An evaluation of the effect of greenhouse gas accounting methods on a marginal abatement cost curve for Irish agricultural greenhouse gas emissions

D O'Brien[1], L Shalloo[1], P Crosson[2], T Donnellan[3], N Farrelly[3], J Finnan[4], K Hanrahan[3], S Lalor[5], G Lanigan[5], F Thorne[6], R Schulte[5]
[1]Teagasc, Moorepark, Fermoy, Co. Cork, Ireland, [2]Teagasc, Grange, Dunsany, Co. Meath, Ireland, [3]Teagasc, Athenry, Co. Galway, Ireland, [4]Teagasc, Oak Park, Co. Carlow, Ireland, [5]Teagasc, Johnstown Castle, Co. Wexford, Ireland, [6]Teagasc, Ashtown, Dublin 15, Ireland *Email:donal.o'brien@teagasc.ie*

Introduction Marginal abatement cost curve (MACC) analysis is a tool that can be used to assess opportunities to reduce agricultural greenhouse gas (GHG) emissions relative to some baseline scenario e.g. "business as usual" scenario and their associated costs or benefits. Generally, MACC analysis follows the Intergovernmental Panel on Climate Change (IPCC) methodology to estimate the abatement potential of mitigation measures. There is however an alternative to the IPCC method, known as life cycle analysis (LCA), which is the preferred method to assess the GHG intensity of food (kg of GHG per unit of food). The purpose of this study was to compare the effect of using the IPCC and LCA methods when completing a MACC analysis of Irish agricultural GHG emissions.

Material and methods Donnellan and Hanrahan (2012) previously projected the impact of realising the Irish agricultural industries 2020 production and value targets (Food Harvest 2020) on GHG emissions, by comparing a 'business as usual' baseline to a 'Food Harvest 2020' scenario. For this study, the 'Food Harvest 2020' scenario as outlined by Donnellan and Hanrahan (2012) was adopted as the reference scenario in the MACC analysis. Mitigation measures were then selected, following a scoping and screening exercise. The criteria used to screen measures was: 1) mitigation measures must be applicable to typical Irish farming systems, 2) scientific data, from completed research, must be available on the relative cost/benefit of each mitigation measure as well as their relative abatement potential, 3) for each measure, activity data must be available to assess the total national abatement potential and associated cost/benefit. The result of the screening phase was a list of 10 mitigation measures suitable for Irish agriculture. The abatement potential of mitigation measures was estimated using the IPCC and LCA methods. The IPCC method was employed to assess the effect mitigation measures have on national and sectoral GHG emissions. The sources of GHG attributed to the agriculture sector using the IPCC method are enteric fermentation, manure management and agricultural soils. The LCA method was applied to quantify GHG emissions from all on and off-farm sources associated with agriculture up to the farm gate. Thus, the approach includes upstream emissions from the manufacture of inputs e.g. fertiliser even if they occur outside national boundaries. For this analysis, downstream emissions from the processing and distribution of agriculture produce were not included, because few if any Irish studies presently exist that consider the life cycle impacts of GHG mitigation strategies for agricultural produce up to the retail stage. The abatement potential of mitigation measures was only constrained by the biophysical environment. The rate of adoption of mitigation measures by farmers was not constrained, because it depends on multiple factors in addition to the economic cost/benefit of a mitigation measure, which are difficult to quantify. Most of the mitigation measures assessed, when applied in combination can interact. Consequently, the abatement potential of a group of mitigation measures may not be additive. Where possible, interactions between measures were accounted for.

Results Table 1 shows that the total abatement potential that can be attributed to Irish agriculture using the IPCC method amounts to about 1.1 Mt of CO_2 equivalents per annum by 2020, compared to the Food Harvest 2020 reference scenario. Almost all of this abatement potential can be achieved without financial cost by adopting measures that reduce the ratio of inputs to agricultural produce (improve productive efficiency). The LCA results show that the total abatement potential was approximately 3.4 Mt of CO_2eq, 73% of which could be achieved without financial cost**.** The large difference between methods was because the IPCC method attributes partially or fully the abatement from some mitigation measures to sectors or regions other than the Irish agriculture sector.

Table 1 The annual abatement potential in Mt of CO_2eq of cost effective (cost is less than the forecasted price of carbon), and cost prohibitive mitigation measures of Irish agriculture compared to the Food Harvest 2020 reference scenario.

Measure	IPCC method		LCA	
	Annual Abatement	Attributed to Irish Agriculture	Annual Abatement	Attributed to Irish Agriculture
Cost effective	2.49	1.10	2.81	2.81
Cost prohibitive	0.18	0.02	0.62	0.62

Conclusions The comparison between GHG accounting methods showed that the IPCC and LCA methods did not agree on the abatement potential of several of the mitigation measures evaluated. This finding has important implications as demonstrates that it will be difficult to incentivise farmers to adopt mitigation measures where the abatement may not be attributed to the Irish agricultural sector.

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Reduction of GHG emissions by reduced livestock production resulting from dietary changes in the EU

J P Lesschen[1], H J Westhoek[2], S Wagner[1], T Rood[2]
[1]Alterra, Wageningen UR, Wageningen, The Netherlands, [2]PBL Netherlands Environmental Assessment Agency, Bilthoven, The Netherlands *Email:janpeter.lesschen@wur.nl*

Introduction The contribution of livestock production in the European Union (EU) to GHG emissions is well established (Lesschen *et al*., 2011). Although various technical options are available to reduce GHG from livestock production, the effect of these options have their limits. Another pathway to reduce GHG emissions and other environmental effects is through dietary changes. Current EU diets are relatively rich in animal protein compared to other regions and to WHO recommendations (Westhoek *et al.*, 2011). GHG emissions might be significantly reduced when animal protein are replaced by plant-based alternatives. However, given the complex interactions within the agricultural system, the effects of such a drastic change cannot be properly quantified by upscaling the GHG emissions per unit of produce from LCA analyses (Stehfest *et al.*, 2013). We used a more integrated approach to assess the consequences for the environment if EU consumers were to replace part of the meat, eggs and dairy products with plant-based foods.

Material and methods We developed six alternative diets for the EU27, in which the consumption of meat, dairy and eggs is lowered by 25% or 50% compared to 2004 as the reference situation. We compensated for the reduced consumption of livestock products by increasing cereal consumption. We assumed that the reduction in consumption of livestock products is followed by a parallel reduction of livestock production in the EU. Consequently, the effects on the use of imported and domestic feed (including roughage from grasslands) were estimated. The reduction in feed use is expected to have consequences for European land use, freeing up land for other uses. Two contrasting land use change scenarios were therefore examined; i) a high-prices scenario with maximum cereal production, and ii) a greening scenario with extensification of grassland use and production of bio-energy on excess arable land. The environmental effects were assessed using MITERRA-Europe (Lesschen *et al.*, 2011), a deterministic model for the EU territory, which calculates emissions of reactive nitrogen and GHGs on an annual basis, using emission factors.

Results When reducing meat and dairy consumption in the EU by 50%, which would still direct GHG emissions form the EU agricultural sector will be reduced by 25-43%, depending on the consequences for land use (Figure 1). In this scenario the total food intake of protein would reduce by 10% and for saturated fats by 40%. In the high-prices scenario the CO_2 emissions increase due to the conversion of excess grassland to arable land for cereal production, whereas in the greening scenario CO_2 is sequestered by perennial bioenergy crops. The emission reduction in the whole food system might be higher, as less inputs (e.g. mineral fertilizer) and processing energy are needed. Depending on the land use scenario, more bio-energy crops may be grown as well. In addition, significant GHG emission reduction might occur outside the EU, as the EU imports of soybean and beef is reduced, while the exports of cereals increases, leading to lower crop production and land use conversion rates outside the EU.

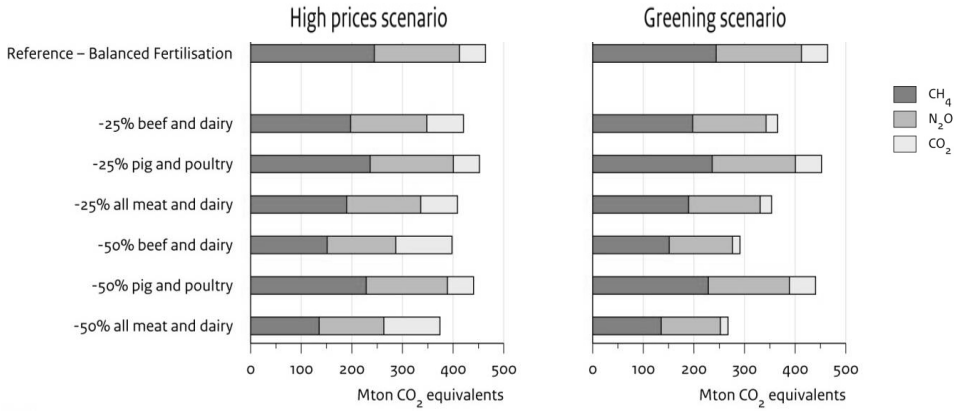

Figure 1 Effect of alternative diets and land use scenarios on direct GHG from EU agriculture.

Conclusions Our study shows that a 50% reduction in the livestock component of EU diets, with corresponding changes in agriculture, would have substantial environmental and health benefits. The calculated impact on GHG emissions is generally larger than the estimated mitigation potentials from technical mitigation measures. While further, more detailed, analysis is warranted, our study has made it clear that food choices matter, both for our health and our environment.

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A methylotrophic methanogen isolate from the Thermoplasmatales affiliated RCC clade may provide insight into the role of this group in the rumen

J Padmanabha, J Liu, C Kurekci, S E Denman, C S McSweeney
CSIRO Animal, Food and Health Sciences, Brisbane, Queensland, Australia *Email:chris.mcsweeney@csiro.au*

Introduction. Methane is an important atmospheric greenhouse gas. Around 500-600 Tg of CH_4/yr is emitted globally. Of this, about 74% is biologically generated almost exclusively by methanogens that belong to the phylum *Euryarchaeota* and grouped under six orders. Ruminant methanogens generate 13-19% of global methane output which is the largest of anthropogenic emissions. Among the rumen methanogens, Rumen Cluster 'C' (RCC), an unclassified and uncultured archaeal clade, represents 15.8-80.9% of rumen archaea (Janssen and Kirs, 2008), which has the potential to contribute significantly to ruminant derived methane. Phylogenetically, RCC distantly affiliate to a non-methanogenic order - *Thermoplasmatales*. Analysis of 16S rDNA identified members of RCC in diverse habitats including digestive tracts of insects, birds, cattle, pigs, camels, macropod marsupials and man. However, their position as methanogens was unresolved because of a lack of pure cultures, despite methane producing, non-axenic archaeal cultures being enriched from these sites. Here, we report the isolation and characterisation of the first methanogen from the RCC clade, strain DOK-1 in pure culture, that in turn could help understand the role of this large group in the rumen. We propose the nomenclature *Methanoplasma gallocaecorum* strain DOK-1, for this novel methanogen.

Material and methods Caecal contents from broiler chickens were anaerobically pooled and inoculated into 10 ml Balch tubes containing anaerobic BRN enrichment media (Rea *et al.*, 2007). Methylotrophic methanogens were enriched by adding methanol, mono-, di- and trimethylamine HCl. Cultures were grown at 39^{o}C under 150 kPa of 80:20 H_2:CO_2 and monitored for CH_4 production by GC and sub-cultured every 3-5 days for several transfers. Cultures of a 10-fold serial dilution with CH_4 production at the highest dilution were further purified by antibiotics. Methanogens were also monitored by phase-contrast microscopy and a qPCR using *mcr*A and total bacterial primers. Finally, a persistent bi-culture consisting of a methanogen and a bacterium was obtained. Isolation was achieved by inoculation of serial dilutions of cultures into agarose containing solid media. Several, single colonies were picked and cultured in liquid media to determine purity. Genomic DNA from these cultures were amplified using archaeal 16S primers 86F-1340R; direct sequencing of PCR products confirmed sequence alignment with *Thermoplasmatales* affiliated novel methanogens. Nutritional and physiological requirements and morphologic features of the isolate were studied; cells in late exponential phase were freeze-dried, fixed and analysed by transmission electron microscopy.

