4 combinations of each) mixtures.
All data were analyzed using the MIXED procedure of SAS software (SAS Institute, Inc.) with factorial treatment design: $Y_{ijr} = \alpha_i + \beta j + e_{ijr}$; where Y_{ijr} is the variable studied, α_i is the cereal grain type effect, βj is the DDGS level effect, and e_{ijr} is the residual standard deviation used as the error term.
When a significant difference was detected ($P < 0.05$), means were separated using the Tukey-Kramer post test. Grain, DDGS level (DDGS), and Grain×DDGS were set as fixed effects and DDGS plant set as a random effect.
Orthogonal contrasts were used to compare linear and quadratic and cubic effects of including 0, 25, 50, 75 or 100% DDGS. Each sample was considered the experimental unit.

Results The results show that the effect of wheat DDGS inclusion had significant effects (P<0.05) on CP fractions in oat, barley and corn. Within wheat DDGS inclusion rate increased (0:4, 1:3, 2:2, 3:1), soluble protein PA fraction increased (P<0.05) with a range from 227-308 (oats), 156-301 (barley), 162-308 (corn) g/kg CP. But rapidly degradable protein PB1 fraction decreased (P<0.05) with a range from 181-85 (oats), 89-20 (barley), 144-77 (corn) g/kg CP. Intermediately degradable protein PB2 fraction also decreased (P<0.05) with a range from 496-182 (oats), 579-213 (barley), 490-175 (corn) g/kg CP. The slowly degradable protein PB3 fraction increased (P<0.05) with a range from 83-294 (oats), 153-300 (barley), 181-308 (corn) g/kg CP. Undegradable protein PC fraction increased (P<0.05) with a range from 24-132 (oats), 24-92 (barley), 24-132 (corn) g/kg CP. True protein decreased with a range from 349-560 (oats), 821-568 (barley), 815-560 (corn) g/kg CP.

Conclusions Current study showed that the increasing inclusion of wheat DDGS in feed mixtures linearly increased their soluble, slow degradable, and undegradable subfractions of protein and carbohydrate, while decreasing their rapid and intermediately degradable fractions of protein and carbohydrate. Considering these findings, we concluded that through varying inclusion rate of wheat DDGS in feed mixture, rumen degradation rate of protein and carbohydrate (energy) of grain based diets can be manipulated. This can be used to create more optimal and synchronized carbohydrate fermentation and protein degradation in the rumen and can be used in the formulation of feed rations. This further should be used to avoid adverse effects to the animal which occurred during feeding beef cattle using grain based diets. In conclusion, the protein fraction profiles of grains can be significantly modified when combination with wheat DDGS. These changes will result in significant impact on degradable kinetics and nutrient availability.

Acknowledgements Funding provided by the Science Cluster AAFC and BCRC-Beef Cattle Research Council (Project # FED.02.09). The authors wish to express their gratitude to Z. Niu (Department of Animal and Poultry Science, University of Saskatchewan).

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Evaluation of the main lamb feeding system in the Central regions of Tunisia

N Moujahed, Y Bouazizi, M A Ben Romdhane, C Ben Mostfa, C Darej
INAT, LRAA, Cité Mahragène, Tunis/Tunis, Tunisia
Email: nizar.moujahed@yahoo.fr

Introduction Qualified animal products (labels, controlled origin products) needs a crucial understanding of their producing factors and practices. In Tunisia, meat of lamb from central areas of the country is highly appreciated by the consumers for its presumed organoleptic characteristics. Meat quality is mainly related to individual factors such as genetic, age and sex (Revue of Geay *et al*., 2002) but also to the feeding and nutritional factors which may contribute to the determination of the sensory characteristics (Priolo *et al*., 2002). In preliminary investigation (unpublished) carried out in the region of Kairouan (Central Tunisia), we found that the main lambs feeding system (about 50% of the heard) practiced by farmers was composed of 3 periods corresponding respectively to grazing native pasture or stubbles and to hay supplemented with barley during a fattening period. The aim this study was to characterize this feeding system through measurements of lamb performances in site and during the identified feeding periods, in connection with carcass characteristics determination at some possible ages of sale and/or slaughtering.

Materials and methods Twenty Barbarine breed lambs (initial age and live weight averaged 6 months and 29.9 kg respectively in April the 11th 2009) were followed in a native pasture from the area of El Ouslatia (Central region of Tunisia, Kairouan). The study focused on the feeding system composed of 3 previously identified periods based respectively on grazing native pasture (P1: from 6 to 8 months of age) or stubbles (P2: from 9 to 10 months of age) and hay supplemented with barley grain during a fattening period (P3: from 11 to 12,5 month of age). Lambs were doubly weighed at the beginning and the end of each period and their live weight (LW) variation (LWV) and daily live weight gain (DLWG) were determined. Simultaneously, 5 lambs randomly sampled were slaughtered at 3 possible ages of sale and/or slaughtering (A1, A2, A3 respectively at 8, 10 and 12.5 months of age) to determine carcass characteristics (weights of hot carcass and tail and carcass yield). Analysis of variance was employed to analyze data using General Linear Model procedure (GLM) of Statistical Analysis System (SAS 1999, version 6.12). The model included feeding period or slaughtering age effects. LSMEANS test was used to separate treatment means.

Results Results are presented in Table 1**.** Lambs were marked by a decrease in performances at the second period of the study which coincided with the grazing on low-feeding quality barley stubbles. Indeed, LW decreased averagely by about 2.5 kg ($P < 0.05$) and DLWG value became negative ($P < 0.0001$) compared with the first period which was based on herbaceous native- species grazing. In the third period, when animals were fed in stalls (hay supplemented with barley grain), animal performances were improved and weight increased by about 7.2 kg and DLWG became positive again. Changes in carcass characteristics according to the age of slaughtering showed that all parameters (hot carcass weight, carcass yield and tail weight) were the lowest (respectively $P<0.001$, $P<0.001$ and $P<0.05$) at the age of 10 months (A2), which coincided with the end of stubble grazing period. This may be related to the low stubble feeding value, which could not cover even maintenance requirements of lambs. By another hand, The highest carcass weight was observed at A3, while the lowest value was noted at A1. These tow slaughtering ages corresponded respectively to the ends of stall-feeding and grazing pasture periods.

Table 1 Lamb performances and carcass characteristics

	P 1	P 2	P 3	SEM
Final LW (kg)*	39.8 b	37.3 a	44.5c	0.9
LWV (kg)**	9.8 c	- 2.5 a	7.2 b	1.3
DLWG (g/day)***	128 b	- 36 a	127 b	13
	A1	A2	A 3	
Hot carcass weight (kg) **	17.6ab	16.5b	19.3a	0.41
Carcass yield (%) ***	46.5a	39.4b	44.6a	0.6
Tail weight (kg) *	2.3a	1.3ab	1.7b	0.27

a, b, c. values with different subscripts in the same line are statistically different. SEM : Standard error of the mean, * P< 0,05 ; ** P<0,01 ; ***P<0,0001

Conclusions It was concluded that the feeding period as associated to the season influenced lamb performances according to the available feed resources quality. Equivalent growing performances were obtained when lambs were grazing native pasture or fed hay supplemented with barley grain. The ends of these two periods coincided with the ages resulting in the best carcass parameters. Dry feeding period based on stubble resulted in body weight losses and induced lower carcass characteristics. The study is currently being completed in our laboratory by the analysis of the sensory characteristics of meat.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effect of feeding differently processed sweet sorghum bagasse based complete diet on growth and carcass traits in growing ram lambs

N Nalini Kumari[1], Y Ramana Reddy[1], M Blummel[2,1], D Nagalakshmi[1], T Monika[1], M Pavani[1], M Sudhakar Reddy[1], Ch Ravinder Reddy[3], B V S Reddy[3]
[1]S.V. Veterinary University, Andhra Pradesh, India, [2]ILRI, Hyderabad, Andhra Pradesh, India, [3]ICRISAT, Hyderabad, Andhra Pradesh, India *Email: ramanayr19@yahoo.co.in*

Introduction Sweet sorghum (*Sorghum bicolor* L. Moench), which is similar to grain sorghum but the stalks are juicy and rich in fermentable sugars as high as 15-18 per cent with cane yield of 40 t/h. The sweet sorghum stalks are used an alternate stock for ethanol production. Sweet sorghum bagasse (the leftover stalks after juice extraction for ethanol production) and the leaves can be used as fodder resource for livestock. These residues are generally considered to be low in protein, energy and have poor digestibility mostly due to highly lignified cell walls. It can represent a large potential source of energy for ruminants provided their nutrients are fully exploited with suitable processing technology. Thus scientific and judicious combination of these residues with concentrates to produce a well-balanced complete ration to meet the nutritional requirements for various physiological functions has a great significance. In light of the stated fact, the present investigation was carried out to evaluate the effect of processing sweet sorghum bagasse (SSB) based complete diet on growth performance and carcass traits in growing ram lambs.

Materials and methods The SSB based complete diet containing 50:50 roughage to concentrate ratio was processed into mash (SSBM), expander extruded (SSBP) and chop form (1.5-2.0 cm) with supplementation of concentrate mixture at 50:50 (SSBC) and evaluated in comparison to sorghum stover based complete diet (50:50) in mash form (SSM). Twenty four growing Nellore x Deccani cross ram lambs of 3 months old were distributed into four groups of six animals each with similar initial body weights in a completely randomized design (CRD) and conducted a growth trial by feeding the four diets for a period of 180 days. The fortnightly body weights and the daily feed intake and leftover of the lambs were recorded during the trial period. At the end of the trial, three representative ram lambs were randomly selected from each group for slaughter and studied the carcass traits. Stripping, legging, dressing and evisceration were performed by adopting the standard procedures described by Gerrand (1964). The meat samples were collected from *Longissmus dorsi* muscle immediately after slaughter and these were analyzed for moisture, protein, fat and ash content (AOAC, 1997).The data generated in the experiment was statistically analyzed (Snedecor and Cochran, 1994).

Table 1 Effect of feeding differently processed SSB based complete diets on growth rate, feed intake, feed efficiency and cost of feeding in growing Nellore x Deccani cross ram lambs

Parameter	SSM	SSBC	SSBM	SSBP	SEM
Initial body wt. (kg)	10.57	10.57	10.65	10.53	0.23
Final body wt. (kg)**	24.13^{b}	23.53^{b}	25.37^{b}	28.77^{a}	0.51
Weight gain (kg)**	13.57^{b}	12.97^{b}	14.72^{b}	18.23^{a}	0.53
Average daily gain (g)**	75.37^{b}	72.04^{b}	81.76^{b}	101.30^{a}	2.94
Feed Intake (g/d)	804.70	790.31	847.53	910.791	19.00
DMI (g/d)	727.26	710.84	754.46	829.82	17.80
DMI (% b. wt.)	4.20	4.17	4.21	4.23	0.05
DMI (g/ kgw$^{0.75}$)	85.73	84.78	86.67	88.97	0.79
Cost/kg feed (INR)	7.03	6.43	6.53	6.73	-
Feed conversion efficiency (kg feed/kg gain)*	10.69^{b}	11.13^{b}	10.57^{b}	9.05^{a}	0.28
Cost/kg gain (INR)*	75.15^{b}	71.56^{b}	69.00^{ab}	60.89^{a}	1.84

^{a,b} values bearing different superscripts in a row differ significantly, **P<0.01; *P<0.05 ; INR - Indian rupee

Results The lambs fed SSBP diet have shown higher (P<0.01) body weights than the other rations at 5th and from 7th to final fortnight of growth trial. The ADG of ram lambs fed SSBP was significantly (P<0.01) higher than SSM, SSBC, SSBM diets and it was comparable among the SSBM, SSM and SSBC diets (Table 1). Processing SSB based complete diet into different forms did not influence the DMI of lambs. The SSBP fed lambs efficiently (P<0.01) utilized the feed to gain 1 kg of body weight when compared to other rations and it was comparable among SSM, SSBM and SSBC diets. Feeding SSBP diet was more (P<0.01) economical to gain one kg of body weight than the SSBC diet and SSM diet and it was comparable between SSBM and SSM diets. In carcass traits studies, the pre slaughter weight was significantly (P<0.05) higher in SSBP ration compared to other three rations. The empty body weight and carcass weights were not significantly different among the rations, but numerically higher in lambs fed SSBP ration. The differences in dressing percentage either on slaughter weight or empty body weight basis among the rations were, comparable. Processing of SSB based and sorghum straw based diets did not significantly influence the wholesale cuts of lambs, yield of organs, bone and meat yield (%) and their ratios in various wholesale cuts. The moisture, protein, fat and ash contents of meat were not affected by the processing of complete diets and roughage source in the diet.

Conclusions It is concluded from the present study that, the SSB based complete diet with 50:50 roughage to concentrate ratio is better utilized when processed into expander extruded pellets by the growing ram lambs for economic meat production.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Sensory qualities of *Longissimus* muscle from finished young bulls offered diets enriched in linseed and antioxidants supplements.

D Micol[1], D Bauchart[1], C Jurie[1], K Meteau[2], A Thomas[1], D Durand[1], J F Hocquette[1]
[1]INRA-URH1213, Theix, F-63122 Saint Genes Champanelle, France, [2]INRA-EASM1206, Le Magneraud, F-17700 Saint Pierre d'Amilly, France
Email: micol@clermont.inra.fr

Introduction Consumers are becoming increasingly aware of the relationship between diet, health and sensory qualities. Supplementation of diets in finishing beef cattle with plant oils can enhance tissue concentrations of polyunsaturated fatty acids (PUFA), especially PUFA of n-3 series, beneficial for human health like their anti atherogenic properties. However, in relation to the high sensitivity of PUFA to peroxidation, antioxidants are needed to prevent these deleterious effects in muscle tissue in live animal but also during meat processing and retail. Improvements in the nutritional quality of beef must not result in a deleterious effect on sensory characteristics. The objective of this study was to investigate the effects on beef sensory qualities of dietary linseed (rich in 18:3n-3) and antioxidants (vitamin E, plants extracts rich in polyphenols).

Materials and methods The experiment was performed with 74 young bulls of Angus, Limousin and Blond d'Aquitaine breeds. Animals (12 month-old), selected for their liveweight and age, were assigned for a 100 day finishing period at random to four diets (n=18/ group). Diets consisted in straw (25%) and concentrate (75%) based i) without lipid and antioxidant supplements (diet C), ii) with a lipid supplement from extruded linseed (providing 40g oil/kg diet DM) (diet L), iii) with the lipid and vitamin E (250IU/kg diet DM) supplements (diet LE), iv) with the lipid and vitamin E + plant extracts rich in polyphenols (provided by a mixture of rosemary, grape, citrus fruit and marigold extracts) supplements (diet LEP). Animals were slaughtered at 16 months of age. Samples of *Longissimus thoracis* (LT) muscle were prepared and stored for ageing under vacuum for 14 d at +4°C. Meat lipids were extracted by the Folch method and their fatty acids (FA) were determined by GLC on CP Sil 88 glass capillary column. Each loin sample was aged 14 days, frozen and stored (-20°C) until sensory evaluation after cooking at 55 °C. Taste panel comprised 12 trained assessors. Panellist rated tenderness, juiciness, beef flavour intensity, abnormal flavour intensity, residue, overall liking on a continuous from 0 to 10 scale and abnormal aromas. All data were analyzed by using GLM procedure of SAS. The model included the diet as fixed effect.

Results *Ad libitum* DM and energy intakes were the same between diets. Average daily gain reached 1.5 kg/d. Carcass weights at slaughter achieved 400 kg for the same fat score (14.5 % of fat tissues in the carcass). Total lipids and FAs contents of LT muscle were not influenced by the linseed (± antioxidants) diets. Health value of saturated FA was improved by linseed diets by decreasing concentration of 16:0 ($P<0.0001$) to the benefit of 18:0 (Bauchart *et al*., 2010). Concerning monounsaturated FA, dominated by 18:1, linseed diets increased proportions of *trans* 18:1 to the detriment of Δ9 cis18:1 (Bauchart *et al*., 2011). Expressed in mg of FA/100g of LT muscle, FAs were influenced by linseed enriched diets which led to a lower deposition of 18:2n-6 ($P<0.0005$), and a higher deposition of 18:3n-3 ($P< 0.0005$). Proportion of CLA in total FA was higher ($P< 0.0001$) in LT of bulls given the linseed supplement ($P<0.04$). Supplementation of vitamin E alone or associated with plant extracts rich in polyphenols strongly decreased the level of MDA resulting of the lipoperoxidation metabolic pathway (Durand *et al*., 2010).
Tenderness attribute of the meat was the same between diets. The shear force measurements were in agreement with these scores (Table 1). Residue of chewing was also in accordance with tenderness appreciation. Beef flavour was expressed identically between experimental diets. No abnormal flavour appeared with linseed and antioxidants supplementations. No specific flavour attributes (rancid, metallic...) were cited significantly by panellists in accordance with Moloney *et al*., (2010) using oil supplements. Overall appreciations, although low, were also no different between diets.

Table 1 Effects of linseed and antioxidants supplements on sensory qualities and shear force in *Longissimus thoracis* muscle in beef

	C	L	LE	LEP	SEM	*P value*
Tenderness	5.16	4.71	4.75	5.13	0.16	ns
Juiciness	4.71	4.73	4.76	4.75	0.10	ns
Beef flavour	3.94	4.06	3.96	4.09	0.09	ns
Abnormal flavour	2.25	2.36	2.42	2.27	0.10	ns
Residue	3.18	3.42	3.38	3.19	0.12	ns
Overall liking	2.65	2.82	2.68	2.93	0.13	ns
Shear force (N/cm^2)	40.2	43.8	44.7	40.7	2.6	ns

Conclusions It can be concluded that differences on sensory qualities between these linseed and antioxidants supplementations in diets of finishing beef were very small and unlikely to be significant during meat consumption.

Acknowledgement This work was supported by the ProSafeBeef program, FOOD-CT-2006-036241

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Species and strain characterisation of different silage inoculants

I Hindrichsen[1], E Augustsson[1], J Holliday[1], B Lund[1], M Mørk Jensen[1], M Raun[1], J Jatkauskas[2], C Ohlsson[1]
[1]Chr. Hansen A/S, Hørsholm, Denmark, [2]Institute of Animal Science, Baisogala, Lithuania
Email: dkidh@chr-hansen.com

Introduction Growth of clostridia and fungi is disadvantageous to the nutritive value of silage because they may result in loss of dry matter (DM) and protein, high concentrations of butyric acid, acetic acid and ammonia, and reduced aerobic stability resulting in warm silage. Collectively, these spoilage organisms will result in less palatable silage and reduced animal performance.
In addition to good management, the inoculation of lactic acid bacteria (LAB) will help to secure high quality silage by rapidly reducing pH and thereby limiting growth of spoilage organisms. The LAB for silage vary significantly in their functionality. Therefore, it is important to know the individual characteristics of LAB both at the species and strain level. In the current laboratory trial, LAB were tested for their ability to remove oxygen, reduce pH and inhibit clostridia. Furthermore, strains were tested for aerobic stability in clover-grass silage by using mini silos.

Materials and methods The LAB were tested in an artificial silage medium (Woolford and Wilkins, 1975) when determining oxygen scavenging capability and pH reduction over time. Clostridia were grown anaerobically in Hungate tubes by using a modified buffered BHI agar and antagonistic assay used to test the effect of antimicrobial compound. A 3-L-mini silo experiment was conducted and arranged in a randomized complete block design (n=5) at the Animal Nutrition and Feed Department, Institute of Animal Science of LVA, Lithuania.

Results *Lactococcus lactis* and *Enterococcus faecium* are clearly faster at removing oxygen compared to *Lactobacillus plantarum*, *Lactobacillus buchneri* and *Pedicoccus pentosaceus*. However, there are also differences between bacterial strains, e.g. *L. lactis* D2 is superior compared to *L. lactis* D1, and E. *faecium* A2 is superior compared to A1 (fig. 1). *L. buchneri* was much slower to reduce pH compared to the other bacteria. The antagonistic assay testing the effect of antimicrobial compound of five LABs against four Clostridia strains was conducted and showed that *L. lactis* D2 was inhibiting clearly all four Clostridia strains, while only a small inhibition in *C. perfringens* was observed with *L. lactis* D1 and *E. faecium* A2.
Next to the laboratory experiments, a minisilo experiment was conducted with seven bacteria. *L. buchneri* was superior in improving aerobic stability (fig. 2). All seven strains improved the silage quality parameters (DM loss, pH, lactic acid concentration, butyrate concentration, alcohol concentration) after 90 days of preservation. Differences in the reported parameters were both related to differences in bacterial species and *L. plantarum* strains.

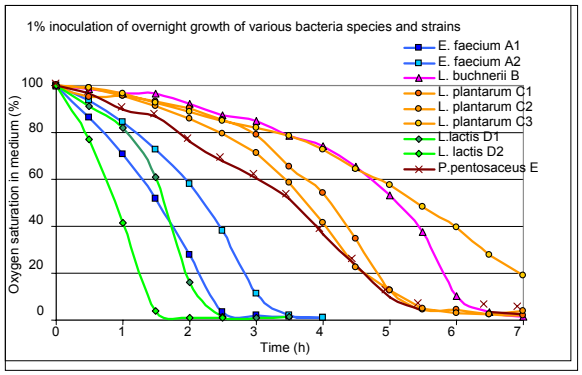

Figure 1 Change in oxygen saturation over time with different different silage inoculants.

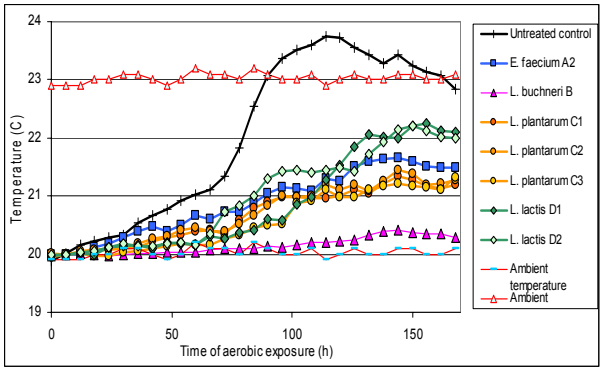

Figure 2 Change in temperature over time with silage inoculants.

Discussion The results illustrate the importance of combining the appropriate LAB species and strains to be able to optimize the fermentation of silage and thereby reducing many of the practical problems that the farmers face. Removal of oxygen is of great importance to ensure good silage quality. *L. lactis* D2 was explicitly engineered for increased oxygen scavenging through natural mutation. The oxygen scavenging capability of *E. faecium* was not previously known until this experiment was conducted. The superior inhibition of clostridia by *L. lactis* D2 was attributed to the production of nisin Z as previously reported in the Swedish patent no. 511828.

Conclusions Oxygen scavenging and clostridia inhibition differed significantly among LAB species and strains. *L. lactis* D2 was superior in removing oxygen, while *L. lactis* D1 was the only strain inhibiting all of the tested clostridia. *L. buchneri* reduced pH much slower, but improved aerobic stability compared with other LAB. All silage inoculants improved silage quality parameters, but the magnitude differed depending on species and strains.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Effects of fattening lambs on rapeseed cake or DDGS diets with hay or pasture grazing

B Borys
National Research Institute of Animal Production, Krakow, Poland
Email: bronislaw.borys@onet.eu

Introduction Due to the rapidly developing production of biofuel components from field crop seeds (e.g. rape, maize), large amounts of by-products from biofuel production appeared on the market (e.g. rapeseed cake, DDGS). They should be efficiently used, mostly as livestock feeds. Recent studies have shown that rapeseed cake and DDGS are completely suitable as protein and energy feeds for ruminants, including sheep. There are no studies with fattened lambs to compare the effects of using these feeds in diets with hay or pasture grazing. The study undertaken in 2010 evaluated the fattening results and slaughter value of lambs.

Materials and methods Subjects were 40 ram-lambs fattened semi-intensively in 4 feeding groups with 10 animals per group. Seven lambs of prolific-dairy Koluda sheep (KS) and 3 F_1 crosses (Ile de France meat rams × KS) were randomly assigned to each group. The experiment used twin- and triplet-born lambs (2.4 lambs born on average), with an equal proportion of twins and triplets in the feeding groups. The experimental fattening was conducted from weaning at 8 weeks of age to 30-35 kg body weight. at the end of fattening, 6 ram-lambs from each group (3 KS and 3 IF×KS) were slaughtered and assessed for slaughter value. Lamb fattening systems and feeds varied according to the group. Lambs from all the groups received a concentrate mixture (3% of body weight) and grass hay (H) *ad libitum* or pasture grazing for 5 h/day (P). The concentrate mixture contained rapeseed cake (RC; 50%) and linseed (5%) in groups RC+H and RC+P, and maize distillers' dried grains with solubles (DDGS; 50%) and linseed (5%) in groups DDGS+H and DDGS+P.

Results Feed intake in DDGS+H lambs was lower by 6.0% on average and hay intake higher by 6.5% in relation to the comparable group MR+H. The type of biofuel by-product component had no effect on feed and pasture forage intake in groups RC+P and DDGS+P. The rations consumed by lambs from the comparable groups had similar content of fat in dry matter, ranging from 8.80% (DDGS+S) to 9.54% (MR+S). The analysed dietary factors caused significant differences in the growth rate of the lambs, with DDGS lambs growing faster than RC lambs, and hay-fed lambs (H) growing faster than grazed lambs (P) by 14.8% ($P \leq 0.05$) and 10.8% ($P \leq 0.05$), respectively. Breed origin had no significant effect on body weight gains, but compared to KS lambs, IF×KS lambs had lower body weights both at the beginning of fattening (by 10.0%, NS) and at the end of fattening (by 9.3%) ($P \leq 0.01$). With the uniform mean body weight of the ram-lambs subjected to experimental slaughter (30.3 kg), neither dietary factor caused significant differences in the post-slaughter value of the lambs. With similar dressing percentage (43.85% on average), more noticeable differences were only found between RC and DDGS groups for loin eye area (greater in DDGS lambs by 8.5%) and for fatness over the ribs (greater in RC lambs by 8.2%). Breed origin of the lambs had a pronounced effect on the slaughter value parameters analysed. With similar dressing percentage, IF×KS compared to KS ram-lambs were awarded better EUROP grades: 3.50 vs. 2.33 pts for conformation and muscling ($P \leq 0.01$) and 2.67 vs. 2.00 pts for fatness ($P \leq 0.05$). These scores were reflected in greater loin eye area (by 10.5%, NS) and thicker layer of fat over the ribs (by 32.0%, $P \leq 0.05$) in the crosses.

Conclusions In the semi-intensive fattening of the lambs to 30-35 kg body weight, using the concentrate with 50% RC had no considerable effect on the daily intake of concentrate and on nutrient concentration in the diet with grass hay and when pasture grazing, while a 50% proportion of DDGS in the diet had no effect on the daily intake of the diet and nutrients when pasture grazing but reduced daily intake by about 6% when grass hay was fed. The analysed dietary factors caused significant differences in the growth rate of fattened lambs (higher for DDGS compared to RC feeding and higher for hay feeding compared to pasture grazing), whereas the crossing of KS and Ile de France rams clearly improved carcass meatiness and increased carcass fat content.

INNOWACYJNA GOSPODARKA
NARODOWA STRATEGIA SPÓJNOŚCI

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Research was realised within the project "BIOFOOD – innovative, functional products of animal origin" no. POIG.01.01.02-014-090/09 co-financed by the European Union from the European Regional Development Fund within the Innovative Economy Operational Programme 2007 – 2013

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Lipid profile of raw and grilled lamb meat when using rapeseed cake or DDGS diets with hay or pasture grazing

B Borys[1], S Grzeskiewicz[2], U Siekierko[2], A Borys[2], E Grzeskowiak[2]
[1]National Research Institute of Animal Production, Krakow, Poland, [2]Institute of Agricultural and Food Biotechnology, Warszawa, Poland
Email: bronislaw.borys@onet.eu

Introduction Until recently research on modification of lamb meat health quality through nutrition has concentrated on optimizing the fat content, increasing the proportion of unsaturated fatty acids in meat and decreasing the cholesterol content. Most studies in this area were performed on individual raw muscles and depot fat. From the consumers' point of view, it is important to investigate the effect of different factors on the quality of whole culinary cuts, both raw and after heat treatment. The present study was therefore undertaken to determine the effect of selected feeding methods on the dietary quality of culinary lamb meat, both raw and after heat treatment.

Material and methods Subjects were 24 ram-lambs fattened semi-intensively to 30-35 kg body weight in 4 groups, each having 3 lambs of prolific-dairy Koluda sheep (KS) and 3 F_1 crosses (Ile de France meat rams × KS). Lambs received a concentrate mixture (about 3% of body weight) and grass hay (H) *ad libitum* or pasture grazing for 5 h/day (P). The concentrate mixture contained rapeseed cake (RC; 50%) in groups RC+H and RC+P, and maize distillers' dried grains with solubles (DDGS; 50%) in groups DDGS+H and DDGS+P. The study was conducted with slices of boneless roast leg, 1.5 cm thick. The feed rations and the raw and grilled meat were analysed for fat content by the Soxhlet procedure and for fatty acid (FA) composition by gas chromatography. The meat was also assayed for cholesterol content. The results were analysed statistically by four-factor analysis of variance using STATISTICA 8 package.

Results Marked differences were found in the FA content of the diet and daily FA intake between MR and DDGS, with no appreciable effects of hay and pasture grazing (groups H and P). Compared to DDGS, RC groups consumed less SFA (by 29.7%), more MUFA (by 72.6%) and overall less PUFA (by 30.2%), including 47.5% less C18:2, and 18.0% more C18:3. Compared to DDGS, the fat of lambs from RC groups contained less SFA (45.01 vs. 46.45%, $P \leq 0.05$) and more UFA (54.49 vs. 53.13%; $P \leq 0.05$), including more MUFA (46.36 vs. 44.20; $P \leq 0.01$) and less PUFA (8.13 vs. 8.93; $P \leq 0.05$). The higher MUFA content in RC groups was due to the higher content of C18:1 acids dominating in rape fat, while the higher PUFA content in DDGS groups was due to the higher content of C18:2 acid, the main acid in maize fat. The fat of RC lambs contained more C18:3 (0.82 vs. 0.64%; $P \leq 0.01$) and less CLA (0.76 vs. 0.90%; $P \leq 0.05$). The use of RC and DDGS had no effect on the fat and cholesterol content of meat and the PUFA:SFA ratio while causing differences in n3 PUFA content (1.31 vs. 1.07%; $P \leq 0.01$)) and the n6:n3 PUFA ratio (3.84 vs. 3.58; $P \leq 0.01$). Pasture grazing (P) compared to hay feeding (H) reduced SFA content (44.74 vs. 46.72%; $P \leq 0.01$). P lambs had higher MUFA levels (46.03 vs. 44.54%; $P \leq 0.01$) and similar content of C18:1 c9 (31.50%). With the similar PUFA content in groups P and H, the fat of P lambs contained more C18:2 (5.37 vs. 4.84%; $P \leq 0.05$) and C18:3 (0.81 vs. 0.66; $P \leq 0.01$), and less C 20:4 (0.90 vs. 1.12%; $P \leq 0.01$). The grazed lambs were characterized by better PUFA:SFA ratio (0.196 vs. 0.179; $P \leq 0.05$) and higher CLA and n3 PUFA content in the FA pool as well as higher cholesterol content of meat. Crossing IF with KS had no effect on the content of SFA, MUFA and PUFA and CLA in the FA pool, with significant differences in the content of C14:0, C16:0; C16:1, C20:4 (IF×KS < KS) and C18:0, C18:1 T, C18:1 c11, C18:3 (IF×KS > KS). Overall, the meat of IF×KS animals had a more favourable n6:n3 PUFA ratio (4.40 vs. 5.03 in KS; $P \leq 0.01$) but a higher cholesterol content (77.5 vs. 71.2 mg/100 g; $P \leq 0.01$). Grilled meat compared to raw meat contained more fat (8.58 vs. 5.49%; $P \leq 0.01$) and cholesterol (82.1 vs. 66.7 mg/100 g; $P \leq 0.01$). Thermal treatment had no effect on the content of SFA and MUFA in the FA pool but decreased the proportion of PUFA (8.16 vs. 8.90; $P \leq 0.05$), thus having a negative effect on the health attributes of meat, n3 PUFA content (1.12 vs. 1.26%; $P \leq 0.05$) and n6:n3 PUFA (4.90 vs. 4.53; $P \leq 0.05$) and PUFA:SFA ratios (0.18 vs. 0.20; $P \leq 0.05$).

Conclusions The effect of the biofuel by-products components (rapeseed cake and DDGS) on the lipid profile of lamb fat was clear but inconclusive in terms of the health quality of meat. Temporary grazing of fattened lambs on pasture compared to indoor feeding of hay generally had a beneficial effect on the lipid profile of their meat. Crossing prolific-dairy Koluda sheep with Ile de France meat rams generally had a positive effect on the composition of fatty acids, with increased cholesterol content of meat. Grilling roast lamb leg caused a significant increase in fat and cholesterol content, had no significant effect on the proportion of SFA and MUFA in fat, and decreased the proportion of PUFA, thus adversely affecting the health attributes of the meat.

INNOWACYJNA GOSPODARKA
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Research was realised within the project "BIOFOOD – innovative, functional products of animal origin" no. POIG.01.01.02-014-090/09 co-financed by the European Union from the European Regional Development Fund within the Innovative Economy Operational Programme 2007 – 2013

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Effects of Supplementation of Crushed Linseed in Combination with Varying Forage Type and Forage to Concentrate Ratio on Milk Fatty Acid Profile in Lactating Dairy Cows

A-R Sterk[1,2], H Taweel[2], A Van Vuuren[1], J Dijkstra[1]
[1]Wageningen University, Animal Nutrition Group, Wageningen, The Netherlands, [2]Agrifirm Innovation Center, Apeldoorn, The Netherlands
Email: attje-rieke.sterk@wur.nl

Introduction The milk fatty acid (FA) profile largely depends on FA intake and FA metabolism in the rumen (Jenkins *et al*., 2008). Characteristics of the basal diet, such as forage type and forage to concentrate ratio (F/C ratio), affect ruminal metabolism of poly unsaturated FA from supplemental fat sources (Kliem *et al*., 2008, Loor *et al*., 2005). Few direct comparisons exist between the basal diet and lipid supplementation and to our knowledge, effects on milk FA profile of a combination of different forage types, F/C ratio, and crushed linseed supplementation have not been reported. The objective of this study was therefore to investigate the effects of forage type and F/C ratio in combination with supplementation of crushed linseed (CL) on milk FA profile of lactating dairy cows.

Materials and methods The effect of type of forage (grass silage or maize silage), F/C ratio, and proportion of CL were studied using a 3-factor Box-Behnken design (Box and Behnken, 1960). Thirty-six Holstein and Swedish Red cows (2.1 ± 0.9 parity; 72 ± 17 days in milk) were assigned to 4 groups and were fed different treatment diets during 4 experimental periods of 21 d each. Treatment diets were formulated from combinations of the 3 main factors with 3 levels each according to the Box-Behnken design. Type of forage was 20%, 50%, and 80% (DM-basis) grass silage, with the remainder being maize silage. Forage to concentrate ratio was 35:65, 50:50, and 65:35 (DM-basis) and CL was supplied at 1%, 3%, and 5% of DM. Thirteen treatments were formulated, including the centre point treatment (50% grass silage, 50:50 F/C ratio, 3% CL). During every period 4 treatments were tested including the centre point treatment. The centre point treatment was therefore repeated four times. Cows were housed in cubicle stalls and had *ad libitum* access to automated feed bins with weighing equipment. Each group of 9 cows had access to 5 automated feed bins. Cows had free access to water and were milked thrice daily at 0600, 1300, and 2100h. The DMI and milk production were recorded during the last 3 days of each experimental period. Milk samples were collected over 9 consecutive milkings during the last 3 days of each period, pooled, and analysed for fat, protein and FA profile. Milk FA methyl esters were quantified using gas chromatography (Trace GC Ultra™, Thermo Fisher Scientific, Waltham MA, USA) with a fused silica capillary column (SP2560, Supelco, Bellefonte PA, USA). Results were analysed using the PROC MIXED procedure of SAS (SAS version 9.2; SAS Institute Inc., Cary, NC, USA) according to the model described by St-Pierre and Weiss (2009). The model included linear and quadratic main effects and all 2-way interactions as fixed effects. Random effects included cow group, period within cow group, and cow nested within cow group. Non-significant fixed effects ($P > 0.10$) were removed from the model.

Results and discussion Mean DMI and milk yield were 23.0 ± 3.6 and 41.2 ± 7.3 kg/d, respectively. The proportion of *cis*-9,*cis*-12-C18:2 in milk fat increased ($P < 0.001$) and the proportion of *cis*-9,*cis*-12,*cis*-15-C18:3 in milk fat decreased ($P < 0.01$) when shifting from 80% grass silage to 80% maize silage in the diet. These relationships reflect the FA composition of the forages with a higher proportion of *cis*-9,*cis*-12-C18:2 in maize silage and a higher proportion of *cis*-9,*cis*-12,*cis*-15-C18:3 in grass silage, and the change in proportions of these FA in milk fat are in line with those observed by Kliem *et al*. (2008). The F/C ratio in the diet showed a quadratic relationship with *cis*-9,*cis*-12-C18:2 ($P < 0.05$) and *cis*-9,*cis*-12,*cis*-15-C18:3 ($P < 0.05$) proportions in milk fat, with the lowest levels of both achieved when the diet contained a 50:50 to 65:35 F/C ratio. The proportion of CL in the diet also showed a quadratic relationship with *cis*-9,*cis*-12-C18:2 ($P < 0.05$) and *cis*-9,*cis*-12,*cis*-15-C18:3 ($P = 0.07$) proportions in milk fat. The proportion of *cis*-9,*cis*-12-C18:2 reached a maximum at approximately 3% crushed linseed, and that of *cis*-9,*cis*-12,*cis*-15-C18:3 reached a plateau at 4 to 5% of crushed linseed in the diet. The proportion of *cis*-9,*cis*-12,*cis*-15-C18:3 showed a significant interaction between F/C ratio and CL. The proportion of *cis*-9,*cis*-12,*cis*-15-C18:3 reached a higher level when feeding 5% crushed linseed in combination with a 35:65 F/C ratio than with a 65:35 F/C ratio, irrespective of forage composition. This response was in agreement with results of Loor *et al*. (2005) who observed a higher *cis*-9,*cis*-12,*cis*-15-C18:3 proportion in milk fat when feeding linseed oil in combination with a high-concentrate diet compared to a low-concentrate diet. The proportions of various *trans*-FA in milk fat were also significantly ($P < 0.05$) affected by the interaction between F/C ratio and CL. The *cis*-9,*cis*-12-C18:2 and *cis*-9,*cis*-12,*cis*-15-C18:3 proportions in milk fat were not affected ($P > 0.10$) by the interaction between forage type and CL.

Conclusions Substituting grass silage with maize silage in the diet of dairy cattle is reflected in the proportions of *cis*-9,*cis*-12-C18:2 and *cis*-9,*cis*-12,*cis*-15-C18:3 in milk fat. The interactions between F/C ratio and CL proportion for *cis*-9,*cis*-12,*cis*-15-C18:3 and various trans-FA suggest that rumen metabolism of poly unsaturated FA is affected. The proportion of *cis*-9,*cis*-12,*cis*-15-C18:3 reached the highest level when feeding 5% CL in combination with 35:65 F/C ratio, irrespective of forage type.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Intensification alternatives for pasture-based systems in Australia: nitrogen balance and greenhouse gas emissions

S Fariña, S C Garcia, W Fulkerson
The University of Sydney, Sydney, NSW, Australia
Email: santiago.farina@intelact.com

Introduction The increasing price for dairy land and volatile cost of purchased feeds in Australia will need the industry to rely more on increasing milk production from home-grown feed per unit of land. The complementary forages system (CFS) was developed to maximize milk production per ha from home-grown feed by combining forage crop rotations and pasture (Fariña *et al*., 2011). This study assesses the potential environmental impact of the CFS at the whole farm scale, in comparison to other intensification options.

Materials and methods The approach involved an initial 2-year whole farm study with 100 cows on 21.5 ha to evaluate the bio-physical aspects of the implementation of the CFS. Actual data from the CFS whole farm study (Fariña *et al*., 2011) were used as a basis for modelling two additional systems with similar stocking rate but no use of forage crops. Cows in the 'Base' system received similar amount of concentrate lactation^{-1} as the CFS, whilst cows in the 'Pasture+Grain' system received as much concentrate as needed to produce the same amount of total milk per ha as the CFS. Systems were simulated using the Farmax Dairy-Pro decision support model (Bryant *et al.*, 2010). For each of the farm systems modelled, the nitrogen (N) balance at the farm-gate level and the emission of greenhouse gases (GHG) according to the guidelines of the Intergovernmental Panel on Climate Change for National Greenhouse Gas Inventories (IPCC, 1997) were estimated for a complete year.

Results The intensification systems evaluated showed a trend for an increase in total N surplus per ha and total GHG (Table 1). However, the CFS appeared to have a lower environmental impact per unit of milk, both in terms of N surplus (0.097 kg N surplus kg of milksolids^{-1}), and total GHG (10.39 t CO_2 eq t of milk solids^{-1}).

Table 1 Mean utilised yields of forage crops and pasture (t DM ha^{-1} year^{-1}), concentrates fed (t DM cow^{-1} year^{-1}), stocking rate (cows ha^{-1}), annual milk yields (L) per cow and per ha, milk solids (%), N balance (kg ha^{-1} year^{-1}) greenhouse gas emissions (t CO_2 eq year^{-1}) for the Base, CFS and Pasture+Grain farm systems modelled from the Complementary Forage System (CFS) whole farm study

			Base	CFS	Pasture+Grain
Feed source	Pasture	(t DM ha^{-1} year^{-1})	17.29	18.9	18.77
	Forage crops	(t DM ha^{-1} year^{-1})	-	36.73	-
	Concentrate	(t DM cow^{-1} year^{-1})	1.33	1.35	2.27
	Stocking rate	(cows ha^{-1})	4.01	4.02	4.01
Milk and components	Milk solids	(g/kg milk)	73.5	77.6	73.7
	Milk per cow	(L 305-d lactation^{-1})	6,232	7,475	7,381
	Milk per ha	(L year^{-1})	30,226	36,144	36,132
N balance	Total N input	(kg ha^{-1} year^{-1})	401	495	497
	Total N output	(kg ha^{-1} year^{-1})	172	223	205
	N surplus	(kg ha^{-1} year^{-1})	229	271	292
	N surplus/ kg milk solids	(kg kg^{-1})	0.103	0.097	0.110
Greenhouse Gas Emissions	Pre-farm[a]	(t CO_2 eq year^{-1})	337	333	538
	On-farm CO_2	(t CO_2 eq year^{-1})	240	240	240
	On-farm CH_4	(t CO_2 eq year^{-1})	2,493	2,654	2,604
	On-farm N_2O	((t CO_2 eq year^{-1})	902	852	899
	Total farm	((t CO_2 eq year^{-1})	3,972	4,078	4,280
	Total per t milk solids	((t CO_2 eq t^{-1})	12.77	10.39	11.48

[a] comprises greenhouse gas emissions embedded in inputs to the farm system (diesel, fertiliser, purchased feed) and from electricity

Conclusions The use of highly N use efficient sources of home-grown feed in the CFS enabled to increase milk production per ha with lower N surplus per unit of milk produced in comparison with intensification alternatives based on pasture plus concentrates. The lower GHG per unit of milk in the CFS was due to the lower use of brought-in feed in this system, which in turn led to lower pre-farm emissions than the system based on feeding more concentrates. These results suggest that intensification of dairy production systems through use of complementary forage systems can reduce rather than increase N surplus and GHG per unit of milk produced.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Methane inhibition by different mitigation agents in a batch *in vitro* rumen fermentation system

J M Castro Montoya[1,2], S De Campeneere[2], B De Baets[3], V Fievez[1]
[1]Laboratory of Animal Nutrition and Animal Product Quality, Ghent University, Melle, Belgium, [2]Institute of Agricultural and Fisheries Research, Animal Science Unit, Melle, Belgium, [3]Department of Applied Mathematics, Biometrics and Process Control, Ghent University, Ghent, Belgium
Email: joaquinmiguel.castromontoya@ugent.be

Introduction In recent years many researchers were involved in finding appropriate methane (CH_4) mitigation strategies. Options like immunisation, biological control, probiotics, elimination of protozoa, manipulation of dietary ingredients and mitigation through dietary additives have been considered to reduce CH_4 production in ruminants. Regarding dietary additives, different working mechanisms for CH_4 inhibition have been studied, e.g. inhibition of protozoa (e.g. saponins, in some cases poly- and mono-unsaturated fatty acids (PUFA and MUFA)); stimulation of propionate (e.g. fumaric acid); reduction of hydrogen production (e.g. monensin, PUFA and medium chain fatty acids (MCFA), cinnamaldehyde), direct inhibition of methanogens (e.g. garlic oil). Diverse studies have tested the potential of such additives for CH_4 inhibition, showing that their effectiveness is related to the basal substrate and the concentrations applied, hence it is difficult to compare additives from results coming from different studies. Furthermore, in order to test inhibitors intended to be applied in dairy cattle industry, the formers should be tested in association with feedstuffs normally used as dairy cattle feed. This study aimed at evaluating the effects of different methane mitigation agents and their possible interaction with different substrates on *in vitro* CH_4 production.

Materials and methods Seven inhibitors [cinnamaldehyde (1.5 and 3 mg/ml), fish oil (1 and 2 mg/ml), fumaric acid (1.67 and 2.33 mg/ml – 10 and 20 mM –), garlic oil (0.15 and 0.3 mg/ml), MCFA (0.6 and 1.2 mg/ml), monensin (0.0015 and 0.003 mg/ml – 2.5 and 5 μM –) and quillaja saponins (0.5 and 1 mg/ml)], and five substrates [concentrate (CONC), grass silage (GS), maize silage (MS), GS+MS+CONC in a ratio of 35:35:30 (MIX 1) and GS+MS+CONC+Sugar beet pulp in a ratio of 28:28:30:14 (MIX 2)] were incubated in an *in vitro* batch fermentation system. Additionally, a PUFA rich concentrate (60% extruded linseed) and a PUFA + MUFA rich concentrate (30% extruded linseed and 26% extruded rapeseed) were tested for CH_4 production. The substrate (250 mg) and the inhibitor were incubated during 24 h in airtight glass flasks at 39°C under anaerobic conditions. Rumen fluid (5 ml) from two fistulated hay fed sheep and 20 ml of phosphate buffer solution (pH adjusted to 6.8) were used as incubation medium (Hassim *et al*., 2010). Ethane (1 ml) was added to each flask as marker gas for CH_4 determination by gas chromatography (3000 micro-GC, Agilent, USA) (Hassim *et al*., 2010). Samples of incubation medium were taken, processed and stored for volatile fatty acids (VFA) analysis using gas chromatography (Shimadzu GC-14A) (Van Ranst *et al.*, 2010). The experimental design was completely randomized and the data were analyzed using the GLM procedure of SAS 9.2 considering the dose, the additive and the substrate as fixed factors.

Results Cinnamaldehyde and MCFA reduced *in vitro* CH_4 production by 70 to 95% ($P < 0.001$), but also decreased the production of VFA (by *ca*. 80 and 50% respectively), reflecting the inhibition of the fermentation process. Garlic oil also decreased CH_4 production ($P < 0.001$) but not affecting the VFA ($P = 0.23$). Fumaric acid, fish oil and monensin reduced CH_4 production by *ca.* 40% ($P < 0.001$), the first two showed numerical increases in the VFA ($P = 0.13$), while monensin showed numerical decreases ($P = 0.99$). Quillaja saponins inhibited CH_4 production by *ca.* 30% were observed, with no effect on VFA ($P = 0.99$). Finally, the incubation of PUFA + MUFA rich concentrate did not reduce CH_4 production ($P = 0.78$) compared with CONC, whereas the incubation of PUFA rich concentrate decreased CH_4 production by 47% ($P < 0.001$). These substrates did not reduce the total VFA production ($P = 0.73$).

Table 1 Total methane (mmol/flask) production after 24 h *in vitro* incubation (n = 6) (highest concentrations of each additive)

Additive	Control	Monensin	Garlic oil	Quillaja saponins	MCFA	Fish oil	Fumaric acid	Cinnamaldehyde	SEM
mg/ml	0	0.003	0.3	1	1.2	2	2.33	3	
CONC	280^{e}	155^{c}	24.0^{ab}	200^{d}	44.6^{b}	161^{c}	160^{c}	13.6^{a}	8.73
GS	368^{g}	177^{d}	23.5^{ab}	249^{f}	48.0^{b}	191^{c}	230^{e}	13.8^{a}	11.63
MS	325^{f}	169^{b}	23.8^{a}	225^{e}	18.6^{a}	234^{d}	200^{c}	14.0^{a}	11.12
MIX 1	320^{e}	179^{b}	22.3^{a}	232^{d}	13.8^{a}	203^{c}	217^{c}	13.7^{a}	10.48
MIX 2	277^{e}	163^{b}	22.1^{a}	208^{d}	13.5^{a}	196^{c}	209^{c}	14.5^{a}	10.21

Different superscript within a row indicates significant differences ($P < 0.05$)

Conclusions *In vitro* and at the concentrations tested, garlic oil showed the strongest inhibitory effects on CH_4 production without reducing VFA production. The tested additives interacted with the different substrates.

Acknowledgements PhD project jointly funded by the Institute for Agriculture and Fisheries Research (ILVO) – Belgium and the Laboratory of Animal Nutrition and Animal Product Quality (LANUPRO) – Ghent University – Belgium.

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The effect of lipid sources on the methane emission of beef cattle at pasture using the SF6 tracer technique

I P C Carvalho[1], T T Berchielli[1,2], A Berndt[3], R T S Frighetto[4], G Fiorentini[1]
[1]UNESP, Jaboticabal, Brazil, [2]INCT/CA Member, Brazil, Brazil, [3]EMBRAPA, São Carlos, Brazil, [4]EMBRAPA, Jaguariúna, Brazil
Email: carvalho_isabela@yahoo.com.br

Introduction Methane is known to be a potent greenhouse gas and its release to the atmosphere contributes to global warming. The losses of dietary energy average about 5-12% in methane (Van Soest, 1991) and it contributes to 68 % of the Brazilian emission of this gas. The methane production in the rumen is intrinsic to the rumen fermentation, acting as a hydrogen (H_2) drain, so the reduction on methane emission requires an alternative route for the H_2, avoiding its accumulation. Lipid sources can reduce methanogens and protozoa through a toxic effect and also act as an H_2 drain due to the biohydrogenation process.

Materials and methods Twenty Nellore steers (initial average body weight of 440kg) were assigned to five treatments of a completely randomized design. The animals were divided in to 10 paddocks (2 paddocks per treatment) of *Brachiaria brizantha* cv. Xaraés. Linseed oil, palm oil, soy bean grain and by-pass fat (Lactoplus®) were added to a supplement offered to the animals once a day (an amount of 1,0% of the body weight). The control treatment was composed of an energy-protein supplement with no additional fat. All the concentrate containing 20% CP and 10% EE (except the control supplement, which contained 3% EE). The animals were weighed every 28 days and slaughtered at 495.6 kg. The methane emissions were evaluated using the SF6 tracer gas technique (Johnson *et al*., 1994; Westberg *et al*., 1998, adapted by Primavesi *et al*. 2004). Permeation tubes with known release rates for SF6 were placed in the rumen, 72 hours prior to the start of gas sampling. Each tube was charged with 500-600 mg of SF6 and incubated at 39°C for calibration. Release rates of SF6 were determined by measuring the weight loss of tubes for 6 wk to establish a steady pre-determined rate. Release rates of the permeation tubes used in this study ranged from 930 to 1600 ng min–1. Gases exhaled from the nose and mouth were drawn into pre-evacuated collection tubes, through a capillary tubing. The collection system was designed to deliver half its volume during a 24-hr collection, ensuring a uniform collection rate. Five consecutive 24-hr gas samples were collected from each animal. The fixed effect was the treatment. The paddocks were used as the experimental unit. The methane emission and average daily gain were analysed using the tukey test, with a 5% probability.

Results There was no effect (P>0.05) of lipid sources on the average daily gain (ADG) of beef steers at pasture fed with 1% body weight supplement. The methane emissions were expressed in kilograms of methane emitted per year (kg $CH_4.yr^{-1}$), gram of methane emitted per day (g $CH_4.day^{-1}$), milligram of methane emitted per day per kilogram of body weight (mg $CH_4.day.BW^{-1}$), gram of methane emitted per day per kilogram of metabolic body weight (g $CH_4.day.MBW^{-1}$) and kilograms of methane emitted per kilogram of carcass produced (kg $CH_4.kg\ CAR^{-1}$). The methane emission was also not different (P>0.05) among the treatments in any unit expressed .

Table 1 Average daily gain and methane emission of animals receiving lipid sources on supplement

Variables	Treatments					CV (%)
	Control	Palm oil	Linseed oil	By pass fat	Soy bean	
ADG	0.564	0.567	0.661	0.556	0.561	12.123
kg $CH_4.yr^{-1}$	41.515	41.115	25.615	37.190	30.100	19.443
g $CH_4.day^{-1}$	113.735	112.645	70.180	101.880	82.470	19.446
mg $CH_4.day.BW^{-1}$	238.651	228.516	147.044	208.547	180.847	11.770
g $CH_4.day.MBW^{-1}$	1.115	1.077	0.687	0.980	0.836	13.627
kg $CH_4.kg\ CAR^{-1}$	0.236	0.245	0.144	0.227	0.180	22.604

Means within each variable followed by different letters differ by tukey test (P < 0.05), n=2.
ADG = average daily gain; BW= body weight; MBW = metabolic body weight; CAR= carcass

Conclusions Lipid sources such as palm oil, linseed oil, soy bean and by pass fat were not effective in mitigating methane emissions in Nellore steers, when included in a supplement with 10% EE, offered at a quantity of 1.0% BW to animals at tropical pasture.

Acknowledgements Bellman® Animal Nutrition**;** FAPESP

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Construction and installation of six modular open-circuit chambers for monitoring gas emissions from large ruminants

N Peiren[1], B Sonck[1], J Castro Montoya[1,2], D De Brabander[1], V Fievez[2], S De Campeneere[1]
[1]Institute for Agricultural and Fisheries Research, Animal Sciences Unit, Melle, Belgium, [2]Ghent University, Laboratory for Animal Nutrition and Animal Product Quality, Melle, Belgium
Email: nico.peiren@ilvo.vlaanderen.be

Introduction Global warming is one of the most important political, sociological and scientific topics in recent years. Since industrialisation, global atmospheric concentrations of the most important greenhouse gases have enormously increased. In animal husbandry, methane is the most causing agent. Due to its short lived time in the atmosphere, methane mitigation has a great potential to reduce the greenhouse gas effects in the short term (Steinfield *et al*. 2006). To study the effect of mitigation strategies like methane reducing additives, the Animal Sciences Unit of ILVO in collaboration with LANUPRO of Ghent University has constructed six open-circuit chambers for monitoring gas emissions from large ruminants.

Construction chambers The chambers are constructed from 50 mm thick polypropylene (PP) copolymer panels around an internal stainless steel frame. The chambers have an outside dimension of 400x155x280 cm (LxWxH). The panels are welted together to make the construction airtight. Each of the six chambers can house one large ruminant (dairy or beef cattle), but can also be adapted to house smaller ruminants and monogastric animals. The chambers are grouped two by two which makes a modular construction of the ventilation system possible. Each chamber has three doors: an entrance door in the back, a lateral door that serves as exit door and for milking the cows, and a front door for feed supply. To reduce the feeling of captivity and improve visual contact between cows natural lighting in the chambers was maximized with large windows of polyethylene terephtalate glycol in each door and in the side panels. In the chamber the floor is raised by 350 mm, this makes it possible to add a manure shelf in the rear part of the chamber. Above the shelf a metal slatted grid is installed. In the front on that floor a feed bin is placed. A drinking bowl, with non-spill edge and water meter is attached to the side wall. A thick lying mat is present for optimising the cow's comfort. Cows are tied with a vertical strap tying system.

Construction ventilation The ventilation system is a mechanical central flow system. The fresh air enters the stable compartment through two windows equipped with windbreak netting. The air enters the chambers via an adjustable opening in the lower panel of the front door. The air outlet is situated in the roof panel at the rear end. In that opening a module with a diameter of 350 mm is placed. This module makes the connection with a 12.6 m long central ventilation duct made of coated plywood. Finally the air is evacuated by an axial exhaust fan. One central exhaust fan makes the air flow through the six chambers, however each chamber has his own module with a control damper that regulates the amount of air and an integrated full size free-running impeller that continually measures the airflow. The chamber with the highest demand determines the speed of the central fan. To achieve the right volume of air in chambers with a lower demand, the vortex damper damps the air exhaust in these chambers. The unique aspect of the central flow control is that it doesn't simply take the section with the highest demand into account, but also continually checks the positions of all the dampers. By this, all chambers have enough fresh air, without any unnecessary damping. It is the first time a central flow ventilation system common to pig and poultry stables is introduced in a chamber system for individual measurement.

Gas measurement The gas concentrations of CH_4, CO_2, N_2O and NH_3 can be measured every 8 min. with an infrared laser optical-feedback cavity-enhanced absorption spectrometer in the exhaust channels of each chamber. The principles of this technique are described by Morville *et al*. (2005). At the entrance of each exhaust channel a stainless steel sampling probe is placed, containing an inline filter and a sonic nozzle. The probe is connected via a PFA tubing to an eight channel multi sampler connected with the detector. The whole measuring system functions at continue underpressure of 120 mbar. This gas analysis system is recently commercialised and is for the first time used in an agricultural environment. It has a few advantages: excellent compositional sample representativity, low response time, no false positive responses, high selectivity, enables measuring range from ppt to ppm using a single calibration, simultaneous measurement of several gases using the same laser spectrometer, no condensation and no heated lines.

Conclusions To our knowledge this is an unique system for measuring gas emissions in open-circuit chambers for large ruminants, not only due to the combination of the chambers, the ventilation system and the gas analyser, but also due to the innovative aspects of each these individual parts.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Molecular analysis of methanogenic archaea in the forestomach of the alpaca (*Vicugna pacos*) reveals differences in population structure between individual hosts

B St-Pierre, A-D G Wright
The University of Vermont, Burlington, Vt, USA
Email: bstpierr@uvm.edu

Introduction Complex gastro-intestinal microbial communities are responsible for digestion of plant biomass into energy molecules that can be assimilated by herbivore hosts. This process also generates by-products such as methane, a greenhouse gas produced by methanogenic archaea that is released into the environment. Methane emission is detrimental because it not only contributes to climate change but also results in reduced animal production. The South American camelid species alpaca (*Vicugna pacos*) is a good model to study methanogens because it is efficient with feed and produces low amounts of methane. Our study aimed to 1) identify and determine the phylogeny of alpaca forestomach methanogens; 2) investigate methanogen population structures in individual alpacas; and 3) compare alpaca methanogen population structures with other host species.

Material and methods Microbial genomic DNA was extracted from forestomach fluid sampled from 5 alpaca animals, ranging from 19 months to 7.5 years of age, and maintained under the same diet and environmental conditions in Washington, Vermont (USA). PCR-amplification of methanogen 16S rRNA genes from each individual DNA sample was performed as previously described (Wright & Pimm, 2003). Amplicons were cloned and sequenced to generate 5 independent libraries. Based on a 98% species-level sequence identity cutoff, methanogen 16S rRNA library clones were grouped into operational taxonomic units (OTU) by the open-source program MOTHUR (Schloss *et al*, 2009), using distance data according to the Kimura two-parameter model (Kimura, 1980) in PHYLIP (Version 3.69, Felsenstein 2006). MOTHUR was also used to determine the Shannon and LIBSHUFF diversity indices. For phylogenetic reconstruction, representative alpaca methanogen 16S rRNA sequences were combined with methanogen 16S rRNA gene sequences from major archaeal phylogenetic groups. PHYLIP (Version 3.69, Felsenstein 2006) was used to construct a neighbor-joining tree, which was bootstrap resampled 1 000 times.

Results Each library contained between 179 and 201 clones, for a combined total of 947 methanogen 16S rRNA gene sequences from the alpaca forestomach, which were distributed into 51 species-level OTUs. The number of OTUs represented in each individual library ranged from 21 to 29. We found 47.3% of the combined library sequences with species-level identity to *Methanobrevibacter millerae*, 15.0% to *Methanobrevibacter ruminantium* and 4.3% to *Methanobrevibacter smithii*. For the remaining clones: 21.1% had genus-level (95-97.9%) sequence identity to *Methanobrevibacter* species, 7.3% had genus-level identity to *Methanobacterium* species, 2.4% had genus-level identity to *Methanosphaera* species, and 2.5% belonged to a group of uncultured methanogens distant from the Methanobacteriales order. Phylogenetic analysis highlighted 5 major groups of alpaca forestomach methanogens: SGMT (related to *Methanobrevibacter* species *smithii*, *gottschalkii*, *millerae* or *thaurei*), RO (related to *Methanobrevibacter* species *ruminantium* or *olleya*), related to *Methanospharea* (Msp) species, related *Methanobacterium* (Mba) species, and the clade of novel uncultured (Unc) methanogens. The representation of each methanogen group in individual animals is shown in Figure 1.

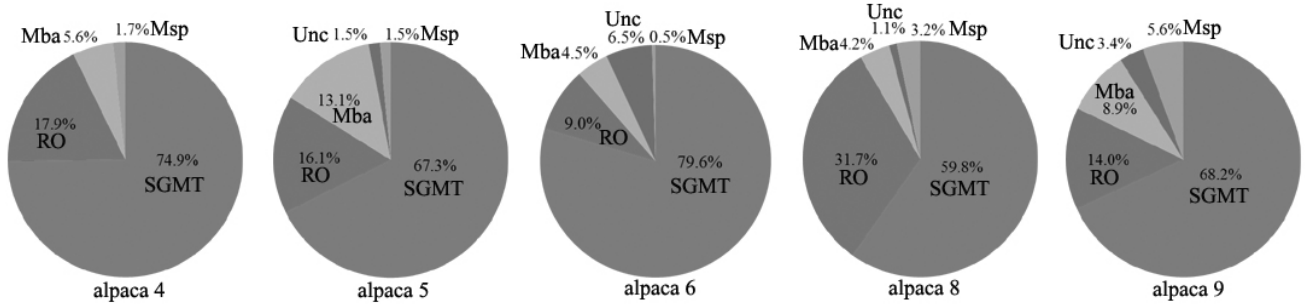

Figure 1 Distribution of major methanogen groups in sampled alpaca animals.

Conclusions Consistent with other host species, methanogen 16S rRNA clones from the alpaca forestomach with genus-level sequence identity to *Methanobrevibacter* species were highly represented at 88.3%. However, the alpaca is distinct because sequences with species-level identity to *Methanobrevibacter millerae* were the most abundant. While they followed a similar pattern, methanogen population structures varied significantly between each alpaca, highlighting an additional level of complexity in the analysis of microbial populations. LIBSCHUFF analysis confirmed that library composition was different between animals.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Optimization of Roughage to Concentrate Ratio in Sweet Sorghum Bagasse Based Complete Ration for Efficient Microbial Biomass Production in Sheep Using *In vitro* Gas Technique

N Nalini Kumari[1], Y Ramana Reddy[1], M Blummel[2], D Nagalakshmi[1], T Monika[1], M Pavani[1], M Sudhakar Reddy[1], Ch Ravinder Reddy[3], B V S Reddy[3]
[1]S.V. Veterinary University, Andhra Pradesh, India, [2]ILRI, Hyderabad, Andhra Pradesh, India, [3]ICRISAT, Hyderabad, Andhra Pradesh, India *Email: ramanayr19@yahoo.co.in*

Introduction The sustainability of number-driven growth in livestock output would be severely constrained by declining per capita land availability and feed and fodder scarcity in developing countries like India. To meet the increasing demand for the feedstuffs, it is necessary to search and exploit the newer feed resources like Sweet Sorghum Bagasse (SSB). The SSB is a by-product of ethanol industry after extracting the juice from the plant, can compensate for fodder loss. These low quality roughages are deficient in critical nutrients like protein, energy, minerals and vitamins. Addition of concentrate ingredients to the diets can improve the utilization of low quality roughages. The objective of the present study was to determine the digestibility, gas production and ME of SSB based complete diets with different ratios of SSB and concentrate and optimize the SSB to concentrate ratio for efficient microbial biomass production in sheep.

Materials and methods *In vitro* gas production (IVGP) technique (Menke and Steingass, 1988) was used to describe the extent of gas production from the experimental SSB based complete diets with roughage to concentrate ratio of 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60 and 30: 70 on DM basis. The rumen fluid was collected from three Nellore sheep, kept on straw based ration. Rumen fluid–media mixture (inoculum) was prepared under continuous flushing with CO_2 and a 30 ml of inoculum was transferred into the pre-warmed glass syringes containing 200 mg sample of experimental SSB based complete diets and incubated in the water bath at 39^{o}C for 24h. The gas produced was recorded at 8 and 24 hours of incubation. Gas production at 24 h, corrected for blank and standards, was used for determination of OMD (Krishnamoorthy *et al*., 2005) and ME (Menke and Steingass, 1988). Partitioning factor (PF) was calculated as the ratio of substrate truly degraded and gas volume produced. The efficiency of microbial biomass production (EMBP) of experimental rations was determined by measuring the ratio of truly digested organic matter (TDOM) and gas production as described by Blummel *et al.* (1997). Feed samples were analyzed for proximate principles (AOAC, 1997) and fiber fractions (Van Soest *et al.,* 1991). Statistical analysis of the data was carried out according to Snedecor and Cochran (1994).

Results Significantly (P<0.01) higher *in vitro* gas production volume (ml) at 24 h incubation, IVOMD (mg) and ME (MJ/kg) were recorded for the rations 80R:20C to 30R:70C compared to complete rations with 100 and 90 per cent SSB proportion. Where as, TDOM was significantly (P<0.01) higher for the rations 90R:10C to 30R:70C compared to ration with sole SSB. Among all the rations, 30R:70C has shown highest (P<0.01) IVOMD, ME and TDOM and the trend observed in ME, TDOM values reflected that, as the concentrate proportion increased in the ration, these values were also proportionately increased. The partitioning factor (PF, mg/ml) values obtained were ranged from 2.79±0.01 to 3.18±0.01 for the experimental rations. The rations from 90R:10C to 30R:70C were significantly (P<0.01) higher in PF, microbial biomass production (MBP) and efficiency of microbial biomass production (EMBP) compared to the ration contained 100 per cent SSB. There was no significant difference observed for PF and EMBP among the rations from 60R:40C to 30R:70C, wherein the SSB proportion decreased from 60 to 30 per cent in the rations. Where as for MBP, no significant difference was observed in the rations from 50R:50C to 30R:70C. These diets have also shown higher IVOMD resulting in higher microbial biomass synthesis. The less intense microbial activity was reflected by low volumes of gas produced in the diets of sole SSB to 70R:30C.

Table 1 Effect of SSB to concentrate ratio in SSB based complete rations on *in vitro* gas production parameters

Ration	Gas volume (ml)	IVOMD (mg)	ME (MJ/kg)	PF	TDOM (mg)	MBP (mg)	EMBP (%)
100R:0C	42.67^{e}	93.87^{e}	8.13±0.02^{g}	2.79^{d}	119.08^{h}	25.21^{e}	21.17^{d}
90R:10C	43.00^{e}	94.60^{e}	8.27±0.04^{g}	2.94^{c}	126.60^{g}	32.00^{d}	25.28^{c}
80R:20C	44.67^{d}	98.27^{d}	8.60±0.06^{f}	2.94^{c}	131.12^{f}	32.85^{d}	25.06^{c}
70R:30C	44.83^{d}	98.63^{d}	8.74±0.05^{e}	3.06^{b}	136.95^{e}	38.32^{c}	27.98^{b}
60R:40C	47.67^{c}	104.87^{c}	9.21±0.06^{d}	3.12^{ab}	148.93^{d}	44.06^{b}	29.59^{ab}
50R:50C	48.17^{bc}	105.97^{bc}	9.37±0.04^{c}	3.18^{a}	153.21^{c}	47.25^{a}	30.84^{a}
40R:60C	49.00^{b}	107.80^{b}	9.61±0.04^{b}	3.18^{a}	155.80^{b}	48.00^{a}	30.81^{a}
30R:70C	50.17^{a}	110.37^{a}	9.90±0.06^{a}	3.16^{a}	158.43^{a}	48.07^{a}	30.34^{a}
SEM	0.57	1.24	0.13	0.04	3.39	2.27	1.15

$^{a, b,c,d,e,f,g,h}$ values bearing different superscripts in a row differ significantly (P<0.01)

Conclusions The results of the present study suggested that, sweet sorghum bagasse, an agro- industrial by product can be included from 50-60 per cent level in the complete rations of ruminants for economic production of meat, milk and wool.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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The effect of incremental levels of Sandalwood oil on methane emissions and animal performance in sheep

J Veneman[1], J R Newbold[1], H Perdok[1], W Gerrits[2], J Dijkstra[2], S van Zijderveld[1]
[1]Provimi Holding B.V., Research Centre De Viersprong, Velddriel, The Netherlands, [2]Animal Nutrition Group, Wageningen University, Wageningen, The Netherlands
Email: jveneman@nl.provimi.com

Introduction Sandalwood oil, originating from trees from the genus *Santalum*, is commonly used in the perfume industry. Sandalwood oil was selected from a large database comprising almost 2500 compounds to be one of the most promising in reducing methane production and reducing the rate of bacterial breakdown by protozoa *in vitro* (Newbold *et al*., 2010). In the current experiment the effects of feeding incremental levels of Sandalwood oil on methane production and performance in sheep were investigated.

Materials and methods Twenty male Texel crossbred lambs (32.7 ± 2.1 kg) were blocked according to body weight and, within a block, randomly assigned to one of four treatments for a period of four weeks. Diets consisted of 69% maize silage, 15% barley straw, 15% soybean meal and 1% mineral mix on a DM base. Treatments consisted of 0%, 0.18%, 0.36% and 0.72% Sandalwood oil on a silica carrier which was mixed into the soybean meal. During the first two weeks animals were housed in individual calf hutches and fed *ad libitum*. At the beginning of the third week animals were moved to individual wooden boxes to adapt to a restricted environment. In the last week the animals were placed in individual respiration chambers to determine methane production. During the third and fourth week, the amount of feed offered was limited to 95% of the feed consumed by the animal consuming the least feed within a block during the *ad libitum* feeding period. Water was available *ad libitum* throughout the experiment. Animals were weighed weekly and feed intake was measured on a daily basis. Parameters were analyzed by ANOVA with block and treatment as fixed effects using GenStat (VSN International Ltd., 2009). The data were analyzed for a linear and quadratic response.

Results Methane production per day was linearly reduced with increasing levels of Sandalwood oil (Table 1). There was a trend ($P<0.1$) for reduced methane production per kg DMI but not for methane production per kg BW. Although feed intake was restricted during the methane measurements, there was a reduction in DMI ($P<0.006$) with increasing levels of Sandalwood oil. Gain was significantly reduced with increasing levels of Sandalwood oil during the *ad libitum* fed period, but not DMI. No quadratic responses were found for any of the parameters ($P>0.5$).

Table 1 LS means of methane production (week 4), DMI and gain in sheep fed incremental levels of Sandalwood oil.

	Sandalwood oil (% of DM)					P-value
	0	0.18	0.36	0.72	s.e.d.	linear
Methane production (g/sheep/day)	17.2	17.6	15.6	15.0	1.10	0.027
Methane (g/kg DMI)	24.3	24.7	22.2	22.0	1.52	0.088
Methane (g/kg BW)	0.47	0.50	0.46	0.44	0.030	0.111
DMI (g/sheep/day, week 4)	710	715	700	679	10.3	0.004
DMI (g/sheep/day, week 1-2)	867	868	796	799	54.9	0.279
Gain (kg, week 1-2)	2.88	2.94	1.90	1.52	0.416	0.002

Conclusions Increasing levels of Sandalwood oil reduced daily methane production and tended to reduce methane per kg of DMI, but not methane production per kg of LW. Effects of Sandalwood oil on methane production may have been confounded by effects on dry matter intake and live weight gain.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Potential methane production using different feed formulation by *in vitro* rumen fermentation

S-H Kim[1], L Mamuad[1], Md. J Alam[1], Y-K Oh[3], C-O Jeon[2], S-S Lee[1]
[1]Sunchon National University, Suncheon, Jeonnam, Republic of Korea, [2]Chung-Ang University, Seoul, Republic of Korea, [3]National Institute of Animal Science, Suwon, Gyeonggi-do, Republic of Korea
Email: mhs0425@hanmail.net

Introduction Methane production from ruminants is one of the important issues in global ecology and animal nutrition. Investigating formulated diet is an efficient way to reduce methane in ruminants (Lee, 2003). Thus, this study aimed to evaluate, select and formulate diets for ruminants pertaining to methane production using *in vitro* fermentation.

Materials and methods Nineteen concentrate ingredients were grouped into three which were bran (corn gluten feed, palm kernel and wheat from China and Korea), vegetable protein (cottonseed meal, soybean meal and others) and cereals (barley, wheat and corn). These concentrates were individually evaluated for their capability to produce methane using *in vitro* fermentation. From the evaluated concentrates, feed formulation was done which was based on methane production of each concentrate ingredient in a group. They were categorized into high (corn gluten feed, soybean and perilla meal, corn), medium (corn gluten feed, rapeseed and coconut meal, wheat) and low (corn gluten feed, palm kernel, distillers dried grains and barley). The formula diets were subjected for *in vitro* fermentation to determine the methane production and its effect on digestibility. Rumen fluids were collected from cannulated Holstein cow and mixed with the buffer (Asanuma and Hino, 2000) for the basal medium. Each serum 50ml bottle contains one percent DM of each diet. Then, 25 ml basal medium was dispensed and capped anaerobically under O_2-free N_2 gas. After that, all were immediately transferred to the shaking incubator (39°C, 80rpm) for 0, 2, 4, 8, 12, 24 and 48 hrs of incubation. Total gas production, methane, carbon dioxide, VFA concentration and pH values were determined after completion of incubation.

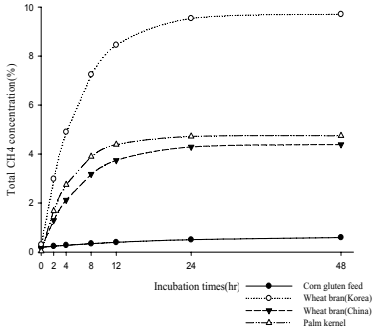

Figure 1 Brans methane concentrations at different incubation period

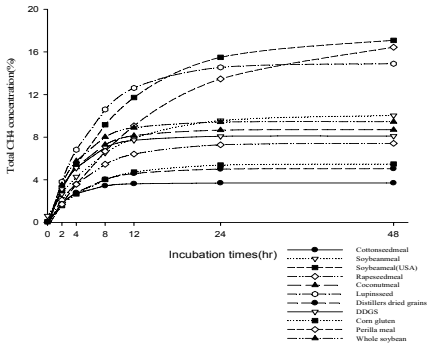

Figure 2 Vegetable protein methane concentration at different incubation period

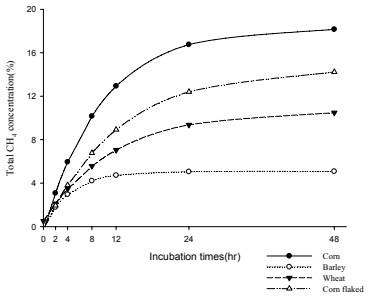

Figure 3 Cereals methane concentration at different incubation period

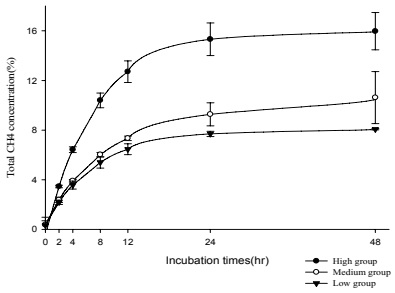

Figure 4 Formulated diet methane production at different incubation period

Results Lowest total gas production ($P<0.05$) was found in wheat (Korea) for brans, cottonseed meal for vegetable protein and barley for cereals (data not shown). Moreover, lowest methane production ($P<0.05$) was found in corn gluten feed for brans (Fig. 1), cotton seed meal for vegetable protein (Fig. 2) and barley for cereals (Fig. 3). On the other hand, lowest carbon dioxide production was found in wheat (China) for bran, distillers dried grains for vegetable protein and barley for cereals (data not shown). For VFA production, acetic acid was found highest ($P<0.05$) in wheat bran, soybean meal and wheat cereals while butyric acid was found highest ($P<0.05$) in wheat bran, corn gluten and corn cereals, and propionic acid was found highest ($P<0.05$) in wheat bran, lupin seeds and wheat cereals (data not shown). pH values were gradually decreased from 0 to 72h incubation and not differed among the same group (data not shown). For formulated diet *in vitro* fermentation, methane production is directly proportional to the formula diet.

Formula diet categorized with high methane production at the first study confirms to produce also high methane production (Fig. 4). In addition, methane production showed inversely proportional to total digestible nutrient (TDN) wherein the higher the methane production and the lower the TDN (data not shown).

Conclusion It is concluded that total gas production is directly proportional to carbon dioxide and methane production wherein as the total gas production becomes higher, the carbon dioxide and methane also becomes higher and vice versa. Moreover, formulated diet is also directly proportional to methane production wherein the high formulated diet produced higher methane and vice versa. In addition, total digestibility and methane production is inversely proportional to each other wherein as the methane production becomes higher, the total digestibility becomes the lower and vice versa. This research is currently under investigation.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effects of stearidonic acid-rich Echium oil on methane production in a RUSITEC system

P Amaro[1], M R G Maia[1], R J Dewhurst[2], A J M Fonseca[1], A R J Cabrita[3]
[1]REQUIMTE, ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Campus Agrário de Vairão, Rua Padre Armando Quintas, 4485-661 Vairão VC, Portugal, [2]Teagasc, Animal & Grassland Research and Innovation Centre, Grange, Dunsany, County Meath, Ireland, [3]REQUIMTE, Departamento de Geociências, Ambiente e Ordenamento do Território, Faculdade de Ciências, Universidade do Porto, Campus Agrário de Vairão, Rua Padre Armando Quintas, 4485-661 Vairão VC, Portugal *Email: rita.cabrita@mail.icav.up.pt*

Introduction Strategies to reduce ruminal methane production are needed both to decrease the negative impact of ruminant production on the environment and to increase feed utilisation. Polyunsaturated fatty acids are known to decrease methane production; earlier studies have mainly used C18:3 n-3 or C18:2 n-6, a more recent study (Amaro *et al*., 2011) showing that stearidonic acid (C18:4 n-3; SDA) decreased methane proportion without negatively affecting fermentation parameters *in vitro*. This fatty acid has only been identified in a small number of plant families (mainly Boraginaceae and Primulaceae), one of the richest sources of SDA known being Echium oil (up to 15% SDA; Guil-Guerrero, 2007). Therefore, the present study aimed to evaluate the effect of Echium oil supplementation on methane production in RUSITEC, a long-term fermentation system.

Methods The RUSITEC system had eight independent fermentation vessels placed in a water-bath at 39ºC and artificial saliva buffer solution (McDougall, 1948) continually infused at a dilution rate of 4.5% h^{-1}. Strained ruminal fluid (300 mL) obtained from three dairy cows at a slaughter house was incubated with 500 mL of saliva and a nylon bag containing 12 g dry matter (DM) of a commercial dairy total mixed ration (TMR). After a five-day adaptation period, four treatments were allocated to each of two fermenters for the next seven days. Treatments comprised TMR with: no supplementation (control, C); 5mM of bromoethanesulfonic sodium salt (BES; methanogens-specific inhibitor); 4% Echium oil (EO4), and 8% Echium oil (EO8). Total gas and methane production, volatile fatty acid (VFA) and ammonia (N-NH3) concentrations, pH, redox, and DM and neutral detergent fibre (NDF) digestibility were measured. Total DNA extraction was performed in freeze-dried effluent and feed residues (after incubation), using a bead beater method. To quantify methanogens and total bacteria PCR primers were used to target DNA sequences and a qPCR SYBR Green assay was performed. Results were analyzed using the mixed procedure of SAS (SAS Institute, Inc., Cary, NC), with the measurements from the adaptation period used as covariates. Orthogonal contrasts were constructed in order to compare treatments.

Results The Echium oil contained 15% of C18:4 n-3. Total gas production was significantly reduced by BES and EO8 treatments. Compared to the control, methane proportion was significantly decreased for all treatments. However, when expressed as mmol per g of DM incubated, only BES and EO8 significantly decreased methane production. Total VFA, DM and NDF digestibility and pH were not affected by any of the treatments. Ammonia concentration and redox were significantly reduced by BES addition. The acetate: propionate ratio (C_2:C_3) was significantly reduced by the EO8 treatment. BES and EO8 did not affect the total microbial population, but decreased the proportion of methanogens.

Table 1 Effect of Bromoethanesulfonic sodium salt and Echium oil supplementation on ruminal fermentation parameters.

	Treatments				SEM	Contrast			
	C	BES	EO4	EO8		C vs BES	C vs EO4	C vs EO8	EO4 vs EO8
Total gas volume, mL	2110	1773	2311	1679	123.1	0.054	0.256	0.015	0.001
CH_4,%	6.06	0.99	4.64	2.23	0.49	<0.001	0.005	<0.001	<0.001
CH_4, mmol/g DM	0.450	0.049	0.423	0.108	0.0561	<0.001	0.654	<0.001	<0.001
pH	6.67	6.68	6.68	6.71	0.023	0.583	0.711	0.221	0.392
Redox, mV	-322	-310	- 322	-321	2.5	0.001	0.997	0.772	0.777
Digestibility DM, %	75.9	74.7	74.7	75.3	1.74	0.656	0.635	0.820	0.804
N-NH_3, mg/mL	130	105	139	122	6.4	<0.001	0.234	0.226	0.018
Total VFA, mM	65.9	62.5	68.2	69.2	4.12	0.570	0.698	0.579	0.861
C_2:C_3	2.38	2.32	2.24	1.94	0.103	0.646	0.292	0.002	0.035
ΔCt effluent	1.96	0.33	2.27	0.69	0.252	0.001	0.409	0.004	0.001
ΔCt feed residues	1.38	0.29	1.01	0.75	0.195	0.002	0.212	0.043	0.359

C- Control (no supplementation); BES - Bromoethanesulfonic sodium salt 5mM; EO4- Echium oil 4%; EO8- Echium oil 8%;
ΔCt-Methanogens estimated as a proportion of total rumen prokaryotes ($2^{-(Ct\ methanogenes\ -Ct\ total\ bacteria)}$) x 10^3.

Conclusions Echium oil supplementation decreased methane production and the methanogen population at the 8% level of inclusion. Effects were obtained without negatively affecting rumen fermentation parameters. Thus, supplementation with Echium oil, one of the richest sources of stearidonic acid, is a promising strategy to reduce methane production.

Acknowledgments P. Amaro and M.R.G. Maia gratefully acknowledge scholarships from FCT, Portugal (SFRH/BD/47857/2008 and SFRH/BDP/70176/2010, respectively). The authors gratefully acknowledge funding provided by Projectos em Co-Promoção QREN – Sistema de Incentivos à Investigação e Desenvolvimento Tecnológico (SI I&DT); Projecto N.º 5343, LEITE SAUDÁVEL.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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The effect of different physical forms of rapeseed as fat supplement on rumen NDF digestion and methane emission in dairy cows

M Johannes, P Lund, M R Weisbjerg, A L Hellwing, T Hvelplund
Aarhus University, Foulum, Denmark
Email: maike.johannes@agrsci.dk

Introduction Fat has been reported as a promising feed component to reduce enteric methane (CH_4) production. However, fat can also reduce fibre digestibility in the rumen. Nutritional strategies to reduce CH_4 output are therefore associated with decreased feed intake or feed digestibility and any effect on methane production should therefore be related to the concurrent effect on e.g. intake, digestibility and milk production. Fat can be added to the diet in different forms. Rapeseed meal and rapeseed cake are by-products of the plant oil industry and common feedstuffs in Denmark, but it is also possible to feed whole seeds or oil. The release pattern in the rumen is related to the physical structure of feed. Purified oil is freely available whereas fat in rapeseed cake and whole seeds is stored intracellular so that cell walls need to be degraded before the fat is liberated. The purpose of this experiment was to study if the physical form of rapeseed fat has an impact on its methane-reducing properties and on feed digestion.

Materials and Methods The experiment was conducted as a 4 x 4 Latin square design with four rumen, duodenum and ileum fistulated Holstein dairy cows (1 primiparous and 3 multiparous) and four diets. The cows were 193 days in milk (sd 67) and had a milk yield of 28.7 kg (sd 7.2 kg). The diets were a control diet with rapeseed meal with 4% fat and three diets supplemented with rapeseed cake (RSC) with17% fat, whole crushed rapeseed (WCR) with 49% fat and rapeseed oil (RSO), respectively. In the diets, the fat content was 3.5% in control, RSC 5.4%, WCR 6.2% and RSO 6.5%. The fat-free rapeseed content was constant for all diets. Forage percentage of the diet was 50% with maize and grass silage (maize:grass 46%:54%). All compositions refer to DM. The experiment consisted of four periods; during the third week of every period, samples were taken from the different fistulae in order to determine digestibility where chromic oxide (Cr_2O_3) was used as flow marker (2 x 10 g/d). Methane production was measured in four open-circuit respiration chambers during the fourth week of the period. Samples were analysed for ash-free NDF in a Fiber-Tec system according to Mertens (2002). Gross energy (GE) in feed samples was determined by bomb calorimetry. The data was analyzed using the SAS proc GLM procedure with cow, treatment and period as effects.

Results Additional fat reduced methane production per kg DM intake and as proportion of GE intake by 11% (P=0.02) and 14% (P=0.01), respectively. Feed intake and NDF intake were numerically reduced for RSO but this did not reach significance probably due to cow treatment interactions, although this cannot be documented. Neither total tract nor rumen digestibility of organic matter (OM) or NDF were affected by treatment. Relating CH_4 emission to total tract digestibility resulted in a tendency for less CH_4 per kg digested OM (P=0.09) for fat supplemented vs. control diet. There was still a numerical difference in CH_4 per kg digested NDF, but this did not reach significance (P=0.19). Methane production per kg digested OM or NDF was lowest for RSC and WCR diets while RSO values were closer to the control diet. However, the contrasts did not show a significant difference between RSC and WCR vs. RSO.

Table 1 Effect of the different rapeseed feeds on CH_4 production and digestion

	Diets					Contrasts P-value[1]		
	Control	RSC[2]	WCR[2]	RSO[2]	SEM	2	3	4
DM intake kg	18.3	18.9	17.9	16.1	1.63	0.33	0.24	0.42
NDF intake kg	6.1	6.2	5.8	5.3	0.61	0.57	0.33	0.61
Ruminal NDF digestibility	61.7	65.5	62.7	63.9	1.44	0.15	0.90	0.14
Ruminal OM digestibility	42.5	44.6	38.1	37.8	2.61	0.51	0.61	0.07
Total NDF digestibility	61.7	63.4	60.8	60.4	1.96	0.53	0.25	0.39
Total OM digestibility	73.2	74.3	72.0	71.5	1.28	0.60	0.19	0.44
CH_4 L/DM intake	29.6	26.9	25.8	26.2	1.04	0.02	0.94	0.37
CH_4 % of GE	6.3	5.6	5.3	5.4	0.24	0.01	0.77	0.32
CH_4/kg digested OM	46.1	40.3	39.8	42.9	2.60	0.09	0.38	0.87
CH_4/kg digested NDF	154	135	137	147	10.8	0.19	0.42	0.85

[1]Contrasts 2=control vs fat diets, 3=RSC and WCR vs RSO, 4=RSC vs WCR, [2]RSC rapeseed cake, WCR whole crushed rapeseed, RSO rapeseed oil

Conclusions The results confirm that fat supplementation has a certain potential to reduce enteric CH_4 production per kg feed intake and per kg milk without affecting OM rumen digestibility negatively. The physical form of fat did not influence the methane reducing effect of rapeseed fat.