Results and Conclusion Cells did not fluoresce under UV and appeared round (cocci) by both phase contrast and TEM microscopy measuring ~650-1200 nm in diameter. They are non-motile and non-flagellated with a fragile proteinaceous cell wall with no detectable S-layer. EM showed a delicate cell wall, a simple plasma membrane and a nondescript cytoplasm of moderate electron density (Fig 1). DOK-1 is a methylotrophic methanogen with a strict requirement of H_2 for growth. They cannot grow autotrophically on H_2:CO_2 alone and require rumen fluid and yeast extract. DOK-1 stoichiometrically converted methanol, mono-, di- and trimethylamine to CH_4 with equimolar H_2 consumption (Table 1). Ammonia was produced when methylamines were used as substrates [$(CH_3)_3$-N + $3H_2$ → $3CH_4$ + NH_3]. It does not utilize formate, acetate, betaine, choline, pyruvate, spermidine, butanol, propanol, vanillate or nitrite and lacks the disproportionating pathway of *Methanosarcinales*. Growth is completely inhibited by 2-bromoethanesulfonic acid, only partially by mevinolin and not at all by lumazine. It is insensitive to several antibiotics like penicillin, ampicillin, kanamycin, vancomycin, monensin and cefotaxime. DOK-1 is phylogenetically (16S rRNA) closely related to similar methylotrophic non-axenic methane producing archaea from the rumen/fore-stomach of cattle, camel, kangaroo and wallaby, all of which cluster distantly to the *Thermoplasmatales*. They belong to a new seventh order of methanogens, 'Methanoplasmatales', proposed by Paul *et al.* (2012). We have recently sequenced the entire genome of DOK-1 and its analysis is underway.

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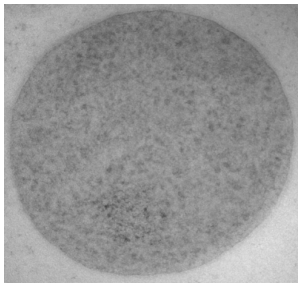

Fig 1. EM of strain DOK-1 (~ 650 nm)

Table 1 Methane production by strain DOK-1 on TMA and Hydrogen

Time (h)	CH_4 produced-µmol (s.e.)	H_2 utilized-µmol (s.e.)	OD_{600} (s.e.)
0	6	0	0.018
12	56 (8.2)	36 (10.9)	0.032 (0.001)
24	80 (6.9)	82 (1.4)	0.061 (0.004)
36	98 (24.8)	123 (18.7)	0.144 (0.014)
48	246 (20.9)	168 (24.9)	0.230 (0.021)
72	501 (51.7)	362 (46.3)	0.482 (0.032)
96	794 (33.9)	645 (45.1)	0.653 (0.027)
120	998 (16.5)	899 (15.8)	0.719 (0.021)

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Effects of feeding 3-nitrooxypropanol on methane emissions and productivity of lactating dairy cows

J Haisan[1], Y Sun[1], K Beauchemin[2], L Guan[1], S Duval[3], D R Barreda[1], M Oba[1]
[1]University of Alberta, Edmonton, Alberta, Canada, [2]Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada, [3]DSM Nutritional Products, Ltd, Kaiseraugst, Switzerland *Email:haisan@ualberta.ca*

Introduction Ruminants lose 2-12% of their ingested energy as enteric methane (CH_4) thus reducing animal efficiency as well as contributing to global greenhouse gasses. As such, finding methods to reduce enteric CH_4 production, without detrimental effects on animal performance, is of great importance. It has been found that 3-nitrooxypropanol (NOP) can reduce CH_4 production from the rumen. The objective of this study was to evaluate the effects of feeding NOP on ruminal CH_4 production, and productivity of lactating dairy cows.

Materials and methods Twelve ruminally-cannulated lactating Holstein cows (6 multiparous and 6 primiparous) were used in a crossover design study with 28-d periods. Cows were fed either the NOP compound (10% 3-nitrooxypropanol on SiO_2, DSM Nutritional Products Ltd., Switzerland) or SiO_2 (CON) at 25 g/d. The NOP compound or SiO_2 was first mixed with 80 g ground barley grain, 50 g molasses and 45 g canola oil to improve its adhesion to feed particles and palatability, and then hand-mixed into the ration immediately after feeding to allow animals to consume treatment mixtures continuously throughout the day. All cows were fed a 38%-forage diet that was formulated to meet nutrient requirements for a 650-kg cow producing 40 kg milk per day (NRC, 2001). After a 21-d adaption period, dry matter intake (DMI) and milk yield were recorded daily (d 22-28). Rumen fluid was collected on d 22 and 28 to determine volatile fatty acid profile. Enteric CH_4 production was measured for 5 d (d 23-27) using the sulfur hexafluoride tracer gas technique (Johnson *et al*., 1994). Data were analyzed by the fit model procedure of JMP (version 10, SAS Institute, Inc., Cary, NC) using the model with fixed effects of period and treatment, and random effect of cow.

Results Cows fed the NOP compound decreased CH_4 production (7.5 vs. 18.8 g/kg DMI), increased body weight gain (1.07 vs. 0.30 kg/d) and tended to increase feed efficiency (1.79 vs. 1.60) compared to CON cows. When feeding the NOP compound, there were tendencies towards decreased acetate concentration (52.1 vs. 55.5 mol/100 mol) and increased propionate concentration (26.4 vs. 23.9 mol/100 mol) as well as a decreased acetate-to-propionate ratio (2.02 vs. 2.35) when compared to CON cows.

Table 1 Effect of feeding the NOP compound on productivity of lactating dairy cows and ruminal volatile fatty acid profile.

	CON	NOP	s.e.	P
Dry matter intake (DMI), kg/d	19.7	19.3	0.31	0.35
CH_4, g/d	371	146	15.7	< 0.0001
CH_4/DMI, g/kg	18.8	7.5	0.75	< 0.0001
4% fat corrected milk (FCM) yield, kg/d	31.5	34.1	1.45	0.23
Milk fat, g/kg	33.1	39.6	2.62	0.11
Milk crude protein, g/kg	31.3	31.1	0.18	0.73
Milk lactose, g/kg	46.5	46.5	0.18	0.96
Body weight change, kg/d	0.30	1.07	0.214	0.03
Feed efficiency, 4% FCM/DMI	1.60	1.79	0.069	0.08
Volatile fatty acids				
Total, mM	103.2	109.2	5.18	0.39
Acetate, mol/100mol	55.5	52.1	1.11	0.10
Propionate, mol/100mol	23.9	26.4	0.84	0.06
Butyrate, mol/100mol	14.3	14.8	0.87	0.59
Acetate-to-propionate ratio	2.35	2.02	0.107	0.08
Total bacteria, $\times 10^{10}$/g	34.4	20.5	5.86	0.12
Methanogen, $\times 10^{8}$/g	2.43	1.29	0.282	0.02

Conclusion Feeding the NOP compound significantly reduced CH_4 emissions and ruminal methanogen counts of dairy cows, and increased body weight gain without negatively affecting milk production.

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Effects of incremental rumen doses of 3-nitrooxypropanol on methane production, digestion, and rumen parameters in lactating dairy cows

C K Reynolds[1], D J Humphries[1], P Kirton[1], M Kindermann[2], S Duval[3], W Steinberg[2]
[1]University of Reading, Reading, UK, [2]DSM Nutritional Products, Basel, Switzerland, [3]DSM Nutritional Products, Saint-Louis Cedex, France *Email:c.k.reynolds@reading.ac.uk*

Introduction There is currently a massive global research effort exploring potential nutritional, genetic, and management options for reducing greenhouse gas emissions from ruminants, focusing primarily on methane. Earlier work showed significant inhibitory effects of chloral hydrates on methane production both *in vitro* and *in vivo* (Trei *et al*., 1972), and numerous bioactive compounds have shown promise as potential inhibitors of rumen methane production based on studies *in vitro* or in sheep (McAllister and Newbold, 2008). More recently 3-nitrooxypropanol (3NP) reduced methane emission of sheep when dosed into the rumen for 30 days (Martinez-Fernandez *et al*., 2013). However, supplements effective at reducing methane production in sheep have in some cases been ineffective in lactating dairy cows (e.g. Foley *et al*., 2008). The objective of the present study was to determine the incremental effects of 2 doses of 3NP on methane emissions of lactating dairy cows.

Method Six rumen cannulated mid-lactation Holstein-Friesian dairy cows were fed twice daily for *ad libitum* dry matter (DM) intake (DMI) a total mixed ration with maize silage as the primary (430 g/kg DM) forage source. Cows received each of one of 3 treatments using an experimental design based on two 3x3 Latin Squares with 5 week periods. Treatments were a control or 500 or 2500 mg/d of 3NP into the rumen in two equal doses before feeding. Measurements of methane production and energy and nitrogen balance were obtained during the last week of each period using respiration calorimetry and digestion trials. Rumen volatile fatty acid (VFA) and ammonia concentrations were measured at the end of the 4th week of each period. Data were analyzed using Mixed models procedures of SAS® and a model testing fixed effects of square and treatment (3NP level), random effects of period and cow, and 3NP by period interaction, along with Dunnett's comparisons of treatment and control means (0 *vs* 500 and 0 *vs* 2500 mg/d).

Results Methane production and yield (CH_4/kg DMI) were reduced by both doses of 3NP (Table 1). It was notable that when added to the rumen before the afternoon feeding there was a pronounced, transitory (2-3 h) effect on methane concentration in exhaust air (Figure 1 shows example 4 day averages for 2 animals), whilst dosing the product before the morning feed delayed the postprandial increase in methane production observed for the control treatment. Fat-corrected (4%) milk yield (FCM) was not affected, but digestibility of dry matter (Table 1), N, OM, and ADF (data not shown) tended to be reduced (P < 0.08) by the higher dose. There was no effect on rumen ammonia concentration, but acetate concentration and thus acetate to propionate ratio (C2:C3) was reduced.

	Daily 3NP dose, mg/d				P <		
	0	500	2500	s.e.m.	3NP	500	2500
DMI, kg/d	18.9	18.8	18.5	0.7	0.74	0.83	0.47
CH_4, L/d	594	555	536	15.3	0.02	0.03	0.01
CH_4, L/kg DMI	31.3	29.9	29.2	1.2	0.01	0.03	0.01
FCM, kg/d	28.5	27.1	26.4	2.7	0.43	0.41	0.22
DM Digested, kg/d	14.24	13.79	13.48	0.516	0.34	0.39	0.16
DM Digested, g/g	0.753	0.736	0.726	0.009	0.18	0.23	0.08
C2:C3, mM	2.93	2.60	2.74	0.05	0.01	0.01	0.01

Table 1 Effects of 3NP on DMI, methane production, milk yield and rumen C2:C3.

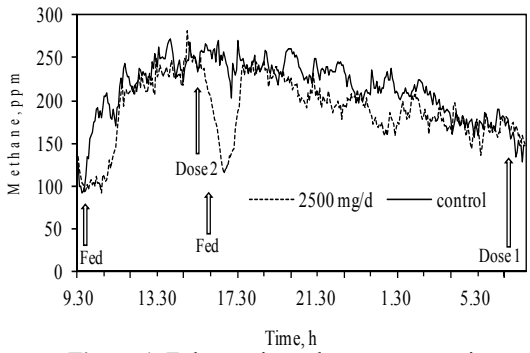

Figure 1 Exhaust air methane concentration.

Conclusions As observed in sheep, after 4 weeks of treatment 3NP added to the rumen reduced methane production of lactating dairy cows (to 93 and 90 % of control for 500 and 2500 mg/d, respectively). It was notable that a 5-fold increase in dose did not have a 5-fold greater effect. Changes in rumen VFA concentration suggest a shift in microbial metabolism, which was associated with a decrease in feed DM digestibility. The pronounced but transitory effect of 3NP on methane production suggests that although highly effective at inhibiting methane production, 3NP may be absorbed, metabolized, or pass out of the rumen after dosing and thus a continuous infusion or feeding the product could be more effective at reducing methane production.

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Fungal secondary metabolites from Monascus spp. reduce methane production by affecting rumen methanogenic Archaea diversity in wethers

M Popova[1], N Parisot[0,2], J Denonfoux[0,2], D Graviou[1], Y Rochette[1], E Dugat-Bony[0,2], E Peyretaillade[0,2], D Boucher[0,2], D Morgavi[1], P Peyret[0,2]
[1]INRA, Theix, France, [2]EA 4678, Conception, Ingénierie et Développement de l'Aliment et du Médicament, Clermont Université, Université d'Auvergne, Clermont-Ferrand, France *Email:milka.popova@clermont.inra.fr*

Introduction In a previous study metabolites produced by *Monascus* spp. appear to have an inhibitory effect upon methanogens and decreased methanogenesis *in vitro* and in short-term *in vivo* trial without any apparent negative effect on rumen fermentation (Morgavi *et al*., 2013). The aim of this study was to assess changes in methanogens diversity in order to better understand the mode of action of these secondary metabolites in the rumen.