Acknowledgments The work was founded by the Danish Ministry of Agriculture and the Danish Cattle Federation

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effect of decreasing phosphorus intake and structure value on intake, milk production and phosphorus status of dairy cows

W van Straalen[1], B Tas[1], I Kok[1], T Schonewille[2]
[1]Schothorst Feed Research, Lelystad, The Netherlands, [2]Faculty of Veterinary Medicine, Department of Farm Animal Health, Division of Nutrition, Utrecht, The Netherlands
Email: btas@schothorst.nl

Introduction In dairy cow diets phosphorus (P) content generally exceeds requirements resulting in a high phosphorus output to the environment. Phosphorus is an essential mineral for production and health of the dairy cow and its rumen microbes. A reduced dietary P intake results in a higher P efficiency but also in a lower recycling of P via saliva and this might reduce fermentation rate and microbial growth in the rumen. More structure in the diet may stimulate rumination activity and hence flow of saliva P to the rumen. In this study, the effect of a low and high phosphorus level in combination with a low and high structure value on intake, milk production and P status of dairy cows in mid lactation is determined.

Materials and methods The experimental design was a 2x2 factorial design with a low P (LP = 2.8 g/kg DM) and high P (HP = 3.5 g/kg DM) level in combination with low structure (LS = low alfalfa) and high structure (HS = high alfalfa) in the diet. The experiment lasted 10 weeks with a pre-period of 3 weeks and a main period of 7 weeks. Forty eight dairy cows in mid lactation were used and assigned based on parity, days in milk and milk production in pre-period to one of the four treatments. The difference in P level was obtained by feeding two concentrate feeds with a low P level (2.1 g/kg) and high P level (3.5 g/kg) with almost the same ingredient composition but addition of 0.62% monocalcium phosphate. Two basal diets (HS and LS) were fed consisting of 18.5% vs. 29.6% grass silage, 59.1% corn silage, 14.8% vs. 3.7% dried alfalfa, 7.3% bypass soybean meal and 0.3% feed urea. Daily feed intake, milk production and composition was measured. In week 3 and 10, apparent DM and P digestibility was determined by marker administration in concentrate feed (1% chromium oxide) and faecal spot sampling (three times a day, two days). P content in saliva, rumen fluid and plasma were determined. by sampling saliva and blood in week 3, 5, 8 and 10, and rumen fluid with an oral probe in week 3 and 10. Data were analysed with data in pre-period as covariate and main effects Block, Phosphorus and Structure level and interaction of P x S using GenStat 10.2.

Results Phosphorus and structure level did not affect DM intake, milk production and apparent DM digestibility (Table 1). Milk protein and lactose contents were lower on diets with low P. High structure increased protein but decreased lactose content. Apparent DM digestibility did not differ among treatments. Phosphorus intake and excretion in faeces were 10 to 16 g/d higher of HP than LP, whereas excretion of P in milk was similar (assuming 1 g P/kg milk). Apparent P digestibility ranged from 36% on HP to 46% on LP. The P balance was negative and tended to be lower for the high phosphorus levels. P content tended to be lower in saliva and rumen fluid of the LP than HP diets. Plasma P content was lower of LP than HP, but remained above levels of P deficiency (1 mmol/L). The LPHS diet had the lowest P contents. More structure in the diet did not result in a difference in P content in saliva and rumen fluid as was hypothesised by increased rumination activity. Maintaining the P content in the rumen by recycling of P via saliva for microbial growth seems to be prioritised above plasma P content.

Table 1 Effect of low (L) and high (H) phosphorus (P) and structure (S) level in the diet on DM intake, fat and protein corrected milk production (FPCM), DM digestibility, P intake, excretion in faeces and milk, balance, and P content in saliva, rumen fluid and plasma

	Pre-period	Treatment				lsd[1]	Significance		
		HPHS	HPLS	LPHS	LPLS	P<0.05	P	S	PxS
DM intake (kg DM/d)	23.9	23.9	24.4	24.1	23.6	0.96	0.382	0.994	0.086
FPCM (kg/d)	37.9	36.0	36.6	37.1	36.1	1.19	0.409	0.549	0.041
Fat (%)	3.68	3.93	3.84	3.88	3.92	0.20	0.828	0.703	0.319
Protein (%)	3.18	3.23	3.33	3.20	3.25	0.08	0.080	0.015	0.304
Lactose (%)	4.70	4.70	4.66	4.67	4.64	0.03	0.031	0.005	0.941
DM digestibility (%)	65.3	63.1	64.7	65.1	66.3	4.20	0.167	0.167	0.749
P intake (g/d)	77.9	79.1	82.1	67.8	66.6	3.19	<0.001	0.431	0.054
P faeces (g/d)	49.9	51.9	51.7	36.5	40.1	7.75	<0.001	0.886	0.710
P milk (g/d)	37.5	35.2	34.7	35.7	34.3	2.09	0.812	0.621	0.001
P balance (g/d)[2]	-11.1	-6.2	-5.7	-4.0	-6.2	5.53	0.102	0.927	0.794
Saliva P (mmol/L)	7.90	8.62	9.11	6.57	8.82	2.42	0.097	0.110	0.264
Rumen fluid P (mmol/L)	5.16	6.69	6.62	4.83	6.01	1.93	0.055	0.440	0.317
Plasma P (mmol/L)	1.52	1.96	1.97	1.50	1.62	0.34	0.002	0.596	0.620

[1] lsd = least significant difference; [2] P balance = P intake - P faeces - P milk

Conclusions Feeding a low phosphorus content of 2.8 g/kg DM during 7 weeks to dairy cows in mid lactation did not result in a lower DM intake or milk production, although and milk protein content was numerically and lactose content was significant lower. Lower dietary P contents resulted in decreased excretion of P in faeces and 10% higher apparent P digestibility, lower plasma P contents and a tendency to lower P contents in saliva and rumen fluid.

https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Methane emissions using taniferous native plants in ruminal fermentation *in vitro*

E Andrade-Rivero, O A Castelán-Ortega, A R Martinez-Campos, J G Estrada-Flores
Universidad Autonoma del Estado de Mexico, Estado de Mexico, Mexico
Email: jgestradaf@uaemex.mx

Introduction Methane (CH_4) is emitted during microbial ruminal fermentation from carbohydrates in rumen. Currently, feeding strategies are being developed for minimizing these CH_4 emissions. The use of forages rich in condensed tannins (CT) in ruminant diets is a strategy that has served to mitigate CH_4 emissions; however, the way in which such compounds act in the rumen has not been clarified. Tavendale *et al*. (2005) report that using forage with high tannin content may reduce methane emissions. The aim of this experiment was to evaluate the effects of *in vitro* ruminal fermentation when native plant species containing condensed tannins (CT) from the southern part of the State of Mexico are added to a diet.

Materials and methods A base diet was used, with 60% forage (*Cynodon plectostachyus*) and 40% concentrate (López-González *et al*., 2010). Three inclusion levels [low level (-1), middle level (0) and high level (1)] were added to the follow species in the base diet: condensed tannin of quebracho (CTQ) extract (*Schinopsis lorentzii*) (1%, 2% and 3%); oregano oil (OO) (*Lippia graveolens*) (450, 500 and 550 ppm), and the native species *Mimosa diplotricha* (0.5, 1.5 and 2.5% of CT) and *Tagetes erecta* (0.5, 1.5 and 2.5% of CT). Total tannins (TT) and condensed tannins (CT) (Makkar, 2003) of *T. erecta* and *M. diplotricha* were obtained. The Theodorou *et al*. (1994) *in vitro* gas production technique was used to obtain methane gas production at 8, 16, 24, 32, 40 and 48 hours. A 100µl of gas was obtained from the bottles and transferred to SRI chromatograph. The FID temperature was 170°C and 130°C for the oven, and the carrier gas used was nitrogen with a flux of 25ml/min. An external standard was used with a mix of CO, CO_2, CH_4, C_2H_6, C_2H_4, C_2H_2 and N_2 in a 1% concentration. Three samples of each species and each treatment were used as replicates. A completely randomized experimental design was used, in which the treatments were the inclusion levels (-1, 0, 1), and the variable analyzed was CH_4 production. Results were analyzed using analysis of variance and expressed in means with their respective standard errors. The statistical differences between the means ($P<0.05$) were determined through a Tukey test; the Minitab Statistical Package (version 14) (2000) was used.

Results Table 1 shows the effects of Diet+*Mimosa* and Diet+*Tagetes*; significant differences were obtained in CH_4 production in diet+*Mimosa* at 16h ($P<0.01$), in diet+*Tagetes* at 8h ($P<0.05$) and 16 h ($P<0.01$). In both cases total methane production was reduced in comparison with the base diet. *M. diplotricha* had 4.3% of CT and 5.2% of TT, whereas *T. erecta* contained 1.3% of CT despite its TT content (7.3%). Significant effects ($P<0.05$) were obtained when OO was included in the diet, because methane was reduced at 8 (0.5mlg/MS) and 40 hours (0.2mlg/MS), and in the total methane production, especially at the highest inclusion level (27.3 mlg/MS at the lowest level *vs* 5.0 mlg/MS at the highest level). In this case, the inclusion of OO acts as a bactericide, especially at the highest level of inclusion, due to the drastic reduction of methane. These results show that methane emissions may diminish in an *in vitro* system using plants with tannin contents.

Table 1 Effect of the treatment on methane production (ml/g MS) in *in vitro* fermentation

	Base diet	Diet + *Mimosa*					Diet + *Tagetes*				
Time (h)		-1	0	1	SE	P	-1	0	1	SE	P
8	11.9	10.2	7.8	6.6	1	NS	8.1[a]	10.7[a]	2.8[b]	1.7	*
16	13.6	1.4[b]	9.3[a]	13.9[a]	1.3	**	6.2[b]	12.0[b]	21.4[a]	2.2	**
24	23.8	13.8	13.1	14.9	1.9	NS	10.6	11.9	16.5	3.4	NS
32	4.5	2.1	4.1	5.4	1.7	NS	5	2.7	4.5	1.8	NS
40	6.0	5.7	5.8	5.5	1.9	NS	3.2	3.5	8.1	2	NS
48	3.6	5.2	4.3	2.5	1.3	NS	5.8	3.7	1.9	1.2	NS
Total	63.9	38.3	42.3	48.9	3.2	NS	39	44.5	55.3	5.5	NS

[abc] Different letters in the same column means significant differences ($p<0.05$); NS, Not significant; *($p< 0.05$); **($p<0.01$); -1: low, 0: middle, 1: high inclusion levels.

Conclusions Native plants containing tannins and included in a diet with different inclusion levels may reduce methane emissions in ruminal fermentation.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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The role of genetic improvement for productivity traits in reducing enteric methane relative to the amount of lamb carcase produced from the Welsh national sheep flock

C Nakielny[2], J Roden[1], C J Newbold[1]
[1]The Institute of Biological, Environmental and Rural Sciences, Aberystwyth, Ceredigion, UK, [2]KN Consulting, Llandeilo, Carmarthenshire, UK
Email: catherine@knconsulting.co.uk

Introduction Reducing enteric methane emissions is one of the key challenges currently facing the sheep sector and requires a number of different approaches. Genetic improvement is one approach that could provide long term, cost effective solutions as a result of the permanent and cumulative changes achieved. These genetic changes could result in reductions in enteric methane emissions through improvements in production and reproductive efficiency within existing sheep production systems. However, whilst it has been suggested that genetic improvement in livestock could help play a role in decreasing greenhouse gas emissions on a UK wide basis (Wall *et al*, 2008) the relative importance of this approach in reducing methane emissions in sheep production in Wales has not yet been quantified. The aim of this study was to provide estimates of likely changes in methane emissions as a consequence of current genetic improvement programmes and to identify opportunities for decreasing emissions from the Welsh sheep flock while also improving economic efficiency.

Materials and methods A model, written in MS Excel, was developed to estimate the changes in methane production per kg of carcase produced that would be expected as a result of the genetic improvement resulting from the current breeding programmes available to Welsh sheep producers. The role of genetic selection for single traits associated with increased production was also considered. The effect of the likely genetic improvement throughout the stratified structure of the sheep sector was modelled for a self-replacing hill flock; an upland flock in which hill ewes are mated to a crossing sire to produce crossbred ewes and a lowland flock in which crossbred ewes are mated with a terminal sire. Genetic improvement within each of a hill breed, a crossing sire breed and a terminal sire breed was modelled over a 20 year period using gene flow techniques. For each of the twenty years the mean breeding values of flock rams, flock ewes, ewe hogs (replacement females for the breeding flock), lambs and rams for sale as breeding stock were calculated for each trait, taking into account the age structure of each class of livestock. The appropriate breeding values for each stock class and breed were then used to calculate mean performance in a model of flock performance. The effects of heterosis on ewe and lamb performance were incorporated where appropriate. Methane emissions from enteric fermentation were estimated using the Tier 2 methodology published by the Intergovernmental Panel on Climate Change (IPCC 2006). Energy requirements were estimated for four different classes of stock: ewes, rams, ewe hogs and lambs. Data relating to breed and industry structure was drawn from the Welsh Sheep Strategy breed survey of 2000, the national sheep breed survey of 2003 (Pollott and Stone, 2004) and the most recent estimates of the ewe numbers in Wales (Welsh Assembly Government, 2010).

Results The results showed that genetic improvement programmes for terminal sire breeds are expected to result in a significant decrease in methane emissions per tonne of carcase produced. Genetic improvement of hill and crossing sire breeds is expected to result in slight decreases in methane emissions. Across the industry as a whole genetic improvement in all breed types combined is expected to result in a decrease of 0.03% per year in methane emissions per kg carcase produced. If correlated changes in ewe weight are restricted a greater annual reduction in methane emissions of 0.08% per year is expected. The potential benefits of genetic improvement of both prolificacy and ewe longevity were significant in terms of reducing methane emissions in all sectors of the industry. Across the industry as a whole the potential decrease in methane emissions is 0.6% to 0.7% per year if selection was based on prolificacy. The numerical dominance of hill ewes in the national flock means that selection for prolificacy in hill breeds contributes most to reductions in emissions across the industry but genetic improvement in this trait in the crossing breeds also has a significant effect, potentially contributing an annual reduction of 0.08% in methane per tonne of carcase produced. Genetic improvement of carcase quality by selection on traits such as muscle depth is also expected to have a positive effect on reducing emissions in hill flocks due to correlated improvements in carcase weight and therefore result in an annual reduction in methane emissions per kg carcase in both hill and lowland flocks. Genetic improvement of lamb survival is expected to be low in all flocks because of the low heritability of this trait, but nevertheless it is expected to result in a small annual decrease in methane emissions in lowland flocks.

Conclusions Current genetic improvement programmes used in the Welsh sheep industry are expected to achieve small but significant reductions in methane emissions if widely applied across the industry. Of the single traits examined in this study prolificacy, ewe longevity and muscle depth (through its correlated effect on carcase weight) are most likely to have a beneficial effect on methane emissions through genetic selection or breed substitution.

Acknowledgements This project was funded by the Rural Development Plan for Wales 2007 - 2013 and carried out on-behalf of Hybu Cig Cymru – Meat Promotion Wales.

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Evaluation of volatile fatty acid stoichiometry models to predict enteric methane production in lactating Holstein cows

J Dijkstra[1,3], A Bannink[2], J Ellis[1,3], J France[3], E Kebreab[4], Y Morvay[1]
[1]Animal Nutrition Group, Wageningen University, Wageningen, The Netherlands, [2]Livestock Research, Animal Sciences Group, Wageningen University and Research Centre, Lelystad, The Netherlands, [3]Centre for Nutrition Modelling, Department of Animal and Poultry Science, University of Guelph, Guelph, Canada, [4]Department of Animal Science, University of California, Davis, USA
Email: jan.dijkstra@wur.nl

Introduction The type of volatile fatty acid (VFA) formed in the rumen depends on the type of substrate fermented, the microbial population, and the rumen fermentation conditions (Bannink *et al.* 2008). Changes in VFA molar proportions are related to enteric methane production. In the last few decades, a number of models predicting rumen fermentation stoichiometry have been developed. The aim of the present study was to evaluate methane production calculated from rumen VFA stoichiometry coefficients using an independent dataset of lactating Holstein cow digestion trials.

Materials and methods Six VFA prediction models were independently evaluated, *viz*. Murphy *et al.* (1982), Argyle and Baldwin (1988), Friggens *et al.* (1998), Sveinbjörnsson *et al*. (2006), Bannink *et al.* (2006) and Bannink *et al.* (2008). A dataset consisting of 101 treatments from 24 peer-reviewed publications was collected. All papers reported full chemical composition, rumen pH and rumen VFA composition. The data and results of the VFA evaluations are described by Morvay *et al.* (2011). Methane (mmol/mol VFA) was calculated based on stoichiometric principles, assuming complete hydrogen recovery, for observed and predicted VFA profiles. Model errors were determined by root mean squared prediction error (RMSPE). In addition, the accuracy and precision of the models were evaluated using concordance correlation coefficient (CCC) analysis. CCC values range from -1 to +1, where values closer to +1 indicate a more precise and accurate model.

Results Mean methane production was accurately predicted by all models, except for the model of Sveinbjörnsson *et al*. (2006) (Table 1). The largest difference in mean predicted value between the other models was 13% of observed mean (Argyle and Baldwin, 1988 compared with Friggens *et al*., 1998). The predictive performance (RMSPE; CCC) was highest for the models of Bannink *et al*. (2006) and Bannink *et al*. (2008), lowest for the model of Sveinbjörnsson *et al*. (2006), with the other models in between. Using the pH dependent stoichiometry of Argyle and Baldwin (1988) compared with the original derivation by Murphy *et al*. (1982), and that of Bannink *et al.* (2008) compared with the original of Bannink *et al*. (2006), did not result in an improvement in the prediction of methane production.

Table 1 Evaluation of the predictive performance of VFA stoichiometric models for methane production (mmol/mol VFA). RMSPE, root mean square prediction error (% of mean observed); ECT, error due to bias (% of MSPE); ER, error due to deviation of the regression slope from 1 (% of MSPE); ED, random error (% of MSPE); CCC, concordance correlation coefficient; C_b, bias correction factor; ρ, Pearson's correlation coefficient; μ, location shift. Observed mean methane production is 303 mmol/mol VFA.

	Murphy *et al.* (1982)	Argyle and Baldwin (1988)	Friggens *et al.* (1998)	Sveinbjörnsson *et al*. (2006)	Bannink *et al.* (2006)	Bannink *et al.* (2008)
Mean	289	283	321	240	297	296
RMSPE	11.7	12.2	10.8	23.9	8.6	8.9
ECT	16.5	29.2	28.7	77.2	5.4	7.3
ER	3.9	4.0	0.4	7.1	1.4	0.0
ED	79.5	66.8	70.9	15.7	93.1	92.7
CCC	0.51	0.52	0.56	0.31	0.69	0.68
C_b	0.88	0.84	0.80	0.46	0.91	0.93
ρ	0.57	0.62	0.70	0.67	0.76	0.74
μ	0.43	0.57	-0.56	1.52	0.19	0.22

Conclusions The VFA stoichiometry models evaluated varied considerably in their precision in predicting methane. The model of Bannink *et al*. (2006) and Bannink *et al*. (2008) showed improved predictive performance over the models of Argyle and Baldwin (1988), Friggens *et al*. (1998), Murphy *et al*. (1982) and Sveinbjörnsson *et al*. (2006).

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effects of lipid sources in steers performance and methane emission finished in feedlot

I P Carvalho de Carvalho[1,2], G Fiorentini[1,2], T T Berchielli[1,2], A Berndt[4], R Frighetto[3], R C Canesin[1,2], J F Lage[1,2]
[1]FCAV-UNESP, Jaboticabal, São Paulo, Brazil, [2]FAPESP, São Paulo, São Paulo, Brazil, [3]Embrapa Meio Ambiente, Jaguariúna, São Paulo, Brazil, [4]Embrapa Pecuária Sudeste, São Carlos, São Paulo, Brazil
Email: ttberchi@fcav.unesp.br

Introduction One important aspect in the beef cattle industry refers to the enteric methane production, and this issue may represent losses that range between 4 and 12% of total gross energy intake (IPCC, 1996). Thus, the objective of this was to evaluate the effect of the different lipid sources in enteric methane production.

Materials and methods Twenty Nellore steers (average body weight at the experiment 471.6 ± 28.08 kg and 18 months age) were feed with 60% corn silage and 40% concentrate, with 7.0% of ether extract on total diet. The lipid sources were: soybean grain, by pass fat (Lactoplus®), linseed oil and palm oil plus a control treatment, without additional fat. The supplements were based in corn and soybean meal. The animals were housed in individual stalls, weighed for 90 days (every 30 days) and slaughtered at 497.96 kg. The animals were assigned to a completely randomized design, with five treatments and four replications.The methane emission was evaluate using the SF6 tracer gas technique (Johnson *et al*., 1994; Westberg *et al*., 1998, adapted by Primavesi *et al*. 2004). Permeation tubes with known release rates for SF6 were placed in the rumen, 72 hours prior to the start of gas sampling. Each tube was charged with 500-600 mg of SF6 and incubated at 39°C for calibration. Release rates of SF6 were determined by measuring the weight loss trough the tubes for 6 weeks to establish a steady pre-determined rate. Release rates of the permeation tubes used in this study ranged from 930 to 1600 ng min^{-1}. Gases exhaled from the nose and mouth were drawn into pre-evacuated collecting tubes, through a capillary tubing. The collecting system was designed to deliver half of its volume during a 24h collect, ensuring an uniform collecting rate. Four consecutive 24h gas samples were collected from each animal. The fixed effect was the treatment. The methane emission and average daily gain were analysed using the Tukey test, with 5% probability.

Results There was statistical effect (P<0.05) of different lipid sources on the average daily gain (ADG), methane emission expressed in kilogram of methane emitted per year (kg $CH_4.yr^{-1}$), gram of methane emitted per day (g $CH_4.day^{-1}$) and gram of methane emitted per day per kilogram of metabolic body weight (g $CH_4.day.MBW^{-1}$) in steers finished at feedlot. The ADG was higher in animals fed with the control and bypass fat when compared to other treatments. The treatments kg $CH_4.yr^{-1}$, g $CH_4.day^{-1}$, and $CH_4.day.MBW^{-1}$ where fat sources were added to the basis of unsaturated fat acids had lower methane emissions per year. However the kilogram of methane emitted per kilogram of carcass produced (kg CH_4.kg CAR^{-1}) did not differ (P>0.05) among the treatments in any unit expressed, although there was a tendency (P=0.070) for the treatments with linseed oil and soy bean showed be more efficient than others.

Table 1 Average daily gain and methane emission of steers finished in feedlot

Variables	Treatments					P[1]	CV[2] (%)
	Control	Palm oil	Linseed oil	By pass fat	Soy bean		
ADG	1.15 [a]	0.36 [c]	0.79 [b]	1.03 [a]	0.86 [b]	0.040	11.2
kg $CH_4.yr^{-1}$	53.75 [a]	24.37 [b]	22.91 [b]	40.39 [ab]	23.35 [b]	<.001	24.2
g $CH_4.day^{-1}$	147,25 [a]	66.70 [b]	62.77 [b]	110.65 [ab]	63.98 [b]	<.001	24.8
g $CH_4.day.MBW^{-}$	1.40 [a]	0.68 [b]	0.63 [b]	1.01 [ab]	0.66 [b]	<.001	23.0
kg CH_4.kg CAR^{-1}	0.22	0.29	0.16	0.20	0.17	0.070	22.4

Means within each variable followed by different letters differ by Tukey test (P < 0.05).
ADG = average daily gain; BW= body weight; MBW = metabolic body weight; CAR= carcass
[1] Probability; [2] Coefficient of Variation

Conclusions Lipid sources with 7.0% of ether extract on total diet were effective in reduced the emission of methane. Results of methane emissions expressed in kilogram of dry matter and energy consumed per day are important and necessary in the interpretation and discussion of results.

Acknowledgements Bellman® Animal Nutrition**;** FAPESP

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Potential differences on methane emissions between lactating suckler cows of different breeds grazing extensive diverse pastures

P Ricci, C Umstätter, A Waterhouse
SAC, Edinburgh, UK
Email: patricia.ricci@sac.ac.uk

Introduction Breeds differ on their live weight (LW), energy use efficiency and performance (AFRC, 1993). In addition, many studies have demonstrated breed differences in grazing intake and foraging behaviour in cattle on rangelands and semi-natural pastures. Other authors have considered potential variation in methane (CH_4) due to difference in performance, live weight, but have not considered the capacity of different breeds to modify their diet, its digestibility or to modify requirements through different activity patterns in their foraging behaviour. This study aims to assess potential differences in CH_4 emissions among breeds of free ranging cows and calves.

Materials and methods A database from a large scale 4 year study of grazing behaviour (Umstatter *et al*., unpublished) containing LW, performance (Body weight change– BWC, pregnancy and lactation) of lactating, pregnant spring calving cows wearing GPS collars with integrated activity sensors of three diverse breed types (Aberdeen Angus cross Limousin (AxL, n = 44), Charolais (CH, n = 45) and Luing (LUI, n = 42)) grazing extensive diverse hill pastures during summer periods was used to predict cow metabolic energy requirements (ME) (AFRC, 1993). Hill grassland digestibility was estimated based on values from the literature of the plant communities actually selected by each breed, using monthly means per breed. Dry matter intake (DMI) was then estimated per cow as the amount needed to cover the energy requirements (AFRC, 1993), and CH_4 emissions using the prediction equation of Yan *et al*. (2009) [CH_4 (kg/d) = (35.1 * DMI + 14.7)/1400]. Results were analysed in a completely randomised design with breeds as factor, using the General Linear Model procedure of SAS.

Results Cows LW was significantly higher for CH, followed by AxL and LUI (785.1 ± 2.43; 711.9 ± 2.19 and 622.3 ± 2.29 kg, respectively, $P<0.05$). Therefore, ME for maintenance, estimated from LW, had the same trend (65.8 ± 0.31; 61.7 ± 0.28 and 56.3 ± 0.31 MJ ME/cow/day, respectively, $P<0.05$). Calves LW also differed between breeds, being higher for AxL and CH than LUI (183.3 ± 3.4; 181.2 ± 3.6 and 168 ± 3.8, respectively, $P<0.05$). Although the estimated digestibility of the diet selected by each breed tended to be higher for CH, followed by LUI and AxL (0.520 ± 0.024; 0.495 ± 0.020 and 0.486 ± 0.022 kg/kgDM, respectively), these differences were not significant. The calculated ME for physical activity derived from GPS one minute interval measurements and activity data and estimated from AFRC (1993) was significantly higher for CH, followed by AxL and finally by LUI (22.8±0.28; 18.8±0.19 and 15.5±0.24, respectively, $P<0.05$). The vertical GPS accuracy is known to be not as precise as the horizontal accuracy (Swain *et al*., 2011), therefore only horizontal distances walked per cow were considered in this analysis. The total ME for production (BWC, pregnancy and lactation) was higher for LUI, followed by AxL and finally for CH (45.5±0.82; 40.9±1.08 and 32.9±1.22 MJ/cow/day, respectively, $P<0.05$). Luing cows were the only ones gaining weight across the experiment and their calves had the highest BWC hence the highest ME for lactation. When the total ME for production plus maintenance and activity was accounted, this was higher for AxL compared with LUI, but both were similar to CH, as CH and AxL required more energy for maintenance and activity than LUI. Differences in total ME observed between AxL and LUI were not followed by the same response for DMI, which differed significantly between AxL and CH, as the energy required by CH was counteracted by a slightly better quality diet compared with AxL. As expected, the amount of CH_4 predicted to be produced per cow followed the same trend as the results of DMI, these were higher for AxL compared with CH but both were similar to LUI. However, when these emissions are expressed per unit of metabolic body weight ($kg^{0.75}$, MBW) they were significantly higher for AxL and LUI compared with CH (Table 1).

Table 1 Mean ± standard error of total metabolisable energy requirements (Total ME), dry matter intake and predicted methane emissions (CH_4) per cow and per metabolic body weight (MBW). Different superscripts means significant differences ($P<0.05$).

	AA x Limousin	Charolais	Luing
Total ME (MJ/cow/d)	123.4 ± 1.26^{a}	122.8 ± 1.40^{ab}	119.0 ± 1.06^{b}
Intake (kgDM/cow/d)	17.4 ± 0.31^{a}	16.0 ± 0.25^{b}	16.4 ± 0.29^{ab}
CH_4 per cow (g/cow/d)	445.9 ± 7.87^{a}	411.5 ± 14.95^{b}	422.8 ± 7.15^{ab}
CH_4 per MBW ($g/kg^{0.75}$/d)	3.2 ± 0.06^{a}	2.8 ± 0.04^{b}	3.4 ± 0.05^{a}

Conclusions Cattle of CH breed required more energy for activity and tended to select a better quality diet. Luing cows had lower activity requirements and higher production requirements through higher performance. Physical activity, energy requirements and DMI varying between breeds can potentially be translated into different CH_4 outputs. Heavier breeds diluted the CH_4 produced per unit of body weight and significant differences were observed between breeds. This analysis illustrates how differing grazing and foraging behaviour among breeds has a potential impact on the total energy requirements, DMI and CH_4 production.

Acknowledgements authors acknowledge funding from the Scottish Government and Scottish Natural Heritage.

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Voluntary intake, apparent digestibility and urinary nitrogen excretion by hair sheep fed increasing levels of foliage of *Leucaena leucocephala* mixed with *Cynodon nlemfuensis* grass

A Ruiz-González, S Albores-Moreno, E Briceño-Poot, A Ayala-Burgos, F Solorio-Sánchez, J Ku-Vera
University of Yucatan, Merida, Yucatan, Mexico
Email: kvera@uady.mx

Introduction In the tropical regions, extensive systems of sheep production are based on the grazing of species such as *Panicum maximum*, *Cynodon nlemfuensis* and *Brachiaria brizantha*. Breeds of hair sheep (Pelibuey, Blackbelly) are usually exploited under commercial farming conditions. Growth rate of sheep is low due to variation in grass availability and quality resulting in low profitability for the farmer. Silvopastoral systems are claimed to be an alternative under such conditions, with *L. leucocephala* being one of the preferred legume species throughout the tropics, due to its high crude protein content. However, nitrogen losses from the gastrointestinal tract may occur if an excess of rumen degradable nitrogen is present in the mixture (grass:legume) consumed (Poppi and McLennan, 1995), this suggests the existence of a loss of nitrogen in the rumen (Calsamiglia *et al*., 2010) as the consumption of the legume increases. The aim of the present work was to assess the effect of increasing the level of *L. leucocephala* mixed with *C. nlemfuensis*, on intake, digestibility and urinary nitrogen excretion by hair sheep.

Materials and methods Five male Pelibuey sheep with a liveweight of 30 kg were used in the experiment. Sheep were randomly assigned to a 5 x 5 Latin Square design with 7 days adaptation period and 5 days for sample collection. Sheep were dewormed and given an injection of vitamins ADE before the start of the experiment. Treatments were five levels of incorporation of foliage (young leaves) of *L. leucocephala*: 0, 15, 30, 45 and 60% (DM basis) mixed with fresh foliage of *C. nlemfuensis.* Both forages were cutted, chopped and mixed manually on a daily basis. Sheep were kept in wooden metabolic cages to measure DM intake and feces and urine output. Fresh water was freely available. During the collection period feces and urine samples were taken. Ten percent of daily fecal output was kept in the freezer and urine was received in trays containing sulphuric acid (10%) and samples (100 ml) were kept for N analysis. Amount of food offered was adjusted every two days in accord with the actual DM intake of each sheep. Data was analyzed by ANOVA with the statistical software SAS. Orthogonal contrasts were performed to analyze the linear, quadratic and cubic terms of the response.

Results Table 1 shows DM intake and apparent digestibility by Pelibuey sheep of rations containing increasing levels of *L. leucocephala* mixed with *C. nlemfuensis* grass. Significant differences ($P < 0.05$) were observed for DM intake. The highest DM intake was observed when *L. leucocephala* was incorporated at 30% of ration DM, showing a quadratic response. The same trend was observed for DMI when expressed per metabolic body weight. DMI was higher than that reported by Kaitho *et al*., (1998) of 60 to 66 g DM/kg$^{0.75}$/day, for Ethiopian sheep fed Leucaena at 15 and 60% of ration DM. DM digestibility and intake of digestible DM showed a slightly increase when *L. leucocephala* was incorporated at 30% of ration DM, however no statistically significant differences were observed for those response variables. Daily N intake was increased as the level of Leucaena in the ration was augmented. ME intake showed significant differences ($P < 0.05$) among treatments being higher when leucaena was included at 30% of ration DM. Urinary N excretion of sheep was linearly increased ($P < 0.05$) as the level of *L. leucocephala* in ration DM was augmented.

Table 1 DM intake, apparent DM digestibility, digestible DM intake and urinary N excretion by Pelibuey sheep fed increasing levels of foliage of *L. leucocephala* mixed with *C. nlemfuensis* grass.

	Percent incorporation of foliage of *L. leucocephala* in the ration (DM basis)					SEM
	0	15	30	45	60	
DM intake g/sheep/d	950^{b}	1010^{ab}	1200^{a}	1115^{ab}	1089^{ab}	119.78
DM intake g/kg$^{0.75}$/d	71^{b}	75^{ab}	89^{a}	82^{ab}	81^{ab}	8.56
DM digestibility %	58^{a}	54^{a}	62^{a}	61^{a}	56^{a}	7.70
Digestible DM intake g/sheep/d	581^{a}	571^{a}	755^{a}	687^{a}	633^{a}	126.78
ME intake MJ/sheep/d	7.60^{b}	8.24^{ab}	9.97^{a}	9.43^{ab}	9.07^{ab}	1.17
ME intake MJ/kg$^{0.75}$/d	0.56^{a}	0.61^{a}	0.74^{a}	0.70^{a}	0.67^{a}	0.09
Intake N g/sheep/d	17.68^{b}	21.42^{b}	30.58^{a}	31.56^{a}	33.30^{a}	4.37
Urinary N excretion g/sheep/d	4.13^{b}	6.34^{ab}	8.0^{ab}	9.16^{ab}	10.18^{a}	2.56

Rows with different literal differ significantly ($P< 0.05$).

Conclusions The highest DM intake, digestibility of DM and digestible DM intake by Pelibuey sheep were recorded when *L. leucocephala* was incorporated at 30% of ration DM. Urinary nitrogen excretion was increased linearly as the level of inclusion of leucaena in ration DM increased.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effect of time duration of ruminal urea infusions on ruminal ammonia concentrations and portal-drained viscera extraction of arterial urea-N in lactating Holstein cows

B A Røjen, N B Kristensen
Dept. of Animal Health and Bioscience, Aarhus University, Tjele, Denmark
Email: betina.amdisenrojen@agrsci.dk

Introduction Physiological regulation of urea-N recycling and its potential impact on nitrogen (N) utilization in dairy cattle remains an area of interest (Reynolds and Kristensen, 2008). The aim of the experiment was to investigate the impact of asynchronous N supplementation on the ability of cattle to buffer ruminal N supply by recycling of urea-N to the gastrointestinal (GI) tract and on the portal-drained visceral (PDV) extraction of arterial urea-N.

Materials and methods Three Danish Holstein cows (120±4 DIM) fitted with ruminal cannulas and permanent indwelling catheters in the major splanchnic blood vessels were randomly allocated to a 3 x 3 Latin square design with 21-d periods. Cows were fed a N deficient basal diet and treatments were ventral ruminal infusion of water for 24 h (water INF), 24 h infusion of 15 g of urea/kg of DMI (24-h INF), and 6 h infusion of 15 g of urea/kg of DMI (6-h INF). Cows were fed equally sized portions at 8 h intervals. The 6-h INF was initiated 0.5 h after the afternoon feeding at 1630 h and ran until 2230 h. Eight sets of blood and ruminal fluid samples were obtained at 0.5 h before morning feeding starting at 0730 h, and at 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, and 6.5 h after feeding i.e. 9 to 15.5 after the 6-h infusion was terminated. Data was analyzed using the mixed model procedure of SAS with treatment, period, sampling time, and the interaction treatment x time as fixed effects. Cow was considered a random effect. Variables with only one observation within cow and sampling day were analyzed using a reduced model, not including the effect of time.

Results Dry matter intake decreased (P=0.05) with 6-h INF compared with 24-h INF and water-INF (10.6, 18.0, and 17.4 kg/d, respectively). For arterial blood urea-N concentration a treatment by time interaction (P=0.03) was observed (Figure 1a). With 6-h INF the urea-N concentration decreased steadily with time, whereas the concentrations of urea-N with water-INF and 24-h INF remained steady. Conversely, the ruminal ammonia concentration and ammonia absorption with 6-h INF was not different from water INF, but less compared with 24-h INF (P<0.01). Net uptake of arterial urea-N from the blood to the GI tract was not affected (P=0.31) by treatment. Arterial urea-N extraction across the PDV was greater with water INF from 0.5 to 3.5 h after feeding compared with urea infusion treatments (P=0.03; Figure 1b).

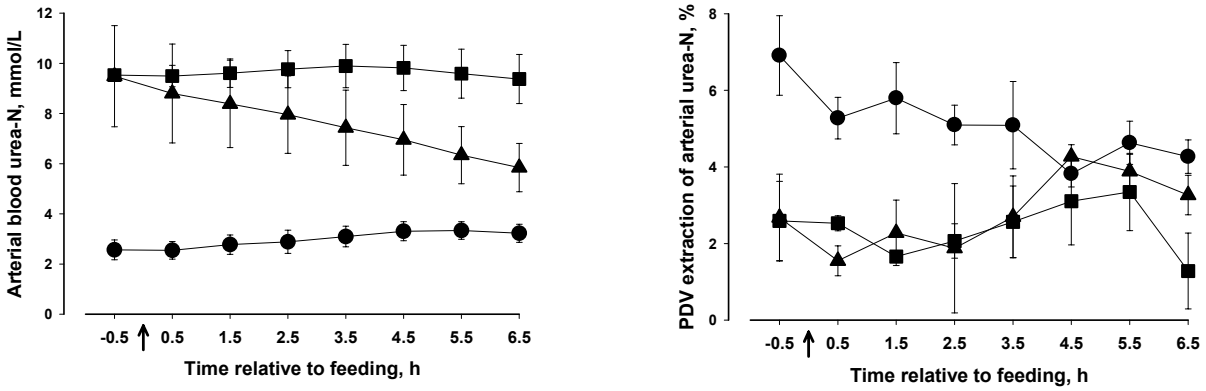

Figure 1 a and 1b. Arterial blood urea-N concentrations (a) and PDV extraction of arterial urea-N (b) relative to time of feeding in lactating Holstein cows. Treatments were 24 h ruminal infusion of water (circles), 24 h ruminal infusion of 15 g urea/kg DMI (squares), and 6 h ruminal infusion of 15 g urea/kg DMI (triangles). The arrow indicates time of feeding. Data points represent the means of 3 cows ± SEM.

Conclusions This study showed a sustained, increased arterial urea-N concentration with 6 h urea infusion on a 24 h basis indicating a carryover effect for arterial urea-N concentration but the cows were unable to make use of circulating urea-N as ruminal N source to compensate low ruminal ammonia concentrations during the period of the day without intraruminal infusion of urea. One of the study objectives was to assess the regulatory impact of daily minimum N status relative to daily maximum N status on urea-N transport. The low ruminal ammonia concentration for 6-h INF within the sampling window did not increase the PDV extraction of urea-N, implying that ruminal peak concentrations of ammonia or blood urea-N concentrations overruled potential signals from low ruminal ammonia concentration during the sampling window.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effect of progressive inoculation of fauna-free sheep with holotrich protozoa and total-fauna on rumen fermentation and methane emissions

A Belanche, G de la Fuente, S E Doran, J M Moorby, C J Newbold
IBERS, Aberystwyth University, Aberystwyth, UK *Email: aib@aber.ac.uk*

Introduction Methane emissions are about half of total greenhouse gases released by the UK livestock sector with ruminant enteric methane production being the most important source, representing about 80% of these emissions (Gill *et al*., 2010). Up to 25% of rumen methanogens are associated with protozoa (Newbold *et al*., 1995), and it has been suggested that elimination of rumen protozoa would decrease methane production by about 10% (Morgavi *et al*., 2010). Rumen protozoa can also modify rumen fermentation patterns, increasing the proportion of methanogenic substrates (i.e. acetate and butyrate), while bacterial predation by protozoa has a substantial negative effect on the efficiency of N utilization in the rumen (Eugène *et al*., 2003). However, it remains unknown which protozoal species are most closely related to methane emissions and/or to the inefficiency of N utilization in the rumen. This study was carried out to investigate the effect of absence of rumen protozoa, the presence of holotrich protozoa, and the presence of a mixed protozoa population on rumen fermentation and enteric methane emissions by sheep.

Material and methods Eight mature Texel-crossbreed sheep (approximately 4 years old) were used in a straight-through design experiments with three consecutive periods, with a 3 months adaptation phase between each period. All sheep were naturally fauna-free by separation from the ewes within 24 h of birth and had been kept isolated from adult animals. The sheep were kept in individual pens and fed twice per day with a diet designed to meet maintenance requirements (66% ryegrass hay and 33% grounded barley). For the first period animals remained fauna-free; for the second period they were inoculated orally with a mixed holotrich population, and for the third period animals were inoculated with rumen fluid obtained from control animals (with a natural protozoal population). In each period, rumen fluid (100 ml/sheep) was extracted by oesophageal tube to verify the absence/presence of rumen protozoa, and the microbial population was homogenized among animals by re-inoculation of the pooled rumen fluid. In each period methane emissions were determined from each animal using respiration chambers for 4 days. Airflow and methane concentrations were measured in the intake and exhaust ducts of each chamber (T^a range 10-15ºC) every 30 min using an ADC MGA3000 gas analyser. Enteric methane emissions were calculated as the differences in the methane fluxes entering and leaving each chamber. Rumen contents were sampled before morning feeding when sheep were introduced and released from the respiration chambers to determine rumen fermentation parameters (pH, ammonia and volatile fatty acids). Data were analyzed by ANOVA using Genstat software.

Table 1 Effect of protozoal populations on rumen fermentation and methane emissions.

	Fauna-free	Holotrich	Total-fauna	SEM	P
Body weight, kg	93.8^a	92.9^a	90.9^b	0.49	<0.01
Total DMI, kg/d	1.58	1.59	1.68	0.040	NS
Total protozoa, 10^4/ml	-	5.26	75.6	5.50	<0.001
Isotricha, 10^4/ml	-	1.12	0.57	0.153	<0.05
Dasytricha, 10^4/ml	-	4.14	1.82	0.406	<0.01
Entodinium, 10^4/ml	-	-	67.4		
Epidinium, 10^4/ml	-	-	1.73		
Diplodiniinae, 10^4/ml	-	-	4.02		
Rumen pH	6.94^a	6.89^a	6.73^b	0.035	<0.01
Ammonia-N, mg/dl	1.29^b	1.39^b	4.85^a	0.412	<0.001
Total VFA, mmol/l	78.1^b	87.3^{ab}	94.4^a	3.28	<0.05
Acetate, %	66.9	68.8	66.9	1.55	NS
Propionate, %	21.8^a	16.3^b	17.5^b	1.04	<0.01
Butyrate, %	7.82^b	11.0^a	12.0^a	0.80	<0.01
Acetate/Propionate	3.23^b	4.26^a	3.83^{ab}	0.021	<0.05
Butyrate/Propionate	0.35^b	0.69^a	0.69^a	0.043	<0.001
CH_4, l/d	30.3^b	49.5^a	53.5^a	2.24	<0.001
CH_4, l/kg body weight	0.32^b	0.53^a	0.59^a	0.027	<0.001
CH_4, l/kg DMI	19.2^b	31.2^a	31.8^a	1.38	<0.001

Results Animals remained in good condition with similar DM intakes throughout the experiment (Table 1). Presence of holotrich protozoa promoted a substantial increase (+65%) in methane emissions compared to fauna-free sheep. The holotrich population did not substantially modify the fermentation pattern (pH, ammonia and total VFA), although significantly increased the acetate/propionate and butyrate/acetate ratios. Holotrich concentrations in total-fauna were half that observed in monofaunated animals, representing 3.2% of the total population. Total faunated animals had a 14-times greater total protozoal concentration than monofaunated animals; however, no differences in methane emissions nor in VFA profile were detected between them. On the other hand, a significant decrease in rumen pH, accompanied with an increase in ammonia and total VFA concentrations, was observed in total faunted sheep compared with the two other groups. Within rows, values with different superscripts differ ($P < 0.05$).