Material and methods Four adult Texel wethers fitted with rumen cannulae were used in this study. Wethers were fed 1.2 kg of a hay:wheat grain diet 1:1 on a DM basis during the control period. During treatment 0.4 kg of wheat was replaced by an equal amount of *Monascus*-fermented wheat for 12 d. Methane production was measured using the SF_6 tracer technique. Rumen contents were sampled at the end of the control period (n=4) and at the end of the treatment period (n=4). Total DNA was extracted as described in Morgavi *et al.,* 2013 and pyrosequencing was performed targeting the *mcrA* gene (molecular marker of methanogens) to assess the diversity of methanogens. Sequences were imported and analysed using the quantitative insights into microbial ecology (QIIME) pipeline. Sequences were trimmed with the following parameters and any sequence not matching these criteria was excluded from downstream analysis: minimum quality score of 25, minimum sequence length of 200 bp, maximum ambiguous bases in the sequence of 6, no mismatches in the primer sequence. Sequences were grouped in OTUs with an identity threshold of > 87% (Popova *et al*., 2013). Sequences were aligned and clustered using QIIME default parameters. Representative sequences for each OTU were selected and taxonomic identity was assigned to each sequence using a homemade *mcrA* taxonomic classifier at 90% confidence. A *mcrA* database was constructed by extracting approx. 12500 sequences identified as *mcrA* from Genbank. QIIME was used to compute the between community diversity for each of the eight microbial communities and generate Principal Coordinate Analysis (PCoA) plots representing the relationships among the microbial communities.

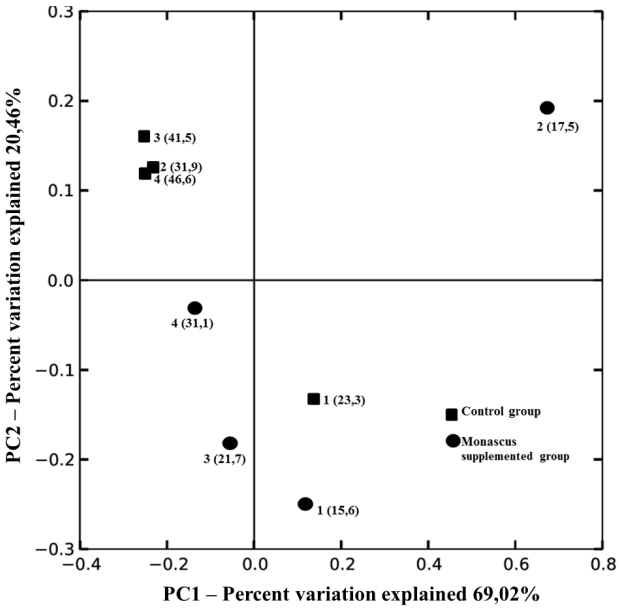

Figure 1 Principal coordinate analysis of weighted UniFrac distances from *mcrA* gene barcoded pyrosequecing. Numbers identify the sampled animal; numbers between brackets give individual methane production (g / day).

Results and discussion Wethers fed the *Monascus* supplemented diet produced 40 % less methane ($P < 0.05$) (expressed as g /day) compared to wethers from the control group. Pyrosequencing provided a total of 25 539 high quality sequences with mean distribution of 3 192 (±1 012) sequences per sample. A total of 78 OTUs were constructed. All eight samples were dominated by a non-identified group of methanogens. In all four animals wethers the proportion of *Methanobacteriales* decreased after supplementation of the diet with Monascus secondary metabolites (data not shown). Results of PCoA are shown in Figure 1. Three of the four wethers grouped tightly together during the control period - the wether that separated from others based on the methanogens diversity (animal n°1) had the lowest methane production during this period. In contrast, when fed Monascus-fermented wheat there was a greater spatial separation among wethers indicating a different evolution of the methanogenic community.

Conclusion These results suggest that *Monascus* secondary metabolites influenced methanogens diversity in the rumen of wethers. Further studies should help to precise the relationship between the amount of methane produced and methanogens diversity.

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Manure management technologies and mitigation of greenhouse gases: opportunities & limitations

J W De Vries, I J M De Boer
Animal Production Systems Group, Wageningen University, Wageningen, The Netherlands *Email:jerke.devries@wur.nl*

Introduction Manure management (i.e. storage, processing, transport and field application) contributes up to roughly 17% of EU agricultural greenhouse gas (GHG) emissions (EEA, 2012), but also to emissions of nitrogenous compounds, including NH_3 and NO_3^- leading to acidification and eutrophication. Manure processing technologies were developed to reduce environmental impact of manure management, including GHG emissions, and to produce valuable by-products, such as bio-energy. Such technologies include: liquid and solid separation, filtration, drying and composting, and anaerobic digestion. Our aim here was to provide an overview of several manure management technologies and their opportunities and limitations to mitigate greenhouse gas emissions. We highlight trade-offs with other environmental impacts: acidification, eutrophication, particulate matter emission, and fossil fuel depletion.

Material and methods We used life cycle assessment (LCA) to assess the GHG mitigation potential in the whole management chain of three technologies: segregating fattening pig urine and faeces inside the housing system, anaerobic mono- and co-digestion of pig manure, and separation of liquid manure into a solid and liquid fraction with further de-watering of the liquid fraction. We assessed and compared the environmental impact of these technologies to a reference based on conventional liquid manure management. Segregation of fattening pig urine and faeces occurred within the housing system by a V-shaped belt under a slatted floor. As the faeces composition depends on the use of bedding material and, therefore, may become more liquid or solid, we included two scenarios: one with low DM faeces, anaerobically stored, and one with high DM faeces, aerobically stored (De Vries *et al.*, 2013). For anaerobic digestion we compared mono-digestion of pig manure to co-digestion with co-substrates: maize silage, maize silage and glycerine (10%), beet tails, wheat yeast concentrate (WYC), and roadside grass. As these co-substrates compete with other applications on the market, we included the environmental impact of producing a substitute for the initial use of these co-substrates (De Vries *et al.*, 2012a). Finally, separation of liquid manure and de-watering of the liquid fraction included two scenarios: one with only separation and purification and one including anaerobic digestion of the solid fraction (De Vries *et al.*, 2012b).

Results Segregating pig urine and faeces showed a significant reduction in GHGs compared to conventional manure management (up to 82%). Furthermore, segregation reduced acidification and particulate matter formation up to 49% (both significantly in the scenario with low DM faeces), whereas eutrophication increased up to 11% (not significant), as more N was emitted during storage and field application. Anaerobic mono-digestion and co-digestion with roadside grass reduced GHGs (16 and 89 kg CO_2-eq per ton substrate, respectively), whereas co-digestion with maize silage, glycerine, beet tails, and WYC increased GHGs (up to 105 kg CO_2-eq per ton substrate). This increase in GHGs resulted from emissions related to land conversion for producing a substitute for the used co-substrates. Separation and de-watering of liquid fraction increased GHGs with 9% compared to conventional manure management, but also acidification (19%), particulate matter emission (23%) and fossil fuel depletion (33%) as a result of N emissions from storage and processing and increased energy use. Anaerobic digestion again led to reduced GHGs (-117%) and fossil fuel depletion (-59%) compared to conventional manure management.

Conclusions LCA revealed essential for determining opportunities & limitations for mitigating GHGs, as it shows all related impacts and changes in the manure management continuum. Several technologies had opportunity to reduce GHGs, however, were also limited due to shifting of N emissions leading to increased acidification and eutrophication. The most promising manure management technologies for mitigating GHGs were found to be: segregation of urine and faeces and anaerobic mono- or co-digestion with wastes, such as roadside grass.

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Greenhouse gas fluxes from experimental dairy barnyards

J M Powell, B D Duval, P A Vadas
United States Department of Agriculture, Agricultural Research Service, Madison, Wisconsin, USA
Email:b.d.duval@gmail.com

Introduction Dairy production systems are well-established sources of greenhouse gas (GHG) emissions. Dairy cows emit CH_4 directly, and contribute to CO_2, N_2O and NH_3 emissions via manure. The substrate on which manure is deposited is likely a control on GHG flux to the atmosphere, because substrate quality (e.g., C and N content, porosity, water holding capacity) controls microbial activity and microbial communities responsible for GHG production from manure decomposition. In Wisconsin, USA many dairy cows spend considerable time in outside holding areas (Powell *et al*., 2005; Gourley *et al*., 2012). In these areas manure goes uncollected resulting in significant build-up of organic carbon and nitrogen. To examine potential GHG mitigation strategies in components of dairy production systems, we constructed experimental barnyards with different surface substrates, and measured GHG fluxes from those barnyards over a two year period.

Materials and methods We constructed replicated (n = 3) barnyard plots (6.1 m x 6.1 m corrals) with surface (0 to 50cm) substrates comprised of silt loam soil, sand, and shredded bark, at the research farm of the US Dairy Forage Research Center in Prairie du Sac, central Wisconsin, USA. Holstein heifers were put on the plots, 3 to 4 per plot, for approximately 28 days per year (approximately 7 days each during spring, summer, fall and winter). Measurements of CO_2, CH_4, N_2O and NH_3 were made using a portable FTIR multi-component gas analyser (Gasmet, Model DX-4030). Gas measurements were made the day before putting the heifers on the plots, and during the days just after the heifers were moved off the plots. Before starting the gas measurements, all plots were mechanically pumped to remove drained leachate (the barnyards were lined with rubber membrane to capture all leachate).

Results There were no GHG emissions differences among the different surface substrates just prior to introducing cows. However, after cows were in the barnyards for 7 consecutive days, we observed significant increases in CO_2 (1-way ANOVA, $F_{1,77} = 8.66$, $P = 0.004$) and a trend for increased N_2O efflux ($F_{1,77} = 3.27$, $P = 0.07$) from the soil plots. The presence of heifers increased CO_2 emissions from sand ($F_{1,78} = 7.42$, $P < 0.05$). We measured significantly higher emissions of CO_2, ($F_{1,75} = 11.07$, $P = 0.001$) and CH_4, ($F_{1,75} = 5.37$, $P = 0.02$), and trends for higher emissions of N_2O ($F_{1,75} = 2.71$, $P < 0.10$) and NH_3 ($F_{1,75} = 3.33$, $P = 0.07$) from the barnyards that had shredded bark as a surface substrate. Comparing the surface substrates to each other after introducing heifers, there was very high variability in GHG flux, but most striking was the observation of an order of magnitude increase in N_2O flux from bark-covered barnyards compared to either sand or soil.

Conclusion These results highlight that differences in barnyard surface substrate are a significant control on GHG emissions. Using a high carbon-containing and porous medium like shredded bark appears to be a poor GHG mitigation strategy for barnyards on dairy farms. Coupling these emissions data with estimates of run-off losses of C and N, leached nitrate, and microbial community activity will be useful in understanding GHG flux from barnyards and in developing mitigation practices. Furthermore, these data can serve as baseline values for whole-farm GHG estimates, and will be relevant to simulation modelling and life-cycle analysis of cost-effective GHG mitigation strategies for dairy production systems.

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Effect of dietary protein concentration on utilization of dairy manure nitrogen for plant growth, leachate nitrate losses, and ammonia emissions from lysimeters

C Lee[1], G W Feyereisen[2], A N Hristov[1], C J Dell[3], J Kaye[1], D Beegle[1]
[1]Pennsylvania State University, University Park, PA, USA, [2]USDA-ARS-SWMRU, St. Paul, MN, USA, [3]USDA-ARS-PSWMRU, University Park, PA, USA *Email:anh13@psu.edu*

Introduction Animal diet can have a significant impact on manure composition and nutrient losses during storage and following soil application. This lysimeter experiment was designed to investigate the effects of dietary protein concentration on nitrate and ammonia losses from dairy manure applied to soil and manure nitrogen utilization for plant growth. Our hypothesis was that manure from dairy cows fed dietary protein below requirements will result in lower leachate nitrate and ammonia N volatilization from soil and similar plant N uptake compared with manure from cows fed a protein-adequate diet, when manure is applied at similar N application rates. Further, we hypothesized that urinary N would have a greater contribution to leachate nitrate and ammonia N pools, which would be exacerbated by higher dietary protein concentration.

Materials and methods As part of a larger experiment, lactating dairy cows were fed diets with 16.7 or 14.8% crude protein content. Feces and urine were labeled with ^{15}N by ruminal pulse-doses of $^{15}NH_4Cl$. Unlabeled and ^{15}N-labeled feces and urine were used to produce manure for a study with 21 lysimeters. Treatments were: (1) no manure (control); (2) unlabeled feces and urine from cows fed the 16.8% crude protein diet (HighCP); (3) ^{15}N-labeled feces and unlabeled urine from cows fed the 16.8% crude protein diet (High$CP^{15}F$); (4) unlabeled feces and ^{15}N-labeled urine from cows fed the 16.8% crude protein diet (High$CP^{15}U$); (5) unlabeled feces and urine from cows fed the 14.8% crude protein diet (LowCP); (6) ^{15}N-labeled feces and unlabeled urine from cows fed the 14.8% crude protein diet (Low$CP^{15}F$); and (7) unlabeled feces and ^{15}N-labeled urine from cows fed the 14.8% crude protein diet (Low$CP^{15}U$). Lysimeters (61 × 61 × 61 cm) were filled with soil (a Hagerstown silt loam; fine, mixed, mesic Typic Hapludalf) and randomly arranged in a 3-row × 7-column pattern in a greenhouse facility. Air temperature in the greenhouse was maintained at 20 to 26°C throughout the experiment with exhaust fans. Lysimeters were blocked based on location in the greenhouse. Manure application rate corresponded to 277 kg N/ha. Soil ammonia-N emissions and ^{15}N enrichment of ammonia-N were measured from the surface of manure-amended soil of each lysimeter at 3, 8, 23, 28, 54, and 100 h after manure application. Following the ammonia emission measurements, manure was incorporated into the soil and a leaching event was simulated. Leachate was analyzed for nitrate and ^{15}N enrichment of nitrate-N. Spring barley was planted (387 plants/m^2) 7 days after the leaching event and plant samples for analysis of N and ^{15}N-enrichment were randomly collected weekly throughout the experiment. Whole barley plants were harvested at senescence, 86 days after planting. The whole plant and barley kernels were analyzed for total N and ^{15}N-enrichment. Soil samples were collected from each lysimeter at various depths and analyzed for total N and ^{15}N-enrichment. Data were analyzed as a complete randomized block design using the MIXED procedure of SAS (2002-2003; SAS Inst., Inc., Cary, NC).