Conclusions Holotrich protozoa had a limited effect on rumen fermentation pattern; however, their presence and the methanogens associated with them seem to act as a very efficient H_2 sink in the rumen leading to a considerable increase in the methanogenic/non-metanogenic VFA ratios and methane emissions. Conversely, the presence of a total mixed fauna promoted substantial changes in the rumen fermentation pattern, such as increase in the ammonia and total VFA concentrations and a decrease in the rumen pH. Nevertheless the complete protozoal population modified neither the methane emissions nor the VFA profile with respect to those observed in holotrich monofautanted sheep.

Acknowledgements This study was funded by the Commission of the European Communities FP7, KBB-2007-1.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effects of condensed tannin extracts from sainfoin (*Onobrychis viciifolia* Scop) on rumen *in vitro* methane production and fermentation characteristics

B Hatew[1], W F Pellikaan[1], W H Hendriks[1], I Mueller-Harvey[2], E Stringano[2]
[1]Animal Nutrition Group, Wageningen University, Wageningen, The Netherlands, [2]Chemistry & Biochemistry Laboratory, Animal Science Research Group, Reading University, Reading, UK
Email: bayissa.chuko@wur.nl

Introduction Methane emission from enteric fermentation of feeds by ruminants forms about 15% of global production (Moss *et al*., 2000). Plants secondary metabolites such as tannins are considered to have potential to reduce methane production in ruminants. These polyphenolic compounds are perceived as natural by the consumers as compared to chemical feed additives. Besides its feeding value, sainfoin contains relatively high amounts of condensed tannins (CT; up to 100g/kg DM) with tannin-forming cells evenly distributed throughout the plant (Scharenberg *et al*., 2007). However, the effectiveness of CT varies depending upon the source, concentration and its structure (Min *et al*., 2003). The objective of this study was to investigate the efficacy of CT extracts from different sainfoin varieties on rumen *in vitro* methane (CH_4) production and fermentation kinetics.

Materials and methods CT extracts from different varieties of sainfoin were characterized by direct thiolysis and analysed on HPLC. Substrate (0.250 g) was weighed into single 250 ml bottles per treatment within run, with duplicate runs. Rumen *in vitro* fermentation kinetics of substrate was assessed using an automated technique of gas production (Cone *et al*., 1996) by incubating lucerne (*Medicago sativa*) in 30 ml of buffered rumen fluid for 72 h. Rumen fluid was collected from the same two rumen cannulated lactating Holstein-Friesian dairy cows. Four CT were added to the substrate each at concentrations of 40, 80 and 120 g/kg at the time of incubation. To determine CH_4, 17 gas samples from the headspace of each bottles were taken at defined time points and analysed on gas chromatograph (GC). A modified nonlinear Michaelis-Menten model was fitted to the CH_4 concentration patterns and model estimates were used to calculate total cumulative CH_4 production (Pellikaan *et al*., 2011). At the end of incubation supernatant from bottle content was sampled for volatile fatty acid (VFA) analysis on GC. Data on total gas and CH_4 productions, VFA and others were subjected to analysis of variance using the General Linear Model procedure of SAS. Difference among treatment means were tested using Tukey-Kramer's multiple comparison test and differences were declared significant at a probability value of $P<0.05$.

Results Tannin composition of extracts differed considerably (mDP = 19 to 84; PD/PC ratio = 66 to 83). There were highly significant effects ($P<0.0001$) of types and concentrations of CT on gas and methane production (Table 1). Inclusion of CT at 40 g/kg had no effect on cumulative gas and methane production. However, addition of higher concentrations of CT was negatively correlated with methane production. A significant difference in per cent reduction of methane production was observed upon addition of 120 g/kg CT from CPI 63763 ($P=0.007$), CPI 63767 ($P=0.0002$), Rees "A" ($P<0.0001$) and Cotswold Common ($P=0.001$) as compared to control (Table 1). Both cumulative and asymptotic gas productions decreased significantly ($P<0.05$) and were highly correlated ($r=0.93$ to 0.99; $P<0.001$) with increasing concentrations of CT. The rate of total gas produced at 12 h of incubation was reduced. Addition of different types and concentrations of CT gave a decrease ($P<0.0001$) in total VFA, individual straight- and branched-chain fatty acids, and the acetate to propionate ratio.

Table 1 Rumen *in vitro* gas and methane production kinetics of lucerne incubated with different types and concentrations of condensed tannins.

Condensed tannin (CT)	Concentration (g/kg)	Total gas (ml/g OM)	CH_4 (ml/g OM)	Half-time CH_4 (h)
CPI 63763	0	308.45^{a}	58.59^{a}	8.89^{a}
(mDP = 20	40	309.66^{a}	70.42^{a}	13.70^{a}
PD/PC = 81)	80	299.28^{a}	53.88^{a}	14.90^{a}
	120	245.91^{b}	32.65^{b}	27.81^{b}
CPI 63767	0	308.45^{a}	58.59^{a}	8.89^{b}
(mDP = 84	40	313.51^{a}	65.36^{a}	12.99^{b}
PD/PC = 83)	80	258.44^{b}	44.03^{b}	29.65^{a}
	120	211.93^{c}	23.43^{c}	13.08^{b}
Cotswold Common	0	308.45^{a}	58.59^{a}	8.89^{a}
(mDP = 26	40	295.17^{a}	63.87^{a}	13.24^{a}
PD/PC = 67)	80	nd	nd	nd
	120	206.83^{b}	30.96^{b}	46.58^{b}
Rees "A"	0	308.45^{a}	58.59^{a}	8.89^{a}
(mDP = 19	40	296.73^{a}	50.86^{b}	13.88^{a}
PD/PC = 66)	80	292.75^{a}	55.00^{a}	27.19^{ab}
	120	218.61^{b}	30.54^{d}	70.58^{b}

Different superscripts within the same CT indicate significant difference ($P<0.05$) among concentrations of CT; nd= not determined.

Conclusions CT extracts from sainfoin showed favourable properties in reducing methane production. The differences observed in the biological activity of CT is related to the high proportion of procyanidin (PC) and lowest molecular size. The decrease in proteolytic activity as indirectly shown by changes in VFA composition can be seen as potential advantage in terms of improvement of N-use by ruminants. This suggests that CT from sainfoin have a potential to be used as feed additives for rumen manipulation to reduce methane emission.

Acknowledgement This study was funded by EU HealthyHay project (MRTN_CT-2006-035805).

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Faecal phosphorus emission from organic and conventional dairy farms in Sweden

M Nordqvist, R Spörndly, K Holtenius
Swedish University of Agricultural Sciences, Uppsala, Sweden
Email: maria.nordqvist@slu.se

Introduction Emission of phosphorus (P) from dairy farms in Sweden is under increasing focus because of the environmental problems it causes. The P fraction that is soluble in water causes eutrophication in oceans and lakes, due to its motility with the run-off water from agricultural land. Since P supply is limited, an economical use of this natural resource is of great importance for several reasons. The soluble fraction (sP) of P in faeces from dairy cattle can be used as a tool to estimate overfeeding of P. The soluble fraction increases proportionally with increasing P feeding as all excess P is excreted with the faeces in soluble form (Dou *et al*., 2007). In organic farming commonly used protein feeds like soybean meal are not accepted and are therefore replaced by example given rapeseed cake. However the P concentration in rapeseed cake is higher compared to that in soybean meal. The purpose of the study was to screen the level of P excretion at Swedish conventional and organic dairy farms. The hypothesis was that P excretion from animals in the organic herds would be larger than that from the conventional herds.

Material and methods Dairy farms, both organic (n=14) and conventional (n=15), were visited during winter 2009-2010 when the cows were housed indoors. From each herd, ten cows in different stages of lactation were selected for faecal sampling. The feed consumption of the selected cows was estimated during one day, feed samples were taken and the feed and faeces samples were analysed for P, using plasma emission spectrometry by an accredited commercial laboratory.
The sP in the faeces was measured using a modified method from Dou *et al*. (2007), where 5 g of the faeces was mixed with 95 ml of 0,04 % HCl and shaken for 1 h. Therafter the mixture was centrifuged and filtered, and P was measured in the clear solution obtained, using a commercially available colorimetric kit (RANDOX, Crumlin, UK). The difference between organic and conventional herds in total P (tP) and sP was evaluated using a two-tailed Student´s t-test. Equality of variance was tested (SAS 9.2, SAS Institute 2008).

Results The organic herds had a lower mean value for tP and sP (7,2 versus 8,8 g/kg DM (P=0,001) and 4,3 versus 5,2 g/kg DM (P=0,001), respectively) as well as a smaller variation of tP content in faeces compared to the conventional herds (SD=1,8 versus 2,4, P=0,002). The relation between tP and sP in faeces from conventional and organic herds is shown in

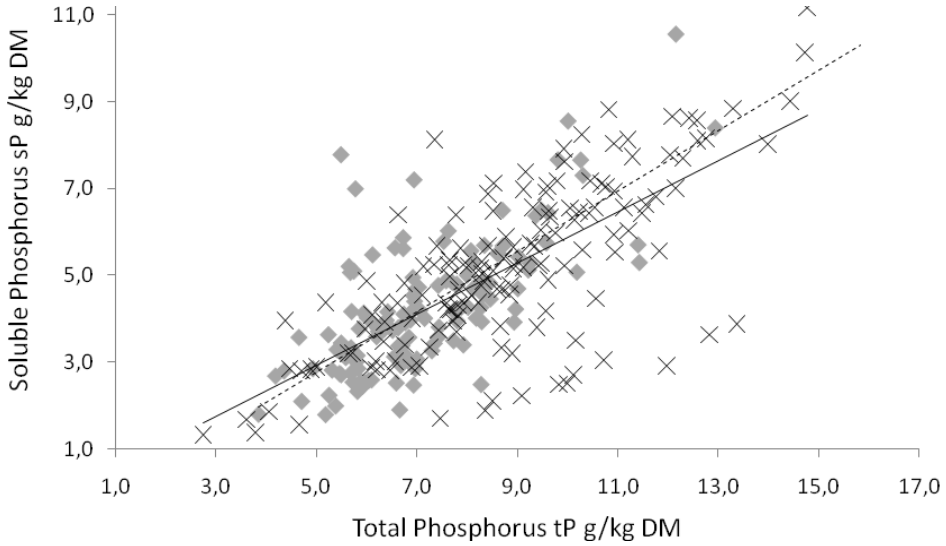

Figure 1 The relationship between tP and sP in faeces from conventional cows X (—conventional, r^2=0,506; P<0,001) and organic cows ♦ (---organic , r^2=0,558; P<0,001)

Conclusion The hypothesis that organic dairy farms release more P in soluble form to the environment than conventional dairy farms could not be supported by the data from this study. Instead the results show that the faecal P concentration is higher in conventional herds. Data analysis of the consumed feed in the different farms is of importance for further knowledge.

Acknowledgements This work was funded by Formas.

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On-farm evaluation of the effect of coffee pulp supplementation on milk yield and dry matter intake of dairy cows grazing tropical grasses in Central Mexico

O Castelán-Ortega[1], J Estrada-Flores[2], P Pedraza-Beltrán[1], I Estrada-López[1], A Rayas-Amor[1], G Yong-Angel[1]
[1]Facultad de Medicina Veterinaria y Zootecnia of the Universidad Autónoma del Estado de México, Toluca, Estado de México, Mexico, [2]Instituto de Ciencias Agropecuarias y Rurales of the Universidad Autónoma del Estado de México, Toluca, Estado de México, Mexico
Email: oaco2002@yahoo.com.mx

Introduction Coffee pulp (COP) is considered the major polluting agent of rivers and lakes located near the coffee-processing regions. The use of COP to supplement dairy cattle would be an environmentally safe alternative for the appropriate disposal of COP. The COP can be a good source of energy for ruminants as it is rich in soluble carbohydrates. The objective of the present study was to evaluate the effect of COP supplementation on milk yield and forage intake by dairy cows in a tropical region of Central Mexico.

Materials and methods The study was carried out in a smallholder dairy farm in the tropical region of central Mexico at 18º 45´30" N and 99º 59´ 07" W, with an altitude of 1,327 m, 24 ºC of average temperature and 1000 mm of rain. The experiment took place from August to November 2008. Four multiparous crossed Holstein-Brown Swiss-Cebu cows of similar weight at the beginning of their third lactation stage were used. Their mean body weight was 435 ± 27 kg, and their mean milk yield was 12.6 ± 2 kg d^{-1}. The duration of the experiment was 84 days, divided into four experimental periods of 21 days each. The first 15 days were used for diet adaptation and the last six days for measuring milk yield and collecting samples of milk, faeces and food. The cows were milked by hand and the yield weighed twice a day. Their body weight was measured at the beginning and at the end of each period. Samples of milk were tested in the laboratory for fat, protein and total solids content. The cows remained 10 hrs/day in a native sward dominated by Bahia Grass (*Paspalum notatum)*, with a stocking rate of three cows per hectare. Experimental concentrates were composed of grounded maize grains (*Zea mays* L.), canola cake (*Brassica napus L.*), molasses, urea and 0, 10, 15 and 20% of coffee pulp for concentrate 1 to 4 respectively. The levels of grounded maize and canola cake varied to determine the effect of their partial replacement with coffee pulp on milk yield. The replacement rates of maize were 6%, 10% and 13% for concentrates 2, 3 and 4 with respect to the concentrate 1 (control treatment). The replacement rates for canola cake were 3%, 5% and 7% for concentrates 2, 3 and 4 also with respect to the concentrate 1. Each cow received 6 kg DM of concentrate per day divided into two rations, each cow received, each concentrate, one in each of four periods. The n-alkanes technique (Dove and Mayes, 2000) was used to measure grass dry matter intake (DMI). Samples of grass (hand plucking), concentrates and COP were analyzed for DM, ashes, OM, crude protein, NDF, ADF and *in vitro* digestible dry matter contents. Results were analysed by analysis of variance for 4x4 Latin square design.

Results The results in Table 1 show that there was no difference (P>0.05) in the average daily milk yield and milk composition between concentrates. Results also demonstrated that COP, at an inclusion level of up to 20%, can replace expensive ingredients in concentrates like maize and canola cake without compromising milk yield or milk composition. In the same way the COP did not have effect on DMI as the forage DMI in cows feed concentrate without COP is not different from those with COP (P>0.05). These results are in line with early observations of Cabezas *et al*. (1987) who indicated that feeding dairy cows for several years with commercial concentrates that contain up to 20% ground COP does not produce detrimental effects on milk production, nor apparent physiological disturbances in the cows. More recent investigations suggest (Lima de Souza *et al*. 2005) that the adequate level of inclusion of COP is between 10.5% to 20% of the total DMI, which is similar to what was found in the present work.

Table 1 Mean daily milk yield, milk composition, grass and total DMI for the different COP treatments

Concentrate	Milk yield (kg d^{-1})	Fat	Protein g kg^{-1}	Total solids	Grass DMI	Total DMI (kg DM day^{-1})
1 (0% COP)	6.8^{ns}	47.2^{ns}	34.3^{ns}	136.5^{ns}	3.2^{ns}	9.2^{ns}
2(10%COP)	7.9^{ns}	43.0^{ns}	31.8^{ns}	128.8^{ns}	3.1^{ns}	9.1^{ns}
3(15%COP)	6.9^{ns}	49.4^{ns}	31.7^{ns}	134.7^{ns}	3.0^{ns}	9.0^{ns}
4(20%COP)	6.7^{ns}	43.3^{ns}	31.4^{ns}	123^{ns} □	3.3^{ns}	9.3^{ns}
sem	0.5	3.3	0.9	5.0	0.4	0.06

Means between rows with different superscripts are significantly different (P<0.05)

Conclusions It is concluded that COP can be included at levels of 20% in the concentrates of dairy cows without compromising significantly milk yield and DMI. Its use will constitute an environmentally safe alternative for the disposal of COP.

Acknowledgements Funding of Universidad Autónoma del Estado de México and ICAMEX grant No. UAEM2386/2006E is acknowledged

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Milk fatty acid profile from dairy cows grazing a diverse pasture

S Greenwood, V Totty, G Edwards
Lincoln University, Lincoln, New Zealand
Email: sabrina.greenwood@lincoln.ac.nz

Introduction Strategies to decrease the environmental impact of N-based emissions from the New Zealand dairy industry include dietary manipulation. Inclusion of condensed-tannin -containing plant species in the diet, such as lotus species (*Lotus corniculatus* and *Lotus pedunculatus)*, decreases the urine: faeces N ratio (Woodward *et al*., 2009). Inclusion of other plant species, such as plantain (*Plantago lanceolata*) and chicory (*Cichorium intybus*), has also increased milk production (Waugh *et al*., 1998) while altering dietary CP content. There is a need to examine milk profile resulting from these N-mitigating diets to verify their effects on milk output. The objective of the current study is to examine the milk fatty acid profile of milk from dairy cows grazing a diverse pasture.

Materials and methods Thirty-six lactating Friesian-Jersey crossbred dairy cows were randomly assigned to one of three treatments, being offered approximately 16 kg DM/cow/d of either 1) a ryegrass (*Lolium perenne)*-clover (*Trifolium repens*) pasture diet (RC; ME, 12.3 MJME/kg DM; CP, 26.2 % of DM), 2) a high sugar ryegrass-clover pasture diet (HSRC; ME, 12.3 MJME/kg DM; CP, 26.3% of DM), or 3) a diverse pasture diet including high sugar ryegrass, clover, lotus (*Lotus pedunculatus),* chicory, and plantain (HSD; ME, 12.2 MJME/kg DM; CP, 23.7 % of DM). Milk volumes were recorded daily, while milk samples were collected at 0700 h and 1500 h on d 6, 8 and 10. A subsample of each milk sample was preserved with 2-bromo-2-nitropropane-1,3-diol and stored at 4˚C until component analysis (LIC, New Zealand). A second milk subsample collected on d 10 was frozen at -20˚C until milk fatty acid methyl esters (FAME) analysis. Subsamples were thawed to 37˚C, and proportions of am and pm subsamples were pooled by cow based on milk volumes recorded per cow. FAME GC analysis (GC-2010, Shimadzu) was performed as previously described (Lee and Tweed, 2008), with minor modifications. Peaks were identified by comparison of retentions times with reference standards (Larodan, Sweden). Data were analyzed using one way ANOVA (GenStat v. 12.2), including treatment and block as fixed effects.

Results Total milk fat yields were 0.46, 0.43 and 0.41 kg/day from cows grazing RC, HSRC, or HSD pasture, respectively (P = 0.166). Fatty acid composition (g/100 g fatty acid) of milk collected from cows grazing RC, HSRC, and HSD is listed in Table 1. The total content of medium chain saturated fatty acid (MCSFA) in milk collected from cows in all treatments did not differ (P = 0.104); however, total amount of fatty acids below 16C were significantly different between treatment (P = 0.014). Content of both vaccenic (18:1 *trans*-11) and rumenic acid (18:2 *cis*-9, *trans*-11) was lower in milk from HSD cows (P = 0.025 and P = 0.067, respectively) compared to that from RC and HSRC cows; however, content of all other determined 18:2 was higher in milk from HSD cows (P = 0.008) compared to cows receiving other treatments. Content of both linoleic acid (18:2 *cis*-9, *cis*-11) and alpha-linolenic acid (18:3 *cis-9*, *cis*-12, *cis*-15) were higher in milk from HSD cows (P < 0.001) compared to milk from RC and HSRC cows.

Table 1 Milk fatty acid content (g/100 g fatty acid) of milk from cows grazing either RC, HSRC, or HSD pasture

Fatty Acid (g/100 g fatty acid)	HSRC	HSD	RC	SEM	P
C18:2 *cis*-9, *cis*-12	0.87	1.36	0.78	0.03	<0.001
C18:3 *cis*-9, *cis*-12, *cis*-15	1.15	1.69	0.99	0.04	<0.001
CLA *cis*-9, *trans*-11	2.33	1.51	2.00	0.23	>0.05
LCSFA	17.45	16.12	17.97	0.53	>0.05
MCSFA	54.98	56.91	56.54	0.65	>0.05
Fatty acids above16C	41.97	40.15	40.37	0.60	>0.05
Fatty acids below16C	25.15	26.65	26.21	0.34	<0.05
Total MUFA	25.71	23.68	25.14	0.50	<0.05
Total PUFA	7.67	7.57	6.74	0.33	>0.05
Total SFA	72.44	73.04	74.51	1.02	>0.05

Conclusions Though both the vaccenic and rumenic acid content were lower in milk from HSD-fed cows relative to the RC and HSRC-fed cows, inclusion of multiple plant species in a diverse pasture resulted in lower diet-derived LCSFA and higher amounts of linoleic and alpha-linolenic acids present in milk from HSD cows. In addition, the higher content of 4-15C fatty acids identified in milk from HSD cows suggests higher *de novo* synthesis. These results provide information regarding changes in milk FAME profile as a result of a diverse pasture compared to a simple ryegrass-clover or high sugar ryegrass-clover pasture.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Variability of SF_6 and CH_4 gas concentrations in the breath samples of sheep and cattle: defining criteria for the systematic exclusion of data when using the SF_6 tracer technique to measure CH_4 emissions

N Swainson[1], I Brookes[2], S Hoskin[1], H Clark[1]
[1]AgResearch Ltd, Palmerston North, New Zealand, [2]Massey University, Palmerston North, New Zealand
Email: natasha.swainson@agresearch.co.nz

Introduction The sulphur hexafluoride (SF_6) tracer technique is widely used to calculate the methane (CH_4) emissions from ruminant livestock. SF_6 is used as a marker for CH_4 and the ratio of the two gases is used to calculate daily CH_4 production (CH_4 g/day), after adjustments for SF_6 release rate and ambient gas concentrations (SF_6 and CH_4). The technique assumes the emission of SF_6 simulates that of CH_4. Therefore, the dilution rate of the two gases should be identical and there is equal mixing of both gases in the exhaled breath (Johnson *et al.* 1994). However, highly variable SF_6 concentrations were observed in breath samples collected during three experiments with sheep and one with cattle (Swainson, 2011). In these studies, concentrations of SF_6 (parts per trillion, ppt) were found to be highly variable, both between-days and within-animals, and/or at unusually low concentrations that were near ambient concentrations of SF_6. This is despite CH_4 concentrations (parts per million, ppm) being consistent, both between-days and within-animals, and at greater than ambient concentrations. This indicates that the collection of the breath samples was not problematic. As a result, criteria were sought to define a systematic method 'decision tree' for including or excluding data, based on the concentrations of SF_6 and CH_4 in the breath samples from sheep and cattle.

Materials and methods The data were sourced from the New Zealand Methane Database, from 2002 to 2007, encompassing 15 independent sheep experiments, totalling 3542 SF_6 and CH_4 data points, and 17 independent cattle experiments totalling 5170 SF_6 and CH_4 data points. The ranges and variability of each gas was compared. From this, final criteria were developed for the decision tree (Figure 1) for each gas and their ratios based on the mean gas concentrations, plus or minus the standard deviation of the mean.

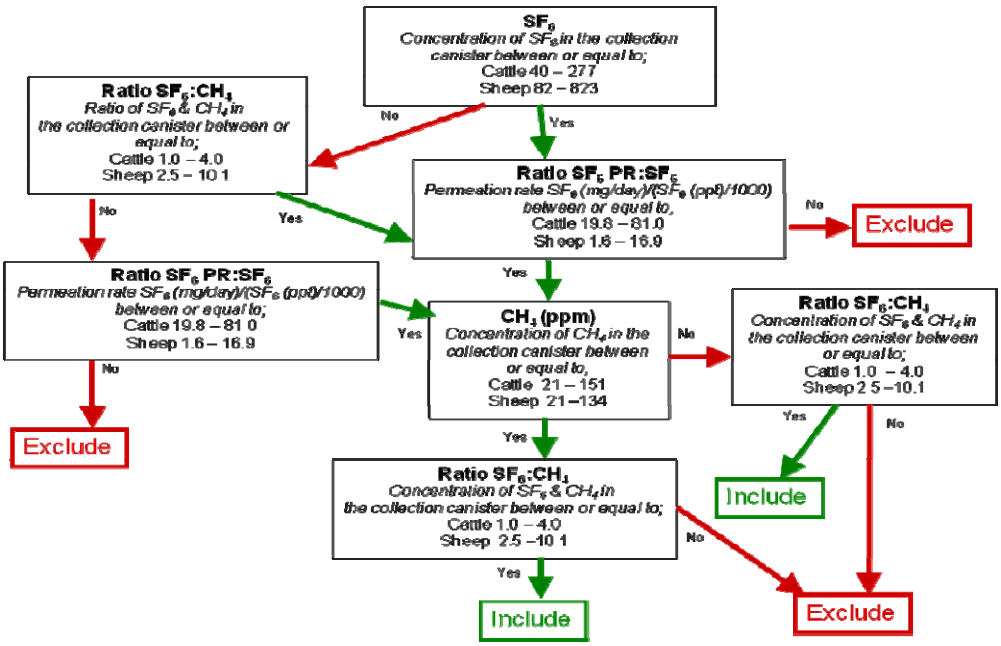

Figure 1 Decision tree for the inclusion or exclusion of data based on the concentrations of the gases sulphur hexafluoride gas (SF_6, expressed as ppt) and methane (CH_4, expressed as ppm) from collection canisters of sheep and cattle.

Results The application of the decision tree to data reduced the range in coefficients of variation (CV, standard deviation divided by the mean) from 1506% to 39% (n = 3 datasets) for sheep and from 894% to 33% (n = 1 dataset) for cattle the experiments. Nevertheless, these new CVs are still greater than those reported by Vlaming *et al.* (2008) for sheep of 2.2 to 42% within-animal and 17% between-animal and for cattle of 8 to 18% between and within-animal CV, respectively.

Conclusion The use of the decision tree to exclude data reduced the variability of estimated CH_4 yield, for both sheep and cattle, between and within-animals, but still allowed for a wide range of CH_4 emission estimates and did not appear to create a bias towards high or low CH_4 emissions. It is recommended that the decision tree or a similar such approach should be used as part of a systematic step-wise process for ensuring data quality, when using the SF_6 technique to calculated CH_4 production.

Acknowledgements Authors would like to thank AgResearch for the use of the New Zealand Methane Database.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Hydrogen emissions from sheep: A spill-over for methanogenesis?

N Swainson[1], C Martin[2,1], S Muetzel[1], C Pinares-Patiño[1]
[1]AgResearch Ltd, Palmerston North, New Zealand, [2]INRA UR1213 Herbivores, Clermont-Ferrand/Theix, France
Email: natasha.swainson@agresearch.co.nz

Introduction The transfer of hydrogen (H_2) is fundamental for digestion processes of feed consumed by ruminants. It is assumed that all the H_2 released in the rumen during microbial fermentation is used, predominately by methanogens, with no H_2 gas emitted from the animal. Noteworthy H_2 emissions have been measured in recent studies conducted by our group with sheep in respiration chambers. Results indicated that as H_2 yield (g/kg DMI) increased with diets containing starch, but methane (CH_4) yield (g/kg DMI) decreased (Pinares-Patiño *et al.* 2010; unpublished). It is hypothesized that methanogens are unable to utilise all the H_2 released during rapid fermentation. This would result in a spill-over of H_2 produced in the rumen, measurable as gaseous H_2 emissions released during eructation. The aim of this study was to test this hypothesis by manipulating the rate of fermentation in the rumen by changing the meal frequency.

Materials and methods Sixteen 3-year old wether sheep were split into two groups, balanced for live weight. Sheep were individually fed at a rate of 1.5 times metabolisable energy requirements for maintenance per day either two (M2) or eight (M8) times per day at equal intervals between feedings. The diet consisted of pelleted lucerne hay (60%) and wheat grain (40%), with an additional amount of straw (35g DM/day). After a 22 day-adaptation period to the diet and feeding frequency, gaseous emissions from sheep were measured using open-circuit respiration chambers for 3 consecutive days. Methane was measured using an infrared Servomex 4900 gas analyser (Servomex Group Ltd., East Sussex, UK) fitted with an electrochemical analyser for H_2 detection. The accuracies for the detection of CH_4 and H_2 were 0.5 and 5 ppm, respectively. Data were analysed using a mixed linear model with meal frequency as a fixed effect and repeated measurements (days in chambers) within sheep. Means are presented plus or minus the standard error (SEM), unless otherwise stated. A significant difference was declared if $P < 0.05$ and a trend discussed if $P > 0.05$ and ≤ 0.10.

Results Feeding frequency tended to impact on the production of H_2, with the M2 group having lower emissions than the M8 group (M2, 0.20 vs. M8, 0.27 ± 0.030 g/day, $P = 0.10$). In addition, the mean CH_4 production from the M2 group was 33% lower than the M8 group (M2, 21.6 ± 1.33 vs. M8, 33.7 ± 1.36 g/day; $P < 0.001$). There was a trend for meal frequency to influence H_2 yield, as the M2 group yielded less H_2 than the M8 group (M2, 0.14 vs. M8, 0.20 ± 0.021 g/kg DMI, $P = 0.07$). The yield of CH_4 from the M2 group was also found to be lower ($P < 0.001$) than the M8 group (15 ± 0.92 vs. 24.8 ± 0.92 g/kg DMI). Figures 1 and 2 illustrate the daily emission kinetics of H_2 and CH_4 for both groups and show that the mean peak of both H_2 and CH_4 occurred approximately 30 minutes after each meal event. The mean H_2 peak of the M2 group (Figure 1) was greater than for the M8 group (Figure 2; M2, 35.8 ± 6.62 vs. M8, 9.3 ± 5.77 (SD) ppm), whereas the mean peak in CH_4 after each meal event appears similar (M2, 163.4 ± 38.85 vs. M8, 147.0 ± 35.72 (SD) ppm).

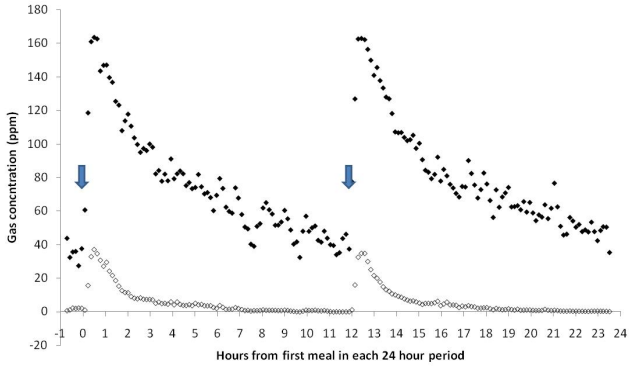

Figure 1 Daily mean emission kinetics (ppm, v/v) of methane (♦, SD 38.9) and hydrogen (Δ, SD 6.6) for sheep fed twice a day. Arrows denote the meal events.

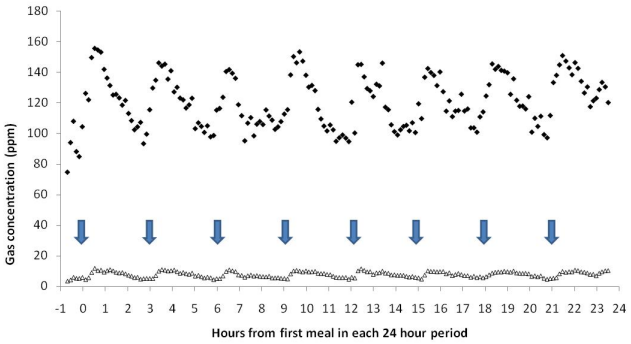

Figure 2 Daily mean emission kinetics (ppm, v/v) of methane (♦, SD 35.7) and hydrogen (Δ, SD 5.8) for sheep fed eight times a day. Arrows denote the meal events.

Conclusions Feeding frequency tended to influence both H_2 production and yield, with the M2 group having lower emissions than the M8 group. In addition, the peak of H_2 after each meal event was higher for the M2 group than for the M8 group. Despite the post-feeding peak in CH_4 appearing to be similar between both groups the mean production and yield of CH_4 was reduced for the M2 group compared with the M8 group. These results suggest that there was a greater spill-over of H_2 from microbial fermentation of feed after each meal event for the M2 group and a lower availability of H_2 for methanogenesis, therefore supporting our hypothesis. Further research is currently being undertaken to confirm these results.

Acknowledgements Thanks are given to Drs. David Pacheco and Peter Janssen for the helpful discussion and advice around H_2 emissions, and to the Ruminant Nutrition technical staff for their assistance throughout the experiment.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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The impact of animal and diet factors on enteric methane conversion factor in dairy cows

T Yan, D Wills, C Ferris, A Carson
Agri-Food and Biosciences Institute, Hillsborough, UK
Email: tianhai.yan@afbini.gov.uk

Introduction In countries/regions where methane (CH_4) data is unavailable to estimate Tier 2 CH_4 emission inventories for enteric fermentation from dairy cattle, enteric CH_4 conversion factor (i.e., CH_4 energy/gross energy intake, CH_4-E/GEI) is assumed to be fixed at proportionally 0.065 (IPCC, 2006). However, this fixed value does not take account of variations in animal, dietary and management factors which exist in practice. Thus, the objectives of the present study were to use calorimeter data from this Institute to examine the effects of animal and dietary factors on CH_4-E/GEI in dairy cows.

Materials and methods The data used in the present study were obtained from 20 energy metabolism studies (conducted at this Institute between 1993 and 2009) involving non-lactating Holstein-Friesian cows (n = 43), lactating Holstein-Friesian cows (n = 511), lactating Norwegian Red cows (n = 36) and lactating Jersey-Holstein cows (n = 32). Milk yields ranged from 3.2 to 49.1 kg/d, while parity ranged from 1 – 9. The majority of cows were offered grass silage-based diets (n = 566) while the remaining cows (n = 56) were offered fresh grass-based diets (zero grazed), with forage components of the diets harvested from perennial ryegrass swards. While 83 cows (from 4 studies) were offered either grass silage or zero grazed grass as the sole feed, the remaining cows were offered mixed diets comprising forage and concentrates (forage proportion = 0.181 to 0.869 kg/kg DM). The CH_4 emissions from enteric fermentation were measured using indirect open-circuit respiration calorimeter chambers. Experimental effects on relationships between CH_4-E/GEI and animal and dietary factors were removed.

Results and discussion In the present study, CH_4-E/GEI ranged from 0.041 to 0.108, with a mean of 0.070 (s.d., 0.012). This mean value is marginally higher than that (0.065) proposed by IPCC (2006) for use when developing Tier 2 CH_4 inventories. The large range in CH_4-E/GEI obtained in the present study indicates that using the fixed CH_4 conversion factor contained within IPCC (2006) to calculate CH_4 inventories can result in considerable and systematic errors. A range of animal and dietary factors were evaluated to quantify their effects on CH_4-E/GEI. These relationships are presented in Table 1 and Figure 1. All relationships are significant ($P < 0.001$), with R^2 values ranging from 0.49 to 0.56. These relationships indicate that increasing levels of production (milk yield or DM intake) can reduce CH_4-E/GEI, and CH_4-E/GEI is also negatively related to diet crude protein and metabolisable energy (ME) concentration. However, CH_4-E/GEI increased with increasing forage proportion in the diet and increasing fibre content of the diet (ADF and NDF). The findings of this study clearly demonstrate that CH_4-E/GEI is not a fixed factor, but rather varies with a number of animal and diet factors. These relationships can be used to predict enteric CH_4 emissions for dairy cattle which are applicable to local farming conditions than the fixed default factor contained within IPCC (2006).

Table 1. Equations for prediction of CH_4-E/GEI

Equations *	R^2	Eq. No	No of data
CH_4-E/GEI = -0.00058 Milk yield + 0.083	0.49	1	579
-0.00128 DM intake + 0.091	0.58	2	622
0.0179 Forage prop + 0.061	0.53	3	622
0.0392 ADF content + 0.062	0.55	4	487
0.0346 NDF content + 0.057	0.56	5	487
-0.146 CP content + 0.096	0.55	6	622
-0.00312 ME content + 0.109	0.53	7	622

* Forage prop - forage proportion; Unit - kg/d for milk yield, MJ/kg DM for ME content, and kg/kg DM for other variables

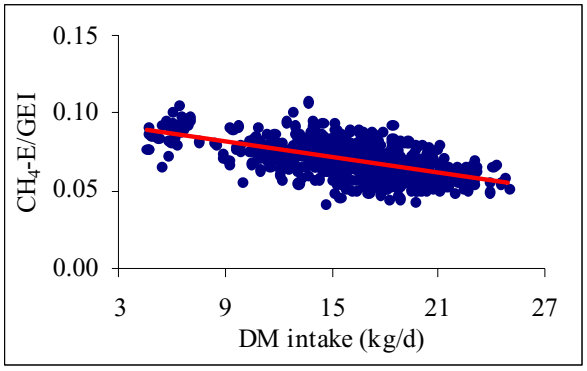

Figure 1. Relationship between CH_4-E/GEI and DMI

Conclusions The findings of this study clearly demonstrate that CH_4-E/GEI is not a fixed factor, but varies with a number of animal and diet factors.

Acknowledgements This study was funded by the Department of Agriculture and Food of the Republic of Ireland and Department of Agriculture and Rural Development of Northern Ireland.

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Whole farm net greenhouse gas abatement of establishing Kikuyu-based perennial pastures in south-western Australia

D Thomas[1], J Sanderman[2], S Eady[3]
[1]CSIRO, Floreat, WA, Australia, [2]CSIRO, Glen Osmond, SA, Australia, [3]CSIRO, Armidale, NSW, Australia
Email: dean.thomas@csiro.au

Introduction Kikuyu-based (*Pennisetum clandestinum* Hochst.) pastures are being established in southern Australia to improve year round feed supply in grazing systems and to increase perennial ground cover to stabilise soils (Sanford *et al*. 2003). These pastures may also have a role in carbon sequestration because soil carbon has been found to increase in areas where kikuyu has been established. However, because farm feed supply is improved by establishing kikuyu pastures it is likely that there will be a corresponding increase in farm stocking rate and enteric emissions. The aim of this study was to examine the value of establishing kikuyu pastures for carbon emissions mitigation when this practice change has an inherent leakage risk with potential increases in livestock-produced methane and nitrous oxide.

Materials and methods A series of soil carbon surveys were conducted on five properties with kikuyu pastures established in the south-west of Western Australia. The kikuyu pastures ranged from 3 to 16 years in age. Carbon sequestration rates were estimated as the difference between the soil organic carbon stocks to 30 cm in each Kikuyu-based paddock and an adjacent paired annual paddock divided by the number of years since conversion. A one-sample *t*-test (one-tailed) was used to assess whether the rate of increase in soil carbon in kikuyu pastures was greater than zero.
A simulation experiment was conducted using the GrassGro biophysical model to evaluate the farm system response for a Merino sheep enterprise by establishing kikuyu pasture. The simulation experiment was located at Gnowellen, Western Australia (34° 24' S, 118° 36' E), which corresponds with one of the experimental sites where soil carbon data was collected. Models were built for two farm systems, these were 1) prior to (Current) and 2) after (Improved) kikuyu pastures were established on land that was allocated to pasture. These models were based on production and management parameters for a mixed cropping and self-replacing Merino sheep enterprise in the Albany Eastern Hinterland in Western Australia (Masters, 2006). Reporting of carbon values (carbon dioxide equivalents) was standardised by winter grazed area of farm land, which refers to the area of the farm that is retained for grazing (not cropped) during the winter/spring growing season.

Results Soil carbon stocks were higher in paddocks where kikuyu was sown, with soil carbon accumulating at an estimated rate of 1.79 ± 0.55 t $CO_{2\text{-}e}$ ha^{-1} yr^{-1} (P=0.016). Greenhouse gas emissions produced per animal were comparable in both Current and Improved farm models. However, due to a higher stocking rate in the Improved farm model, an additional 0.250 t $CO_{2\text{-}e}$ winter grazed ha^{-1} yr^{-1} of methane and an additional 0.023 t $CO_{2\text{-}e}$ winter grazed ha^{-1} yr^{-1} nitrous oxide (a total increase of 0.273 t $CO_{2\text{-}e}$ winter grazed ha^{-1} yr^{-1} of livestock GHG emissions) was produced in the Improved farm scenario. Values for carbon storage, and emissions for Current and Improved farms are reported in Table 1. The net carbon offset value of establishing kikuyu pasture in the Improved farm model was determined to be 0.88 t $CO_{2\text{-}e}$ winter grazed ha^{-1} yr^{-1}, or 0.61 t $CO_{2\text{-}e}$ farm ha^{-1} yr^{-1}.

Table 1 Carbon balance and livestock production efficiency of Current and Improved farm models

Farm model component	Current	Improved
Carbon storage (t $CO_{2\text{-}e}$ winter grazed ha^{-1} yr^{-1})	0.00	1.15
Methane emissions (t $CO_{2\text{-}e}$ winter grazed ha^{-1} yr^{-1})	0.96	1.21
Nitrous oxide emissions (t $CO_{2\text{-}e}$ winter grazed ha^{-1} yr^{-1})	0.10	0.12
Net greenhouse gas emissions(t $CO_{2\text{-}e}$ winter grazed ha^{-1} yr^{-1})	1.06	0.18
Meat production (kg liveweight winter grazed ha^{-1} yr^{-1})	131	168
Wool production (kg clean fleece winter grazed ha^{-1} yr^{-1})	25	33
Meat production efficiency (t farm $CO_{2\text{-}e}$ t $liveweight^{-1}$)	8.1	1.1
Wool production efficiency (t farm $CO_{2\text{-}e}$ t $clean\ fleece^{-1}$)	43	5.5

Conclusions Based on likely practice change associated with establishing kikuyu pastures, net carbon sequestration can be achieved on farms by this method. There was a marked increase in the efficiency of producing livestock products, in terms of the quantity of wool and meat produced per unit of farm carbon emissions. This study may have wider implications for establishing perennial forage species in livestock systems. Provided other perennial species are able to accumulate soil carbon at a rate similar to Kikuyu-based pastures, then planting perennial forage plants on permanent pasture land deserves attention for emissions mitigation.

Acknowledgements This project was supported by the Australian Government Department of Climate Change and Energy Efficiency, and the CSIRO Sustainable Agriculture Flagship.

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Effect of diet protein level on nitrogen excretion and greenhouse gases emissions in lactating dairy cows

N Edouard[1], M Hassouna[2], P Robin[2], P Faverdin[1]
[1]INRA UMR1080 Production du Lait, Saint-Gilles, France, [2]INRA UMR1069 Sol Agro et hydrosystème Spatialisation, Rennes, France
Email: nadege.edouard@rennes.inra.fr

Introduction Increasing concerns regarding greenhouse gas (GHG) emissions from dairy cattle encourage producers to develop feeding strategies to reduce nitrogen and methane excretion. Many studies have shown that reducing N intake decreases N in faeces and urine (Castillo *et al.* 2000). However, a decline of N supplies could affect performances, and increase CH_4 emissions through a modification of rumen degradation characteristics as suggested by Bannink *et al.* (2010) simulating reduction in N grassland fertilisation. The aim of this study is therefore to test the influence of two contrasting dietary N levels on milk production, nitrogen excretion and their consequences for GHG (N_2O, CH_4) emissions in lactating dairy cows.

Materials and methods Two groups of 3 Holstein dairy cows in late-lactation were fed *ad libitum* mixed rations (80:20 maize silage:concentrate) offering either a low (L; CP: 12%DM) or a high (H; CP: 18%DM) dietary crude protein level. Diets were formulated to provide similar metabolizable protein contents (95 and 97 g PDIE/kg DM respectively for L and H) due to higher by-pass protein content in L diet but with degradable N either deficient or in excess (PDIN/PDIE of 0.86 and 1.18 for the L and H respectively, INRA 2007). The experiment followed a split-plot design with 2 periods corresponding to the inversion of the groups between the 2 experimental rooms and 2 sub-periods corresponding to the succession of L and H treatment offered to the animals within each period, leading to 4 sub-periods of 2 weeks each. Milk yields and dry matter (DM) intakes were measured each day throughout the experiment. Measurements of gas emissions (N_2O, CH_4) were performed on the last 10 days of each sub-period with an infra-red photoacoustic analyser (INNOVA 1312), in controlled isolated experimental rooms (temperature, ventilation flow). Digestibility was estimated through the total collection of faeces ([intake – faecal output] / intake), together with a total collection of urine, during four days at the end of each sub-period. Blood samples were collected once per sub-period after the morning ration distribution to assess uraemia. Statistics were performed using the mixed procedure of SAS and a model testing diet N levels as a fixed effect with the sub-period and the cow random effects.

Results Feed DM intake and diet digestibility were reduced on the low diet N level while milk yields were maintained (Table 1). All components of nitrogen balance were higher on the H treatment except faecal N which was not affected. Urinary urea N was 8 times more elevated on the higher diet N level than on the lower one which allowed a higher nitrogen efficiency (N milk / N intake: 0.35 vs 0.23 for L and H). Ruminal kinetics showed a high concentration of rumen liquor N-NH_3 after the morning ration distribution for the H diet (Figure 1). The treatment did not seem to affect N_2O and CH_4 emissions; however, N_2O emissions significantly decreased when urines were totally collected (0.62 vs 0.50 +/-0.23g/d for 'no collection' and 'collection' days respectively, P<0.01).

Table 1 Components of intake, nitrogen balance and greenhouse gas emissions in late-lactating cows fed two diet N levels

	Low N	High N	P	SEM
DM Intake kg/d	21.0	22.0	<0.001	0.6
DM Digestibility	0.70	0.72	<0.001	0.1
Milk Yield kg/d	25.4	26.1		1.4
Urine L/d	10.6	17.6	<0.001	0.5
N Balance g/d	19	56	<0.01	10
N Intake g/d	372	588	<0.001	12
N Milk g/d	129	136	<0.01	7
N Faecal g/d	159	159		5
N Urine g/d	65	237	<0.001	8
Urinary urea N g/d	22	185	<0.001	8
Uraemia (mg/dl) mean 8-11h	10	45	<0.001	2
N_2O g/d	0.54	0.58		0.23
CH_4 g/d	497	507		51
CH_4 g/kg DM intake	23.9	23.7		2.2

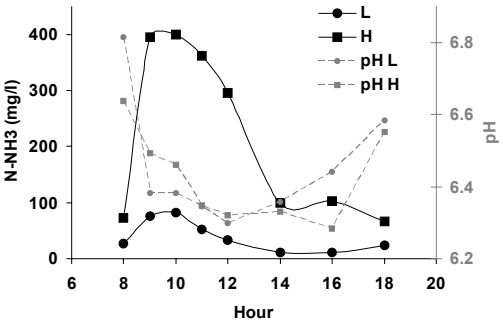

Figure 1 Ruminal liquor N-NH_3 content (mg/l) and pH kinetics

Conclusions Feeding a low degradable CP diet was found to reduce digestible DM intake for the cows. However, due to the metabolizable protein content, this treatment resulted in similar milk yields than the high CP level and improved performances in terms of efficiency of dietary N utilization for milk N production without affecting GHG emissions. The use of low degradable N content of indoor feeding diet could thus be an interesting mitigation option both for climate change and eutrophication.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Rate of intake and crop utilisation by non-lactating Friesian x Jersey dairy cows grazing two allowances of swedes (Brassica napus L) in late pregnancy during winter

D Stevens, B Thompson, A Wall
AgResearch Ltd, Mosgiel, New Zealand
Email: david.stevens@agresearch.co.nz

Introduction Dairy cow are often wintered outdoors on brassica crops such as kale and swedes in grazing systems in the South Island of New Zealand (Judson & Edwards 2008). Ambient conditions during grazing are often relatively cold and wet with often saturated soils. Cows are continually exposed to these conditions during late pregnancy. The feed intake and digestion rates on kale are very rapid (Judson *et al*. 2010) leading to prolonged periods during the day, post-digestion, when the animal is in negative energy balance. This experiment aimed to determine the feed intake rate of dairy cows fed swedes in late pregnancy and to determine if a higher feed allowance would alter the rate of intake and hence the potential rate supply of heat of digestion.

Materials and methods Two hundred and forty five multiparous dry Friesian x Jersey dairy cows (live weight 467 kg +/- 27.9)were allocated to one of two feed allowances as swedes (6 kg DM/cow/d, n=121 and 8 kg DM/cow/d, n=124) during late pregnancy just prior to calving (mean calving date 30 August+/-12.8 d). Each herd was also offered supplements in the form of hay and baleage in equal proportions at 4.0 and 5.5 kg DM/cow/d for the Low and High treatments respectively. Feed was allocated on a daily basis. Feeds were analysed for ADF, NDF, CP, soluble sugars and starch, and ME concentration using near infrared reflectance (Corson *et al.* 1999). The rate of disappearance of leaf, bulb and supplement was measured during four 24 h periods over 10 days between days 220 and 230 of pregnancy. Measurements of the amount of feed on offer and the residual remaining at 1, 3, 5 and 24 hours post allocation were done harvesting all crop in four randomly placed 1.0 m^2 quadrats and separating into leaf and bulb. Leaf found on the ground at 3 hours was presumed to be lost to grazing thereafter. The amount of feed consumed at each time after feed allocation was analysed by a simple ANOVA with each harvest day being a replicate. The rate of feed intake post allocation was analysed by a repeated measures multi variate model using REML variance components analysis with fixed effect being the brassica allowance and the repeated measure being time after daily feed allocation.

Results The allocation of High and Low allowances of swedes and supplement resulted in significantly different total intake of feed at each of the four time points recorded, being 6.03 and 5.29, 8.33 and 6.50, 9.01 and 7.38, and 12.97 and 8.98 kg DM for high and low allowances at 1, 3, 5 and 24 hours post allocation respectively, (SED: 0.43, $P<0.05$). Feed intake rate (kg DM/h) was higher on the High allocation than the Low allocation (Figure 1, SED: 0.28, $P<0.05$) and was highest during the first hour after allocation than during the rest of the grazing period (Figure 1, SED: 0.26, $P<0.01$). These effects were also apparent in the rate of swede intake ($P<0.05$), but there was only an effect of time after allocation on the rate of supplement intake ($P<0.01$). Feed nutritional analysis estimated the metabolisable energy concentration as 11.1, 14.3, 7.9 and 11.4 MJME/kg DM for the swede leaf, bulb, hay and baleage respectively (SED: 0.15).

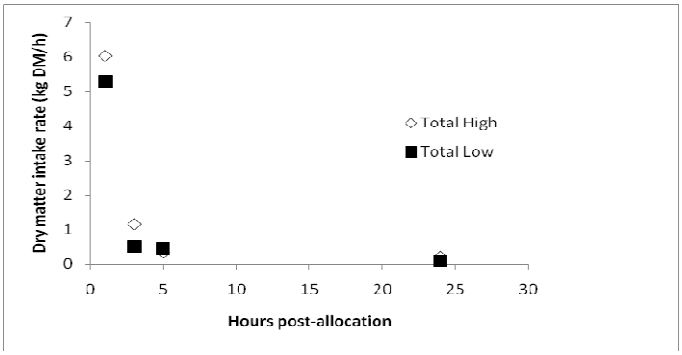

Figure 1 Intake rate of swedes and supplements by non-lactating dairy cows offered two allowances in late pregnancy

Conclusions The intake of swedes by non-lactating dairy cows in late pregnancy, estimated by crop disappearance, was not significantly affected by allowance at one hour after allocation, being approximately 4 kg DM. However, the intake of the cows on the Low allowance was then limited by the availability of the forage having eaten approximately 80% of the full allowance. The intake of cows offered the high allowance did not achieve 80% utilisation of the swedes until approximately 5 hours after allocation. The consumption of the supplements was similar on both allowances until 5 hours post allocation, irrespective of swede intake, indicating that the harvestibility of the crop may have influenced the rate of intake. The cows continued to consume forage as baleage and hay with intake diverging with time. The concept that a higher allowance would change the rate of feed intake was unproven in this experiment. This data provides a base line to understand the intake dynamics of non-lactating dairy cows in late pregnancy when offered different allowances. Metabolisable energy intakes were calculated as 103 and 149 MJME/cow/d for the Low and High allowances respectively, demonstrating the significant requirements for dairy cows wintered outdoors, grazing crops *in situ*.

Acknowledgements The authors thank Telford Rural Polytechnic for hosting the experiment and managing the cows and Dairy NZ for funding.

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Effect of the addition of reductive acetogenic bacteria on *in vitro* ruminal fermentation and methane emission

M-J Gu[1], H-I Lee[1], L Mamuad[1], C-O Jeon[2], Y-K Oh[3], S-S Lee[1]
[1]Department of Animal Science & Technology, Sunchon National University, Suncheon, Jeonnam, Republic of Korea, [2]Department of Life Science, Chung-Ang University, Seoul, Republic of Korea, [3]National institute of Animal Science, RDA, Suwon, Gyeonggi-do, Republic of Korea
Email: mjvictress@sunchon.ac.kr

Introduction Reductive acetogenesis is an alternative hydrogen-utilizing pathway to methanogenesis in the rumen and has potential as a strategy for reducing ruminant greenhouse gas emissions and inhibiting feed energy loss. Homoacetogens use the Wood-Ljungdahl pathway for growth on hydrogen and carbon dioxide as the sole energy source (Diekert, G., and G. Wohlfahrt. 1994). The objective of this study was to isolate and identify new homo-reductive acetogens from rumen contents of Holstein cows and Korean black goats, evaluate the characteristics of these strains and determine the effectiveness on *in vitro* rumen fermentation patterns and methane output by feed substrates.

Materials and methods The ruminal samples were collected from Holstein cows fitted with cannula at the experimental farm and slaughtered Korean black goats. Rumen fluid was transferred to an anaerobic chamber [gas phase, 90% N_2-5% CO_2-5% H_2 (vol%)] for inoculation into AC-B1 medium (T.D. Le Van *et al*. 1998), pressurized to 300 kPa with 80% H_2-20% CO_2 (vol%) and incubated for 10 days at 39°C and 200 rpm. Isolates were analyzed the 16S rRNA gene sequence using the NCBI-BLAST search. To measure the presence of rumen homoacetogens, we examined sequences of formyltetrahydrofolate synthetase (FTHFS) and constructed the Phylogenetic tree. Acetate production and other fermentation end products by FTHFS-containing isolates supplied H_2 and CO_2 or glucose as substrates were determined using the high performance liquid chromatography (HPLC). Serum bottles of 50 mL volume were filled under a stream of CO_2 gas with rumen fluid and Coleman buffer (1:2, vol: vol) for *in vitro* fermentation. The bottles containing 0.2 g of Casein and soluble starch, respectively, were inoculated with 5 mL of the 96 h cultured acetogens and placed in the 39°C shaking incubator for 24 and 48 h at 100 rpm. After incubation, pH, volatile fatty acids (VFA), total gas production and methane emission were measured using a pH meter (Panicles), HPLC (Agilent), press and sensor machine (EA-6) and gas chromatography (Agilent), respectively. All data were subjected to use the procedures (SAS, 2003)

Results Of fifty-two colonies, four acetogenic bacterial strains (DA01, GA01, GA02, and GA03) were selected and clustered as *Proteiniphilum acetatigenes* TB107 (AY742226) and *Alkaliphilus crotonoxidans* B11-2 (AF467248). These strains confirmed the presence of FTHFS gene and showed a lower similarity with sequences of known homoacetogens. Isolates produced more acetate from H_2 and CO_2 and glucose compared with reference acetogenic strains. The highest amount of acetic acid was detected with the strain of GA02 derived from Korean native goat's rumen and butyrate produced only when added the glucose. Most of the isolated acetogens reduced the production of total gas and methane (figure 1 and 2) with increased effect on total VFA, acetate and propionate in comparison to the control ($p<0.05$) and added reference strain (*Eubacterium limosum* ATCC 8486). The methane formation was greatly inhibited, when DA01 and GA03 were added to the cultures of mixed rumen microorganisms in each of the treatment of casein and soluble starch.

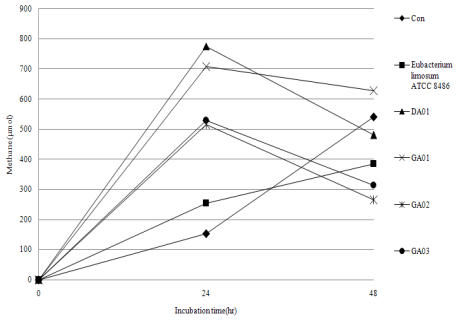

Figure 1 Amounts of methane produced per ml of *in vitro* culture in casein treatment after incubation

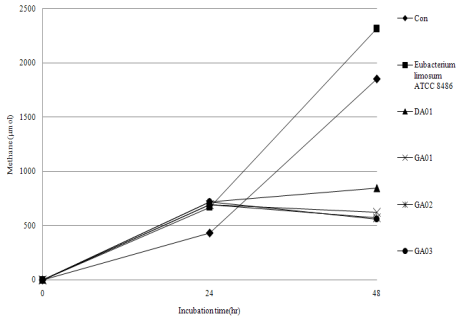

Figure 2 Amounts of methane produced per ml of *in vitro* culture in starch treatment after incubation

Conclusions Identified Sequences of 16S rRNA gene from rumen were diverse but only 4 strains contained FTHFS gene and showed the lower sequence similarity. The highest amount of acetic acid was detected with the strain of GA02 isolated from Korean black goat's rumen and butyrate produced only when glucose added. Addition of DA01 in casein and GA03 in soluble starch treatment reduced methane production and increased total VFA. This results need to compare in qPCR assay and PCR-DGGE pattern using methanogen and reductive acetogen specific primers and may guide further *in vivo* studies to develop effective mitigation of methane emission from ruminants.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Repeatability of the methane production of sheep fed *ad libitum*

S Bickell[1,3], A Toovey[2,3], D Revell[1], P Vercoe[1,3]
[1]School of Animal Biology, The University of Western Australia, Crawley WA 6009, Australia, [2]CSIRO Livestock Industries, Wembley, WA 6913, Australia, [3]Sheep Cooperative Research Centre, Armidale, NSW 2351, Australia
Email: samantha.bickell@uwa.edu.au

Introduction Total methane production increases with feed intake, but the rate of increase declines for each extra unit of feed consumed (Blaxter and Clapperton 1965). Thus the linear relationship between methane production (MP) and feed intake (FI) is weaker at higher levels of FI. In order to minimise the variability in MP due to FI, many studies investigating MP of ruminants restrict the amount of feed offered. The decision on the level of feeding to use is important, especially in studies that aim to identify individuals that consistently produce less methane than their counterparts (Hegarty *et al*. 2010). It has been suggested that feeding conditions that maximize feed intake favour the expression and persistence of between-sheep differences in MP (Pinares-Patiño *et al*. 2003) and thus having access to *ad libitum* feed may enable animals to express their 'true' MP. This suggests that although MP at higher levels of FI is more variable between animals, MP may be more repeatable over time (with the same animals) than MP at lower levels of feed intake. This study aimed to determine the repeatability over four weeks in MP of sheep fed *ad libitum*.

Materials and methods The methane production (MP) of Merino wethers (n = 20; 2 years old) at 6h, 12h and 23h were measured twice, 4 weeks apart using respiration chambers as described by Klein and Wright (2006). Sheep were fed a mixed ration (90% chaffed hay and 10% cracked lupins) *ad libitum* for 10 weeks before the first methane measurements, and throughout the four weeks between the next methane measurements. Food and water were offered *ad libitum* in the respiration chambers and feed intake was determined from digital scales remotely logging the weight of each animal's feed container every 5 min. Correlations of MP and MP per unit of feed intake at 6h, 12h and 23h between the two measurements periods were performed with a Pearson's Correlation Test.

Results A significant correlation between MP measurements at 10 and 14 weeks was found when MP was measured over 23 hours ($r = 0.553$, $p < 0.001$) and 12 hours ($r = 0.401$, $p = 0.03$) but not over 6 hours ($r = 0.213$, $p = 0.18$; Figure 1) whilst sheep were in the respiration chambers. However, when MP is expressed per unit of feed intake, the correlation between the two MP measurements was only significant when measured over a 23-hour period ($r = 0.376$, $P = 0.05$) but not over a 12-hour ($r = 0.248$, $p = 0.14$) or 6-hour period ($r = 0.219$, $p = 0.18$).

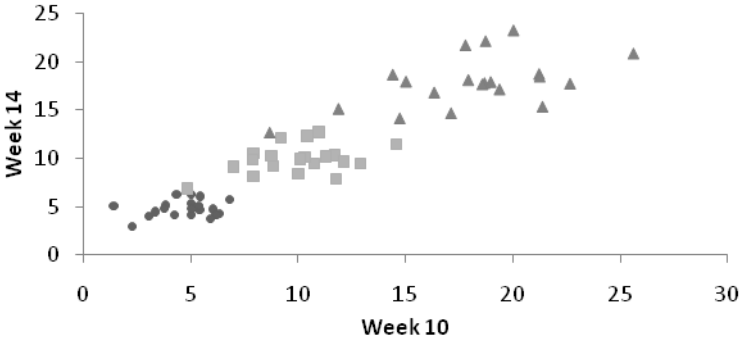

Figure 1 Correlation of methane production (g/hours in chamber), measured 4 weeks apart, of 20 sheep fed ad lib after 6h (●), 12h (■) and 23h (▲) in a respiration chamber.

Conclusions Methane production was found to be repeatable over two measurements made four weeks apart, indicating that MP is a trait that can be quantified in individuals to identify animals with lower (or higher) MP. Importantly, our data show that for animals fed *ad libitum,* which resembles the situation in some commercial situations, MP should be measured over 23-hour, or possibly longer, periods to obtain a reliable measurement. The repeatability of measurements made over the 12- or 6-hour periods that sheep were in the respiration chambers was not as strong, suggesting that it will be harder to make progress in identifying individual animals with lower MP if we measure MP over short time periods (<12 hours) whilst being fed *ad libitum*. While our results provide valuable information about the repeatability of MP of sheep fed *ad libitum*, further investigations into the best methodology for measuring and predicting an animal's 'true' methane production are still needed.

Acknowledgements This research was supported by funding from Meat and Livestock Australia and the Sheep Cooperative Research Centre.

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Plant-mediated proteolysis among red clover (*Trifolium pratense* L.) cultivars and the relationship with the polyphenol oxidase activity

M Seebeck, N Weiher, P Voss, F Taube, M Gierus
Grass and Forage Science/Organic Agriculture - Christian Albrechts University, Kiel, Germany
Email: mgierus@email.uni-kiel.de

Introduction In red clover, the activity of the polyphenol oxidase (PPO) is high compared to other forage legumes. Several studies indicate that the specific PPO activity may decrease the protein degradation rate in the silo and in the rumen. Recent investigations demonstrated that plant proteases are involved in protein degradation as well (Theodorou *et al*., 1996). Although the microbial activity is primarily responsible for protein degradation in the rumen, information concerning the variation caused by PPO activity on plant mediated proteolysis is poorly described. In this study the response of three red clover cultivars with distinct PPO activities and white clover as control were tested using an *in vitro* system to simulate the ruminal environment without microbes. The objective was to identify the influence of the specific PPO activity on the reduction of protease activity, which may be used in future breeding programs.

Materials and methods Samples were collected from a field experiment in Northern Germany at the first cut of 2009. Three red clover cultivars (cv. Harmonie, Montana and Milvus) and white clover (cv. Vysokan) were grown in a randomized complete block design with three replicates. Leaf blades were rinsed with dist. water, chopped and incubated under anaerobic conditions at 39°C for different time periods (0, 6, 24 hours). The true protein concentration of the leaves was measured according to the method described by Bradford (1976). The degradation rate was calculated with the following equation: [Kd (%/h) = (ln B_0 –ln B_{24})/24], whereas B_0 and B_{24} are the protein contents after 0 hours and 24 hours. The specific PPO activity after Eickler *et al.* (2011) was measured in leaves. The data were submitted to the analysis of variance. With a significant F-test, means were separated using pre-planned contrasts with the Tukey-Kramer-Test (P<0.05).

Results The protein content declined as the incubation period increases (Tab. 1). Differences among red clover cultivars and white clover were observed at 24 h. The degradation rate differed only when the whole incubation time from 0-24 h (P<0.07) was considered. The highest degradation rate was for white clover leaves.

Table 1 Changes in the protein content (mg/g DM), degradation rate (%/h, 0-24 h) and specific PPO activity (IU/µg prot./g DM)

hours	Red clover			White clover	SE
	Harmonie	Montana	Milvus	Vysocan	
0 h	1.54	1.55	1.50	1.51	0.04
6 h	0.25	0.20	0.21	0.27	0.01
24 h	0.18 [a]	0.15 [ab]	0.15 [ab]	0.09 [b]	0.01
Deg. rate	9.2 [b]	10.0 [ab]	9.8 [ab]	12.2 [a]	0.01
PPO	1.81 [a]	1.16 [ab]	1.59 [a]	0.06 [b]	0.30

Conclusions The decline in true protein content over the incubation period indicates that the plant derived proteases are involved in the process of proteolysis. However, the degradation rates of the tested cultivars and the specific PPO activity were not related.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Lipid supplementation and basal diet influence oxidative and lipogenic metabolism in muscle and adipose tissues in cattle

M Bonnet[1], I Cassar-Malek[1], D Bauchart[1], Y Chilliard[1], C Jurie[1], A Thomas[1], J Normand[2]
[1]INRA, UR1213, Saint-Genès-Champanelle, France, [2]Institut de l'Elevage, Lyon, France
Email: isabelle.cassar-malek@clermont.inra.fr

Introduction Lipids are added to diets of bovine as a source of energy and some specific fats are used to alter the fatty acid profile of meat and milk toward creation of value-added bovine products. Fatty acids provided by the dietary fats have been shown to regulate gene expression in adipose tissue (AT) and muscle which ultimately may affect their metabolism and growth (Hausman *et al*. 2009). Muscle growth relatively to AT growth determines the lean-to-fat ratio of the carcass which is an economic challenge for the beef industry. However, the impact of dietary fatty acids on the metabolic pathways crucial for the growth of both AT and muscles remains incompletely known. In this study, we analysed the influence of lipid supplementation (*n*-3 polyunsaturated *vs.* saturated fatty acids) in interaction with the basal diet on oxidative and lipogenic metabolisms in the *Longissimus thoracis* (LT) muscle and in two AT (subcutaneous and intermuscular). Our objective was to evaluate if changes in the nutrient profile could impact nutrient partitioning and oxidation between ATs and muscle.

Materials and methods Forty-five dry Normand cows (687 kg mean live weight) were given during a 100-day finishing period six experimental diets (n = 7 to 8 per diet) formulated to meet energy and protein requirements and to be isoenergetics. Diets were based either on maize silage (70%) plus concentrate (30%, MS) or on concentrate (70%) plus straw (30%, C) supplemented with no additional lipids (experimental diets named MS and C) or with either 5% of lipids in total dry matter, from either extruded linseeds (rich in C18:3 *n*-3; MSL or CL diets) or palmitostearin (rich in C16:0 and C18:0; MSP and CP diets). The percent (relative to the total fatty acids) of C16:0, C18:0 and C18:3 *n*-3 were assayed in LT by gas liquid chromatography. The oxidative metabolism was assessed by measuring the activities of isocitrate dehydrogenase (ICDH, involved in the tricarboxylic acid cycle) and phosphofructokinase (PFK, involved in glycolysis). Lipogenesis was assessed by measuring the abundance of transcripts (qPCR experiments) encoding the fatty acid synthase (FAS, involved *in de novo* lipogenesis) and lipoprotein lipase (LPL, responsible for the hydrolysis and captation of circulating triglycerides by AT and muscle) as previously described (Bonnet *et al.* 2007; Jurie *et al*. 2007). Data were analysed using the MIXED procedure of SAS (1989). Fixed effects included the type of basal diet, the type of lipids and their interaction.

Results The basal diet and lipid supplements had no significant effects on the carcass weight (328 kg in average), lipid content in the LT (3.2 g/100 g fresh tissue) and soluble protein content of AT and LT. *Effect of basal diet*: C basal diet compared to the MS basal diet decreased (- 10%, $P = 0.05$) the absolute and relative weights of perirenal AT and tended ($P = 0.07$) to increase the activity of PFK in intermuscular (+ 21%) and subcutaneous (+ 13%) AT. The weights of the other visceral and subcutaneous AT as well as the percent of fatty acids in LT and the metabolic pathways were not modified by the composition of the basal diet. *Effect of lipid supplementation:* Whatever the basal diet, extruded linseeds (MSL or CL compared to MS or C) decreased C16:0 (- 8%, $P < 0.01$) and increased ($P < 0.05$) C18:0 (+ 10%) and C18:3 *n*-3 (+ 212%) percentage in LT, while palmitostearin (MSP or CP compared to MS or C) only slightly increased C18:0 (+ 9%, $P = 0.05$). Whatever the basal diet, extruded linseeds and palmitostearin decreased the abundance of mRNA encoding FAS (- 27% in average, $P = 0.05$) in the intermuscular and subcutaneous AT, as well as the activity of PFK (- 40% in average, $P = 0.01$) in LT. Conversely, lipid supplements did not affect neither activities of PFK and ICDH and abundance of mRNA encoding LPL in AT, nor ICDH activity and abundances of mRNA encoding FAS and LPL in LT muscle.

Conclusions The two basal diets allowed significant transfer of C18:3n-3 from the dietary extruded linseeds to the LT muscle. Palmitostearin had a minor effect on the saturated fatty acid content of muscle. Altogether, these results pointed out a tissue specificity of the effects of lipid supplementation on metabolism: reduction of glucose catabolism and maintenance of lipogenic activities in muscle *vs.* decrease in *de novo* lipogenesis in ATs. Similarly, the basal diet composition affected the metabolism of ATs but not that of the LT muscle. These data confirm the generally negative effect of lipid supplementation on lipogenic activities of AT in growing cattle (Chilliard, 1993) and highlight that lipid supplementation reduces glucose oxidation in LT muscle and ultimately may allow glucose sparing which could be thus available for other tissues or organs.

Acknowledgements This work was carried out in the “Lipivimus” Project with the financial support of the French National Research Agency (ANR) - Project ANR-06-PNRA-018.

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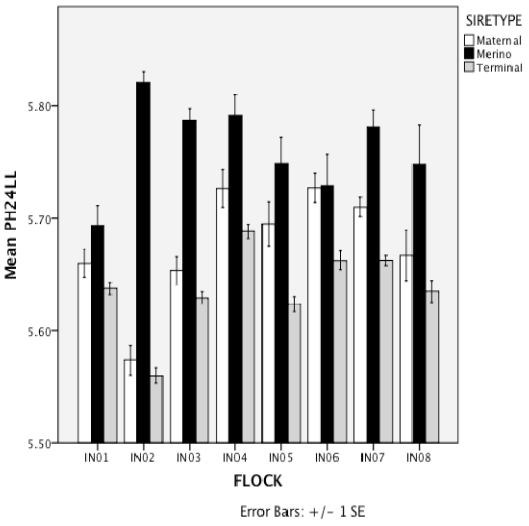
Genetic and non-genetic effects on ultimate meat pH of lamb meat

K Pearce[1], R Warner[3], R Jacob[2], D Hopkins[4], S Mortimer[5], D Pethick[1]
[1]Murdoch University, Murdoch, WA, Australia, [2]Department of Agriculture and Food Western Australia, Perth, WA, Australia, [3]CSIRO, Werribee, Victoria, Australia, [4]Industry and Innovation NSW, Cowra, NSW, Australia, [5]Industry and Innovation NSW, Trangie, NSW, Australia
Email: k.pearce@murdoch.edu.au

Introduction The ultimate pH of meat (measured at approx. 24 hours post slaughter) is a key quality determinant of sensory attributes of sheep meat (Warner *et al* 2010). Between a pH of 5.4-5.7 sheep meat is tender, juicy and light in colour with better keeping qualities. Muscle glycogen levels at slaughter are governed by the quality of pre-slaughter nutrition and depletion results from stress and/or exercise prior to slaughter. Breed type may also play an important role, with Australian studies indicating that the Merino sheep breed has a higher incidence of elevated ultimate pH compared to first and second cross lambs (sired by Border Leicester and Poll Dorsets) (Hopkins *et al* 2005). This is attributed to a greater sensitivity to stress and therefore a greater glycogen depletion rate than crossbred lambs (Gardner *et al* 1999). The objectives of this study are to better quantify the genetic and non-genetic factors and relevant interactions influencing ultimate pH levels in pure merino and cross bred lambs representing diverse genetic backgrounds.

Materials and methods This experiment was completed as part of the CRC for Sheep Industry Innovation Information Nucleus flock (further details refer to van der Werf *et al* 2010). Briefly, approximately 2000 lambs were produced in 2009 from AI mating to Merino and crossbred ewes located at 8 research sites across Australia (Katanning WA, Trangie NSW, Cowra NSW, Kirby NSW, Struan SA, Turretfield SA, Hamilton VIC, and Rutherglen VIC) representing a broad cross section of Australian production systems. These lambs were the progeny of 93 key industry sires representative of the major production types in the Australian sheep industry. The sires can be classified by sire type as either merino, maternal or terminal resulting in either pure merino or crossbred lambs. Within each sire type there are a number of sire breeds of which merino and poll merino are included under the merino sire type and crossbred lambs would be under maternal and terminal type. At each of the 8 sites there are pure merino and cross bred lambs all being run under the same feeding and environmental conditions. Lambs were slaughtered at their target average carcase weight of 21.5kg. The pH and temperature of each carcass was measured about 19-24 hours after slaughter taken in the left portion of the m. *longissimus thoracis et lumborum* (loin or pH24LL) muscle at the 12th rib site (further details refer to Pearce *et al* 2010). A linear mixed effects model was used to analyse the data and included fixed effects (and their relevant interactions) for site, sex, birth type-rear type, sire type, dam breed within sire type, sire breed within sire type and date of kill. Sire and dam identification were included as random terms.



Results There were significant (P<0.01) main effects for site, kill date, sire type and sire breed within sire type effect. Within sire type the average ultimate pH for the merino sire breeds was 5.73 with a range of 5.65 to 5.90 (se of 0.013). For the maternal sire type the average ultimate pH across the sire breeds was 5.67 (Range of 5.63-5.72, SE of 0.024) and terminals the ultimate pH was 5.63 (range of 5.59-5.67, se of 0.03). Within each site there were marked differences between kill dates which varied by as much as 0.15 pH units with kills containing higher proportion of merinos having a higher ultimate pH. There was a significant variation in ultimate pH across sites with site IN02 having the lowest overall ultimate pH but the highest merino sire type ultimate pH. This finding reflects differences in finishing treatments between sites. Site IN07 had the highest ultimate pH overall which may be because these lambs were finished for slaughter on a maintenance ration, which resulted in lower muscle glycogen levels, and therefore a higher ultimate pH compared to other sites that were finished on above maintenance rations.

Figure 1 Ultimate pH of the loin (pH24LL) for the different sire types across sites (mean ± SE).

Conclusions This study has shown that despite similar finishing conditions within site, the ultimate pH of the loin is significantly different between sire types and sire breeds within sire type. The ultimate pH of loins from merino sire types was significantly higher than maternal and terminal sire type lambs. Maintaining sufficient levels of glycogen in merino lamb meat through adequate pre slaughter nutrition is essential to maintain the sensory appeal for consumers.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Effect of ground flaxseed and exogenous hormones on plasma and muscle fatty acid composition, growth performance, and carcass characteristics of finishing cattle

C Van Bibber, L Litton, K Miller, J Drouillard
Kansas State University, Manhattan, Kansas, USA
Email: cadlvabi@ksu.edu

Introduction Flaxseed is a rich source of secoisolariciresinol diglycoside (SDG) and omega-3 fatty acids. Linseed meal (LSM) is a protein meal derived through solvent extraction of oil, resulting in a more concentrated form of SDG. Gastrointestinal bacteria can convert SDG in the hull of flaxseed to estrogen-like compounds that are metabolically active in mammals (Clavel *et al*., 2005). Dunn *et al*. (2003) reported that feeding diets with flaxseed resulted in poorer average daily gain of steers implanted with exogenous steroids compared to those fed diets without flaxseed, but the opposite was true in steers without implants, suggesting a possible hormonal effect of the flaxseed. Therefore, the objective of the present study was to further evaluate interactions between flaxseed, or its derivative, LSM, and exogenous steroid growth promotants in fattening cattle fed cereal-based diets.

Methods and materials This study was completed in two trials. In trial one, Holstein steers (n=40; 499 ± 46 kg) were used in a randomized complete block experiment with a 2 × 2 factorial treatment arrangement to evaluate the effects of ground flaxseed and steroidal implants on growth performance, carcass quality, and fatty acid composition of plasma and longissimus muscle lipids. Cattle were blocked by individual body weight and assigned randomly to 1 of 4 treatments. Treatments were as follows: soyabean oil with implant, soyabean oil without implant, flaxseed diet with implant, and flaxseed diet without implant. Diets contained similar concentrations of lipid, and ten animals were assigned to each treatment. Every 28-day period cattle were weighed individually, residual feed collected, and blood samples taken from the jugular vein. The heavier blocks were harvested on day 89, and the lighter blocks were harvested on day 119. The primal rib sections (6^{th} through 12^{th} ribs) were collected from one side of each carcass following 48 hours of refrigeration.
In trial 2, 366 heifers (374 ± 0.6 kg) were arranged in a randomized complete block experiment with a 2 × 2 factorial treatment arrangement to evaluate growth performance of diets with and without linseed meal, both in the presence or absence of steroidal growth promotants (estradiol/trenbolone acetate implants and the feed additive, melengestrol acetate). Heifers were housed in pens of 6 to 8 animals each, with a total of 48 pens. Half of the cattle were harvested on day 119, and the remainder on day 120. Carcass data collected for both trials included hot carcass weight; incidence and severity of liver abscesses; intramuscular fat score; subcutaneous fat thickness over the 12^{th} rib; longissmus muscle area; percent kidney, pelvic, and heart fat; USDA yield grades, and USDA quality grades. Dressing percentage was calculated as hot carcass weight divided by shrunk body weight. Data were analyzed using the Mixed model procedure of the Statistical Analysis System (SAS, 2004). Experimental unit was animal in trial 1, and pen in trial 2. For both trials, mean comparisons were protected by an *F*-test with $P \leq 0.05$. Fixed effects consisted of diet, growth promotant status, and their interaction. Random effects consisted of animal in trial 1, and block in trial 2.

Results In trial 1 there was no effect ($P \geq 0.22$) of diet or the interaction between diet and implant status on finishing performance. Implants improved dry matter intake ($P = 0.02$), average daily gain ($P < 0.01$), and feed efficiency ($P < 0.01$). When implanted, cattle fed flaxseed had the highest yield grade of all treatments, though this difference was only significant compared to flaxseed/no implant cattle. A similar effect from the interaction between diet and implant was observed for marbling scores ($P = 0.04$). Hot carcass weights increased ($P < 0.01$) and dressing percentage decreased ($P = 0.04$) when cattle were implanted. There was no effect of diet ($P \geq 0.11$) on carcass quality. With the exception of plasma C18:1n7, interactions between diet and implant status did not affect ($P > 0.05$) fatty acid composition of plasma or longissmus muscle tissues. Plasma total omega-3 increased ($P < 0.01$) when cattle were fed flaxseed. Subsequently, plasma n-6:n-3 fatty ratio also decreased ($P < 0.01$) when cattle were fed flaxseed. Longissimus muscle total omega-3 fatty acids increased ($P < 0.01$) with flaxseed feeding, subsequently decreasing ($P < 0.01$) n-6:n-3.
In trial 2 finishing performance was not affected ($P \geq 0.12$) by the interaction between diet and exogenous hormones or by the main effect of diet, but use of exogenous growth promotants greatly affected performance. The use of anabolic steroids and estrous suppressants increased dry matter intake ($P = 0.02$), average daily gain ($P < 0.01$), and improved feed efficiency ($P < 0.01$) in conventional cattle compared to non-hormone treated cattle. Carcass quality was not affected ($P \geq 0.10$) by the interaction of diet and exogenous hormones or the effect of diet, but hormone-treated cattle had increased hot carcass weight ($P < 0.01$) compared to non-hormone treated cattle.

Conclusion The interaction between flaxseed products and exogenous hormones resulted in small, but non-significant effects on growth performance of fattening cattle. Flaxseed could be fed without negatively impacting response to exogenous steroids. Exogenous hormones improved dry matter intake, average daily gain, feed efficiency, and hot carcass weight of cattle when compared to non-hormone treated regimes. Flaxseed or its derivative, LSM, did not act as a natural growth promoter. However, feeding ground flaxseed does increase the proportion of omega-3 fatty acids in beef without compromising finishing performance.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Effect of diet and CSN1S1 genotype on nutritional, productive and metabolic responses of milking Girgentana goats

A Bonanno[1], A Di Trana[2], V Bellina[1], G Tornambè[1], A Di Grigoli[1], M Todaro[1], P Di Gregorio[2], G Maggio[2]
[1]Università degli Studi di Palermo, Palermo, Italy, [2]Università degli Sudi della Basilicata, Potenza, Italy
Email: abonanno@unipa.it

Introduction Goat polymorphism at α_{s1}-casein locus (CSN1S1) influences several milk production traits. In fact, milk from goats carrying strong alleles, associated to high α_{s1}-casein level, resulted higher in fat and casein and showed longer coagulation time and firmer curds (Mahè and Grosclaude, 1993) compared with milk from goats with weak alleles linked to low α_{s1}-casein content. As these milk properties are also affected by nutrition, it is of some interest to consider the interaction of the CSN1S1 genotype and dietary characteristics. Few studies focusing on this aspect, demonstrated that strong alleles are related to a greater efficiency of protein utilization (De la Torre *et al*. 2009), and to a better response to high dietary energy level compared to weak alleles (Pagano *et al*. 2010). In order to contribute to the knowledge of the relationships between nutrition and CSN1S1 genotype in goats, this study was aimed to investigate the impact of fresh forage based diet and/or energy supplement on feeding behaviour, milk production, metabolic and hormonal parameters and oxidative stress of Girgentana goats with different genotype at CSN1S1 locus.

Materials and methods From a group of milking goats genotyped using specific PRC protocols at DNA level, 12 goats, averaging 37.2±3.5 kg of live weight, were selected for having the same genotype at CSN1S2, CSN2 and CSN3 loci and differing for the CSN1S1 genotype (G): 6 goats were homozygous for a strong allele (AA) and 6 heterozygous for a weak alleles (AF). Goats of each genotype were allocated homogeneously, based on days in milking (DIM, 50 or 120 days), to 3 sub-groups and fed *ad libitum* in individual pen with 3 diets, in a 3 x 3 Latin square design with 3 periods (P) comprised of 14 days for adaptation and 7 days for data and samples collection. The diets (D) were sulla (*Hedysarum coronarium* L.) fresh forage (SFF), sulla fresh forage plus 800 g/d of barley meal (SFB), mixed hay plus 800 g/d of barley meal (MHB), with 130, 95 and 85 protein/energy ratio (P/E, g protein/Mcal net energy), respectively. During experimental period, milk production and feed intake were measured every day, and milk quality was detected three times. Blood samples were collected at the end of pre-experimental and experimental periods. Plasma content of NEFA, glucose, insulin, fT3 and fT4 was detected; also same plasma markers of oxidative stress were measured as Reactive Oxygen Metabolites (ROMs), biological antioxidant potential (BAP) and □-tocopherol. Data were analysed by a mixed model with DIM, P, G, D and GxD as fixed effects, and goat as random effect. Data of pre-experimental period were used as covariates.

Results There was no significant effect of G and interaction GxD on dry matter (DM) and nutrients intake, efficiency of dietary protein utilization for milk casein synthesis (EPU), glucose, NEFA, insulin, ROMs, BAP and □tocopherol content. With regard to D effect, DM intake was lower with MHB than SFF and SFB (1655 *vs.* 1820, 1807 g/d; P<0.01), whereas protein intake increased passing from MHB to SFB and SFF (203 *vs.* 290 *vs.* 321 g/d, P<0.01). NE intake was higher in SFB (3.0 *vs.* 2.3, 2.4 Mcal/d for SFB, MHB, SFF; P<0.01), and ingested NDF was higher in SFF than in MHB and SFB (632 *vs.* 539, 483 g/d; P<0.01). The diet greatly affected milk yield, which increased from SFF to MHB and SFB (1353 *vs.* 1423 *vs.*1664 g/d; P<0.01). The significant effect of GxD on milk yield (P<0.01) was linked to the superiority induced by SFB diet in AA than in AF goats (1720 *vs.* 1608 g/d; P<0.05). Milk composition was affected by D equally in both genotypes. Barley supplement contributed to reduce fat (36 *vs.* 32 *vs.* 30 g/kg in SFF, SFB, MHB; P<0.01) and urea in milk (35 *vs,* 32, 31 mg/dl in SFF, SFB, MHB; P<0.01), whereas the fresh forage increased the casein content (27, 27 *vs.* 26 g/kg in SFF, SFB, MHB; P<0.01). The EPU was the highest in MHD group, due to lower protein intake, whereas it was favoured by energy supplement (191 *vs.* 164 *vs.* 115 g casein/kg protein intake in MHB, SFB, SFF; P<0.01). The milk of AA goats showed longer coagulation time (r: 15 *vs.* 14 min; P<0.05) and higher curd firmness (a_{30}: 36 *vs.* 29 mm; P<0.01) than AF milk. The D affected NEFA, ROMs and BAP. SFF showed the higher NEFA than other diets (0.39 *vs.* 0.23, 0.21 mmol/l in SFF, MHD, SFB; P<0.01). The BAP increased in groups that utilized fresh forage compared to group fed mixed hay (7.68 *vs.* 8.01, 7.99 ln □mol/l in MHB, SFF, SFB; P<0.05). The ROMs level was lower in SFF goats (3.90 ln U.Carr) compared to MHB and SFB. A significant effect of G (P<0.05) was detected on fT4, which was higher by 15% in the AF than in the AA (1.02 *vs.* 0.88 ng/dl; P<0.05). A slight increase of fT3 was detected in AA compared to AF (3.58 *vs.* 3.36 pg/ml; P=0.10). Interaction GxD (P<0.05) was found for fT3, being higher in AA goats than AF goats (3.92 *vs.* 3.03 pg/ml) but only when fed the MHB diet.