Results Manure from cows fed the 14.8% crude protein diet (i.e., LowCP manure) had on average 35% lower ($P < 0.001$) N concentration compared with HighCP manure – entirely due to the lower N concentration in LowCP urine. There was no difference in whole-crop barley dry matter and N yields ($P > 0.11$) between Low- and HighCP manures. Manured lysimeters yielded about 41% more ($P < 0.001$) barley dry matter and about 52% more N ($P < 0.05$) than the control. Delta ^{15}N of the soil collected at 0 to 1.5 cm depth was not affected ($P = 0.91$) by protein content of manure, but was about 53 and 29% lower ($P < 0.05$; HighCP and LowCP manures, respectively) for lysimeters treated with manures, in which urine was ^{15}N-labeled than manures with ^{15}N-labeled feces. Amount of nitrate in lysimeter leachates was not different ($P = 0.08$) between manures, but urinary N had a greater contribution to nitrate than fecal N (Table 1). Ammonia-N emission rates were on average about 100% greater ($P < 0.001$) for HighCP vs. LowCP manures. Ammonia $\delta^{15}N$ was markedly greater ($P < 0.05$) for manures containing ^{15}N-labeled urine compared with manure with ^{15}N-labeled feces. There was no difference ($P > 0.05$) in ^{15}N recovery in barley plants between HighCP and LowCP manures. With both HighCP and LowCP manures, a greater ($P < 0.05$) proportion of leachate ^{15}N was recovered from lysimeters treated with ^{15}N-labeled urine than with ^{15}N-labeled feces. A remarkably greater ($P < 0.05$) proportion of manure ^{15}N was recovered in emitted ammonia from lysimeters treated with ^{15}N-labeled urine than with ^{15}N-labeled feces. The greatest ($P = 0.02$) contribution of fecal or urinary N to whole-crop barley N at the end of the experiment was with High$CP^{15}U$.

Conclusions Manures from cows fed low- vs. high-protein diets, which were adequate or deficient in metabolizable protein supply, resulted in similar whole-plant barley yield and nitrate concentration in leachate. Nitrogen from HighCP urine had the highest recovery in whole barley plants, barley kernels, and leachate nitrate. Applied at equal N soil application rate, HighCP manure resulted in markedly greater ammonia emissions than LowCP manure with urine N being the primary source of the emitted ammonia.

Table 1 Leachate nitrate, ^{15}N enrichment, and manure N contribution to nitrate as affected by dairy cow diet

Item	High$CP^{15}F$	High$CP^{15}U$	Low$CP^{15}F$	Low$CP^{15}U$	s.e.m.	P
Leachate nitrate-N, mg	99	151	151	129	12.8	0.08
$\delta^{15}N$ of nitrate-N, ‰	23^{c}	89^{a}	27^{c}	50^{b}	8.2	0.002
Contribution of manure-N to nitrate-N, %	3.7^{c}	22.9^{a}	4.5^{c}	11.8^{b}	2.13	0.001

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GHG emissions from the storage of the liquid and solid fractions of co-digested pig slurry

E Dinuccio, D Cuk, L Rolle, F Gioelli, P Balsari
Department of Agricultural, Forest and Food Sciences, University of Torino, Grugliasco (TO), Italy
Email:elio.dinuccio@unito.it

Introduction Anaerobic digestion (AD) of feedstocks and animal manures is booming in Europe and among its nations. In the Piemonte region of northwest Italy alone, 37 agricultural AD plants have been constructed in the last five years and 58 more are waiting for approval or are in a start-up phase. Own calculations (DISAFA, 2013, unpublished data) indicate that biogas plants in the region, currently produce a total amount of approximately 800,000 m^3 of digestate per year. Prior to storage, the anaerobically-digested slurry is generally separated mechanically in order to obtain a solid and a liquid fraction. Anaerobic digestion of animal manure is widely recognized to reduce greenhouse gases (carbon dioxide, CO_2; methane, CH_4; nitrous oxide, N_2O) emissions from both storage and land application. However, little information is available on greenhouse gas (GHG) emissions generated during handling (i.e., storage + land application) of the solid and liquid fractions obtained by separation of co-digested slurry. The objective of this study was to investigate CO_2, CH_4, and N_2O losses during the storage of liquid and solid fractions of co-digested pig slurry. The study was partially financed by the AGER Foundation within the SEESPIG project (http://www.seespig.unimi.it) – grant number 2010-2220, in the framework of the pig supply chain.
Material and methods The experiment was carried out at a 0.5MWel. AD plant operating in the Piemonte region, Italy. The latter is made up of two identical 2500m^3 continuously stirred tank reactors (CSTR) heated at 40°C. During the investigation period the plant was fed with pig slurry (70%), farm yard manure (4%) and energy crops (26%). The organic loading rate (OLR) was of 2.20 kgVS m^{-3} d^{-1} and the hydraulic retention time (HRT) resulted of about 40 days. Digested slurry (approx. 120 m^3 per day) was separated prior to storage by means of a one stage rotating (Rota mod. SEP 97) separator. The digested solid fraction (approx. 12 t per day) is stored in heaps on an uncovered concrete platform, whereas the liquid fraction within a 14000 m^3 above ground open storage tank (diameter 60m, walls height 5m). CO_2 and CH_4 emissions from digested liquid fraction were quantified during 200 days of storage in spring-summer conditions (average environmental and slurry temperature of 15,8 and 18.0°C, respectively). Measurements were carried out by means of a floating system, fixed in the middle of the tank, and following the procedure described by Gioelli *et al*. (2011). On the first working day of each month, samples of digested liquid fraction were collected at the outlet of the mechanical separator in front of the storage tank entrance, to be analysed for pH, total (TS) and volatile (VS) solids content. CO_2, CH_4, and N_2O emissions from 3 dynamic heaps of digested solid fraction were also quantified, during 90 days of storage in spring conditions (average environmental temperature of 13,1 °C), by closed chamber method. Internal chamber gas concentrations were measured by gas chromatography. During the storage period, approx. 0.12 t of fresh digested solid fraction was added daily on top of each heap. The necessary manure was collected at the outlet of the mechanical separator. Samples of fresh digested solid and liquid fractions were taken every 15 days for pH, TS, VS, total (TKN) and total ammonium (TAN) nitrogen analysis. Recorded data were converted into CO_2 equivalents (CO_2eq), assuming a global warming potential of 1, 25 and 298 for CO_2, CH_4, and N_2O, respectively (IPCC, 2007).

Results The main GHG emitted from the digested liquid fraction was CH_4 (Table 1). During storage of digested solid fraction the GHG emissions were dominated by CH_4 and N_2O (Table 1). Total GHG emission over the storage period resulted of 36.9 kg CO_2eq t^{-1} for digested liquid fraction and of 104.7 kg CO_2eq t^{-1} for digested solid fraction.

Table 1 Cumulative emissions of carbon dioxide (CO_2), methane (CH_4)and nitrous oxide (N_2O) recorded during the trials.

	Digested liquid fraction	Digested solid fraction
C-CO_2		
kg CO_2eq t^{-1}	0.42	22.5
% of total VS	2.92	13.5
C-CH_4		
kg CO_2eq t^{-1}	36.5	40.5
% of total VS	10.1	1.15
N-N_2O		
kg CO_2eq t^{-1}	-	41.7
% of total nitrogen	-	2.97

Conclusions The experiment demonstrated significant GHG emissions during storage of liquid and solid fractions from co-digested pig slurry. Therefore, measures available for reducing emissions from AD plant storage structures (such as, the coverage of the liquid and solid fraction stores, the collections of the biogas produced during the storage of the liquid fraction) are strongly recommended.

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Predicting greenhouse gas emission reductions potentially achieved by nutrient management practices – RTI International's CLEANEAST™ project case study

M E Deerhake, Z D Hendren, N C Ubaka-Blackmoore
RTI International, Research Triangle Park, NC, USA *Email:med@rti.org*

Introduction The Comprehensive Livestock Environmental Assessment and Nutrient Management Plan (CLEANEAST™) Project was a Congressionally-funded project administered through a cooperative agreement between the U.S. Environmental Protection Agency and RTI International to provide environmental assessments (EAs) and nutrient management planning (NMP) assistance to livestock and poultry operations. Four hundred twenty-ninety (429) farms received on-site services from CLEANEAST™. In this paper, we present an analysis of greenhouse gas (GHG) emissions from the manure storage practices on the 190 dairy farms served by the Project. We compare emissions from actual nutrient management-based storage practices to emissions estimated if 100% of the dairies converted to one of four Intergovernmental Panel on Climate Change (IPCC)-reviewed manure storage practices,

Materials and Methods Data collected for the190 dairies included the number of animals, average weight, and growth stage. These data were input to perform Tier 2 analyses using IPCC guidelines (Dongo *et al*., 2006) and estimations for milk production (UG CAES) to estimate the CH_4 and N_2O production on a per animal basis. Animals were re-classified into one of three categories: animals < 230 kg were denoted "growing dairy cows", those > 230 kg and produced milk were "mature dairy cows", and all other animals > 230 kg were labelled "other dairy cows". Dairies reported their manure storage practices as one of five storage methods. Since a single dairy may use more than one storage practices, a total of 448 systems were reported in the dataset. (See Table 1.)

Table 1 Manure Storage Systems (MS) Reported in the CLEANEAST™ Project Dataset

Manure Storage Practice	Number of Systems	MS (fraction)
1- or 2-stage lagoon system	51	11%
Aboveground liquid storage	70	16%
Deep pit system	28	6%
Dry waste storage\Litter storage	155	35%
Earthen storage\Storage pond	144	32%

Results Because the mass of manure used for each of the manure storage systems was not part of the dataset, the analysis assumes that the quantity of manure generated (and, in turn, the GHG emissions) is directly proportional to the number of manure systems reported. Under this assumption, total emissions of 360,165 kg CH_4/yr and 72,711 kg N_2O/yr were estimated, which is equivalent to 30,122,000 kg/yr of CO_2 (CO_2-eq). All calculations assumed an average annual temperature of 10°C based on farm locations. To compare the impact of manure storage practices on GHG gas generation, emissions were estimated for the total CLEANEAST™ dairy population assuming all dairy farms switched to one of the four IPCC manure storage practices for all of their manure (i.e., the MS fraction = 100%). The aboveground liquid storage, deep pit, and earthen storage in Table 1fall into the liquid/slurry classification listed in the IPCC guidelines. The Tier 2 calculations were made for the four IPCC manure storage practices (Table 2).

Table 2 Comparison of GHG Emission for Different Manure Storage Practices Reported in the CLEANEAST™ Dataset.

Manure Storage Practice	kg CH_4/yr	kg N_2O/yr	kg CO_2-eq
Lagoon	1,367,056	0	28,708,000
Liquid/slurry (no natural crust)	352,121	0	7,395,000
Liquid/slurry (with natural crust)	207,130	37,821	16,074,000
Dry waste storage/litter storage	41,426	151,283	47,768,000

Conclusions Operation of the liquid/slurry manure storage practice without the natural crust cover at dairy farms would emit the least (75% less) GHG (7,395,000 CO_2-eq) compared to the estimated emissions of the actual practices (30,122,000 CO_2-eq) at the CLEANEAST™ Project's 190 dairies.

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Greenhouse gas emissions from a dairy farm with an operational biogas system

C Wagner-Riddle[1], N M Ngwabie[1], L Maldaner[1], G Johannesson[1], A VanderZaag[2], R Gordon[1], L Evans[1]
[1]University of Guelph, Guelph, ON, Canada, [2]Agriculture and Agri-Food Canada, Ottawa, ON, Canada
Email:cwagnerr@uoguelph.ca

Introduction The increasing evidence that current climate change is caused by anthropogenic greenhouse gases has led to interests in alternative options for lowering GHG emissions. Anaerobic digestion (AD) of organic waste is a promising solution that offers wide benefits as it deals with waste management, renewable energy and climate change mitigation. On-farm measurements considering all potential sources of GHG emissions in AD and conventional manure management systems are clearly needed in order to assess environmental attributes associated with AD. Liebetrau el al. (2010) investigated emissions of ten biogas plants located in Germany and concluded that emissions of the cogeneration unit and from the digestate tanks were higher than the assumed by the German LCA model. The objective of this study is to determine the methane emissions from a barn and manure storage tank at a dairy farm with a recently installed AD. These emissions are compared to measurements taken before AD installation.