Conclusions In this study, the diet showed a more important effect than genotype. As expected, energy supplement improved DM and protein intake, milk yield and dietary protein utilization for milk casein, and reduced milk fat and urea, whereas the sulla fresh forage based diets increased casein and showed higher protection against oxidative stress than dry diet. The higher NEFA of the SFF goats indicates that they supported the energy deficit by body fat reserve mobilization. The CSN1S1 genotype interacted with diet only for milk yield, since the SFB diet, due to a better balanced P/E ratio and lower NDF intake, increased milk yield of AA goats, but this improvement was less pronounced in AF goats. Although the genotype AA did not affected casein, the AA milk showed firmer curd than AF milk, suggesting a more favorable repartition of different caseins in AA milk that is worth to be investigated.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Characterization of polyphenol oxidase activity in different forage ecotypes with different phenolic substrates

A Cabiddu[1], M R. F. Lee[3], M Decandia[1], G Molle[1], L Salis[1], M Vargiu[2], A L Winters[3]
[1]Agris-DIRPA, Olmedo, Italy, [2]Agris DIRVE, Cagliari, Italy, [3]IBER University Wales s, Aberystwyth, UK
Email: acabiddu@agrisricerca.it

Introduction Polyphenol oxidase (PPO) is amongst the largest endogenous factor in plants affecting lipolysis, through denaturation of plant lipases. In particular a review by Van Ranst *et al*. (2011) has highlighted the effect of PPO on lipolysis and biohydrogenation both in silo and *in vivo*. Cabiddu *et al*. (2010) highlighted that others molecules, such as certain phenols (e.g. tannins), could interfere with PPO activity with subsequent interaction effects. The aim of this work was to identify the level of PPO activity (using a range of diphenolic substrate), protein bound phenols (PBP) and their main source of variation in different forage ecotypes (FE) cut at different phenological stages (PS).

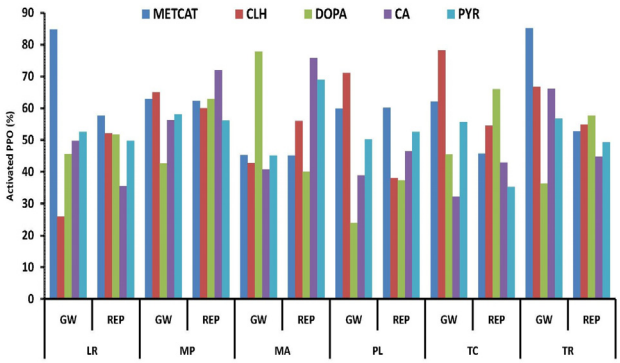
Materials and methods This experiment was carried out at Ussana research station (150 m a.s.l., 39° Lat. N, typic palexeralf soil) during 2009. Six pasture types were established in 2008 in three replicates with the following forage ecotypes: *Lolium rigidum* Gaud. (LR), *Medicago polymorpha* L. (MP), *Medicago arabica* L. (MA), *Plantago lanceolata* L. (PL), *Trifolium cherleri* L. (TC), and *Trifolium resupinatum* L. (TR). Herbage samples were taken on two occasions: during the growing stage (GW April 2009) and reproductive stage (REP May 2009). Leaf samples were taken directly from the plant and stored at -80 °C prior to analysis. PPO and PBP assays were determined according to the method of Winters and Minchin (2005) following freezing and thawing of the plant material to induce PPO activity. PPO activity was detected spectrophotometrically at 420 nm at pH7. Phenolic substrates used to determine PPO activity included: methylcatechol (METCAT), chlorogenic acid (CLH), p-coumaric acid L-3-4- dihydroxyphenylalanine (DOPA), caffeic acid (CA) and pyrocatechol (PYR). Soluble protein extracts were applied to SDS-polyacrylamide gels and protein separated by electrophoresis. Data were analysed by the general linear model (GLM) with forage ecotype (FE) phenological stage (PS) and their interaction as fixed effects. Means were separated by *t* test for multiple comparisons.

Results PPO activity of different FE at different PS was affected by different substrates (Table 1). TR PPO activity was higher with METCAT and CA and was similar to MA with DOPA whilst MA PPO had a higher affinity for CLH. Decreased PPO activity (P<0.01) during GW stage was only observed when METCAT and DOPA substrates were utilised. Potty protein and PBP appear strongly influenced by FE and a relationship between PBP and PPO activity was only observed with CLH or DOPA (P<0.01 and P<0.04, respectively). Despite the low level of PPO activity, PL exhibited higher PBP values which could suggest that factors other than simply enzyme activity are involved in this process (Lee *et al*., 2011). This is further highlighted by the higher efficiency of PBP formation (PBP/mg protein) for PL. The values of active PPO ranged between 25 and 90% of total PPO with no effect from FE and PS (Figure 1). Despite the lack latent PPO in some ryegrass species reported by some authors, our results showed that only 50% of PPO in LR is in the active form suggesting an effect of forage ecotype on the expression of this gene.

Table 1 Effect of FE and PS and their interaction on PPO activity

	LR		MP		MA		PL		TC		TR		Effects			
	GW	REP	GW	REP	GW	REP	GW	REP	GW	REP	GW	REP	SEM	FE	PS	FE*PS
Potty g/kg DM	11.20	2.72	14.19	11.54	16.00	10.84	1.89	2.98	23.62	9.86	18.63	6.70	1.23	**	**	*
PBP g/kg DM	0.81	0.54	0.62	0.98	2.24	2.07	2.58	2.72	2.85	1.16	1.71	0.91	0.16	**	*	*
PBP/mg protein	0.06	0.20	0.04	0.09	0.14	0.20	2.66	0.91	0.13	0.12	0.09	0.14	0.17	**	Ns	Ns
METCAT (ΔOD/mg protein)	0.16	0.41	0.18	0.12	0.08	0.08	1.16	0.78	0.09	0.11	0.22	0.33	1.05	**	Ns	Ns
CLH (ΔOD/ mg protein)	0.22	0.44	0.25	0.22	0.32	0.45	2.20	0.89	0.12	0.24	0.17	0.45	0.13	**	Ns	Ns
DOPA (ΔOD/ mg protein)	0.61	0.93	0.42	0.55	0.95	0.84	8.57	1.83	0.28	0.52	0.85	1.77	0.51	*	Ns	Ns
CA (ΔOD/ mg protein)	0.15	0.25	0.16	0.13	0.12	0.24	3.01	0.42	0.10	0.30	0.17	0.33	0.21	Ns	Ns	Ns
PYR (ΔOD/ mg protein)	0.21	0.37	0.25	0.17	0.18	0.30	1.35	0.42	0.17	0.27	0.15	0.35	0.08	*	Ns	Ns

Figure 1 Active PPO (% total PPO activity)



Conclusion These results confirm that FE and PS strongly govern PPO activity. The exceptionally high level of PBP in PL may suggest that mechanisms are involved other than simply PPO activity. Such factors may include: protein content and amino acid composition, substrate type and potentially non-PPO mediated diphenolic oxidation (Lee *et al*., 2011).

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Beef production under harsh climate conditions in the Eastern Alpine region: effects of using pastures rather than grass silage on growth and meat quality traits

I Gangnat[1], W Pellikaan[1], T van der Poel[1], M Velik[2]
[1]Animal Nutrition Group, Wageningen University and Research, Wageningen, The Netherlands, [2]Lehr- und Forschungszentrum Raumberg-Gumpenstein, Irdning, Austria
Email: isabelle.gangnat@gmail.com

Introduction In difficult climates and landscapes such as in the Eastern Alps (mean temperature 6.7°C; rainfall 969 mm *per annum*), beef cattle are preferably kept in barns on high forage diets while available grasslands are used for making forage. Yet, farmers are showing interest for grazing cattle mainly due to the rising demand for meat from grass fed beef and the increasing cost of feed. Growth performances and meat characteristics of grazing cattle in comparison to cattle fed in barn give contrasting results (French *et al*., 2000; Steen *et al*., 2003; Razminowicz *et al*., 2006) and may not be applicable to less favoured areas like the Eastern Alpine region. Therefore, this study aimed at assessing the suitability of fattening beef heifers on pasture in a harsh mountain climate and compare growth performances and meat quality with that of in barn housed fattening animals.

Materials and methods Twenty Charolais × Simmental heifers, mean initial live weight (LW) 302 (SD=39.5) kg, were paired by LW and age, and distributed over two groups of ten animals. One group (Barn group) was reared in a barn throughout the entire experimental period. The other group (Pasture group) was maintained under a continuous grazing regime (Häusler *et al*., 2008) without additional feed. At LW 500 kg or at latest in October (first snowfalls), heifers from the Pasture group were transferred to the barn for finishing. All animals were slaughtered when reaching LW 550 kg. Animals in barns (both the Barn group and the Pasture group during the finishing period) were fed grass silage and hay *ad libitum* (DM ratio 70:30) supplemented with 2 kg concentrates. Live weight of the animals was recorded weekly and individual feed consumption was recorded daily for the animals in barns. At slaughter, carcasses were classified according to the SEUROP, 1-5 system and stored at 2°C and 70% humidity pending analyses. At day 7 post-slaughter, carcasses were cut and three rib cuts were taken from the 8th to the 11th rib, vacuum packed and stored at 2°C. Meat quality was assessed on the *musculus longissimus dorsi*. Muscle size, intramuscular fat content (IMF) and fatty acid (FA) composition were determined at day 7. Meat colour (fresh cut and after 1 h at room temperature), fat colour, shear force and water holding capacity were assessed at days 7, 14 and 21 post-slaughter. Growth, slaughter and meat characteristics were tested for diet effect (SAS Inc., 2008). In the case of repeated measures (e.g. for meat quality) a mixed model was used to test the ageing effect (days 7, 14, 21) and the interaction between ageing and diet effect. For single measurements, the diet effect was tested by covariate analysis by including LW at the start of the experiment as covariable. The Tukey multiple range test was used for pairwise comparisons and differences considered significant at a probability of P<0.05.

Table 1 Meat characteristics for pasture and in barn reared animals.

Item	Pasture	In Barn	SEM	P-value
Muscle size (cm²)	79.3	93.3	5.42	ns
Marbling (% muscle area)	3.1	4.6	0.42	*
Total fat (g kg^{-1} product)	17.9	28.6	2.95	*
SFA (%)	47.1	50.9	0.80	**
MUFA (%)	43.7	43.2	0.84	ns
PUFA (%)	9.21	5.88	0.658	**
CLA (%)	0.73	0.60	0.028	**
C18:3 n-3 (%)	2.76	1.75	0.212	**
C18:2 n-6 (%)	5.73	3.53	0.467	**

SFA, MUFA, PUFA = Saturated, mono unsaturated and poly unsaturated fatty acids, respectively. *: P<0.05; **: P<0.01; ns: P>0.05.

Results The Pasture group showed similar growth performances as the Barn group regarding average daily gain and feed conversion. Carcass scores also showed similar muscularity between groups since 19 carcasses out of 20 were scored U. The average fatness score was 3. The average muscle size was larger in the Barn group but differences were not significant (P=0.085, Table 1). The meat of the Pasture group had a lower IMF content than the Barn group (P<0.05, Table 1), but IMF remained above the 1.5% threshold for acceptable tenderness as described by Fortin *et al*. (2005). Meat colour was not influenced by the diet. In contrast, subcutaneous fat of the Pasture group was more red (P<0.001) and more yellow (P<0.05) than the Barn group. The Pasture group had higher levels of polyunsaturated FA (P<0.01) and lower levels of saturated FA (P<0.01) compared to the Barn group. Although the Pasture group had more polyunsaturated FA than the Barn group, oxidation measured on meat colour was similar in both groups.

Conclusions Continuous grazing is a suitable alternative to silage based diets regarding growth performance and meat quality traits. Muscle size was not significantly reduced in the Pasture group but the numerical difference could have a practical importance. The fatty acid profile of the meat showed an advantage for the grazing animals regarding human health recommendations.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Causes of variation in fatty acid content and composition in grass silages

N A Khan[1], J W Cone[1], V Fievez[2], W H Hendriks[1]
[1]Animal Nutrition Group, Department of Animal Sciences Wageningen University, PO Box 338, 6700 AH Wageningen, The Netherlands, [2]Department of Animal Production, Faculty of Bioscience Engineering, Ghent University, LANUPRO, Proefhoevestraat 10, 9090 Melle, Belgium
Email: nazir.khan@wur.nl

Introduction Quantifying variations in fatty acid (FA) content and composition in grass silages can help to design management strategies to increase their poly-unsaturated FA (PUFA) content. A relatively small increase in PUFA content in grass silages can lead to substantial increase in the PUFA intake of dairy cows fed on high silage diets. High dietary intake of PUFA can favourably modulate milk fat composition to benefit human health. The present study was designed to investigate the variation in FA content and composition in a large number of grass silages and used multivariate analysis to search for variables that cause these variations.

Material and methods Grass silages (n=101) were randomly sampled from commercial dairy farms in the Netherlands. Data of soil type, N fertilization (kg/year), sward type (grass species), maturity (age and re-growth period), date and number of cuttings, DM yield (kg/ha), plant damage at cutting (bruised, tedded or untreated), weather condition, duration of wilting (in days), kind of additives or acid used to manipulate the fermentation process and heat production during ensiling was recorded for the individual grass silages. Samples were analysed for DM, Ash, crude protein (CP), crude fat (Cfat), sugar, acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL) using near infrared reflectance spectrometry (NIRS). *In vitro* dry matter digestibility (DMD), cell-wall digestibility (CWD), net energy for lactation (NE), digestible CP (DVE), degraded protein balance in the rumen (OEB) and structure index (SI) were also determined using NIRS. The FA content was analysed using gas chromatography as described by Khan *et al*.(2011). To visualize the relationship between the multiple explanatory variables and FA content Redundancy Analysis (RDA) were performed. The bi-plot (Figure 1) displays each variable as vectors (arrows). Correlations between variables are shown by the angle between the arrows, an angle of less than 90° between two arrows implies a positive correlation, whereas an angle above 90° indicates a negative correlation. A stepwise multiple regression procedure was used to obtain regression equations for the estimation of total and major individual FA content in grass silages using statistical analysis system.

Results The total FA content was highly variable (8.10 to 32.47 g/kg DM) in the grass silages. All individual FAs also showed high variations. The contents of predominant FAs, C16:0, C18:2 and C18:3 in grass silages varied linearly with changes in total FA content (Figure 2). The content of C18:3, however, showed the largest variation ranging from 3.57 to 20.53 g/kg DM. The content of C16:0 varied from 1.83 to 5.55 g/kg DM, while C18:2 varied from 1.74 to 4.69 g/kg DM. The RDA ordination bi-plot (Figure 1) visualizes the relationship between the multiple explanatory variables and the FA

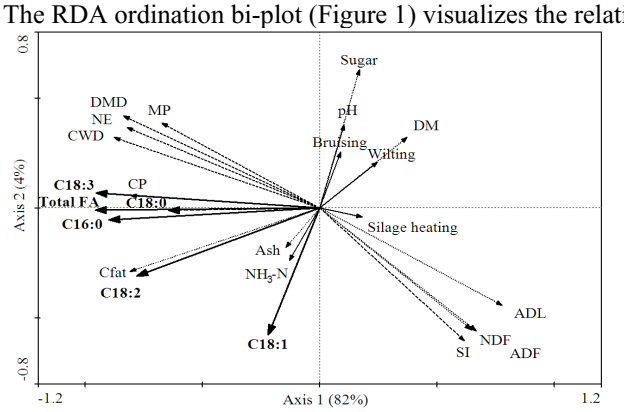

Figure 1 RDA ordination diagram, visualizing the relationship individual fatty- acid in relation to changes in total FA content

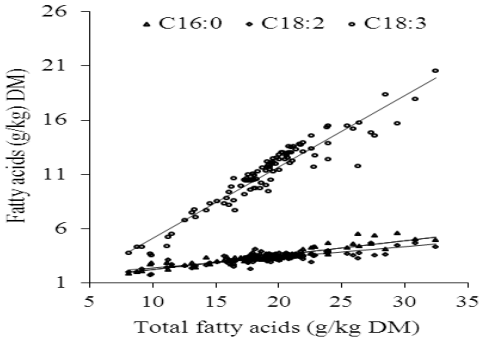

Figure 2 Change in the composition of major between the multiple explanatory variables and FAs contents

content in grass silages. The first axis (grass maturity gradient) of RDA explained 82%, while the second axis (ensiling/fermentation gradient) explained 4% of the total variation in the FA content. The contents of CP, Cfat as well as DMD, CWD, NE and DVE were positively correlated with the FA content in grass silages. In contrast, DM, NDF, ADF and ADL content, as well as a prolonged wilting period and a high SI were negatively correlated with FA contents. Silage heating affected the FA content negatively. This influence was, however, quantitatively much smaller than indicated by the small arrow. Regression analysis gave good estimates for the content of C18:3 ($R^2 = 0.75$) and total FAs ($R^2 = 0.65$).

Conclusions Grass maturity at harvest explained most of the variation in FA content and composition in grass silages. Among the nutrient content and feeding value, variables related to plant maturity were the strongest "predictor" and retained in all the equations. Bruising of grass, silage pH and ammonia N content did not affect the FA content.

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Khan NA, Cone J.W., Pellikan W.F. *et al*., 2011. J. Sci. Food Agric. 91: 1041–1049

https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Milk protein yield in response to post-ruminal supplementation of arginine, isoleucine and valine in dairy cows

M N Haque, H Rulquin, S Lemosquet
INRA, Agrocampus Ouest, UMR1080, Dairy research, 35590 Saint Gilles, France
Email: sophie.lemosquet@rennes.inra.fr

Introduction The conversion efficiency of metabolisable proteins into milk proteins in dairy cows can be improved by supplying a well balanced essential amino acid (EAA) profile in the intestine (Rulquin *et al*., 2007). Very few recommendations are available for Arginine (Arg), Isoleucine (Ile) and Valine (Val) in literature. In addition, these recommendations are variable ranging from 3.1 to 4.8% of PDIE (protein truly digestible in the small intestine) for Arg, 4.6 to 5.3% of PDIE for Ile, and 5.3 to 6.1% of PDIE for Val (Fraser *et al*., 1991; Doepel *et al*., 2004). The aim of this trial was to determine the lactational effect of limiting supply of Arg (-Arg), Ile (-Ile) and Val (-Val) when a well-balanced supply of other EAA is provided.

Materials and methods Four multiparous Holstein dairy cows at 22 ± 6 weeks of lactation received four treatments "EAA+", "-Arg", "-Ile" and "-Val" according to a Latin square design with period length of one week. Treatments were composed of a basal diet given in restricted amount and an infusion of a mixture of amino acids (AA) into the duodenum (445 ± 22.4 g/d). The treatment "EAA+" provided a well balanced supply of EAA close to Rulquin *et al*. (2007) containing: Lysine (Lys) 7.3%, Methionine (Met) 2.5%, Histidine (His) 3.0%, Leucine (Leu) 8.9%, Phenylalanine (Phe) 4.6%, Tryptophan (Trp) 1.4%, Arg 5.0%, Ile 5.2% and Val 5.8% of PDIE. In the three subsequent treatments "-Arg", "-Ile" and "-Val", the concentrations of Arg, Ile and Val were reduced up to 3.6%, 4.3% and 4.6% of PDIE, respectively. Treatments remained iso-nitrogenous by replacing Arg, Ile, or Val in the AA mixtures by non-essential amino acids (NEAA). The basal diet was composed of corn silage (64.6%), concentrate (35%), urea (0.4%) with 300 g of mineral mixture targeted to provide (in kg of DM) 7.1 MJ of NE_L, 125 g of CP and 77 g of PDIE. The preliminary results were analysed with an analysis of variance according to the model: $Y_{ijkl} = \mu + Cow_i + Period_j + AA_k + \varepsilon_{ijkl}$. Three orthogonal contrasts were performed to compare the treatments: C1 (EAA+ *vs*. other treatments), C2 ("-Arg" and "-Ile" *vs*. "-Val") and C3 ("-Arg" *vs.* "-Ile").

Results DMI (19.2 ± 0.1 kg DM) was not affected by treatments. Cows were in positive balances in terms of energy (102 ± 0.7 % of NE_L) and protein (105 % ± 1.5 % of PDIE) requirements and treatments were at 14.8 ± 0.2 g/MJ of PDIE/NE_L. Milk yield and milk protein yield were not modified by treatments (Table 1). However, milk protein content decreased significantly with the treatment "-Val" compared to "-Ile" and "-Arg" (contrast C2: $P < 0.03$). Plasma concentrations of Val, branched chain AA plus Lys, Met, His, sum of EAA and of NEAA were decreased in treatment "-Val", suggesting an increased metabolism of all AA in response to the deficit in Val.

Table 1 Effect of AA profiles on production performance and plasma concentration of amino acid.

	Treatments					P <		
	EAA+	-Arg	-Ile	-Val	SEM	C1	C2	C3
Milk yield, kg/d	30.1	30.0	30.7	30.6	0.3	NS	NS	NS
Milk protein yield, g/d	923	917	926	892	27.7	NS	NS	NS
Milk protein content, g/kg	30.7	30.4	30.3	29.2	0.3	NS	0.03	NS
AA concentration in plasma								
Arg, µMol/L	69.9	52.7	75.7	51.2	8.0	NS	NS	NS
Ile, µMol/L	67.8	76.9	44.6	59.6	7.1	NS	NS	0.02
Val, µMol/L	137	147	137	66.2	8.5	NS	0.01	NS
BCAA + Lys, µMol/L	374	404	365	273	26.0	NS	0.01	NS
Met, µMol/L	43.1	44	43.6	32.0	3.7	NS	0.04	NS
His, µMol/L	81.1	83.4	76.7	61.5	5.6	NS	0.04	NS
EAA, µMol/L	695	711	684	534	49.5	NS	0.03	NS
NEAA, µMol/L	1408	1462	1306	1165	74.9	NS	0.05	NS

Conclusions
Valine could be limiting for milk protein synthesis provided the requirements of Lys, Met, His and Leu are covered and further research is required to establish its precise recommendation. Concentrations of 3.6% and 4.3% of PDIE for Arg and Ile respectively close to those given by Fraser *et al*. (1991) seems sufficient, as performances of cows were not affected by an extra increment of these AA.

Acknowledgement This study was granted by the Commission of the European Communities; project FP7-KBBE-2007-1 "Rednex"

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Chemical composition in milk and cheese goat fed on pasture, supplemented with different protein levels

L R Bernal-Martínez, C Vazquez-Fontes, C A Garcia-Montes de Oca, A Dominguez-Lopez, M Gonzalez-Ronquillo, N Pescador-Salas
Universidad Autonoma del Estado de México, México, Mexico
Email: luzrbm@yahoo.com

Introduction World cheese production has had an increase of 7.5% annually. As part of this is recognized artisan cheese production by its economic potential due to high demand (Serani, 2001). Mexico has an estimated 1.300 establishments that produce cheese, cream and butter. Has been documented that cheeses with higher quality for human health are those from the milk of goats and sheep grazing and a diet high in oil. The aim of this study was to identify the physicochemical characteristics of Manchego cheese made from goat's milk fed on pasture and supplemented with two levels of protein in sunflower seed base.

Material and methods Milk was obtained from 8 dairy goats (60 ± 2 kg BW) fed on pasture (35%), corn silage (20%), supplemented with a concentrate of 12% and 14% CP, based seed sunflower, corn, soybeans and minerals. With 15 days of adaptation to diet and 5 days of sampling. Milk was mixed according to their treatment and manchego cheese was prepared (500 g) matured for two months. Physico-chemical analysis of milk was performed by the Ecomilk (Milk Analyzer. Milkana Kam 98-2, Hillerød, Denmark) was used and the chemical composition of cheese: Fat, Protein, Moisture NMX-26, organic matter (AOAC, 1991) . For each treatment, twelve cheeses produced 500g. The analysis of the results was by a Latin square desing 2 X 2.

Results There were no significant differences (P> 0.05) between treatments with 12% and 14% CP in milk (Table 1). Data obtained from the cheese were compared by the official standard for Manchego cheese (NMX 462). The standard establishes the maximum level of 6.5% OM, at least 26% fat. As a protein at least 22% should have evaluated the cheeses have more than 28% of CP, these values may indicate a correlation between levels of casein and milk production.

Table 1 Chemical composition of milk (g / kg) and cheese (g/100g) type Manchego goat supplemented with two levels of protein (12 and 14%).

Item	12% CP	14% CP	SEM	P<0.05
Milk composition				
Density	1.03	1.03	0.19	NS
CP, g/kg	41.3	41.6	0.52	NS
Fat, g/kg	42.4	43.2	0.12	NS
NSF, g/kg	92.2	92.7	0.62	NS
Cheese composition				
Moisture, g/100g	21.18	21.17	2.39	NS
CP g/100g	28.63	29.33	11.01	NS
Fat, g/100g	41.15	42.20	3.35	NS
OM, g/100g	3.57	3.58	0.08	NS

Conclusions The characteristics of milk found in reports by other authors. In cheese, it was observed that as to meet the highest issued by the NMX 462, there are similar characteristics of the two treatments, suggesting that a diet containing 12% protein, it meets the requirements of the animal. By having a raw material with the characteristics noted above reflect on the physicochemical characteristics of cheese, which meets the parameters set by national standards.

Acknowledgments This Project was founded by UAEMex 2633/2008U

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Milk fatty acids profile of goat milk with different levels of protein in the diet

C A Garcia Montes de Oca[1], M Gonzalez-Ronquillo[1], C Vasquez[1], N Pescador S[1], R Gutierrez T[2], L R Bernal M[1]
[1]Universidad Autonoma del Estado de Mexico, Toluca, Mexico, Mexico, [2]Universidad Autonoma Metropolitana, Xochimilco, Mexico, Mexico
Email: mrg@uaemex.mx

Introduction Corn silage and grazing are the major forage fed to dairy ruminants throughout the world, is energy rich but protein poor. Our objective was to identify the optimal level with which to supplement CP, as soybean meal (SBM),and sunflower seed in a corn silage-based diet to support of production of milk and milk components.Ruminant feed with vegetable oils rich in linoleic acid has proven to be a strategy to enrich milk (Galina *et al*., 2006).

Material and methods Eight milking dairy goats were used (60 ± 2 kg BW), placed in individual pens and were feed with grazing (35%), corn silage (20%) and supplemented with a concentrate with 12% and 14% CP, based on sunflower, corn grain, soybeans and minerals. The animals were distributed in a 2 x 2 Latin square design, repeated with 8 animals. Animals received 15 days of adaptation to diet and 5 days of sampling. Were milked at 08:00 h, and took a sample of 50 ml per animal per day. Physico-chemical analysis was conducted of the milk (Ecomilk, Milk Analyzer. Milkana Kam 98-2 ª, Hillerød, Denmark), and free fatty acid (FA) profile following the method of Folch (1956), by gas chromatography (Perkin Elmer Autosystem 9000). Milk composition and the concentration (g/100g) of FA were analyzed by a Latin square design 2 x 2.

Results There were no significant differences between treatments for any of the variables in the chemical composition of milk and fatty acid profile, the concentration (g/100g) of saturated fatty acids (P> 0.05) was 61.8 ± 0.04 and unsaturated fatty acids 39.5 ± 0.02. However, with 14 %CP in the diet the content of FA unsaturated (C18:1, C18:2 and C18:3) in milk shown a slightly increment.

Table 1 Effect of different concentration levels of protein (12 and 14 %CP) supplemented in the diet, in chemical composition (g/kg) of goat milk.

Item	12% CP	14% CP	SEM	P<
Density	1.03	1.03	0.19	NS
Crude protein	41.60	41.30	0.52	NS
Fat	42.40	43.20	0.12	NS
NSF	9.27	9.25	0.12	NS

Table 2 Efect of the crude protein suplemented on the fatty acids (FA) profile (g/100g) of milk.

FA (g/100g)	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3
14% CP	2.33	3.31	4.05	13.01	5.33	9.81	23.13	10.15	25.36	3.34	0.80
12% CP	2.14	3.06	3.92	13.01	5.34	10.03	24.63	10.47	25.10	3.09	0.76
SEM	0.23	0.28	0.35	0.88	0.33	1.02	1.08	0.79	1.31	0.21	0.10
P<	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Conclusions Supplementation with 12 or 14% CP, fed corn silage and grazing does not affect the quality of milk and fatty acid profile, showing greater concentration of C18:1 , followed by 18:0 and C18:2, so you can meet the nutritional requirements with a 12% CP without affecting the chemical composition and fatty acid profile.

Acknowledgements This project was founded by UAEMex 2633/2008U

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

The value of different dietary fatty acid source of lactating dairy goats: milk yield and fatty acids profile

C A Garcia MO, M Gonzalez-Ronquillo, N Pescador S, J G Estrada F, E Morales-Almaraz
Universidad Autonoma del Estado de Mexico, Toluca, Mexico, Mexico
Email: mrg@uaemex.mx

I**ntroduction** The optimization of milk production can be decisive by dietary supplementation; the concentrate may be the most effective, fast and easy to implement in order to change the composition of milk naturally. Lipid supplements can alter the fat content in milk and fatty acid profile (FA) (Schmidely and Sauvant, 2001). Consumption of fatty acids by ruminants, such as vegetable oils or seeds can affect the quality of milk (Lapierre *et al*., 2006). The aim of this study was to determine the production and chemical composition of goat milk supplemented with three different sources of FA, sunflower seed (18:2 n-6), flax seed (18:3 n-6) and Megalac R (C16: 0).

Material and methods Six lactating Alpine goats (52.5 ± 5 kg BW), arranged in metabolic cages were assigned to one of three treatments, which were fed with MG: Megalac-R 3%, SF: Sunflower seeds 6%, FS: 5% flax seed, supplemented with grass hay (40%), grass silage (20%) and a concentrate based on sorghum, canola, and mineral salts 2%. There were three experimental periods, animals were adapted fourteen days and six days after sampling was recorded daily production per animal, it was collected a sample of milk per day per animal to determine its chemical composition using the computer Eco-Milk (Milk Analyzer. Milkana Kam 98-2 ª, Hillerød, Denmark), FA profile was determined using the technique of Folch (1956), by gas chromatography (Perkin Elmer Autosystem 9000). Experimental design was used in 3x3 Latin square, the data were analyzed using SAS software version. 8.0 and means were analyzed by the Tukey test ($P < 0.05$).

Results By providing different sources of fatty acids in the diet of goats, there were not a significant effect ($P < 0.05$) in milk yield, protein and NFS, fat content was higher ($P > 0.05$) for diet MG with respect to the FS diet. There were not significant differences ($P > 0.05$) in the milk fatty acids C:4, C:6, C:8, C:10, C12:0, C14:0, C18:0 and C:18:3 between treatments. The highest concentration (g/100 g FA) of C16: 0 was for MG ($P < 0.001$), C18:1 was higher ($P < 0.05$) for FS than MG and SF, C18:2 was higher ($P < 0.05$) for MG than FS diet.

Table 1 Effect of different sources of fat in the milk yield production (kg/d) and chemical composition (g/100g) of goat milk.

Item	Megalac-R	Flax seed	Sunflower	SEM	P<
Milk yield (Kg/d)	0.743	0.772	0.782	0.004	NS
Fat	5.35[a]	4.98[b]	5.14[ab]	0.10	0.05
Crude protein	4.5	4.6	4.6	0.02	NS
NFS	9.7	9.7	9.7	0.03	NS

Table 2 Percentages of fatty acids (FA) in milk fat from lactating dairy goats fed with different sources of fat.

FA (g/100g)	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3
Megalac-R	1.78	2.70	3.54	11.73	5.09	9.58	28.24[a]	9.56	24.11[b]	3.38[a]	0.68
Flax seed	1.56	2.35	3.07	10.67	4.81	11.46	22.42[b]	12.02	28.39[a]	2.45[b]	0.76
Sunflower	1.27	2.09	2.84	10.11	4.65	11.28	23.07[b]	10.61	27.58[ab]	3.02[ab]	0.59
SEM	0.21	0.24	0.35	0.98	0.34	0.80	0.89	0.94	1.14	0.23	0.06
P<	NS	NS	NS	NS	NS	NS	0.001	NS	0.05	0.05	NS

Conclusion Supplementation with different sources of fatty acids in the diet of dairy goats has no effect on milk yield production, and components thereof, except the fat content. Supplementation with Megalac R showed a higher concentration of C16:0 and C18:2, but the flax seed supplementation showed a higher concentration of C18:1

Acknowledgements This project was founded by UAEMex 2633/2008U

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The effect of different methods of feeding white clover with contrasting concentrations of water soluble carbohydrate on skatole in the milkfat of grazing dairy cows

G Cosgrove, G Lane, J Burke, D Pacheco
[1]AgResearch Grasslands, Palmerston North, New Zealand, [2]Massey University, Palmerston North, New Zealand
Email: gerald.cosgrove@agresearch.co.nz

Introduction White clover has a high nutritive value but its protein concentration exceeds requirements for milk production and it is inefficiently utilised in the rumen. Excess protein is degraded to ammonia and other metabolites which are subsequently excreted. Selecting white clover for higher concentrations of water soluble carbohydrate should improve protein utilisation. One metabolite arising from protein degradation, specifically of the amino acid tryptophan, is skatole. Following feeding of high protein diets this metabolite appears rapidly in blood, milk and urine but is also cleared rapidly and has recently been shown to be a suitable metabolic indicator of differences in the chemical composition of white clover (Higgs *et al*. 2010). It was hypothesised that the high concentrations of skatole in milkfat at the afternoon milking after feeding on clover during the day and low concentrations in the morning following feeding on ryegrass during the night were a consequence of diet in the period immediately prior to milking. This hypothesis was tested by reversing the order of feeding, by offering cows clover during the night and ryegrass during the day.

Materials and methods Two groups of 5 multiparous Friesian cows in late lactation (238 ± SD 18 days) were assigned to graze perennial ryegrass (*Lolium perenne*) or white clover (*Trifolium repens*) in two different combinations, consisting of clover during the day and grass during the night (CdGn) or clover during the night and grass during the day (CnGd). Two treatments consisted of white clover selected for either high or low concentrations of WSC. A cross-over design with two periods was used and between period 1 and period 2 the groups of cows switched clover treatments. In each period cows grazed the CnGd combination for 24 h and the CdGn combination for 48 h. Cows were offered fresh allocations of the assigned pasture species combination and clover type after each milking and each group was offered an equal amount of dry matter. Cows were milked twice daily at 0700 and 1600 hrs and milk yield recorded and sub samples collected to determine milkfat concentration and the concentration and yield of skatole in the milkfat. The experimental unit was the group of cows and clover treatment, time of milking and feeding combination main effects were compared by analysis of variance.

Results The concentration and yield of skatole was lower in milk sampled immediately following the eating of grass compared with eating clover, irrespective of whether this was during the night (CdGn) or during the day (CnGd) (Table 1). The concentrations and yields were higher immediately following a period of eating clover during the day, but only slightly elevated in the morning when clover was offered during the night (feed combination x time of milking interaction P<0.01). White clover with a high concentration of WSC resulted in lower concentrations of skatole in milkfat.

Table 1 The concentration and yield of skatole in afternoon (pm) and morning (am) milk from cows offered different temporal combinations of ryegrass and white clover containing either high (HSC) or low (LSC) concentrations of water soluble carbohydrate.

		Clover-day/Grass-night		Clover-night/Grass-day		Significance (P)		
	Clover type	pm	am	pm	am	clover	time	feed combination
Skatole concentration mg/kg milkfat	LSC clover	873	157	183	298	0.05	<0.01	.006
	HSC clover	663	94	148	157			
Skatole yield mg/cow/day	LSC clover	324	73	73	149	0.07	<0.01	0.01
	HSC clover	263	42	51	81			

Conclusion The pattern of high concentrations of skatole in milk collected after feeding on clover compared to feeding on grass, previously seen in afternoon milk, was also observed in morning milk after feeding on clover during the night. This confirms the association between skatole concentration in milk and the feed consumed in the interval immediately preceding that milking. The concentration of skatole in milkfat was lower for cows grazing the high WSC selection line, an early indication that higher WSC and the associated lower concentration of protein improves protein utilisation in the rumen. Furthermore, the effect of the contrasting selection lines was seen in the morning milk as well as the afternoon milking, confirming that skatole is a responsive indicator of the carbohydrate and protein composition of white clover even after only short periods of feeding. Skatole is evidently rapidly cleared after a period of feeding on ryegrass containing a lower concentration of protein that degrades more slowly.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Preservative effect of Economas E®, a vitamin E replacer, feeding on the meat quality of Hanwoo (Korean native steers)

D H Lee[1], S B Cho[2], E J Kim[3], J S Chang[4], N J Choi[2]
[1]GRRC center, Hankyong University, Anseong, 456-749, Republic of Korea, [2]Department of Animal Science, Chonbuk National University, Jeonju, 561-756, Republic of Korea, [3]Department of Animal Science, Kyungpook University, Sangju, 742-711, Republic of Korea, [4]Department of Agricultural Science, Korea National Open University, Seoul, 110-791, Republic of Korea *Email: nagjin@jbnu.ac.kr*

Introduction Meat colour is known as an important factor of meat quality which determines customer's choice and shelf life in local market. The bright red colour of fresh meat is gradually changed to dark brown along the storage duration is increased and this change of colour decrease the choice of customer. The degree of colour change can be used as a representative factor of freshness and safety of meat for customer. Therefore, appropriate control to prolong the colour of meat is important. Antioxidant activity is a representative characteristic of vitamin E and it has been reported in many researches that vitamin E can improve the quality and the preservation of beef meat through the suppression of lipid oxidation especially, oxidation of poly unsaturated fatty acid. These effects of vitamin E are notable when the storage period of meat in local market is long. In this study, effect of Economas E®, a vitamin E replacer, on the meat quality of Hanwoo (Korean native steer) was investigated.

Materials and methods Thirty six head of Hanwoo were randomly divided into three groups of experiments, C (without vitamin E and Economas E®), T1 (Economas E®, Alltech Co. USA, 200mg/head) and T2 (vitamin E, α-tocopheryl acetate, Roche Vitamin Co., France, 500 mg/head). A group of experiment consists of three stall and 4 head were located in a stall. All experimental groups were fed same finisher feed (CP 12%, TDN 73%) and for the treatment groups, vitamin E and Economas E® were supplied by top dressing. After 130 days of experiment, all animal was slaughtered and 2 parts of carcass, loin area and rump area, were collected as sample and stored in 4 □ refrigerator for 9 days. For the determination of meat quality, carcass quality, pH of meat, meat colour, TBARS (thiobarbituric acid reactive substances), VBN (volatile basic nitrogen), fatty acids and cholesterol content were analyzed. GLM (general linear model) procedure and Duncan's multiple rage tests by SPSS 18 program was used for statistical analysis.

Result In carcass yield, there were no significant differences between control and treatments except carcass grade. In carcass grade, vitamin E or Economas E® fed treatment groups were significantly lower than that of control group. Meat colour of loin area, the lightness (CIE L*) of treatment groups was significantly higher than that of control group ($P<0.05$) but the significant difference between treatment groups was not found. The significant differences along the day of storage were not found in all experiment groups. Redness (CIE a* value) was significantly higher in control group compared to treatment groups and it might be caused by higher fat content in treatment. But during the storage periods, the redness in control groups were significantly decreased compared to treatment groups ($P<0.05$). Especially, the group fed Economas E® showed stable redness along storage. In meat colour of rump area, the stable redness were found in the treatment groups compared to control groups especially, in 5 and 9 day of storage. In the pH of loin and rump area, significant differences were not found. In fatty acid content in meat, there was no significant difference between control and treatments. TBARS is a representative compound that was increased when lipid oxidation was increased. In this study, TBARS values were increased as storage day was increased in all group of experiments ($P<0.05$). The TBARS of control was significantly changed at 3 day of storage. However, TBARS in Economas E® fed group was not significantly changed until 3 day of storage. Remarkable delayed TBARS increment was found at rump area of Economas E® fed group. VBN is increased when low molecular inorganic nitrogen compound of peptide or amino acid was degraded. VBN is typically used as a criterion to determine the degree of spoiling of meat and below 30 mg% of VBN is regarded as a fresh meat. In this study, all groups showed increased VBN along days of storage. However the VBN values of all groups were under the level that represents fresh meat until final day of storage.

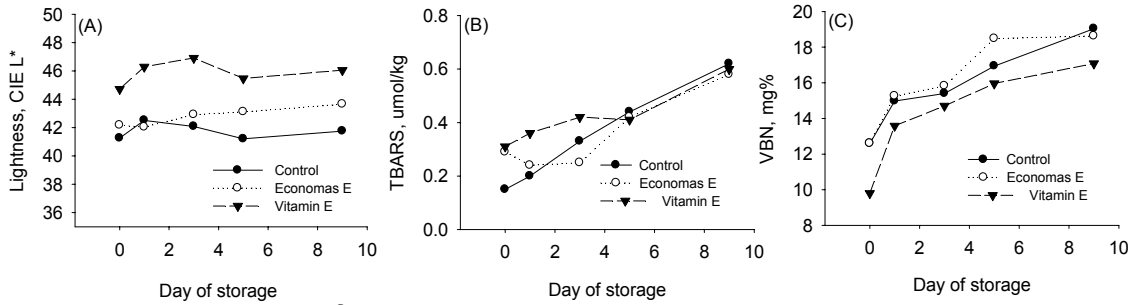

Figure 1 Effect of EconomasE® and vitamin E supplementation on colour, TBARS and VBN of loin area meat of Hanwoo steers. (A) Lightness, (B) TBARS, (C) VBN.

Conclusion Economas E® showed relatively similar effect with vitamin E in colour and preservation of Hanwoo steer meat. And as a result, Economas E® should be appropriate to substitute vitamin E with sufficient for getting stable colour of meat and its preservation.

Acknowledgement This work was supported by Rural Development Administration foundation and by the GRRC program of Gyeonggi province. [GRRCHankyong2010-B04]

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Polymers of triglycerides generated during heating of fat do not protect linoleic acid from ruminal biohydrogenation

M Kaleem[1,2], F Enjalbert[1,2], Y Farizon[1,2], A Troegeler-Meynadier[1,2]
[1]INPT, ENVT, ENSAT, Toulouse, France, [2]INRA, UMR Tandem, Castanet-Tolosan, France
Email: a.troegeler@envt.fr

Introduction Heated oilseeds often induced a decreased of linoleic acid (LA) and linolenic acid biohydrogenation (BH) *in vivo* (Gonthier *et al*. 2005), *in situ* (Troegeler-Meynadier *et al*. 2006) and *in vitro* (Privé *et al*. 2010). A previous study in our laboratory (Privé *et al*. 2010) showed that lipid oxidative compounds could be at least in part responsible of the modifications of ruminal BH of polyunsaturated fatty acids (PUFA) *in vitro*, and this protection of PUFA was probably linked to heating temperature. Our hypothesis was that polymers of triglycerides (TG) could explain this partial protection of PUFA from BH, are polymers of triglycerides (TG), via an impaired lipolysis. The objective of this study was to compare *in vitro* the BH of LA from heated or not heated free FA and TG.

Materials and methods Pure free LA (FLA, ≥99%, Sigma) and pure LATG (≥98%, Sigma) were heated separately for 6 h at 150°C. In Erlenmeyer flasks, 52 mg of LATG (heated or non-heated), or 50 mg of FLA (heated or non-heated) were incubated for 6 or 24 h, with 40 ml of ruminal fluid, taken from a cannulated dry Holstein cow before the morning meal, 40 ml of a bicarbonate buffer gassed with CO_2 and heated at 39°C (pH = 7 ; $Na_2HPO_4.12H_2O$ 19.5 g/l et $NaHCO_3$ 9.24 g/l: NaCl 0.705 g/l ; KCl 0.675 g/l ; $CaCl_2.2H_2O$ 0.108 g/l and $MgSO_4.7H_2O$ 0.180 g/l), and a fermentative substrate (1g of hay, 0.2g of soybean meal and 0.25g of maize starch). Then flasks were filled with CO_2, and incubated in a water-bath rotary shaker at 39°C under anaerobic conditions. Thereafter, fermentations were stopped and the flask contents were frozen and freeze-dried. After extraction, FA of the four fats and of the cultures were analysed by GC. Polymers of TG and hydroperoxides were analysed by HPLC, and aldehydes by GC. Data were analysed using the General linear model of Systat followed by a pairwise comparison (Tukey's Test).

Results Table 1 presents the composition of the different fat sources, including oxidative compounds. Initial LA content was similar among cultures, although slightly lower with heated FLA. As expected, heated LATG contained 27% of TG polymers contrary to other fats. Both heated fats contained high amounts of hydroperoxides and aldehydes; heated FLA contained high amounts of hydroperoxides formed from LA and probably explaining its lower LA content compared to others.
After 6h, the percentage of LA disappearance was higher with free LA than with LATG (Table 2), which could be expected because LA biohydrogenation needs a lipolysis of TG which can delay the isomerisation. Heating did not affect this percentage of disappearance. The amount of disappeared LA was lower with heated FLA than with non heated FLA, due to the lower initial LA amount in flasks.
After 24h, fat source effect was lower than after 6h incubation; heating had a slight but significant effect on LA disappearance of FLA but did not affect LA disappearance in LATG.

Table 1 Quantities and nature of fat and oxidative compounds incubated (by flask)

	LATG	Heated LATG	FLA	Heated FLA
Added LA (mg)	49	48	48	45
TG polymers (mg)	ND	13	-	-
Hydroperoxides (µg)	41	739	ND	1310
Adehydes (µg)	9	100	7	62

Table 2 Disappearance of linoleic acid after 6h or 24h incubations with heated or not heated trilinolein or free linoleic acid

Incubation duration	Disappearance	LATG	Heated LATG	FLA	Heated FLA	S.E.M.	P fat	P heating	P fat*heating
6h	mg	41.7^{b}	43.1^{b}	48.8^{a}	44.2^{b}	0.7	<0.01	0.03	<0.01
	%	75.1^{b}	77.7^{b}	88.7^{a}	85.4^{a}	1.2	<0.01	0.76	0.03
24h	mg	49.7^{ab}	49.1^{b}	51.4^{a}	46.7^{c}	0.4	0.48	<0.01	<0.01
	%	90.0^{b}	88.8^{b}	93.7^{a}	89.8^{b}	0.8	<0.01	<0.01	0.09

^{abc} P<0.05

Conclusion These results showed that TG polymers generated during heating of LATG have no effect on ruminal BH, and in particular do not protect PUFA from BH. Other oxidative products would be investigated.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Effect of increasing maturity in timothy-dominated grass silage offered with or without concentrates on meat quality of finishing Norwegian Red bulls

Å T Randby
Norwegian University of Life Sciences, Ås, Norway *Email: ashild.randby@umb.no*

Introduction Very early harvesting of grass crops for silage may give high-energy- and high-intake silages that support intensive beef production even without concentrate supplementation. Consumers increasingly demand high eating quality and healthy food. Therefore, tenderness, intramuscular fat and α-tocopherol concentrations were measured in M. longissimus dorsi of bulls of Norwegian Red, finished on grass silage alone or supplemented with 4 kg/d of concentrates.

Materials and methods Six dietary treatments, each offered to 6 bulls, included *ad libitum* feeding with timothy, meadow fescue and red clover silage from three maturity stages (harvesting times) of the primary growth in 2006: 30 May to 1 June (H1; very early), 6-8 June (H2; early) and 14-16 June (H3; normal), fed unsupplemented or supplemented with concentrate. From start of experiment (average live weight (LW) 288 kg) the supplemented bulls received 2 kg concentrates daily, increasing to 3 kg at 385 kg LW and to 4 kg at 500 kg LW, individually for each animal. The ingredient composition of the concentrate mixture was: 0.3 oats, 0.18 peas, 0.179 barley, 0.1 wheat, 0.1 wheat bran, 0.06 extracted, heat treated rape seed meal, 0.045 molasses and 0.036 minerals and vitamins, including 0.01 of a micro mineral and vitamin mix. Information on feed quality, intake and performance of the bulls is given by Randby *et al*. (2010). The bulls were slaughtered at 575 kg live weight. Carcasses were subjected to low voltage electrical stimulating. A complete cross section of the M.longissimus dorsi from one side of the carcass, starting at the 10-11th rib and backwards, in total 6-8 cm were removed 45-60 min *post mortem*. The meat pieces were aged at 11^{o}C the first 24 h, thereafter at 4^{o}C the following 13 days, and further stored at -20^{o}C until analyses. Muscle pH was measured by inserting a glass-stick probe connected to a pH meter into both ends of the muscle piece. Ten parallels were sheared perpendicular to the fibre direction with a Warner Bratzler (WB) shear force device attached to an Instron Materials Testing Machine. Alpha-tocopherol was analysed using a HP1100 liquid chromatograph and a HP1100 fluorescence detector. For determination of intra muscular fat (IMF), lipids were extracted from 4 g homogenate using chloroform:methanol (2:1, v/v). Data were analysed as a factorial design with 6 blocks, 3 harvesting times for silage (H), 2 concentrate levels (with or without)(C) and H x C as fixed effects. Analyses were done using PROC GLM in SAS.

Results The quality of the beef meat as assessed by WB shear force, pH, IMF, and α-tocopherol concentrations in M.longissimus dorsi are presented in Table 1, together with some information on the carcasses. Muscle pH was above 5.9 in 6 bulls. These were considered to be stressed during transportation to the abattoir, and were therefore omitted from the pH and WB shear force results. No significant effects of silage harvesting time or concentrate supplementation were found in pH or WB shear force, although bulls fed H3 with concentrates or H2 alone, tended to have higher shear force than bulls fed other diets. Feeding H1 gave higher IMF in M.longissimus dorsi than feeding H2 or H3, and also concentrate supplementation tended to increase IMF. α-tocopherol concentrations in M.longissimus dorsi was higher in bulls fed H1 compared with H2, but was otherwise not affected by dietary treatments, neither when considered on wet muscle basis nor on IMF basis.

Table 1 Effect of harvesting time for silage and concentrate supplementation on carcasses and beef meat quality

	Harvesting time for grass silage								Effect of				
	1 (Very early)		2 (Early)		3 (Normal)				Harvesting time				Inter-
	No C	C	No C	C	No C	C	SEM	P	1 vs 2	2 vs 3	1 vs 3	C	action
Age at slaughter, d	450	427	466	432	543	454	10.2	<0.001	NS	<0.001	<0.001	<0.001	0.004
Carcass gain, g/d	746	825	659	842	465	708	20.8	<0.001	NS	<0.001	<0.001	<0.001	0.002
Cold carc. weight, kg	297	296	291	301	282	293	3.8	0.03	NS	0.05	0.03	0.04	NS
pH	5.51	5.51	5.53	5.49	5.51	5.59	0.04	NS	NS	NS	NS	NS	NS
WB shear force, N	42.5	42.8	56.4	39.7	46.4	58.3	4.6	0.08	NS	NS	NS	NS	0.03
IMF, g/100 g	2.32	3.02	2.03	2.33	2.24	2.15	0.199	0.03	0.02	NS	0.02	0.07	NS
α-toc, mg/100 g	0.19	0.21	0.14	0.13	0.14	0.20	0.021	0.03	0.005	NS	NS	NS	NS
α-toc, mg/100 g IMF	9.09	7.01	7.49	5.79	6.10	9.55	1.08	NS	NS	NS	NS	NS	0.03

Conclusions Neither harvesting time for grass silage nor concentrate supplementation influenced significantly WB shear force. However, M.longissimus dorsi had in general lower WB shear force than often found in bulls (Rødbotten *et al*. 2010), more similar to values often found in steers and heifers. Values from 25 of the 36 bulls were below 50 N, which is considered to be an upper limit for consumers perception of beef to be tender. Concentrations of IMF, which give a perception of juiciness, was highest in beef from bulls offered the very early harvested silage. These carcasses were also classified to be fatter than carcasses from bulls offered H2 or H3. Bulls offered H1 had highest α-tocopherol concentrations, but as proportion of IMF, no differences were apparent.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Methanogen population in Swedish cows fed different levels of high quality forage

R Danielsson[1], J Bertilsson[1], M Griinari[1], A Schnürer[2]
[1]Animal nutrition and management, Uppsala, Sweden, [2]Microbiology, Uppsala, Sweden
Email: rebecca.danielsson@slu.se

Introduction Methane (CH_4) emissions from the ruminants have received increased attention during the recent years as they contribute to global warming. CH_4 is a product of methanogenic archaea in the rumen which represents an energy loss for the cow. As it is known that diet composition has an effect on ruminal CH_4 production, a great number of studies have been performed in an attempt to reduce CH_4 production or redirect the H_2 to other fermentation end-products. However, the relationship between ruminal methanogenic communities and the rate of methane production is not so well understood. The aim of this study was to compare diets with high and low methanogenic potential based on the proportion of forage in the diet with respect to CH_4 production, ruminal methanogenic communities and their interrelationship.

Materials and method Rumen fluid from five rumen-cannulated Swedish Red Breed (SRB) dairy cows (milk yield = 23.2 ± 5.1 kg ECM/d), included in an earlier methane production study (unpublished), were selected for further analysis of the microbial communities**.** Cows were fed two diets with different forage:concentrate ratio (low (L) 500/500 and high (H) 900/100 g/kg dry matter intake (DMI)) in randomized order. The grass silage was a mixture of timothy (*Phleum pratense* L.) and meadow fescue (*Festuca pratensis* L.). The metabolizable energy (ME) of the silage was 11.5 MJ/kg DM and crude protein (CP) 194 g/kg DM. The concentrate was a mixture of oats, barley, peas, rapeseed cake, beet fibre, wheat bran, rapeseed, minerals and vitamins (234, 232, 200, 125, 90, 70, 25 and 24 g/kg DM feed, respectively). The ME of the concentrate was 13.2 g/kg DM and CP 168 g/kg DM. Methane yield was measured using the sulphur hexa fluoride (SF_6) tracer technique. Rumen fluid samples were analyzed with quantitative polymerase chain reaction (qPCR) technique for total numbers of methanogens and *Methanomicrobiales* and *Methanobacteriales*. Analysis of variance was performed on all data using PROC MIXED (SAS 9.2. SAS institute 2002).

Results Mean CH_4 yield was 18.6 and 20.7 g CH_4/kg DMI for L and H diet, respectively. The difference did not reach statistical significance. The total number of methanogens was significantly different between the treatments (Table 1). Difference was also found in the total number of *Methanobacteriales* but the number of the order *Methanomicrobiales* could not be detected (limit of detection $<10^1$). There was no relationship between CH_4 production and total number of methanogens. Further analyses, t-RFLP and clone libraries, specifying the methanogenic strains are in progress.

Table 1 Targeted methanogens in low and high forage diet.

	No. of copies/ml (log. values ± s.e*)*		
Group of microbes	L	H	P value
Methanogens	3.47×10^8 (8.54 ± 0.011)	1.26×10^7 (7.10 ± 0.011)	0.0004
Methanobacteriales	1.59×10^8 (8.20 ± 0.054)	1.62×10^7 (7.21 ± 0.054)	0.002
Methanomicrobiales	$<10^1$	$<10^1$	Undetectable level

Conclusion The number of total methanogens and *Methanobacteriales* were lower in the diet with higher proportion of forage. Interestingly, the CH_4 yield was numerically higher in that diet. The results in this study confirm the findings by Zhou *et al*., (2010), that the CH_4 produced probably is more correlated to the specific methanogenic species present in rumen when cows are fed different diets than to the total methanogen population. Next step in this study is to specify which species that is present in the different treatments.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Muscle and fat colour of early- and late-maturing heifers offered rations designed to enhance beneficial polyunsaturated fatty acids at two slaughter weights in a grass-based beef production system

P G Dunne[1,3], K J Shingfield[2], A P Moloney[1]
[1]Teagasc, Animal & Grassland Research and Innovation Centre, Grange, Dunsany, County Meath, Ireland, [2]MTT Agrifood Research Finland, Jokioinen, FIN 31600, Finland, [3]Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland
Email: aidan.moloney@teagasc.ie

Introduction Meat colour is a key driver of meat purchase decisions and consumers willingness to pay a premium for beef (Killinger *et al*. 2004). Carcass fat colour (Dunne *et al*. 2009) and muscle colour (Priolo *et al*. 2001) are affected by genetic, physiological, nutritional and environmental factors which are reflected in the animal type and production system. These quality characteristics were measured in an experiment, the objective of which was to determine the fatty acid composition and meat quality from early- and late-maturing heifers offered, in a grass-based production system, rations designed to enhance conjugated linoleic acid or n-3 polyunsaturated fatty acids (PUFA), both regarded as beneficial to human health.

Materials and methods Spring-born early-maturing Aberdeen Angus × Friesian heifers (AAF, n=48)) and late-maturing Belgian Blue × Friesian heifers (BBF, n=48)) were randomly assigned, at four months old, to either a standard grass-based production system (Keane and Drennan, 2008) (CO) or within that system to continued supplementation with a safflower oil- (SF) or protected fish oil-containing (FO) ration. Following slaughter, off pasture at 21 months (light) or following indoor finishing at 25 months (heavy), colour of *longissimus dorsi* (LD) muscle (after a 3hour bloom at 0°C), ultimate pH and fat colour were measured at 48 hours *post mortem* (Hunter Lab colour space; D_{65}, 10°, 1inch port). Data were subjected to Analysis of Variance for a split-plot design with block and breed (B) in the main plot and ration (R), slaughter weight (S) and all interactions in the split-plot.

Results Data are presented in Table 1. Increasing slaughter weight increased 'L' (P=0.006; lighter), 'a' (more red), 'b' (more yellow; not shown) and 'C' (more saturated) (all P<0.001) and decreased 'H' (P<0.001) and pH_{48} (P=0.028) of LD. Fat 'L' (data not shown) increased (P<0.001) as slaughter weight increased.. Muscle from BBF had a higher 'H' than AAF (P=0.047). Muscle was less (P <0.05) red, yellow and saturated and fat had lower lightness when FO was offered. Provision of FO, by promoting accretion of membrane PUFA, may have caused structural changes which affected the nature of the interaction of the incident light from the spectrocolorimeter with the muscle matrix. There were B×S (P=0.018) and R×S (P=0.029) interactions for fat 'b' value. AAF 'b' value did not change as slaughter weight increased but BBF increased at the 'heavy' slaughter weight likely due to differences in assimilation of dietary carotenoids. That the 'heavy' heifers had more yellow (higher 'b') fat than light heifers when CO and FO rations were offered but not when SF was offered may relate either to (i) differences in carotenoid metabolism perhaps due to an unknown *de novo* factor in safflower oil or (ii) differential effects of products (e.g. CLA) of its ruminal fermentation or *in musculo* metabolism.

Table 1 Muscle and fat colour of heifers at 48 hours *post mortem*.

	Aberdeen Angus × Friesian						Belgian Blue × Friesian							Effects
	Light			Heavy			Light			Heavy				
	CO	SF	FO	CO	SF	FO	CO	SF	FO	CO	SF	FO	SED	
Muscle														
'L'[1]	34.8	33.5	33.8	34.6	35.3	34.9	34.6	33.5	33.3	34.7	34.0	34.5	0.67	S
'a'	11.8	10.8	10.0	13.2	14.4	13.3	10.4	10.6	9.2	14.0	13.5	13.2	0.66	R, S
'H'	31.2	31.0	31.8	29.2	29.3	29.4	32.2	31.2	32.1	30.2	29.0	29.7	0.68	B, S
'C'	13.8	12.6	11.8	15.1	16.5	15.2	12.2	12.3	10.8	16.2	15.4	15.2	0.75	R, S
pH_{48}	5.53	5.54	5.48	5.51	5.49	5.49	5.59	5.49	5.62	5.49	5.51	5.47	0.0532	S
Fat														
'b'	18.4	19.9	19.3	19.5	17.6	20.5	18.1	18.2	19.1	20.8	19.4	20.9	0.99	B×S, R×S

[1] 'L'=lightness; 'a' = redness; 'H' = hue angle; 'C' = saturation; 'b' = yellowness. Measured using a HunterLab UltraScan XE spectrometer.

Conclusions Finishing to a heavier slaughter weight changed all colour variables in both tissues, largely as expected (Ashmore *et al*. 1972). Differences between early- and late-maturing breed crosses were not as large or widespread as anticipated.

Acknowledgements This research was supported by ProSafe Beef, an EU 6th Framework Programme project (2007-2012). The donation of the rumen-protected fish oil supplement by the Farmright Group, Ltd., UK is gratefully acknowledged.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

In vitro total gas and methane output of ensiled wheat and barley grains as affected by stages of ripeness at harvest and urea treatment

E J.Mc Geough[1,2], D Kenny[2], M O'Brien[1], P O'Kiely[1]
[1]Teagasc Animal and Grassland Research & Innovation Centre, Dunsany, Co. Meath, Ireland, [2]University College Dublin, School of Agriculture, Food Science & Veterinary Medicine, Belfield, Dublin 4, Ireland
Email: emma.mcgeough@agr.gc.ca

Introduction Feeding starch-rich feeds, such as cereal grains to cattle can potentially reduce enteric methane emissions due to their effects on rumen fermentation processes. Although the nutritive value of cereal grains is dependent predominantly on stage of ripeness at harvest, additives may be applied to conserve or further enhance their feeding value. The objectives of this study were to determine the effects of 1) advancing stage of ripeness at harvest and 2) addition of urea, on total gas and methane output associated with wheat and barley grains using the *in vitro* total gas production technique.

Materials and methods Two cereal grain species, wheat and barley, were grown, harvested and stored as described by Stacey *et al*. (2006). Both wheat and barley grains were harvested at three stages of ripeness: 1) Early (630 g DM/kg), 2) Medium (700 g DM/kg) and 3) Late (840 g DM/kg). Each grain subsequently received either 1) no additive (NA) or 2) urea at 50 L/t (Urea) prior to ensiling in laboratory silos for over 100 days. The *in vitro* total gas production technique of Theordorou *et al*. (1994) and modified by Mauricio *et al*. (1999) was used to quantify the total gas and methane produced. Dried, milled (1 mm sieve) silage samples were weighed (0.5 g) into fermentation vessels and inoculated with 10 ml of strained rumen fluid and 40 ml of buffered medium. Rumen fluid was obtained and pooled from four fistulated Friesian steers offered a 60:40 grass silage:concentrate diet. Incubation was terminated at 24 h and gas pressure in the head space of the fermentation vessels measured using a manual pressure transducer, with a gas sample (0.8 ml) extracted from each fermentation bottle for methane determination using gas chromatography. All data were analysed using the MIXED procedure of SAS, with the statistical model including the fixed effects of ripeness, additive treatment, their interaction and the blocking effect of sampling run.

Results A significant stage of ripeness x additive interaction (P<0.001) was observed for total gas output for wheat grain (Table 1). However, total gas output for Early ripeness wheat grain was consistently lower (P<0.001) than Medium and Late ripeness across the two-way interactions. Methane output was higher (P<0.05) for Early ripeness wheat grain than Medium or Late ripeness. The addition of urea to wheat grain did not affect methane output. Total gas and methane output for barley grain were not affected by stage of ripeness at harvest. The addition of urea to barley increased (P<0.05) total gas and methane output per unit of feed DM incubated, however no effects of urea were observed for these variables when expressed per unit of feed DM disappeared.

Table 1 Total gas and methane output for ensiled wheat and barley grains as affected by stage of ripeness and additive treatment

	Early ripeness[1]		Medium ripeness[1]		Late ripeness[1]		s.e.d.	Significance		
Wheat	NA[2]	Urea	NA	Urea	NA	Urea		Ripeness	Additive	R x A
Total gas output										
mmol/g feed DM inc[3]	10.39	9.94	10.70	10.66	10.53	10.48	0.050	***	***	***
mmol/g feed DM dis[4]	11.81	11.13	12.03	11.83	12.10	11.78	0.053	***	***	***
Methane output										
mmol/g feed DM inc	2.21	2.18	2.09	2.17	2.08	2.11	0.043	*	NS	NS
mmol/g feed DM dis	2.51	2.44	2.35	2.41	2.41	2.37	0.050	*	NS	NS
	Early ripeness		Medium ripeness		Late ripeness					
Barley	NA	Urea	NA	Urea	NA	Urea				
Total gas output										
mmol/g feed DM inc	9.84	9.95	9.77	10.10	9.85	10.00	0.110	NS	*	NS
mmol/g feed DM dis	12.30	12.13	12.31	12.15	12.14	12.15	0.196	NS	NS	NS
Methane output										
mmol/g feed DM inc	1.99	2.01	1.97	2.09	2.01	2.08	0.049	NS	*	NS
mmol/g feed DM dis	2.49	2.46	2.48	2.52	2.48	2.52	0.066	NS	NS	NS

*P<0.05, **P<0.01, ***P<0.001, NS = non-significant.
[1] Early ripeness = 630 g DM/kg, Medium ripeness = 700 g DM/kg, Late ripeness = 840 g DM/kg. [2] NA = No additive.
[3] Incubated. [4]Disappeared.

Conclusions Methane output was highest for wheat grain harvested at early ripeness, however methane output for barley grain was not affected by stage of ripeness. The addition of urea to the high moisture grains prior to ensiling did not affect methane output.

Acknowledgements Project funded by the Irish Dept. of Agriculture, Food & Fisheries, Dublin (RSF 05 224).

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

In vitro methane and total gas production as influenced by whole-crop wheat silage grain to straw ratio and level of inclusion of whole-crop wheat silage in grass silage based diets

E J. Mc Geough[1,2], D Kenny[2], M O'Brien[1], P O'Kiely[1]
[1]Teagasc Animal and Grassland Research & Innovation Centre, Dunsany, Co. Meath, Ireland, [2]University College Dublin, School of Agriculture, Food Science & Veterinary Medicine, Belfield, Dublin 4, Ireland
Email: emma.mcgeough@agr.gc.ca

Introduction It is well established that improving the nutritional value of ruminant diets can effectively reduce enteric methane emissions. This may be achieved through the inclusion of starch rich forages such as whole crop wheat (WCW) silage in ruminant feeding regimes. The nutritive value of such diets may be further enhanced by increasing the grain content of the crop. This study investigated the effects of a) increasing the grain to straw ratio of WCW silage and b) altering the level of inclusion of WCW silage with grass silage on total gas and methane production using the *in vitro* total gas production technique.

Materials and methods Winter wheat (*cv.* Einstein) was produced for a beef production study as described by Mc Geough *et al.* (2010), generating four silages differing in their ratio of grain to straw plus chaff: 11:89 (WCWI), 21:79 (WCWII), 31:69 (WCWIII) and 47:53 (WCWIV). Eight samples of each WCW silage, representative of silage fed in sequential three week intervals in the aforementioned *in vivo* study, were used. The five levels of WCW inclusion (using WCW III) with grass silage were (1) 100:0, (2) 75:25, (3) 50:50, (4) 25:75 and (5) 0:100 (on a dry matter (DM) basis). The *in vitro* total gas production technique of Theordorou *et al*. (1994) and modified by Mauricio *et al.* (1999) was used to quantify the total gas and methane produced. Dried, milled (1 mm sieve) silage samples were weighed (0.5 g) into fermentation vessels and inoculated with 10 ml of strained rumen fluid and 40 ml of buffered medium. Rumen fluid was obtained and pooled from four fistulated Friesian steers offered a 60:40 grass silage:concentrate diet. Incubation was terminated at 24 h and gas pressure in the head space of the fermentation vessels measured using a manual pressure transducer, with a gas sample (0.8 ml) extracted from each fermentation bottle for methane determination using gas chromatography. All data were analysed by two-way ANOVA using a model that accounted for treatment and three week time period using the MIXED procedure of SAS. Linear and quadratic polynomial contrasts were also conducted.

Results Increasing the grain content of WCW silage linearly increased (P<0.001) the volume of total gas produced per unit of feed DM incubated, however no differences were observed for total gas produced when expressed per unit of feed DM disappeared. Methane output per unit of feed DM incubated increased linearly (P<0.001) in response to increasing the grain content of WCW silage, with a quadratic response (P<0.05) observed per unit of feed DM disappeared. Total gas and methane production decreased linearly (P<0.001) with decreasing WCW:GS ratio when expressed relative to each unit of feed DM incubated and disappeared.

Table 1 Total gas and methane output as affected by whole-crop wheat silage grain content and by level of inclusion with grass silage

	WCW silage[1]					s.e.d.	Significance		
	I	II	III	IV			Treatment	Linear	Quadratic
Total gas output									
mmol/g feed DM incubated	6.39[a]	7.25[b]	7.97[c]	8.58[d]		0.104	***	***	NS
mmol/g feed DM disappeared	12.64	12.62	12.57	12.55		0.155	NS	NS	NS
Methane output									
mmol/g feed DM incubated	1.14[a]	1.35[b]	1.47[c]	1.56[d]		0.023	***	***	**
mmol/g feed DM disappeared	2.24	2.34	2.32	2.29		0.035	NS	NS	*
	Ratio of WCW:Grass silage								
	100:0	75:25	50:50	25:50	0:100				
Total gas output									
mmol/g feed DM incubated	7.91[a]	7.80[a]	7.59[b]	7.30[c]	6.94[d]	0.053	***	***	**
mmol/g feed DM disappeared	12.64[a]	12.69[a]	12.26[b]	12.01[b]	11.88[b]	0.182	***	***	NS
Methane output									
mmol/g feed DM incubated	1.45[a]	1.40[b]	1.33[c]	1.22[d]	1.10[e]	0.020	***	***	**
mmol/g feed DM disappeared	2.31[a]	2.28[a]	2.14[b]	2.01[c]	1.88[d]	0.043	***	***	NS

Within a row, means without a common superscript differ (P<0.05). *P<0.05, **P<0.01, ***P<0.001, NS = non-significant.
[1]WCW = Whole-crop wheat silage, grain to straw plus chaff ratio I = 11:89, II = 21:79, III = 31:69, IV = 47:53

Conclusions Within the conditions of the present study, increasing the grain content of WCW silage resulted in an increase in methane output. In contrast, decreasing the inclusion level of WCW silage with grass silage reduced methane output. These results contradict *in vivo* responses, perhaps highlighting the inadequacies of this technique to accurately predict methane output.

Acknowledgements Project funded by the Irish Dept. of Agriculture, Food & Fisheries, Dublin (RSF 05 224).

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effect of urine production on milk urea nitrogen as estimator of urinary nitrogen and urea excretion in North America and Europe in dairy cattle. A meta-analysis.

J W Spek[1,2], A Bannink[1], J Dijkstra[2]
[1]Wageningen UR Livestock Research, Lelystad, The Netherlands, [2]Wageningen University, Wageningen, The Netherlands
Email: wouter.spek@wur.nl

Introduction Milk urea nitrogen (MUN; mg N/dL) is positively related to excretion of urinary urea N (UUN; g/d) (Burgos *et al*., 2007) and total N (UN; g/d) (Jonker *et al*., 1998; Bannink and Hindle, 2003) and can therefore be used as an indicator of N-utilization. However, Bannink and Hindle (2003) observed in a meta-analysis on 9 studies that the prediction of UN was substantially improved (R^2 increased from 0.79 to 0.84) when one study, in which the effect of salt intake on urine production was tested, was omitted. A meta-analysis based on dairy cattle studies from North America and Northern Europe was conducted to evaluate whether urine production affects the relationship between MUN and UN or UUN.

Materials and methods Mean treatment data were derived from North American trials (N-Am; 18 trials, 73 observations, and based on maize silage rations) and Northern Europe (EU; 8 trials, 34 observations, and mainly based on grass silage rations) (references available on request). Data were analysed with the MIXED procedure of SAS in which experiment was included as a random factor, whereas the fixed continuous regressors MUN and urine production (URINE; kg/d) were nested within continent (EU or N-Am). Data were weighted as described by St-Pierre (2001).

Results The factor URINE significantly affected the relationship between MUN and UN and between MUN and UUN (Table 1) and largely removed the differences between EU and N-Am with respect to the UN - MUN relationship. Inclusion of the factor URINE substantially improved the AIC value (from 942 to 900) and the experiment non-adjusted model fit (R^2 from 0.70 to 0.82) of UN (models 1 and 2, respectively) whereas the effect of URINE on the model fit of UUN (models 3 and 4) was less obvious. The effect of urine production on the relationship between MUN and UN was largest for the N-Am data. Prediction accuracy of UN and UUN (as judged by the standard error of the estimated UN and UUN) increased by 5 to 23% when, next to MUN, URINE was included in the model.

Table 1 Prediction of urine-N excretion (UN; g/d) and urinary urea N excretion (UUN; g/d) based on urine production (URINE; kg/d) and milk urea N (MUN; mg N/dL)

Model[1]	μ[2]	MUN		URINE		AIC[5]	R-Squared	
	SE	SE		SE			SEE[6]	
	P-value	P-value		P-value				
		EU[3]	N-Am[4]	EU[3]	N-Am[4]		Exp. Adjusted	Exp. Non-adjusted
	30	11.99	15.31			942	0.96	0.70
1. UN	10.9	0.659	0.768				12.2	35.0
	0.012	<.001	<.001					
	- 21	12.07	12.27	1.50	3.45	900	0.98	0.82
2. UN	11.4	0.610	0.859	0.333	0.536		10.0	27.0
	0.078	<.001	<.001	<.001	<.001			
	-44	14.26	16.05			664	0.97	0.84
3. UUN	9.7	0.458	0.743				11.7	27.0
	<.001	<.001	<.001					
	- 80	13.15	13.67	1.68	2.91	643	0.98	0.86
4. UUN	11.8	0.593	1.045	0.475	0.735		9.5	25.7
	<.001	<.001	<.001	<.001	<.001			

[1]UN prediction models 1 and 2 are based on 26 trials and 107 observations whereas UUN prediction models 3 and 4 are based on 18 trials and 78 observations. [2] μ = intercept. [3]EU = Nesting variable of studies carried out in Northern Europe. [4]N-Am = Nesting variable of studies carried out in North America. [5]AIC = Akaike's information criteria; a smaller value indicates a better model fit. [6]SEE = standard error of the estimated UN (model 1 and 2) and UUN (model 3 and 4).

Conclusions Urine production level affects the relationship between MUN and UN and its inclusion in predictive models increases the prediction accuracy of UN and UUN. Results suggest that differences between EU and N-Am with respect to prediction of UN based on MUN can be explained largely by level of urine production.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Effect of niacin supplementation on nitrogen balance and nitrogen efficiency in lactating German Holstein cows fed a ration with a negative rumen nitrogen balance

M Aschemann[1], P Lebzien[1], K-H Südekum[2], S Daenicke[1]
[1]Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute of Animal Health, Bundesallee 50, 38116 Braunschweig, Germany, [2]Institute of Animal Science, University of Bonn, Endenicher Allee 15, 53115 Bonn, Germany
Email: martina.aschemann@fli.bund.de

Introduction Negative ruminal nitrogen balance (RNB = [XP (g/d) – uCP (g/d)] / 6.25) in rations for dairy cows reduced the nitrogen (N) excretion with manure and enhanced the N efficiency (Burgos *et al*., 2007) whereas the flow of microbial crude protein (CP) at the duodenum was declined during a N undersupply in the rumen (Lebzien *et al*., 2006). Niacin is known to increase the flow of microbial CP at the duodenum (Kumar, 2005) and to improve the growth of the rumen microbial population, especially the protozoa (Doreau and Ottou, 1996). The aim of the present experiment was to determine if a niacin supplementation to lactating dairy cow diets can improve the N balance and the efficiency of N utilisation when the RNB is negative.

Materials and methods A total of 9 lactating multiparous German Holstein cows were randomly assigned to one of the three following diets consisting of (dry matter, DM, basis) 10 kg maize silage and 8 kg concentrate: RNB- (n = 6) with energy and CP at the duodenum according to the average requirement of the animals but with a negative RNB (-0.41 g N/MJ ME); RNB0 (n=7) with energy, CP at the duodenum and a RNB (0.08 g N/MJ ME) according to the average requirement of the animals and diet NIA (n = 5) which was the same diet as RNB– except that it was supplemented with 6 g niacin (nicotinic acid)/d. Due to different calving dates not every cow could be used in all periods. Therefore the design was unbalanced. Samples of milk were taken on two consecutive days and faeces and urine were collected completely over five consecutive days. For that purpose the cows were equipped with urine devices which were fitted around the vulva and allowed a separate collection of urine and faeces. N in faeces, urine, milk and feedstuffs was analysed by Kjeldahl method. Statistical analysis was performed using the MIXED procedure of the statistical software package SAS (Version 9.1).

Results As shown in Table1, a negative RNB in the diets RNB- and NIA decreased N excretion with urine, total N excreted with urine and faeces and the N balance. Milk yield and milk N excretion did not differ among diets. Consequently, the RNB - and the NIA diet increased the milk N efficiency compared to RNB 0. The supplementation of 6 g niacin per day reduced the N excretion with the faeces by 11.6 g/d (10%) compared to the diet RNB-. Although the N losses with urine were 9% lower, this difference was not significant. Although the total N excretion was only numerically reduced (16 g/d) by niacin compared to RNB-, this resulted in an improved N balance (+ 19.2 g/d) of cows fed the NIA diet compared to the RNB- diet, thus indicating that more N from the diet was retained as protein in the bodies of the cows.

Table1 Effects of rumen nitrogen balance and supplementation of niacin to dairy cows on nitrogen excretion and nitrogen balance

	RNB 0 (n=7)		**RNB-** (n=6)		**NIA** (n=5)	
Item	LSMeans	SE	LSMeans	SE	LSMeans	SE
Faecal N (g/d)	115.3[a]	2,48	119.4[a]	2,66	107.8[b]	2,85
Urinary N (g/d)	112.6[a]	3,82	48.5[b]	4,21	44.0[b]	4,70
Total N excretion (g/d)	228.2[a]	5,18	168.4[b]	5,72	152.5[b]	6,46
Milk yield (kg/d)	29,3	0,93	29,8	0,99	28,8	1,05
Milk N (g/d)	145,2	5,38	142,2	5,81	142,6	6,25
Balance (g/d)[1]	52.6[a]	2,63	20.6[c]	2,91	39.8[b]	3,12
Milk N efficiency[2]	33.1[b]	1,17	40.6[a]	1,24	41.6[a]	1,31

[1]Balance (g/d) = dietary N (g/d) - faecal N (g/d) - urinary N(g/d) - milk N (g/d).
[2]Milk N efficiency = Milk N (g/d) / N intake (g/d) * 100.
SE, standard error.
[a,b,c] Overall means in the same row with different superscripts differ significantly (P<0.05).

Conclusions The supplementation of 6 g/d niacin to cows fed a diet with a negative RNB increased the N balance. Regarding N losses, the effect of niacin on N excretion with urine was not significant but compared to the RNB- and RNB0 diets the NIA diet reduced the amount of N excreted in faeces. This seems to be an indication for the more efficient use of dietary N in the rumen when niacin was supplemented. However, further evaluation of the experimental data is needed to clarify the effects of the diets on the intra-ruminal processes.

Acknowledgements This study has been carried out with financial support from EU FP7 REDNEX.

Screening of different white rot fungi to improve the feed value of wheat straw

J W Cone[1], V T Dinh[1], J Baars[2], A Sonnenberg[2]
[1]Animal Nutrtion Group, Wageningen University, Wageningen, The Netherlands, [2]Plant Research International, WUR, Wageningen, The Netherlands
Email: john.cone@wur.nl

Introduction One of the main reason for the low feed value of straw and other low quality feedstuffs is the high level of lignin, preventing microbes to access the valuable carbohydrates and causing a low feed intake. In the past many attempts have been published to increase the feed value, using sodium hydroxide, urea and ammonia. Rodrigues *et al*. (2008) showed that enzymes of fungi are capable to increase the digestibility of straw in rumen fluid. Only fungi are capable to degrade lignin and fungi have a strategy to colonize and modify the substrate in such a way that (hemi)cellulose is available when fruit bodies are produced. Some species of white-rot fungi are capable to specifically break down lignin during the vegetative growth. The aim of the present research was to investigate what species of white rot fungi can be best used to unseal straw and to increase its feed value. Wheat straw samples were incubated up to seven weeks with eleven strains of white rot fungi before being fermented in rumen fluid, using the gas production technique.

Materials and methods Wheat straw was chopped to pieces of 3 cm. The white rot fungi *Ceriporiopsis subvermispora* (Cs), *Lentinula edodes* (Le), *Phlebia brevispora* (Pb), *Pleurotus ostreatus* (Po), *Pleurotus eryngii* (Pe), *Bjerkandera adusta* (Ba), *Phanerochaete chrysosporium* (Pc), *Trametes versicolor* (Tv), *Schizophyllum commune* (Sc), *Ganoderma lucidum* (Gl) *and Volvariella volvacea* (Vv) were inoculated for 6 days on 2% malt agar disks (diameter, 7-8 mm) at 24 ºC, to prepare sorghum based spawn. Each 15 grams of chopped wheat straw samples were put into 300 ml plastic containers. The containers were autoclaved twice at 121 ºC for 30 min and, after cooling, inoculated aseptically with 5 g of spawn and incubated at 24 ºC for 0 to 7 weeks. Three replications for each fungal culture of each incubation period were used. After incubation the wheat straw samples were oven dried, ground to 1 mm and analysed for fermentability in rumen fluid. Gas production incubations were performed as described by Cone *et al*. (1996), using rumen fluid from 2 non lactating Holstein Friesian cows receiving 1 kg of concentrate and *ad libitum* grass silage. The chemical composition of the straw was determined after 7 weeks of incubation with the fungi and compared with a control (C), straw not inoculated with fungi.

Results Figure 1 shows the gas production (ml gas/g OM) after 72 h of incubation in rumen fluid of wheat straw after an aerobic incubation with different fungi for 0 to 7 weeks. It is shown that during the first two weeks of incubation with fungi the total gas production is lower than the control (not incubated with fungi). After 2-4 weeks of incubation with the fungi *Ceriporiopsis subvermispora, Lentinula edodes* and *Pleurotus eryngii* total gas production was higher than the control. Other fungi showed only a slight increase in gas production or a lower gas production than the control (data not shown). Figure 2 shows the ratio of cellulose/lignin in the straw after 7 weeks of incubation with the fungi. It is shown that especially *Ceriporiopsis subvermispora* and *Lentinula edodes* degrade the lignin during the aerobic incubation, leaving cellulose relatively unharmed.

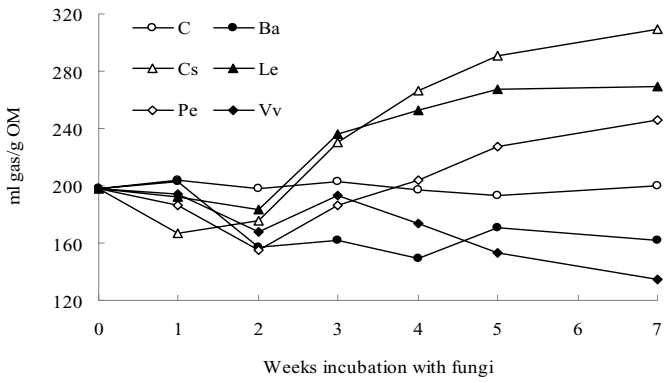

Figure 1 Gas production of wheat straw after 72 h in rumen fluid after a pre-treatment of 0 to 7 weeks with different fungi.

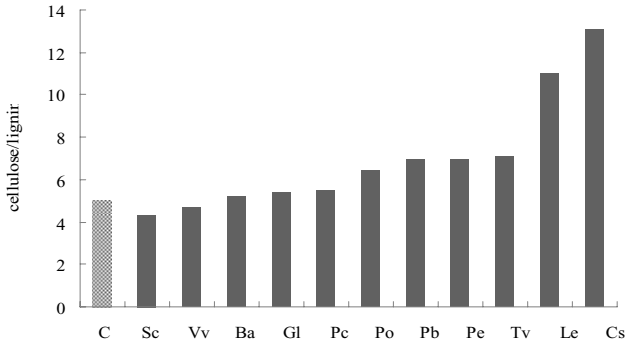

Figure 2 Ratio of cellulose/lignin of wheat straw after 7 weeks of incubation with different fungi, compared to the untreated sample (Control, C).

Conclusions The results show that white rot fungi are capable to specifically degrade lignin during aerobic incubation, resulting in an increased degradability of the straw during anaerobic fermentation in rumen fluid. Especially *C. subvermispora* and *L.edodes* showed a high potency to degrade lignin and to improve the quality of low value feedstuffs.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Buckwheat fractions and rutin incubated with bovine rumen fluid: effects on *in vitro* fermentation and methane formation

F Leiber, C Kunz, M Kreuzer
ETH Zurich, Institute of Agricultural Sciences, Zurich, Switzerland
Email: fleiber@ethz.ch

Introduction Buckwheat is a dicotyledonous crop plant which is cultivated in a broad range of climatic regions and has been shown to be a potential forage plant for ruminants (Amelchanka *et al*., 2010). Compared to typical ruminant forages from temperate climatic regions, buckwheat has a comparably high concentration of phenolic compounds (Kalinova *et al*., 2006). These compounds are possible modifiers of the ruminal fermentation processes and thus may alter microbial productivity and methane release (Szumacher-Strabel and Cieslak, 2010). The main phenolic compound in buckwheat is the flavonoid rutin, which is present in different concentration in different plant parts (Kalinova *et al*., 2006). The present experiment investigated the effects of different parts of the buckwheat herb and of pure rutin on ruminal fermentation *in vitro*.

Materials and methods Amounts of 100 mg each of dried aerial herb (AH), flowers (FL), leaves (LV), stems (ST) and grains (GR) of buckwheat were mixed with 100 mg of pure dried ryegrass. Further, 200 mg of pure ryegrass were supplemented either with 0 (RG0), 0.1 (RG0.1), 1 (RG01) or 10 mg (RG10) of purified rutin. RG0 served as control. These nine different diets were incubated in 30 ml of buffered bovine rumen fluid at 39°C for 24 h in a batch system. Rumen fluid was obtained from a lactating fistulated Brown Swiss cow fed ryegrass clover hay *ad libitum* and 0.5 kg balanced dairy cow concentrate daily. Incubation was carried out in four independent runs, each one comprising two replicates of each diet, resulting in eight replications per diet. After 24h of incubation, the gas volume produced was measured, and gas was analysed for methane concentration by gas chromatography with a flame ionisation detector. In the incubation fluid, volatile fatty acids were quantified by high pressure liquid chromatography; ammonia concentration was measured with a potentiometer and bacteria and protozoa were counted after fixation with Hayem solution and formaldehyde in Bürker counting chambers. Concentrations of total extractable phenol were analysed in all feeds by the Folin-Ciocalteu Method (all methods are described in Amelchanka *et al*., 2010). The resulting data were evaluated by a general linear model using the SAS software, where diet was a fixed and incubation run a random effect. Multiple comparisons among means were performed with Tukey's method.

Results The concentrations (g/kg dry matter) of extractable phenols in the different feeds were: 8.2 (RG), 48 (AH), 88 (FL), 63 (LV), 15 (ST), and 9.0 (GR). Total gas production was lower by 10 to 15% ($P < 0.05$) for all buckwheat plant parts except the grain when compared to ryegrass irrespective of the level of rutin supplementation. The same was true for methane, which was reduced by up to 20% when incubating any buckwheat herb fraction (except the grain) in comparison with the ryegrass-only treatment. The addition of rutin to ryegrass increased gas production (+ 9%; $P < 0.05$). Methane production per unit of fermentation gas was lower ($P < 0.001$) with AH (- 14%), FL (-15%) and RG10 (- 12%) compared to the RG0 control. Total volatile fatty acids were not substantially affected by the diets, but the acetate-to-propionate ratio was shifted ($P < 0.05$) from 3.9 to 3.4 when buckwheat grain was incubated. Protozoa counts were increased ($P < 0.001$) by approximately 80% with the buckwheat grain diet, compared to all other treatments. Bacterial counts were not affected. Ammonia concentration in incubation fluid was decreased ($P < 0.001$) by buckwheat stems (-19%) and grain (-11%); while the proportionate ammonia production per unit of dietary N was declined ($P < 0.001$) with rutin (RG10), buckwheat flowers and leaves, suggesting a certain protein protection capacity of rutin, similar to that of tannins.

Conclusion The study showed that the buckwheat herb may alter the ruminal fermentation processes, especially the flowers, which are rich in extractable phenols. This includes a certain mitigation of ruminal methane production. Related to productivity, also purified rutin showed a potential to mitigate methane; this, however, rather based on an increase in gas production without concomitant increase in methane production. This indicates that rutin could be a substrate for non-methanogenic bacteria. Further, indications for a protective effect of rutin on feed proteins in the rumen environment were found. These results show that buckwheat has an interesting potential to be used as a functional feed for ruminants, as has also been demonstrated with respect to the resulting milk quality of dairy cows (Kälber *et al*., 2011).