Material and methods The research site is located at Clovermead Farm (43.7°N 80.6°W), a 160 cow dairy farm close to Drayton, ON, Canada. Methane emissions from the manure storage tank are being determined a using micrometeorological mass balance (MMB) method and tunable diode laser absorption spectroscopy (Wagner-Riddle *et al*., 2006). For the baseline treatment (conventional manure management practice), methane fluxes from the untreated manure storage tank were measured using MMB method at the same farm in 2011, before the AD system operation. The concentrations of CH_4, NH_3, N_2O, CO_2 and water vapour at three indoor barn locations and two outdoor locations is measured using a photo-acoustic multi-gas analyser. The barn is naturally ventilated and houses 176 dairy cows and 45 replacement heifers in a free-stall. The barn ventilation rate is estimated on an hourly basis using the CO_2 and H_2O mass balance (Ngwabie *et al*., 2011). The activity or movement of the cows needed to calculate the ventilation rate is measured with an activity meter system (DeLaval). Measurements were carried out in the spring (Feb–Apr) and in the fall (Sep-Nov). Straw was used as bedding in the spring and solids extracted from the AD digestate in the fall. Fugitive emissions from all devices that contain biogas are being identified through site inspection with a flame ionization detector and, whenever possible, measured using chamber around the identified source. The flare emission are being calculated based on monitored parameters (biogas flow and flare hours) and flare efficiency. Emissions from the whole farm are being quantified using the inverse dispersion technique based on backward Lagrangian Stochastic (bLS) model (Flesch *et al*, 2005). Untreated manure, co-substrates and digestate samples are being collected monthly and analysed for: organic/inorganic nitrogen, chemical oxygen demand (COD), total solids (TS) and volatile solids (TVS), total organic carbon (TOC), pH and total volatile acids (TVA).

Results The variations in the average hourly concentrations indicated low concentrations in the daytime and high concentrations at night. This shows that a better indoor air quality is achievable in the barn within the daytime as compared to night-time. The lower concentrations in the daytime relative the night-time is related to higher ventilation rates at this time as compared to the night-time. The activity of the cows showed a distinct diurnal pattern with peaks at about 7-9 a.m. and 6-8 p.m. The activity peak at 6-8 p.m. was about 15% higher than the peak at 7-9 a.m. The lowest activity was measured at 4-5 a.m. The average ventilation rates during the entire measurement period were 656 ± 670 m^3 LU^{-1} h^{-1} and 694 ± 705 m^3 LU^{-1} h^{-1} for the CO_2 and H_2O balance methods respectively (1 LU = 500 kg animal mass). A better comparison in ventilations rates using CO_2 and the H_2O balance methods was obtained by restricting the data to the same measurement hours (519 ± 519 m^3 LU^{-1} h^{-1} and 573 ± 575 m^3 LU^{-1} h^{-1}) for the CO_2 and H_2O methods respectively, which were not statistically different ($p = 0.10$). Emission rates of 13.1 g CH_4 LU^{-1} h^{-1}, 0.60 ± 0.29 g NH_3 LU^{-1} h^{-1} and 40.3 ±19.2 mg N_2O LU^{-1} h^{-1} were obtained for the spring period. Fall data are currently being analyzed. Methane emissions from the manure storage tank over one year of measurement in 2011 averaged 272.9 ±5.2 (s.e.) μg m^{-2} s^{-1}. The AD became operational in August 2012, and emissions over the Sep to Oct period averaged 58 ±6.9 (s.e.) μg m^{-2} s^{-1}, in comparison to 442 ±10.4 (s.e.) μg m^{-2} s^{-1} measured over the same period (Sep-Oct) in 2011.

Conclusions Methane emissions from digestate are on-going and will be reported for the Sep 2012 to May 2013 period. In addition, bLS measurements from the whole-farm and manure tank conducted in November 2012 and fall 2012 barn measurements will be presented. Preliminary results show that GHG emissions from the digestate are lower than GHG emissions from an untreated manure storage. Emissions from an on-farm biogas system, considering all potential sources will be reported.

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Release rate of sulphur hexafluoride from permeation-tubes is not affected by submersion in water

M H Deighton[1,2], S R O Williams[1], K R Lassey[3], R J Eckard[2], T M Boland[4], P J Moate[1,2]
[1]Department of Environment and Primary Industries, Ellinbank, Victoria, Australia, [2]The University of Melbourne, Melbourne, Victoria, Australia, [3]Lassey Research and Education Ltd., Lower Hutt, New Zealand, [4]University College Dublin, Dublin, Ireland *Email:matthew.deighton@dpi.vic.gov.au*

Introduction Predictable release of sulphur hexafluoride (SF_6) from permeation-tubes is critical for accurate determination of enteric methane (CH_4) emissions from ruminants using the calibrated SF_6 tracer technique. The accepted practice for determining the SF_6 release rate (RR) from permeation-tubes is measurement of mass loss during dry incubation at 39°C (Lassey *et al*. 2001). Any change in SF_6 release rate from permeation tubes caused by their subsequent submersion within the reticulo-rumen of experimental ruminants would bias estimates of CH_4 emissions. Rochette *et al*. (2012) reported that the rate of SF_6 release from permeation tubes with mean SF_6 RR of 1.7 mg/d decreased following first but not subsequent incubation in a liquid environment, with these authors proposing that tubes should be calibrated following an initial incubation in a liquid medium, preferably in the rumen. The purpose of this investigation was to evaluate if the SF_6 RR from permeation tubes is affected during submersion and hence to determine if the currently accepted practice of dry incubation remains valid without pre-calibration liquid exposure.

Material and methods Twenty-four permeation tubes (Lassey *et al.* 2001) with a mean (± S.D.) SF_6 RR of 7.1 ± 0.53 mg/d and twenty-four Control tubes of identical design, yet containing no SF_6, were supplied by NIWA, Wellington, New Zealand. Control tubes enabled the experiment to determine change in tube mass, independent of treatment effect upon SF_6 RR. Permeation tubes containing SF_6 were blocked (n = 4) by RR determined during the 14 d preceding the experiment and were randomly allocated to treatment within block. Control tubes were randomly allocated to treatment. Two treatments, incubation in air or water, were applied within an incomplete Latin square design. Each tube was sequentially incubated in either air or water for three 14 d periods. Twelve permeation tubes of each type (SF_6 and Control) were placed into one of two pre-heated 500 ml glass bottles containing either air or water sufficient to submerse all tubes. Vented lids were fitted to each bottle to prevent moisture transfer and to ensure the lumen of each bottle was equilibrated with atmospheric pressure. Tubes were incubated at 39°C within a constant temperature incubator (Heratherm Advanced Protocol; Thermo Fisher Scientific, Waltham, USA). Temperature within each bottle was recorded every 10 minutes using logging thermometers (TidbiT v2; Onset Computer Corp., Pocasset, USA). Tube mass was determined by weighing to within 0.1 mg using a digital balance (CP224S; Sartorius AG, Göttingen, Germany) before and after each incubation period. Prior to weighing, tubes were dried by removing surface moisture with a dry cloth and incubation in 39°C air for 45 minutes. The effect of treatment upon SF_6 RR of permeation tubes was determined via analysis of variance using ReML in GenStat 14 and the model;

$$y = \mu + TubeType + Media + \sum_{p=2}^{4} \beta_{pm} t_p + Tube + \varepsilon$$

where y is SF_6 mass change, μ is a constant, *TubeType* is the effect of SF_6 vs. Control tube, *Media* is the effect of air vs. water, *Tube* is a random effect of individual tube, ε is a residual measurement error, t_p is the duration time of period p, and β_{pm} is a the rate of SF_6 loss during period p in media m.

Results Release of SF_6 from permeation tubes did not differ during incubation in either air or water ($P > 0.05$). The mean total SF_6 release (± s.e.) from permeation tubes incubated for 14 d in air was 99.8 ± 2.21 mg while the release of SF_6 from the same tubes during 14 d in water was 97.9 ± 2.21 mg. In order to determine the change in SF_6 mass in either media it was necessary to correct for the change in tube mass due to water exposure as; Δ gross = Δ tube + Δ SF_6 + Δ H_2O. Control permeation tubes (n = 24) increased in mass (5.5 ± 1.06 mg) due to submersion in water, despite removal of surface water and drying at 39°C for 45 minutes. Consequently control tubes assigned to air following water incubation (n = 12) decreased in mass (6.2 ± 6.03 mg) following 14 d incubation in air. Mean incubation temperature (± S.D.) was 38.93 ± 0.013°C and did not differ between treatments during the 42 d experiment ($P > 0.05$).

Conclusions The release of SF_6 gas from permeation tubes was not affected by submersion in water. We conclude that dry incubation of SF_6 permeation tubes is a valid technique to determine the RR of SF_6 from tubes intended to be used for the measurement of CH_4 emissions from ruminants.

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The potential of dietary manipulations in early life to decrease methane production by lambs later in life

K E Waddams[1], N R McEwan[1], D R Yanez-Ruiz[2], C J Newbold[1]
[1]Aberystwyth University, Aberystwyth, UK, [2]Estación Experimental del Zaidín, CSIC, Granada, Spain
Email:kew3@aber.ac.uk

Introduction The importance of reducing ruminant methane emissions has been well documented as ruminal methane production represents a significant energy loss from the animal as well as having a deleterious environmental impact. We have previously shown that changes in diet during the weaning period can influence the bacterial population in the rumen of lambs later in the animal's life (Yanez *et al*., 2010) The aim of this study was to determine whether it was possible to use such an approach to condition the rumen to produce less methane during the ruminant's early life and whether this effect would persist later in the animal's life.

Materials and Methods Two groups of Beulah Speckled Faced lambs (n=40) were weaned onto diets of either hay:concentrate (60:40 respectively) or hay-only diets to establish different microbial populations. Each group consisted of 10 sets of twins; within each set of twins, one twin was treated daily from birth with chloroform (10µl $CHCl_3$ (made up to 1ml with vegetable oil) per kg liveweight per day) added directly to the rumen via the mouth and one with water (1ml/kg liveweight/day) added in the same manner as a control. At 5 months of age (animals were weaned onto solid feed only after 12 weeks), methane emissions were measured in all lambs in open circuit respiration chambers over 3 days and rumen fluid samples collected via stomach tubing (Period 1). After this sampling, chloroform/oil treatment was stopped and all animals grazed on a common pasture for a further 3 months. Following this period, lambs were again housed and all fed a hay:concentrate diet (for 2 weeks prior to measurements but with no chloroform/oil treatment, this whole measurement period took 2 months), methane emissions were measured over 3 days and rumen fluid samples were collected (Period 2). Following this sampling period, all lambs were, again, grazed on a common pasture for a final 8 months before being brought in and sampled (as in Period 2) for a third time (Period 3).

Results In Period 1 and Period 2 there was a significant effect of both the diet (Period 1, P= 0.004, CH_4/L/kg DMI; Period 2, P< 0.001, CH_4/L/kg DMI) and chloroform/oil treatment (Period 1, P= 0.017, CH_4/L/day; Period 2, P= 0.020, CH_4/L/day) in terms of decreasing methane production (Table 1). In Period 3, 13 months after the initial conditioning of the rumen, there was still a significant effect (P= 0.025, CH_4/L/day) of the weaning diet upon the levels of methane produced (Table 1).

Table 1 Effect of diet and chloroform/oil treatment effects on methane emissions from lambs either directly after weaning whilst the treatments were still being applied (Period 1) or 3 months or 13 months later after treatments had been removed (Periods 2 and 3 respectively) (- untreated, + treated)

	Hay:Concentrate		Hay		s.e.d.		
Period 1	-	+	-	+	Diet (D)	Chloroform (C)	D/C
CH_4/L/d	12.2	4.7	8.2	5.9	1.92 ^{NS}	1.92*	2.72 $^{P<0.185}$
CH_4/L/kg DMI	12.0	5.8	19.8	17.2	3.05 **	3.05 $^{P<0.158}$	4.32 ^{NS}
Period 2							
CH_4/L/d	23.8	20.1	20.1	17.1	1.36 *	1.36 *	1.92 ^{NS}
CH_4/L/kg DMI	21.0	18.82	17.4	17.1	0.63 **	0.63 $^{P<0.059}$	0.88 $^{P<0.140}$
Period 3							
CH_4/L/d	29.4	30.6	25.1	25.5	1.98 *	1.98 ^{NS}	2.80 ^{NS}
CH_4/L/kg DMI	19.9	22.0	18.6	18.7	1.19 $^{P=0.06}$	1.19 ^{NS}	1.68 ^{NS}

Conclusions The results from this experiment suggest that it is possible to condition the rumen during the animal's early life and the effects from this conditioning can be carried on into the animal's later life. We are currently investigating the effects of the treatments on the bacterial and archaeal populations within the rumen of the lambs. If additives added to the rumen early in life, as the rumen microbial population develops, continue to have effects on the rumen microbial population and the outputs of rumen fermentation in later life (long after the additive has been removed from the diet), then this could have a significant economical benefit to commercial farmers as well as lowering the environmental impact of ruminant agriculture.