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Dose-dependent additions of *Eremophila glabra* reduce methane production in Rusitec

X X Li[1,2], Z Durmic[1,2], S Liu[1], P Vercoe[1,2]
[1]The Univeristy of Western Australia, Crawley, Western Australia, Australia, [2]Future Farm Industries CRC, Crawley, Western Australia, Australia
Email: lix13@student.uwa.edu.au

Introduction Native plants can be used to modulate ruminal fermentation, especially to reduce methane emission from ruminants. For example, *Eremophila glabra* caused significantly less methane to be produced than common pasture species in batch culture (Durmic, 2010). It also had dose-related effects on ruminal fermentation when mixed with *Medicago sativa* (Li, 2010), suggesting that *E. glabra* has the potential to modulate ruminal fermentation. However, when *E. glabra* was provided as the sole substrate total gas production was inhibited compared with the control, indicating that *E. glabra* may cause adverse effects on rumen microbes at high levels (Durmic, 2010; Li, 2010). We hypothesized that there would be an optimal dose of *E. glabra* that would inhibit methane production without disrupting overall fermentation. We tested three doses of *E. glabra* in the Rusitec for one week. Total gas and methane production were measured daily and compared to productions from a basal control diet.

Materials and methods Leaves from *E. glabra* were freeze-dried and tested as feed supplements. Substrate for fermentation was a grounded basal diet (a mixture of 1 part oaten chaff + 0.25 part lupin + 0.025 part minerals) with or without addition of *E. glabra*. Twelve vessels (1800 ml working volume) were set up and randomly allocated to four treatments with triplicates: the basal diet (control, 60 g basal diet), 0.15 dose of *E. glabra* (Eg 15%; 9 g *E. glabra* + 51 g basal diet), 0.25 dose of *E. glabra* (Eg 25%; 15 g *E. glabra* + 45 g basal diet), and 0.40 dose of *E. glabra* (Eg 40%; 24 g *E. glabra* + 36 g basal diet). Five fistulated Merino sheep were fed the basal diet *ad libitum* for two weeks and rumen fluid samples were collected from them 2 h after morning feeding and strained through cheese cloth. Before inoculation, each vessel was pre-filled with 500 ml of rumen fluid and 1300 ml artificial saliva. Artificial saliva (pH 8.4) was prepared daily and infused into each vessel at a rate of 1350 mL / d. Each vessel had four bags of 15 g basal diet present at any time, and each bag was incubated for 48 h. After a 12 day adaption period, *E. glabra* was supplied on d 13 (recorded as d 0) in nylon bags separately from basal diet for 7 days (d 7). Total gas and methane production were measured daily. Data were analysed using the GLM procedure of SAS for repeated measures (version 9.2; SAS Institute Inc.). The model included the fixed effect of treatment (three doses of *E. glabra* and control), the random effect of individual vessel and the interaction effect between treatment and day. Tukey's test was used for the multiple comparisons of the means.

Results Data from the first two days (d -1, d 0) presented the initial total gas and methane production in corresponding vessels before adding *E. glabra* (Fig. 1). The total gas production varied from 1543 ml to 1699 ml with a mean methane proportion of 0.17. Effects of *E. glabra* on ruminal fermentation were dose-dependent and persisted for 7 days. Eg 15% did not reduce total gas production, but decreased methane production by 0.36 on average compared with control ($P < 0.05$). Eg 25% and Eg 40% caused a persistent depression of both total gas and methane production. Decreases of 0.26 and 0.39 in total gas production, 0.60 and 0.64 in methane production were observed in Eg 25% and Eg 40% treatments respectively. The interaction between treatments and days was significant ($P < 0.001$), and the inhibition effects on methane production accumulated throughout the study ($P < 0.05$).

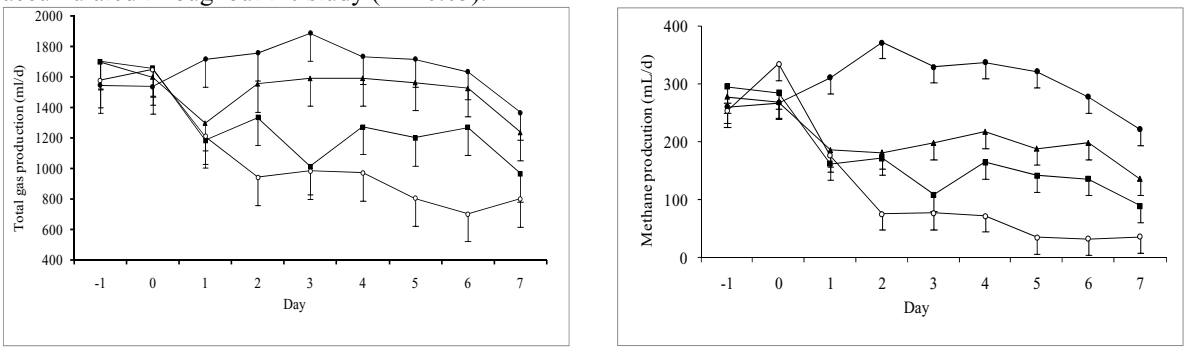

Fig.1 Total gas and methane production in vessels fed with control (●), Eg 15% (▲), Eg 25% (■), and Eg 40% (○) from day -1 to day 7. The error bars represent the standard error of the means.

Conclusions Our expectation that there would be an optimal dose of *E. glabra* for modulating ruminal fermentation is supported by our results. The reductions of methane production were positively related to the doses of *E. glabra.* The inclusion of *E. glabra* at 15% reduced the methane production in total gas while allowing normal fermentation. While Eg 25% and Eg 40% treatments had more general inhibitory effects on overall ruminal fermentation. The 15% inclusion level will be used for future Rusitec studies of persistence and testing the antimethanogenic effects of *E. glabra in vivo*.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Effect of increasing proportion of alfalfa in alfalfa-ryegrass mixed diets on intake, digestion and methane emissions in sheep

V Niderkorn, C Martin, A Le Morvan, Y Rochette, E Brison, R Baumont
INRA, UR1213 Herbivores, Saint-Genès Champanelle, France
Email: vincent.niderkorn@clermont.inra.fr

Introduction Compared to monoculture, grass-legume mixtures have a great potential to increase sustainability and competitiveness of livestock production systems for instance in terms of forage yield and quality and reduction of N fertilization (Nyfeler *et al*. 2009). From a nutritional point of view, feeding ruminants with grass-legume mixtures may increase intake and improve digestive functions (Niderkorn and Baumont, 2009). The aim of the present work was to assess intake, digestive parameters and pollutant emissions (nitrogen and methane) in sheep and their relationships when increasing the proportion of alfalfa in perennial ryegrass based diets.

Materials and methods Four proportions of fresh regrowth of alfalfa cv. *Aubigny* (0, 250, 500, 750 g/kg, DM basis) mixed with a fresh regrowth of perennial ryegrass cv. *AberAvon* were allocated to six ruminally fistulated 1-year-old Texel sheep fed *ad libitum* (10% refusals) during four experimental periods in a partially repeated Latin square design. In average, alfalfa and ryegrass forages contained, per kg DM, 213 g CP, 435 g NDF, 278 g ADF, 58 g ADL, and 107 g CP, 525 g NDF, 275 g ADF, 23 g ADL, respectively. Each experimental period consisted of an 8-days adaptation period followed by a 6-days measurement period during which voluntary intake, digestibility and N balance, methane emissions using the SF6 gas tracer technique, VFA concentration and rumen turnover rate using Cr-EDTA as a marker of the liquid phase were measured. The statistical analysis was carried out using the MIXED procedure of SAS® v.9.1 software. The proportion of alfalfa in the diet, the experimental period and their interaction were considered as fixed effects and sheep as a random effect. The Tukey-Kramer test was used to compare treatments means. Linear and quadratic contrasts were also tested to highlight potential associative effects between forage species across the proportions of alfalfa in the diet.

Results Although DM and NDF intake was not significantly impacted when the level of alfalfa increased, the OM digestibility tended to decrease (P=0.08) linearly and was related to the significant decrease of NDF digestibility (P=0.01) (Table 1). While N retained by animals was similar (P>0.05), N losses in urine increased linearly from 9.1 to 27.0 g/d when the proportion of alfalfa increased from 0 to 750 g/kg (P<0.001). An associative effect occurred between ryegrass and alfalfa on both rumen fluid volume (P=0.03) and fractional turnover rate (P<0.001) as quadratic effects were detected. Total VFA concentration increased linearly (P=0.03) with the level of alfalfa in the diet, whereas methane yield was unchanged.

Table 1 Intake and digestive parameters from sheep fed different proportions of alfalfa in perennial ryegrass

Item	Alfalfa, g/kg DM				SEM	P-value	Contrasts[1]	
	0	250	500	750			Linear	Quadratic
DM intake (kg/d)	1.47	1.50	1.50	1.57	0.059	0.61	NS	NS
NDF intake (kg/d)	0.74	0.73	0.70	0.69	0.033	0.09	NS	NS
OM digestibility	0.79	0.79	0.76	0.74	0.013	0.08	**	NS
NDF digestibility	0.75^{a}	0.75^{ab}	0.71^{bc}	0.68^{c}	0.015	0.01	**	NS
Rumen fluid volume (L)	9.1^{a}	10.1^{a}	9.6^{a}	6.4^{b}	0.81	0.01	*	*
Fractional turnover rate of rumen fluid (/h)	0.11^{b}	0.10^{b}	0.09^{b}	0.15^{a}	0.006	<0.01	***	***
Liquid flow (L/h)	0.98	0.99	0.86	0.97	0.007	0.31	NS	NS
N retained (g/d)	9.0	9.6	7.3	10.6	1.01	0.47	NS	NS
Methane yield (g/kg DM intake)	16.7	18.0	17.8	16.8	1.30	0.98	NS	NS
Total VFA (mmol/L)	93.4^{b}	92.1^{b}	98.5^{ab}	105.3^{a}	3.04	0.03	**	NS

[1]*P<0.05, **P<0.01, ***P<0.001

Conclusions The quadratic effects detected on rumen turnover rate suggest that ryegrass may decrease the outflow rate of alfalfa through the rumen. But as rumen volume decreased with increased proportion of alfalfa, the total liquid flow was not affected by the proportion of alfalfa. The decrease in digestibility could explain that the expected increase of intake was not observed. No synergy between ryegrass and alfalfa was observed on N metabolism as the excess of N from alfalfa was excreted in urine rather being more retained. The methane yield was unchanged for all the mixtures tested.

Acknowledgement
The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/ 2007-2013) under the agreement n°FP7-244983.

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In vitro rumen methanogenesis of different perennial grass species

P Purcell[1,2], M O'Brien[1], T Boland[2], D Grogan[3], P O'Kiely[1]
[1]Teagasc Grange, Dunsany, Co. Meath, Ireland, [2]University College Dublin, Belfield, Dublin 4, Ireland, [3]Department of Agriculture, Fisheries and Food (DAFF), Backweston, Co. Kildare, Ireland
Email: peter.purcell@teagasc.ie

Introduction The predominance of grazed grass in many ruminant diets highlights the need for greenhouse gas mitigation strategies that are grass based. Specific perennial grass species may provide the opportunity to reduce enteric methane production if they change fermentation characteristics in the rumen. Consequently, the objective was to determine the effects of grass species throughout the grazing season on *in vitro* rumen methane output.

Materials and methods Six grasses [perennial ryegrass (*Lolium perenne* L., Portstewart (PR-P) and Navan (PR-N)), cocksfoot (C; *Dactylis glomerata* L., Donata), timothy (T; *Phleum pratense* L., Dolina), tall fescue (TF; *Festuca arundinacea* Schreb., Barolex) and meadow fescue (MF; *Festuca pratensis* Huds., Pradel)] were sampled from plots within each of three replicate field blocks at 8 stages of the growing season (27 April, 21 May, 10 June, 30 June, 27 July, 2 September, 1 October and 30 November, 2009 – defined as Cuts 1 to 8, respectively). Samples were oven dried (40ºC for 48 h) and milled (1 mm). Rumen fluid was pooled from four fistulated steers, and buffer was prepared according to Tilley and Terry (1963). *In vitro* rumen fermentation was carried out according to Theodorou *et al.* (1994) with modifications made as described by Mauricio *et al.* (1999). Rumen fluid and buffer were mixed at a 1:4 ratio, and 50 ml of this mixture and 0.5 g of milled grass were added to each 160 ml fermentation bottle prior to incubation at 39°C for 24 h. The total amount of accumulated gas produced in each bottle after 24 h was estimated using the equation of Mauricio *et al.* (1999): Gas production (ml) = (vh/Pa) x Pt, where vh equals head space volume (millilitres), Pa equals atmospheric pressure (hectoPascals) and Pt equals the bottle head space pressure (hectoPascals). Methane and volatile fatty acids (VFA) were determined using gas chromatography. Data were analysed as a randomised block (n = 3) design with repeat sampling (n = 8) of each plot (n = 6) per block, using the PROC MIXED statement of SAS. Means were separated using Tukey adjusted comparisons.

Results Although species had a significant effect on methane output per gram of dry matter (DM) disappeared (CH_4d; Table 1; $P < 0.05$), there were no differences in CH_4d between individual species when analysed using Tukey adjusted comparisons. Species had no effect on methane output per gram of DM incubated (CH_4i; $P > 0.05$). The acetic acid to propionic acid (A:P) ratio was lower ($P < 0.05$) for PR-N than for C and T, but did not differ from PR-P, TF or MF. The total concentration of volatile fatty acids (tVFA) did not differ between species ($P > 0.05$), except that PR-N was higher ($P < 0.05$) than C. No clear trend was found throughout the grazing season for CH_4d (Table 1) or CH_4i. However, both variables were lower ($P < 0.05$) for Cut 7 than for all other cuts. There was no difference ($P > 0.05$) between the remaining cuts for CH_4d, except that Cut 3 was higher than Cut 5 (Table 1; $P < 0.05$). The CH_4i was higher for Cut 3 ($P < 0.05$) than for all others except Cuts 1 and 4. No clear trend was apparent with advancing maturity for the A:P ratio, but it was lower for Cut 8 than for all other cuts ($P < 0.05$), or for tVFA, which was lower ($P < 0.05$) for Cut 7 than for Cuts 1, 3 and 8. There were significant interactions between species and cut for CH_4d (Table 1), CH_4i, A:P ratio and tVFA ($P < 0.05$). However, the effect of species within individual cuts for all variables was in agreement with the overall effect of species across all cuts when analysed using Tukey adjusted comparisons.

Table 1 Effects of grass species (Sp) and cut on methane output (ml) per gram of dry matter disappeared (CH_4d) after 24 hours incubation

	Species					
	PR-P	PR-N	C	T	TF	MF
Cut			CH_4d			
1	38	39	38	37	40	40
2	37	38	44	41	42	38
3	39	41	43	38	41	39
5	36	37	39	38	40	34
6	37	37	39	40	42	37
7	30	31	34	28	37	31
8	39	38	38	37	41	37

Significance		
	SEM	Sig
Sp	0.7	*
Cut	0.7	***
Sp × Cut	1.8	*

PR-P, perennial ryegrass (Portstewart); PR-N perennial ryegrass (Navan); C, cocksfoot; T, timothy; TF, tall fescue; MF, meadow fescue; SEM, standard error of the mean; Sig, significance; ***, $P < 0.001$; *, $P < 0.05$

Conclusions There was no difference in *in vitro* rumen methane output between perennial grass species, despite differences in the total concentration of VFA and the A:P ratio. Thus, there appears to be limited potential to reduce enteric methane production in ruminants by choosing among these grass species in well managed grassland.

Acknowledgements Dept. of Agriculture,Fisheries and Food (RSF 07517)

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Methane production in pregnant ewes

A Kiani, A Chwalibog
[1]Lorestan University, Khoramabad, Iran, [2]University of Copenhagen, Copenhagen, Denmark
Email: arkashkia@gmail.com

Introduction Besides energy loss, methane production has a great contribution to greenhouse gases and global warming (2). Methane is blamed for at least 15-17 % of the global warming (2, 4). In ruminants, factors such as level of feed intake, composition of diets, quality of diet, age of animal, inclusion of dietary fats and ionophers to diets affect the methane production (3,4). The objectives of this study were to quantify CH_4 production during late gestation in ewes, and to investigate effect of two levels of feed intake on CH_4 production in pregnant ewes.

Material and methods Twenty multiparous Shropshire twin bearing ewes were fed with two feed levels; *ad libitum* group, (silage+200 g barley+200g lactamin) and 60% restriced (silage) from day 90 until parturition. From seven, four and two week's *pre-partum*, daily dry matter and digestible energy (DE) intake were measured in balance trails. CH_4 production (g/day) was measured using an open-air indirect calorimeter. Data were analyzed using MIXED procedure of SAS 9.1 (1). Relationships between CH_4 production, daily dry matter intake (DMI) and digestible energy (DE) were calculated using REG procedure of SAS 9.1 (1).

Results CH_4 production averaged 27.2 (g/d) in pregnant ewes, regardless of gestation period and DMI. The production increased as DMI increased and pregnancy progressed. Ewes in restricted group produced less methane than did *ad libitum* fed ewes ($p<0.05$). The regression equations were:
CH_4 (g/d) = 34.7 DMI (kg/d) - 0.96DE (n=56, R^2=0.96, RMSE=5.9)
CH_4 (g/d) = 22.6 DMI (kg/d) (n=56, R^2=0.95, RMSE=6.0)

Table 1 Dry matter intake, digestible energy and methane production in twin pregnant ewes

	Weeks pre-partum					Feed intake			
	7	4	2	SE	P-value	*Ad libitum*	Restricted	SE	P-value
Dry matter intake (kg/d)	0.88[a]	1.19[b]	1.45[c]	0.04	0.001	1.35[a]	1.00[b]	0.04	0.001
Digestible energy (MJ/d)	9.3[a]	14.8[b]	18.9[c]	0.5	0.001	17.1[a]	11.7[b]	0.4	0.001
Methane production (g/d)	21.5[a]	28.9[b]	31.6[b]	1.4	0.001	29.2[a]	25.2[b]	1.2	0.03

Conclusions The results showed that both pregnancy and feed intake affect the methane production in sheep. The CH_4 production in pregnant ewes was about 23 g/ kg DMI and about 27 g/d/head.

Acknowledgments The authors gratefully acknowledge funding from Iranian Ministry of Science and Technology and University of Copenhagen

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effect of the partial replacement of concentrate with feed blocks including greenhouse wastes on ruminal fermentation and methane production in dairy goats

M Romero, E Ramos, I Martin, R Nogales, E Molina
Estación Experimental del Zaidin (Spanish Research Council), Armilla, Granada, Spain *Email: manuel.romero@eez.csic.es*

Introduction Ruminant production in the Mediterranean area is limited by the poor quality and scarcity of pasture. Concentrates based on cereals are frequently used to overcome this limitation, but increases in cereals prices has driven the attention of ruminant nutritionists toward local alternatives (Ben Salem y Znaidi, 2008). Glasshouse culture is very important in the Mediterranean, producing large amount of wastes, which could be an alternative to cereals for ruminants. The aim of the present experiment was to study, in dairy goats, the effect of substituting 35% of concentrate in diet with feed blocks, including wastes from tomato and cucumber cultivation, on ruminal fermentation and methane production.

Materials and methods Eight Granadina goats (39.6 ± 1.89 kg BW) in the middle of the third lactation were used, and 4 trials were carried out following a 4 × 4 Latin square experimental design. In every trial, 2 animals randomly received 1.0 kg of alfalfa hay (AH) and 1.0 kg of concentrate (diet AC) or 1.0 AH plus 0.65 kg of concentrate plus feed blocks (FB) including greenhouse wastes of tomato (diet ACBI), wastes of cucumber (diet ACBII) or barley (diet ACBIII) with 8 replications per diet at the end of the experiment. The FB were supplied *ad libitum*, resulting in average intakes of 256 ± 31.8, 227 ± 43.9 and 201.8 ± 58.9 g of dry matter/animal/d for ACBI, ACBII and ACBIII diets, respectively. After 25 d of adaptation to the corresponding experimental diet, animals kept in metabolism crates were individually placed into square polycarbonate chambers (1,8 × 1,8 × 1,5 m) to measure CH_4 emissions over a 3-d period. Methane emission was calculated from CH_4 concentration analysed using a gas analyzer ADM MGA3000 (Spurling Works, Herts, UK) and airflows into and out of each chamber. At the end of each trial, rumen content samples from each animal were obtained orally by using a nasogastric catheter before feeding, for pH measurement and concentrations of VFA and NH_3-N analyses by gas chromatography and colorimetric methods, respectively, as described by Molina-Alcaide *et al.* (2009). Individual intakes of diet ingredients were registered throughout the trial. Ground (1-mm) samples of ingredients were analyzed for chemical composition (Table 1) according to the AOAC (2005). The data were analyzed by GLM using the repeated measures ANOVA.

Results Ammonia concentration in the rumen was lower (P<0.001) in animals fed diets including wastes of tomato and cucumber (Table2) than diets AC and ACBIII. The diet affected (P<0.001) total VFA, acetate, butyrate and propionate concentrations, the highest values being for AC and ACBII diets. Differences (P< 0.001) in methane production were promoted by diet, with lower values when 0.35% of concentrate was replaced with feed blocks specially those including greenhouse wastes. Other studied parameters were not affected (P ≥ 0.075) by diet. Lower methane production values with diets ACBI and ACBIII in comparison with AC and ACBII diets could be due to an inhibitory effect of high hydrogen pressure in the rumen on the ruminal microbiota. Decreased methane production in animals fed the ACBII diet was accompanied by increased ruminal propionate concentration, providing the main alternative sink for metabolic hydrogen during fermentation. Higher butyrate concentrations in goat fed diets AC and ACBII than in animals receiving diets ACBI and ACBIII could be associated to increased growth of bacteria producing butyrate since they are involved in the ruminal biohydrogenation, another alternative sink for metabolic hydrogen to a lesser extent.

Table1 Chemical composition (g/kg DM) of diet ingredients

			Feed Blocks		
	Alfalfa hay	Concentrate	Tomato	Cucumber	Barley
DM, g/kg de fresh matter	904	926	919	915	931
OM	880	893	819	818	821
CP	214	170	165	151	152
NDF	419	342	454	514	495
ADF	244	142	250	302	301
ADL	60.9	34.3	42.1	51.4	61.4
Ether extract	13.6	3.39	7.29	8.63	3.01
GE, MJ/kg DM	18.2	18.2	16.1	15.8	15.6

Conclusions The partial replacement of concentrate in diets based on alfalfa hay with feed blocks decreases methane production in dairy goats modifying some ruminal fermentation parameters. More research is needed in order to understand the effects of these diets on milk quality.

Table 2 Effects of partial replacement of concentrate with feed blocks on dry matter intake, ruminal fermentation parameters and methane production in lactating dairy goats

	Diet[1]					
	AC	ACBI	ACBII	ACBIII	*P*-value	*SEM*[2]
DM intake, g/d/kgBW	93	101	98	101	0.255	2.245
pH	6.94	7.05	7.09	7.09	0.232	0.033
N-NH_3, mg/100ml	56.2^{b}	36.5^{a}	36.9^{a}	50.7^{b}	< 0.001	0.995
CH_4, ml/g DM intake	28.2^{c}	17.4^{ab}	17.2^{a}	19.7^{b}	< 0.001	0.555
Total VFAs, mmol/L	42.7^{c}	31.2^{b}	45.9^{c}	27.0^{a}	< 0.001	0.650
Acetate	28.9^{c}	20.3^{b}	30.4^{c}	17.7^{a}	< 0.001	0.498
Propionate	5.49^{c}	4.12^{b}	6.94^{d}	3.35^{a}	< 0.001	0.105
Iso-butyrate	0.93	0.93	1.02	0.90	0.865	0.065
Butyrate	5.89^{c}	4.39^{b}	5.87^{c}	3.73^{a}	< 0.001	0.126
Iso-valeric	1.03	0.97	1.25	0.97	0.075	0.045
Valeric	0.46	0.38	0.43	0.34	0.324	0.013
Acetate/Propionate	5.35	5.01	4.48	5.44	0.273	0.150

[1]AC = Alfalfa hay and concentrate (1:1); ACFBI = alfalfa hay, concentrate (1:0.65) and tomato feed block; ACFBII = alfalfa hay, concentrate (1:0.65) and cucumber feed block; ACFBIII = alfalfa hay, concentrate (1:0.65) and barley feed block; [2] Standard error of the mean.

Acknowledgements The authors wish to acknowledge the financial support from the Junta de Andalucía (Excellence Projects: P05-AGR-00408 and P07-RNM-02746). Manuel Romero is grateful to the CSIC for JAE-CSIC grant. Thanks to J. Fernandez and T. Garcia for technical assistance.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Effect of ricinoleic acid on *in vitro* rumen methanogenesis

E Ramos-Morales[1], N McKain[2], R J Wallace[2]
[1]Consejo Superior de Investigaciones Científicas, Estación Experimental del Zaidín, Granada, Spain, [2]University of Aberdeen, Rowett Institute of Nutrition and Health, Aberdeen, UK
Email: eva.ramos@eez.csic.es

Introduction *In vitro* studies have shown that ricinoleic acid (RA; 12-hydroxy-cis-9-18:1), which is the main fatty acid component in castor oil, inhibits the biohydrogenation of linoleic acid (cis-9,cis-12-18:2) in rumen fluid (Ramos-Morales *et al*., 2010). Fatty acid metabolism is linked to other areas of ruminal metabolism, through a common reliance on H_2 metabolism and/or the microbial species that are involved in multiple metabolic processes; however, the consequences of changing biohydrogenation on other ruminal processes are often not investigated. It has been shown that some fatty acids may be doubly beneficial to ruminal nutrition by decreasing the environmental damage of methane emissions and improving the fatty acid composition of ruminant products (Goel *et al*., 2009). The aim of the present study was to investigate the influence of RA on *in vitro* rumen methanogenesis.

Material and methods Post-mortem samples of rumen digesta were taken from eight mature sheep receiving a mixed hay-concentrate (30:70) diet. Strained ruminal fluid from each sheep was diluted 1:2 in artificial saliva solution and 50 ml were added anaerobically to wheaton bottles containing 0.4 g (1-mm mesh screen) of the ration fed to the sheep. Treatments consisted of control incubations (0.4 g of diet only) and incubations either with 1 ml of 2.5 mM BES (2 bromoethanesulfonic acid-sodium salt), as a positive control, or 1 ml of 10 g RA/l. Two replicate bottles for each treatment and sheep were incubated under CO_2 and at 39 °C for 24 h. Total gas production was then measured and the reaction was stopped by adding 1 ml of saturated mercuric chloride. A gas sample (1 ml) was removed from each bottle and analysed for methane. VFA were determined by using ethylbutyric acid as the internal standard. Data were analyzed by a randomised block analysis of variance with sheep as blocks using Genstat 10th edition (VSN International, UK).

Results A 28% decrease (P< 0.01) in methane production was obtained in 24 h *in vitro* incubations of diluted buffered ruminal fluid with 0.2 g RA/l in comparison with control incubations (Fig. 1). The positive control, 2.5 mM BES, decreased (P< 0.001) methane production by 80.6% (Fig. 1). There was no effect on the total concentration of VFA after 24 h incubation (P> 0.05) as a result of RA addition (Table 1). Mean values of acetate and butyrate concentrations were lower (P< 0.05 and P< 0.001, respectively) whereas those of propionate were higher (P< 0.001) when RA was added (Table 1).

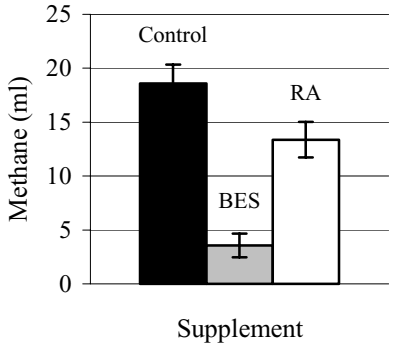

Figure 1 Influence of RA on *in vitro* methane production after 24 h incubation

Table 1 Effect of RA on VFA (mmol/l) in ruminal digesta after 24 h. *P < 0.05; **P < 0.01; ***P < 0.001

	Control		RA	
	Mean	SE	Mean	SE
Total VFA	209.5	15.09	209.6	16.08
Acetate	126.4	7.08	121.2*	8.09
Propionate	49.6	5.16	61.2***	4.88
Isobutyrate	1.39	0.18	1.13**	0.18
Butyrate	26.1	2.91	20.4***	3.14
Isovalerate	3.50	0.55	3.03*	0.55
Valerate	2.60	0.34	2.71	0.34

Conclusion RA decreased methane production *in vitro*. Methane suppression was accompanied by increased propionate and decreased acetate concentrations, the former providing the main alternative sink for metabolic hydrogen during fermentation. These shifts in individual VFA without affecting total VFA concentration would lead to a higher efficiency of energy retention. *In vivo* evaluation of RA is suggested for future studies as it is expected that it would reduce the methane production and enhance fatty acid profile of ruminant products.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Endogenous synthesis of rumenic acid in the mammary gland of dairy ewes

P G Toral[1], E Bichi[1], G Hervás[1], P Gómez-Cortés[2], M A de la Fuente[2], M Juárez[2], P Frutos[1]
[1]Instituto de Ganadería de Montaña (CSIC-ULE). Finca Marzanas s/n, Grulleros, León, Spain, [2]Instituto de Investigación en Ciencias de la Alimentación (CSIC-UAM), C/ Nicolás Cabrera, 9. Campus de Cantoblanco, Madrid, Spain
Email: p.frutos@csic.es

Introduction In ruminants, the enzyme Δ9-desaturase has a key role in the synthesis of milk fatty acids (FA) through the introduction of a *cis* double bond between carbon atoms 9 and 10 (Palmquist *et al*., 2005). In recent years, the study of Δ9-desaturase activity has had a renewed interest, after Griinari *et al*. (2000) demonstrated that endogenous synthesis in the mammary gland of cows is the major source of the potentially health-promoting *c*9*t*11-18:2 (rumenic acid, a conjugated linoleic acid isomer) found in milk. It is now well known that this occurs via desaturation of *t*11-18:1 of rumen origin, but the number of studies quantifying the proportion of *c*9*t*11-18:2 synthesized endogenously is still limited in ruminants (Palmquist *et al*., 2005). Thus, in sheep, there is no information on this subject, despite the high levels of *c*9*t*11-18:2 often found in ovine milk, which might be related with a higher Δ9-desaturase activity in this species (Tsiplakou *et al*., 2009).
The aim of this study was therefore to examine the endogenous synthesis of *c*9*t*11-18:2 in the mammary gland of dairy ewes, via the administration of sterculic acid, a cyclopropenoic FA that specifically inhibits the Δ9-desaturase system (Gomez *et al*., 2003).

Materials and methods Six primiparous Assaf ewes in mid-lactation were housed in individual tie stalls and fed *ad libitum* a pasture diet (in order to supply *c*9*c*12*c*15-18:3 and minimize the amount of milk *c*9*t*11-18:2 coming from ruminal origin). The pasture was harvested prior to commencing the trial and kept frozen at –30ºC until used. During a 5-day treatment period, all the ewes received 0.5 g/d of chemically synthesized sterculic acid, suspended in 6 mL of 10% Intralipid and 0.5 g of Simulsol 5817 and made up to 7 mL with 0.9% (w/v) saline solution. One-fourth of the daily treatment dose was delivered every 6 hours by jugular infusion. The ewes were milked twice daily at 0830 and 1800 hours. One day before the start of sterculic acid administration (pre-treatment), on the last day of the treatment, and 5 days later (post-treatment), milk production was recorded and samples were collected for determination of fat content by infrared spectrophotometry (ISO 9622:1999) and FA composition by gas chromatography (Hervás *et al*., 2008). The content of 14:0 and the product of its desaturation (*c*9-14:1; synthesized almost exclusively in the mammary gland) were used to calculate a correction factor for incomplete inhibition of Δ9-desaturase, in order to estimate the endogenous synthesis of *c*9*t*11-18:2 (Griinari *et al*., 2000). Data were evaluated by one-way analysis of variance using the MIXED procedure of SAS (Version 9.1).

Results Sterculic acid administration had no effect (P>0.10) on milk production (1.14±0.040 kg/d) and milk fat concentration (5.5±0.18%). Although the increases in the content of the potential substrates of Δ9-desaturase were not statistically significant, there was an important decrease in both their products (i.e., *c*9-14:1 and *c*9*t*11-18:2) and the desaturase indexes studied (see Table 1). However, the values observed at the pre-treatment sampling were not always recovered 5 days post-treatment. According to our results, a minimum estimate of 75% of *c*9*t*11-18:2 in milk fat was produced endogenously.

Table 1 Concentration of 14:0, *c*9-14:1, *t*11-18:1 and *c*9*t*11-18:2, and desaturase indexes of milk fat in dairy ewes before, during and after sterculic acid administration

		pre-treatment	treatment	post-treatment	SED	P
FA, g/100 g total FA	14:0	9.85	10.71	9.97	0.321	NS
	*c*9-14:1	0.14[a]	0.04[c]	0.09[b]	0.011	<0.05
	*t*11-18:1	2.73	3.22	2.62	0.562	NS
	*c*9*t*11-18:2	1.29[a]	0.60[b]	0.94[ab]	0.186	<0.05
Desaturase indexes	*c*9-14:1/(14:0+ *c*9-14:1)	0.014[a]	0.004[c]	0.009[b]	0.001	<0.05
	*c*9*t*11-18:2/(*t*11-18:1+ *c*9*t*11-18:2)	0.64[a]	0.38[c]	0.60[b]	0.022	<0.05

[a,b,c] Different superscripts within a row indicate significant differences (P<0.05). NS = non-significant

Conclusions Sterculic acid administration inhibited Δ9-desaturase in the mammary gland of dairy ewes with a strong effect that persisted partially over time. Endogenous synthesis was confirmed as the major source of *c*9*t*11-18:2 in ovine milk, accounting for a minimum of 75% of its content in the milk fat of dairy ewes.

Acknowledgements This work was supported by Project AGL2008-04805 (MICINN). P.G. Toral and E. Bichi were granted fellowships from the CSIC (I3P and JAE Programmes, respectively).

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

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Effect of high sugar grasses on methane emissions simulated using a dynamic model

J L Ellis[1,2], A Bannink[6], J France[1], A J Parsons[3], G R Edwards[4], S Rasmussen[3], E Kebreab[5], J Dijkstra[1]
[1]Centre for Nutrition Modelling, Dept Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada, [2]Animal Nutrition Group, Wageningen University, Wageningen, The Netherlands, [3]AgResearch, Palmerston North, New Zealand, [4]Lincoln University, Lincoln, New Zealand, [5]Dept of Animal Science, University of California, Davis, California, USA, [6]Wageningen UR Livestock Research, Lelystad, The Netherlands
Email: jennifer.st-pierre@wur.nl

Introduction High sugar grass varieties have received considerable attention for their potential ability to reduce nitrogen (N) excretion and increase milk yield in cattle, though responses in the literature have been variable and at times conflicting (Edwards *et al*., 2007). In an attempt to elucidate the patterns in the literature data, Ellis *et al*. (2011) conducted a controlled modelling exercise where grass composition was systematically varied from a basal composition and N and milk yield results were examined. Simulation results showed that the N utilization ratio increased as the water soluble carbohydrate (WSC) content of the diet increased, but to varying degrees depending on the grass composition. The most benefit in terms of N utilization ratio and urine N level was seen when the WSC content of grass increased at the expense of CP, followed by 50:50 CP and NDF, followed by NDF. As well, simulated milk yield decreased slightly as WSC increased at the expense of CP, increased slightly as it increased at the expense of both CP and NDF, and increased most at the expense of NDF. Results were amplified slightly under conditions of low N fertilization and in the absence of grain feeding. To date, however, no *in vivo* studies have examined this strategy with respect to another environmental pollutant, methane (CH_4). Since feeding high WSC grass alters rumen fermentation, it is possible that this feeding strategy has longer reaching impacts in terms of the environment. Thus, the purpose of the present study was to use a modelling approach to evaluate the potential effect of high sugar grasses on simulated CH_4 emissions in dairy cattle.

Materials and methods An extant dynamic, mechanistic model of enteric fermentation and intestinal digestion was used for this evaluation, as in Ellis *et al*. (2011). A simulation database was constructed and analysis of model behaviour was undertaken to simulate the effect of 1.) the level of WSC increase in dietary DM, 2.) change in CP and NDF content of the plant with an increased WSC content, 3.) level of N fertilization and 4.) presence or absence of grain feeding on CH_4 emission levels. A literature database was also constructed, consisting of 4 published studies with 28 treatments that evaluated high sugar grasses for their effect on N excretion (no CH_4 was reported). Water soluble carbohydrate content of the diet (grass + grain) ranged from 95 to 248 g/kg DM, CP content ranged from 115 to 263 g/kg DM, and the NDF content ranged from 400 to 568 g/kg DM.

Results Simulated CH_4 emissions tended to increase with increased WSC content when CH_4 was expressed as MJ/d or % of gross energy intake (GEI), but when CH_4 was expressed in terms of g/kg milk yield, results were much more variable due to the potential increase in milk yield. As a result, under certain conditions, namely if WSC increased at the expense of NDF, CH_4 (g/kg milk) decreased. The largest increases in CH_4 (MJ/d or % GEI) emissions were generally seen as WSC increased at the expense of CP in the diet and this can largely be explained by diet digestibility and volatile fatty acid stoichiometry. Effects were lower when WSC increased at the expense of NDF, and intermediary when WSC increased at the expense of a mixture of CP and NDF. A plot of simulated CH_4 (%GEI) versus urine N (% of N intake) is presented in Figure 1, and simulated CH_4 (g/kg milk) versus the N utilization ratio is presented in Figure 2 where the data represents simulated high WSC grass scenarios.

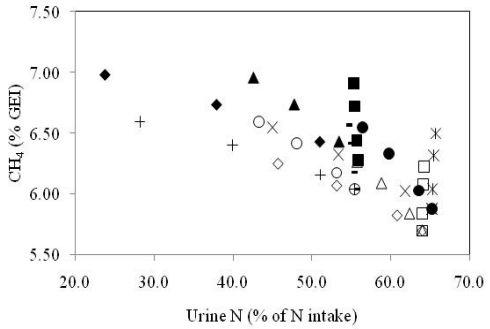

Figure 1 Simulated CH_4 expressed as % GEI versus simulated urine N (% of N intake), where symbols *represent different sugar grass composition scenarios examined.*

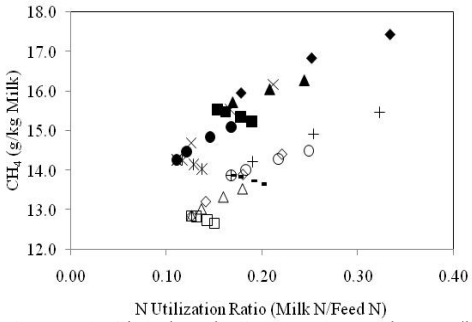

Figure 2 Simulated CH_4 expressed as g/kg milk versus simulated N utilization ratio, where symbols represent different sugar grass composition scenarios examined.

Conclusions Simulation results suggest that high WSC grass as a strategy to mitigate N emission may increase CH_4 emissions, but similar to the variability in N excretion results, CH_4 production levels will depend on the composition of grasses being considered. Overall, this project demonstrates the usefulness of modelling for hypothesis testing in the absence of observed experimental results.

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press

Influence of pasture feeding of dairy cows on the fatty acid composition of protected designation of origin Asiago d'Allevo cheese

M Renna, P Cornale, C Lussiana, A Mimosi, L M Battaglini
Dipartimento di Scienze Zootecniche, University of Torino, Grugliasco (TO), Italy
Email: manuela.renna@unito.it

Introduction Pasture feeding has been shown to consistently ameliorate the fatty acid profile of milk and dairy products from ruminants, by enhancing the content of specific fatty acids able to exert beneficial biological effects on human health (e.g., conjugated linoleic acids, CLA) (Mele 2009). The aim of this study was to investigate the seasonal variations of the fatty acid composition of Asiago d'Allevo, a semi-hard cheese manufactured in North-Eastern Italy from locally produced cows' partially skimmed raw milk and protected with designation of origin (PDO).

Materials and methods Twenty-five Asiago d'Allevo cheese samples manufactured between June 2007 and February 2008 in the alpine mountain territory of the 'Altopiano dei Sette Comuni' (Veneto Region, Italy) were directly collected from thirteen mountain dairies (1,000 to 1,700 m a.s.l.) after four months of ripening. The experimental period covered the variations of the cows' dietary regimen, characterized by pasture and concentrates in summer, and by hay and concentrates during the autumn/winter stabled months. Cheese samples were analysed for their fatty acid composition according to Chistopherson and Glass (1969) by using a gas chromatograph (GC Shimadzu 17A) equipped with a CP-Sil 88 capillary column. Data were submitted to an independent sample Student's t-test considering the milk production period (summer *vs.* autumn/winter) as main effect, and to principal component analysis.

Results If compared to the autumn/winter samples, the summer ones showed lower levels of lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids, which are well known to exert a hypercholesterolaemic effect in humans ($P<0.001$; Table 1). Oleic (C18:1c9), trans-vaccenic (C18:1t11, TVA), and rumenic (C18:2c9t11, CLA) acids were significantly higher in the summer samples ($P<0.001$). These three fatty acids are beneficial since they provide protection against carcinogenesis, diabetes, arteriosclerosis and other inflammatory diseases (Parodi 2009). The favourable lipidic composition observed in the summer samples has to be related to the higher intakes by the dairy cows of α-linolenic acid (C18:3c9c12c15, ALA) during the grazing season. In fact, during rumen biohydrogenation ALA is converted to TVA, which in turn is used by Δ9-desaturase as precursor for CLA synthesis within the mammary gland. The results of fatty acid composition were used for subsequent Principal Component Analysis in order to look at relationships in the dataset. In the PCA bi-plot good separation between summer and autumn/winter samples was achieved (Figure 1). The two principal components explained about 89% of variance. The bi-plot shows up the relevance of trans-vaccenic acid, rumenic acid, oleic acid, and hypercholesterolemic saturated fatty acids, in the definition of the principal components.

Table 1 Effect of period of milk production on selected fatty acids (g $100g^{-1}$ methyl esters) in Asiago d'Allevo PDO cheese

	Summer	Autumn/Winter	SEM	P
C12:0	2.43	3.12	0.08	<0.001
C14:0	9.33	10.77	0.19	<0.001
C16:0	25.84	29.61	0.43	<0.001
C18:1c9	24.47	21.49	0.47	<0.001
C18:1t11 (TVA)	4.49	2.34	0.24	<0.001
C18:2c9t11 (CLA)	1.35	0.69	0.08	<0.001
SFA	61.17	67.25	0.65	<0.001
MUFA	35.00	29.77	0.60	<0.001
PUFA	3.84	2.98	0.13	<0.001
AI	1.60	1.90	0.03	<0.001

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyuns
acids; AI, atherogenicity index

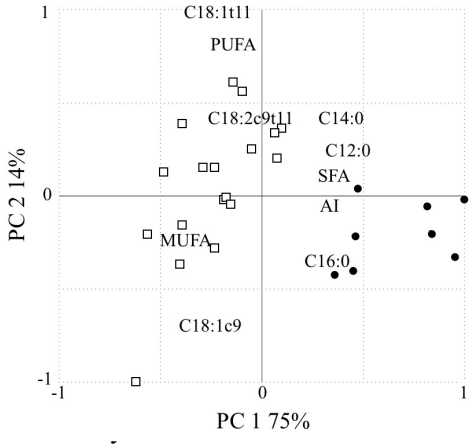

Figure 1 PCA bi-plot: summer cheeses (□) and autumn/winter cheeses (●)

Conclusions Fresh-grass feeding in alpine areas determines improvements of the nutraceutical properties of milk and derived dairy products. Moreover, it allows the reduction of costs for animal feeding and can contribute to the mountain farmers' income through a specific and targeted valorisation of the products (e.g., 'Product of the Mountains' labelling).

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https://doi.org/10.1017/S2040470011002809 Published online by Cambridge University Press