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Sheep fed fresh forage rape (Brassica napus subsp. Oleifera L.) have lower methane emissions compared with perennial ryegrass (Lolium perenne L.)

X Z Sun, D Pacheco, G Molano, D W Luo
Grasslands Research Centre, AgResearch Limited, Palmerston North, New Zealand *Email:xuezhao.sun@agresearch.co.nz*

Introduction Grazing ruminants are the main source of methane (CH_4) emissions in New Zealand. Forage-based mitigation tools would be most likely to be incorporated into the current pastoral systems. We previously reported that feeding brassica forages to sheep reduce CH_4 emissions with the effect being largest for forage rape (*Brassica napus* subsp. *Oleifera* L.; Sun *et al*., 2012). Sheep fed forage rape emitted 25% less CH_4 per unit of dry matter intake compared with perennial ryegrass (*Lolium perenne* L.). However, this result was observed in a single trial only and no information is available for sheep fed forage rape for an extended period of time. The objective of this study is to confirm our previous finding and to examine if this CH_4 reduction is stable for a length of time representative of the use of brassica crops in commercial operations.

Material and methods Forty two healthy 9-month-old Romney male lambs (32.4 ±0.6 kg, mean ± S.D.) were randomly allocated to two feeding groups: forage rape (var. Titan; n=24) and perennial ryegrass (var. Ceres One 50 with AR1 endophyte; n=18). After adaptation in the paddock, they were fed indoors with fresh cut forage twice a day equally at 09:00 and 16:00 h at 1.5 times their metabolisable energy requirements for maintenance. Methane emissions were measured after they ate the designated diets for 7 wks (the first period of measurement). Both rape and ryegrass were in the vegetative state and harvested daily using a sickle bar mower. After the first period of measurement, sheep were returned to graze the same forage as they ate before and then transferred indoors for another CH_4 measurement after they had eaten the designated diets for 15 wks. Measurements of CH_4 emissions were conducted using a sheep respiration chamber facility containing 3 systems with 8 individual chambers each system as described in Pinares-Patiño *et al*. (2011). The animals were transferred to individual chambers in two batches (12 sheep from rape treatment and 9 from ryegrass each batch) for the measurement. The treatments were balanced into the 3 chamber systems, and animals randomly allocated into chambers within system. The measurement of CH_4 emissions lasted for 48 h for each batch. Data were analysed using the linear mixed-effects model with forage treatment as a fixed effect and measurement batch and chamber system as random effects.

Results In both periods of CH_4 measurement, sheep fed rape produced less CH_4 (g/d) than sheep fed ryegrass ($P< 0.001$; Table 1). Relative to ryegrass, feeding rape reduced the methane yield (g CH_4/kg DM intake) by 30% and 20% in the first period and second period, respectively (both $P<0.001$). Sheep fed rape lost less of their gross energy intake in the form of CH_4, compared to ryegrass-fed sheep ($P< 0.001$).

Table 1 Mean methane emissions from sheep fed fresh forage rape or perennial ryegrass

	Period 1 (7 wks)					Period 2 (15 wks)				
	Forage rape		Perennial ryegrass		P	Forage rape		Perennial ryegrass		P
DM intake (g/d)	862	±8.1	792	±25.9	0.006	896	±8.4	929	±20.8	0.116
CH_4 (g/d)	11.7	±0.48	15.4	±0.97	<0.001	16.0	±0.60	21.2	±0.50	<0.001
CH_4 (g/kg DM intake)	13.6	±0.52	19.5	±1.14	<0.001	17.8	±0.64	22.9	±0.45	<0.001
CH_4 energy loss/gross energy intake	0.050	±0.0019	0.063	±0.0039	0.002	0.058	±0.0021	0.073	±0.0014	<0.001

Conclusions Sheep fed forage rape emitted 20-30% less CH_4 per unit of feed eaten than those fed ryegrass and the effect of feeding rape on CH_4 emissions persisted for 15 wks. Forage rape could be a viable CH_4 mitigation tool for pastoral-based sheep production systems. Although this and previous studies both indicated feeding forage rape reduces CH_4 emissions, these studies were conducted indoors. Before translating these effects to practical farming conditions, it is necessary to assess the results under conditions that are representative of grazing conditions, as animal behaviour and eating patterns may differ between indoor housing and outdoor grazing.

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The effect of nitrogen fertilization level and stage of maturity of grass herbage on methane emission in lactating cows

S C Podesta[1], B Hatew[1], G Klop[1], H Van Laar[1], R D Kinley[1], A Bannink[1,2], J Dijkstra[1]
[1]Wageningen University, Wageningen, The Netherlands, [2]Wageningen UR Livestock Research, Lelystad, The Netherlands
Email:sabrina.podesta@wur.nl

Introduction Methane (CH_4) is the most important greenhouse gas in dairy production (FAO, 2010). An average Dutch dairy cow emits about 350 g CH_4/d (Bannink *et al.* 2011). Grass is the main component of the dairy cow ration in the Netherlands. Previous *in vitro* trials (Lovett *et al.* 2004) and simulation studies (Bannink *et al.* 2010) suggest that the chemical composition of grass influences CH_4 production in the rumen. However, studies *in vivo* to evaluate these effects in dairy cows are sparse. Grass quality is largely determined by management practises including nitrogen (N) fertilization level and stage of maturity at cutting. This study examined the effect of N-fertilization and grass maturity on methane emission in lactating dairy cows when fed as fresh herbage.

Material and methods Twenty-eight lactating dairy cows (live weight 606 kg, s.e. 11.3; days in milk (DIM) 206, s.e. 26.3) were assigned to one of four zero-grazed perennial ryegrass diets according to a 2x2 randomized factorial design. Grass had received either a low (L) or high (H) N-fertilization level (20 or 90 kg N ha^{-1}) and was cut after 3 (L3 or H3) or 5 (L5 or H5) weeks of regrowth. In total 12 rumen fistulated cows were used (n=3 per treatment). Cows were blocked by DIM, milk production, parity and presence of rumen fistulae. Cows were adapted to the diet for 12 d in a tie-stall and were subsequently kept in respiration chambers for 5 d to evaluate methane production and animal performance. Diets were supplied ad libitum for the first 8 days. Afterwards feed intake was restricted per block to 95% of the ad libitum feed intake of the cow consuming the lowest amount of feed in that particular block, to avoid confounding effects of possible differences in ad libitum feed intake on methane production. Cows were fed and milked twice daily. Fresh grass was harvested daily in the early afternoon for afternoon feeding and stored in a cooling room overnight for morning feeding. Diets consisted of 15% concentrate and 85% grass (based on dry matter; DM) and all cows received the same concentrate. During the adaptation period (day 10 and 11), rumen fluid was sampled for determination of VFA concentration and pH at 0, 1, 2, 3, 4, 6, 8 and 10h after morning feeding. All other measurements were done during the final 3 days in the respiration chambers. Data were analysed using PROC MIXED in SAS. The model included fertilization level, maturity, and its interaction as fixed effects, and period as random effect.

Results Preliminary results indicate that early cutting and high N fertilization increased fat- and protein corrected milk (FPCM) production compared with late cutting and low N fertilization, respectively (Table 1). Fertilization level did not affect CH_4 production per kg dry matter intake (DMI) or per kg FPCM. Grass maturity did not affect CH_4 production per kg DMI, but CH_4 production per kg FPCM was higher for late cut than for early cut grass. Total VFA concentration in the rumen was higher for high than for low N fertilization. Acetate molar proportion was higher and butyrate molar proportion was lower for high N fertilization than for the low N fertilization. A significant interaction between fertilization level and maturity was observed for propionate and butyrate molar proportion. Rumen fluid pH was not affected by grass maturity or N fertilization level.

Table 1 Dry matter intake, FPCM production, CH_4 production and rumen characteristics in lactating cows fed grass herbage-based diets

	Treatment					P		
	L3	L5	H3	H5	s.e.m.	F[1]	M[2]	F*M
DMI (kg/d)	14.3	14.2	15.0	14.8	0.48	0.058	0.541	0.843
FPCM (kg/d)	21.1	16.8	22.6	21.2	1.35	0.005	0.006	0.124
CH_4 (g/kg FPCM)	14.5	17.3	14.4	15.9	0.85	0.302	0.010	0.374
CH_4 (g/kg DMI)	21.1	20.7	21.3	22.7	0.73	0.094	0.450	0.176
Total VFA (mmol/l)	76.8	76.5	97.4	88.2	3.52	<0.001	0.064	0.082
Acetate (%)	64.7	63.6	66.1	65.6	1.11	<0.001	0.059	0.482
Propionate (%)	16.9	16.3	16.3	17.8	0.35	0.147	0.097	<0.001
Butyrate (%)	16.1	17.7	14.2	14.0	0.93	<0.001	0.012	0.001
Acetate/propionate	3.8	3.9	4.1	3.7	0.14	0.897	0.072	0.008
Rumen pH	6.4	6.5	6.4	6.4	0.04	0.267	0.101	0.556

[1]F: N-fertilization level, [2]M: grass maturity

Conclusions Increased N fertilization level and reduced grass maturity increased FPCM production. Increased grass maturity increased CH_4 emission per kg FPCM but N fertilization level did not have an effect. Acetate molar proportion and total VFA concentration were higher and butyrate molar proportion lower for high N fertilization levels.

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Methane emission from cattle grazed on pasture sprayed with canola oil

C S Pinares-Patino[1], F E Franco[3], G Molano[1], S MacLean[1], H Kjestrup[1], E Sandoval[1], J Laubach[2]
[1]AgResearch Grasslands, Palmerston North, New Zealand, [2]Landcare Research, Lincoln, New Zealand, [3]IVITA Marangani, Cusco, Peru *Email:cesar.pinares@agresearch.co.nz*

Introduction Lipid addition to the diet is one of the most effective dietary manipulations to reduce CH_4 emissions from ruminants, which can be done without compromising animal production (Grainger and Beauchemin, 2011). However, lipid sources that can be feasibly added to the diet in a cost-effective manner remain to be identified. Perennial ryegrass (*Lolium perenne* L) is the dominant forage species in intensively managed temperate pastures. Herbages from this species are high in nitrogen and fibre contents and low in dry mater, lipids and non-structural carbohydrates; hence associated with a lower feeding values and higher CH_4 emission intensities (per unit of animal product) compared to concentrate-based diets. Ulyatt *et al*. (1988) suggested that improving the energy content of forages by raising the concentration of fatty acids would lead not only to improved feeding value and nitrogen utilisation, but also reduced CH_4 emissions. This study was part of a programme that aimed at verifying the ability of micro-meteorological methods to account for differences in methane emissions from grazing cattle when a vegetable oil was sprayed onto the sward. Here we report the CH_4 emissions found using the sulphur hexafluoride (SF_6) tracer technique.

Material and methods A grazing study was carried out with sixty cross-bred (Hereford × Holstein Friesian) steers (1 yr-old, 277.2±21.0 kg liveweight, LW) subdivided into two similar size groups and balanced by LW. Following a 32-d period of acclimatisation to grazing management, one group was randomly allocated to graze canola-oil sprayed pasture and the other group to graze control pasture (no oil spray). Pasture was dominated by perennial ryegrass. The experiment included two periods (Period 1 and Period 2), during which the animal groups were separately grazed in daily grazing strips of 0.1 ha. Period 1 was of 5-d duration, and was used for baseline measurement of emission of CH_4 using the SF_6 tracer technique (Johnson *et al*., 1994), while treatments were not applied. During Period 2 (12-d duration), canola oil was sprayed (12 L per 0.1 ha strip) before grazing to a randomly selected grazing strip. In Period 2, feed dry matter intake (DMI) was estimated using faecal output estimates (TiO_2 dosing) and *in vitro* feed digestibility (Pinares-Patiño *et al*., 2008), whereas CH_4 emission measurement was repeated. Data for mean DMI and CH_4 emissions (absolute and per unit of DMI) were analysed by ANOVA for treatment (oil or no oil spray) effects.

Results The grazed herbage diets were similar in chemical composition (DM basis) from the herbages on offer (17% crude protein, 39% neutral detergent fibre); except the oil-sprayed herbage had twice the lipid content than the herbage on offer (8.9 vs. 3.9%). In Period 1, baseline emission of CH_4 did not differ between the two groups of animals (Table 1). In Period 2, daily CH_4 emission from steers grazing oil-sprayed pasture was 11% lower than that from their counterparts grazing control pasture (Table 1). Daily feed intake by steers grazing oil-sprayed herbage was 10% higher than that by steers on control pasture (Table 1). Consequently oil spraying resulted in 18% reduction of CH_4 yield (emissions per unit of feed intake).

Table 1 Baseline CH_4 emission (Period 1), and DMI and CH_4 emission measured in Period 2. Values are mean ± s.d.

	Oil spray	Control	P
	Period 1 (Treatments not applied)		
CH_4, g/d	137.8±19.0	142.5±17.6	0.250
	Period 2 (Treatments applied)		
DMI, kg/d	8.3±1.4	7.5±0.9	0.020
CH_4, g/d	138.6±17.5	156.1±21.1	0.002
CH_4, g/kg DMI	17.2±3.4	20.9±2.4	<0.001

Conclusions Methane emission was reduced by oil spraying of pasture, which confirms findings reported in the literature (e.g. Grainger and Beauchemin, 2011). The positive effect of oil spraying on herbage intake has no precedent in the literature. Oil supplementation may be a costly method for mitigation of CH_4 emissions from grazed systems. However, longer term studies that validate the desired effects of oil supplementation may prompt forage improvement for higher contents of herbage lipids. Further, oil spray at lower quantities may be an effective method to deliver anti-methanogen agents that are under development.

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Effects of dietary forage to concentrate ratio and sunflower oil supplements on milk yield, rumen fermentation and enteric methane emissions in lactating dairy cows

A R Bayat[1], L Ventto[1], T Stefański[1], I Tapio[1,2], P Kairenius[1], H M Leskinen[1], J Vilkki[1,2], K J Shingfield[1]
[1]Animal Production Research, MTT Agrifood Research Finland, Jokioinen, Finland, [2]Biotechnology and Food Research, MTT Agrifood Research Finland, Jokioinen, Finland *Email:alireza.bayat@mtt.fi*

Introduction Dietary plant oil supplements and increases in the proportion of concentrates in the diet are known to lower ruminal methanogenesis (Martin *et al*., 2010). Both the anti-protozoal properties and inhibitory effects of unsaturated fatty acids on the growth of rumen methanogens have been implicated for the decrease in enteric methane (CH_4) production to dietary lipid supplements. Several mechanisms may account for the changes in CH_4 production following the substitution of forages for concentrate ingredients, including alterations in rumen fermentation, ruminal digestion and microbial ecology. In this study the effects of changes in dietary forage:concentrate (F:C) ratio and supplements of sunflower oil (SO) on enteric CH_4 production, nutrient digestion and rumen fermentation in lactating cows offered grass silage were examined.

Material and methods Four Finnish Ayrshire dairy cows in mid lactation fitted with rumen fistula were used in a 4×4 Latin square with 35d experimental periods comprised 15 d adaptation, 11 d sampling and 9 d washout . Experimental treatments consisted of total mixed rations based on grass-silage containing either high (H; F:C 65:35) or low (L; F:C 35:65) proportions of forage supplemented with 0 (O) or 50 g/kg DM of SO (S). Feed intake, milk yield and milk composition were determined during d 22-25 of each period. Samples of rumen fluid were collected on d 26 of each period at 1.5 h intervals from 06.00 to 16:30 h. Nutrient flow at the omasum was measured using Cr, Yb and indigestible NDF as markers. Enteric CH_4 production was determined during d 16-21 using the sulphur hexafluoride (SF_6) tracer technique (Grainger *et al*., 2007).

Results Decreases in dietary F:C ratio increased ($P < 0.01$) DM intake, whereas SO tended ($P = 0.09$) to lower nutrient intake (Table 1). Treatments had no effect ($P > 0.05$) on milk yield. Rumen pH was lower ($P < 0.01$) for L than H (6.15 vs. 6.56), whereas SO supplements increased ($P < 0.05$ for forage ratio × oil interaction) rumen pH on L but not for H diets (6.03 vs. 6.26). Decreases in the F:C ratio lowered ($P < 0.01$) rumen NH_3-N concentration (5.60 vs. 2.75 mM). Supplements of SO decreased ($P < 0.05$ for forage ratio × oil interaction) total rumen VFA for L but not for H diets (109 vs. 123 mM). Decreases in the F:C ratio and SO supplements lowered ($P < 0.05$) enteric CH_4 emissions by 11.6 and 22.7%, respectively. Corresponding decreases in CH_4 production per kg milk were 17.3 and 17.5%.

Table 1 Effect of dietary forage:concentrate ratio and sunflower oil supplements on intake, milk yield, rumen fermentation and enteric CH_4 production in lactating cows fed grass silage based diets

	Treatment					P		
	HO	HS	LO	LS	s.e.m	Forage	Oil	Forage× Oil
DM intake (kg/d)	19.0	18.6	23.3	20.7	0.60	<0.01	0.09	0.18
Milk yield (kg/d)	26.7	25.7	29.7	28.9	2.50	0.12	0.60	0.97
Milk fat (g/d)	1050	1076	1195	821	94.6	0.38	<0.05	<0.05
Milk protein (g/d)	901	823	1013	1012	60.9	<0.05	0.42	0.43
Ruminal OM digestibility	0.394	0.371	0.433	0.421	0.0154	<0.01	0.14	0.59
Ruminal NDF digestibility	0.537	0.521	0.424	0.338	0.0155	<0.001	<0.05	0.07
Molar proportions in rumen VFA (mmol/mol)								
Acetate	658	648	594	592	5.7	<0.001	0.25	0.43
Propionate	180	185	231	224	11.4	<0.05	0.96	0.71
Butyrate	111	121	125	130	10.0	0.44	0.64	0.88
Enteric methane								
g/d	484	356	408	333	22.8	0.05	<0.01	0.24
g/kg ruminal digestible OM	72.0	57.4	44.6	41.7	3.99	<0.001	0.05	0.16
g/kg milk	18.1	14.3	14.3	12.5	1.35	0.01	<0.01	0.25
% of GE intake	7.08	5.56	4.94	4.80	0.341	<0.01	<0.05	0.07

Conclusions Decreases in the dietary forage:concentrate ratio in the diet lowered enteric CH_4 production, changes that were associated with alterations in rumen fermentation towards propionate at the expense of acetate. Supplements of sunflower oil also lowered enteric CH_4 production, due at least in part, to less extensive digestion of organic matter in the rumen. There was no evidence that the effects of increases in the proportions of concentrates in the diet or oil supplements on ruminal methanogenesis were additive.

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Influence of rumen protozoa on methane emissions in ruminants: A meta-analysis approach

J Guyader, M Eugène, P Nozière, D P Morgavi, M Doreau, C Martin
INRA, Saint Genès Champanelle, France *Email:jessie.guyader@clermont.inra.fr*

Introduction Methane (CH_4) produced by ruminants is the most important greenhouse gas coming from livestock breeding (Steinfeld *et al.*, 2006). In the rumen, CH_4 is produced by methanogenic archaea, mainly from carbon dioxide (CO_2) and hydrogen (H_2) released during fermentation of feeds by other microbes. Protozoa are implicated in methanogenesis through the production of large quantities of H_2 and through their close interaction with archaea, which are the main users of H_2 (Morgavi *et al.*, 2010). However, the relationship between protozoal concentration and the amount of CH_4 emissions is not well quantified. In this study we made a quantitative analysis of the literature to assess this relationship.

Material and methods A database was built from 59 publications reporting data from 76 experiments and 270 treatments. Only *in vivo* experiments giving measured data on both CH_4 production and rumen protozoal concentration on a same group of animals were included in the database. An experiment consisted in one control treatment and at least one experimental treatment testing a CH_4 mitigation strategy on the same basal diet. Quantitative parameters (chemical composition of the diet, intake, total tract digestibility, rumen fermentation and microbial ecosystem) and qualitative parameters (animal species, diet composition, methods of CH_4 and protozoa determination) were considered. Experiments were encoded according to 3 classes of CH_4 mitigation strategies: biotechnological additives (experimental defaunation, probiotics, prebiotics, enzymes), additives (plant extracts, chemical compounds, organic acids) or feed components (forages, concentrates, lipids). Treatments testing associations of two or more strategies were not considered. Within each class, the quantity, source and form of the additive was encoded. Protozoal concentrations were expressed in $\log_{10}$ cells/mL to get a normal distribution of data. Daily CH_4 emissions were expressed as a function of dry matter intake (DMI) to allow interspecies comparisons. The relationship between CH_4 emissions and protozoal concentration was studied with a variance-covariance model allowing dissociation between intra- and inter-experiment variability, with experiment as a fixed effect (Sauvant *et al.*, 2008). Experiments included in the model had a within-experiment variation of protozoal concentration higher than 5.3 $\log_{10}$ cells/mL (2.2 x 10^5/mL) corresponding to the mean s.e.m. of the database for this variable. The influence of potential qualitative and quantitative secondary factors on parameters of the model (slopes, LSMeans, residuals) was tested. Relevant significant factors were finally tested in the model. All statistical analyses were carried out using the GLM model (Minitab, version 16, State College, PA).

Results CH_4 emissions were similar (P=0.365) between animal species and averaged 18.9 ± 5.6 g/kg DMI for cattle, sheep and goat (n_{exp}=67). A significant reduction of both CH_4 emissions and protozoal concentration was observed in 20% of experiments, most of them using lipids. A significant reduction of CH_4 without variation of protozoal concentration was reported in 43% of experiments, most of them using chemical components or essential oil. No variation of CH_4 and protozoal concentration was observed in 28% of experiments, most of them testing the effect of different forage sources. No variation of CH_4 when protozoa decreased was reported in 9% of experiments, most of them testing experimental defaunation. In the model using experiments with a reliable within-experiment variation of protozoal concentration, the average protozoal concentration was 5.9 ± 0.4 $\log_{10}$ cells/mL (10.7 ± 9.4 ×10^5/mL). Within a protozoal concentration ranging between 4.6 and 6.8 $\log_{10}$ cells/mL (0.4 to 63.1 × 10^5/mL), the response law between CH_4 emission and protozoal concentration was linear: CH_4 (g/kg DMI) = -16.6 (s.e. 9.07) + 5.77 (s.e. 1.53; P<0.001) × protozoa ($\log_{10}$ cells/mL) with n_{exp}=25, n_t=75, r.m.s.e.=2.97 and r^2_{adj}=0.75. The model was not influenced by animal species, CH_4 mitigation strategy and CH_4 method of measurement. However, butyrate molar proportion of the rumen volatile fatty acids was significantly correlated with CH_4 LSMeans (P=0.012) and residuals (P=0.016). This shows that for a given level of protozoa, butyrate could partly explain differences in CH_4 between experiments. This factor was thus significant when included in the model instead of experiment effect, leading to the equation: CH_4 (g/kg DMI) = -27.4 (s.e. 10.04; P<0.001) + 6.26 (s.e. 1.77; P<0.001) × protozoa ($\log_{10}$ cells/mL) + 0.778 (s.e. 0.305; P<0.05) × butyrate (mol/100mol) with n_t=64, r.m.s.e.=5.24 and r^2_{adj}=0.27. This meta-analysis indicates that CH_4 emissions are regulated by both protozoal concentration and rumen butyrate which is preferentially produced by protozoa (Brossard *et al.*, 2004).

Conclusions In this database, a reduction in protozoal concentration by lipids or plant extracts always leads to a reduction in CH_4 emissions. The meta-analysis also revealed that for a same change in protozoal concentration, CH_4 emissions are lower when butyrate proportion in the rumen decreases. Nevertheless, protozoal concentration is not the only explanatory factor of CH_4 emissions, as shown by experiments testing chemical components.

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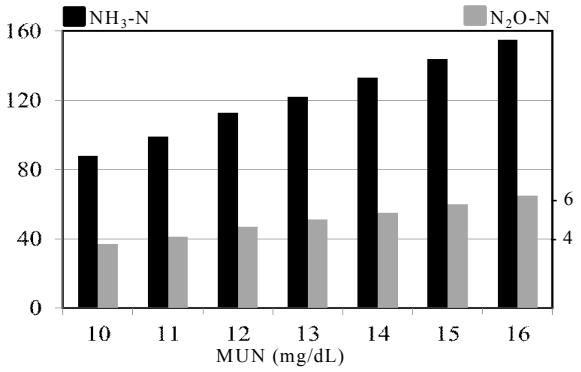
Estimating ammonia and nitrous oxide emissions from dairy farms using milk urea nitrogen

J M Powell[1], M A Wattiaux[2], C A Rotz[3]
[1]USDA-ARS, Madison, Wisconsin, USA, [2]University of Wisconsin, Madison, Wisconsin, USA, [3]USDA-ARS, University Park, Pennsylvania, USA *Email:mark.powell@ars.usda.gov*

Introduction Dairy farms emit ammonia (NH_3) from barns, manure storage and soils, which can be hazardous to human and ecosystem health. Emissions of NH_3 also contribute indirectly to emissions of nitrous oxide (N_2O), a potent greenhouse gas. Direct N_2O emissions occur mostly from soil after application of fertilizer, manure and other nitrogen (N) sources. Urinary urea N (UUN), the most labile N component of manure, is a principal N source of both NH_3 and N_2O emissions from dairy farms. The type and amount of crude protein (CP) fed to a dairy cow impact UUN excretion and therefore NH_3 and N_2O emissions. Recent analyses (Powell *et al*., 2011) showed that the relationship between milk urea N (MUN) and UUN can be used to predict relative NH_3 emissions from dairy barns. The objective of this study was to evaluate the efficacy of using MUN to estimate direct and indirect N_2O emissions from dairy farms in Wisconsin (WI), USA.

Materials and methods MUN records (37,889 cows; 197 herds in WI) over 2 years (2010-11) from the database of AgSource, Cooperative Resource International, a provider of Dairy Herd Improvement (DHI) services were partitioned into five MUN categories: ≤10, 11-12, 13-14, 15-16 and >16 mg/dL. UUN excretion (g/cow/day) was calculated from the equation UUN = MUN *16.23-34.2 (R^2=0.79) derived from 9 lactation trials which included 37 typical diets fed to lactating cows in WI (Wattiaux *et al*., 2011). UUN loss as NH_3 and direct N_2O loss from barns, manure storage and soils were calculated for dairy farms having free stall barns, tie stall barns, and pasture-based farms using the Integrate Farm System Model (IFSM; USDA-ARS, 2012), and indirect N_2O-N emissions were estimated to be 1% of NH_3-N emissions according to the Intergovernmental Panel on Climate Change (IPCC, 2006).

Figure. 1. Estimated N loss (g/cow/day) as NH_3 and N_2O from dairy farms in Wisconsin, USA using relationship between milk urea N (MUN) and urinary urea N (UUN), and calculated UUN loss using the IFSM model.



Results Approximately 23, 24, 23, 16 and 14% of all cows had MUN concentrations (mg/dL) of ≤10, 11-12, 13-14, 15-16 and >16, respectively. Given that a MUN level of 10 mg/dL reflects adequate dietary CP [approx. 165 g/kg of total dry matter intake (DMI)] for high milk production (approx. 9,200 kg/cow/year), these MUN frequency results suggest that about 77% of the surveyed cows consumed dietary CP in excess of requirement. Percentage UUN loss as NH_3-N ranged from 40% (pasture-based farms) to 84% (tie-stall farms using stacked manure storage). Percentage UUN loss as direct N_2O-N ranged from 1.4% when manure was hauled daily on either free stall or tie stall farms to 3.7% from pasture-based farms. Whole farm (barn, manure storage and land application) NH_3-N losses (g/cow/day) were lowest (range of 61 for pasture-based to 86 for free stall) at MUN levels of 10 mg/dL and highest (107 for pasture-based to 172 for tie stall) at MUN levels of 16 mg/dL. Relationships between MUN and total (direct plus indirect) N_2O loss (g/cow/day) were similar, but varied by production system: lowest (3.1 from tie stall to 5.0 from pasture-based) at MUN levels of 10 mg/dL and highest (5.5 from tie stall to 8.8 from pasture-based) at MUN levels of 16 mg/dL. Using the approximate proportion of WI dairy cows on free stall farms (50%), tie stall farms (40%) and pasture-based farms (10%), each decrease of 1 mg/dL in MUN in the range of 16 to 11 mg/dL would result in state-wide decreases in total N emission of approx. 12%, or 15 g of NH_3-N plus N_2O-N/cow/day (Figure 1). Each unit decrease in MUN can be achieved by reducing dietary CP concentrations by approx. 6 g/kg DMI.

Conclusion MUN is used by dairy nutrition consultants and farmers to monitor dietary CP consumption by lactating dairy cows. MUN above 10 mg/dL indicates dietary CP consumption in excess of requirements, which increases feed costs. Elevated MUN levels also indicate increases in UUN excretion, which is the principal source of NH_3 and N_2O emissions from dairy farms. Substantial reductions in NH_3 and N_2O emissions from dairy farms can be obtained through manipulation of dietary CP, which can be monitored and adjusted using MUN.

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Use of stable isotope tracing shows that acetate is the dominant hydrogen sink in the forestomach of the Eastern Grey Kangaroo (Macropus giganteus)

S Godwin[1], A Kang[1], L M Gulino[1,2], M Manefield[3], M L Gutierrez-Zamora Jimenez[3], M Kienzle[1], D Ouwerkerk[1,2], A V Klieve[2,1]
[1]Department of Agriculture, Fisheries and Forestry, Queensland, Brisbane, Queensland, Australia, [2]University of Queensland, Gatton, Queensland, Australia, [3]University of New South Wales, Sydney, New South wales, Australia
Email:a.klieve@uq.edu.au

Introduction The microbial utilisation of hydrogen (hydrogenotrophy) is essential to maintain normal function of the microbial ecosystem in enteric fermentation systems. In the rumen, the dominant hydrogenotrophic process is methanogenesis, but alternate hydrogenotrophic pathways which do not result in the production of greenhouse gas appear to dominate in some other gut fermentation systems. Kangaroos ferment forage material in an enlarged forestomach analogous to the rumen, but in contrast to ruminants, they produce little or no methane[1]. The objective of this study was to use stable isotope tracing techniques to identify the dominant organisms and pathways involved in hydrogenotrophy in the kangaroo forestomach, with the broad aim of understanding how these processes are able to predominate over methanogenesis.

Material and methods Experiment 1: A RNA stable isotope probing (RNA-SIP) approach was used to identify organisms capable of using carbon dioxide and hydrogen in the kangaroo forestomach. A sample of the forestomach content of a single eastern grey kangaroo (*Macropus giganteus*) was used to inoculate anaerobic media containing 100 mM isotopically labelled (^{13}C) bicarbonate as the only carbon source. This inoculated media was incubated at 39 °C in 20 mL Hungate tubes, with the headspace filled with 206 kpa $^{13}CO_2/H_2$. Total RNA was extracted from the samples after 16 hours incubation. Isotopically labelled ('heavy') RNA was separated from unlabelled ('light') RNA by isopycnic centrifugation in caesium trifluoroacetate (CsTFA)[2]. The RNA recovered from both 'heavy' and 'light' fractions of CsTFA gradients was characterised using 454-amplicon pyrosequencing of the V3/V4 region of the bacterial 16S rRNA. χ^2 analysis was used to identify bacterial operational taxonomic units (OTUs) that were statistically significantly more abundant in the 'heavy' fractions. The natural abundance of these OTUs was investigated by querying a database of 16S rRNA gene sequences obtained in a previous survey of the bacterial diversity of the kangaroo forestomach and bovine rumen[3]. Experiment 2: A separate experiment was conducted to track the chemical fates of carbon dioxide in simulations of three different enteric fermentation systems. Forestomach contents of three kangaroos (grazing native pasture) and rumen contents of three grass fed cattle and four grain fed cattle were diluted in media spiked with 5 mM ^{13}C-labelled bicarbonate and incubated in closed 500 mL wheaton bottles at 39 °C for seven days. 2.0 mL subsamples of the gas headspace and fluid portion of each incubation were taken at 0, 3, 6, 24, 48 and 168 hours post inoculation and concentration and carbon isotope ratios ($\delta^{13}C$) of methane, acetate, propionate, butyrate and valerate were determined by gas chromatography-combustion-isotope ratio mass spectrometry (GC-c-IRMS). The data were analysed using repeated measures ANOVA to identify differences between kangaroos, grass fed cattle and grain fed cattle.

Results Experiment 1: RNA-SIP followed by amplicon pyrosequencing and χ^2 analysis identified a total of 19 bacterial OTUs that were statistically significantly associated with CO_2/H_2 metabolism. One of these OTUs was closely related (>97% 16S rRNA gene sequence similarity) to the known reductive acetogen *Blautia coccoides*. The remaining OTUs included members of the genera *Prevotella, Ocillibacter* and *Streptococcus* spp. which have not previously been reported as being capable of CO_2/H_2 metabolism. Close relatives of many of these OTUs were found in high abundance in the forestomach of all macropod species surveyed, and were also present in the rumen of grain fed cattle, but were absent or present in much lower numbers in the rumen of grass fed cattle. Experiment 2: Bovine rumen fermentations produced strongly ^{13}C-labelled methane within 6-24 hours of inoculation. In contrast, kangaroo forestomach fermentations produced a measurable quantity of methane only after 168 hours, and this methane contained statistically significantly ($P<0.05$) less ^{13}C label than in rumen fermentations, indicating that methanogenesis in kangaroos is not only less active than in ruminants, but also occurs via a different biochemical pathway. The acetate produced in kangaroo forestomach fermentations was strongly labelled with ^{13}C, while the acetate produced in bovine rumen fermentations contained statistically significantly ($P<0.05$) less label. This result indicates that reduction of carbon dioxide to acetate is more active in the kangaroo forestomach than in the bovine rumen. No significant differences in ^{13}C incorporation into fermentation end products were observed between grass-fed and grain fed cattle.

Conclusions Together the results presented provide very strong evidence that the activity of bacterial reductive acetogens is a key factor in the reduced methane output of kangaroos. The kangaroo forestomach therefore provides an instructive model system that can yield insights into ways to modify rumen function to reduce greenhouse gas emissions and improve animal productivity.

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Is there a relationship between genetic merit and enteric methane emission rate of lactating Holstein dairy cows?

L F Dong[1,2], T Yan[1], C P Ferris[1], A F Carson[1], D A McDowell[0,2]
[1]Agri-Food and Biosciences Institute, Hillsborough, Co Down, Northern Ireland, UK, [2]Faculty of Life and Health Science, University of Ulster, Shore Road, Newtownabbey, Co Antrim,Northern Ireland, UK *Email:lifeng.dong@afbini.gov.uk*

Introduction Genetic selection for milk production is widely considered to be a cost-effective way to reduce CH_4 emissions from dairy cattle. There is evidence that dairy cows with a high genetic merit for milk yield partition more energy into milk and less into body tissue than cows with a low genetic merit for milk traits (Yan *et al.*, 2006) and consequently produce less CH_4 per kg of milk and feed intake (Yan *et al.*, 2010). The objective was to use a large calorimeter dataset for lactating Holstein dairy cows with a wide range of milk production potentials to investigate if there is any relationship between genetic merit and enteric CH_4 emission rate.

Material and Methods The dataset (n=736) used was collated from 31 calorimeter studies with lactating Holstein cows conducted at this Institute since 2000. The dataset was derived from cows with a wide range in parity (1–9), live weight (384–733 kg), DM intake (6.5–25.0 kg/d), milk yield (1.0–49.1 kg/d) and CH_4 output (138–598 g/d). The cow genetic merit was defined as either Profit Index (PIN) or Profitable Lifetime Index (PLI). These two UK economic indexes are calculated from the financial increase in revenue per lactation, with PIN based on milk production while PLI includes both milk production and functional traits such as fertility, health and longevity. The effects of cow genetic merit on CH_4 emissions were evaluated using the whole dataset of PIN (n=736) or PLI (n=407) by regression of PIN or PLI against CH_4 emissions as a proportion of feed intake or milk production. In addition, effects of cow genetic merit were examined by dividing each dataset of PIN (>£15 (n=265), £15–3 (n=236), <£3 (n=235)) and PLI (>£67 (n=42), £67–23 (n=86), <£23 (n=280)) into 3 subsets categorised as high, medium and low genetic merit, with these representing the top 20%, top 20–50% and bottom 50% of the UK Holstein cow population, respectively. These sub-sets were used to examine if the coefficients of the linear regression of CH_4 energy output (CH_4-E) as a proportion of GE intake (GEI) against GEI between the 3 sub-datasets differed within the PIN or PLI dataset, when a common constant was used in each set of the 3 relationships. The linear regression was undertaken using mixed models with experiment code as blocks to remove effects of variation of individual studies.

Results The values for PIN (£) ranged from -54 to 63 with a mean of 16, and the corresponding values for PLI (£) were -131, 145 and 51, respectively. The statistical analysis demonstrated that with the whole dataset of PIN, there was no relationship between PIN and any proportional CH_4 value, in terms of CH_4 output per kg of feed intake (DM, OM, digestible DM or digestible OM); or CH_4 output per kg of milk yield or energy corrected milk yield; or CH_4-E as a proportion of GEI (Fig. 1), DE intake, ME intake or milk energy output. When the PIN dataset was divided into 3 sub-datasets representing low, medium and high genetic merit, with a common constant, there was no significant difference between the 3 coefficients in the linear relationship between CH_4-E/GEI and GEI (Table 1), CH_4-E/DE intake and DE intake or CH_4-E/ME intake and ME intake. Similar results were also observed with the whole dataset of PLI and 3 sub-datasets of PLI. These results indicate that there is no direct relationship between genetic merit (PIN or PLI) and CH_4 emission rates with lactating Holstein dairy cows.

Table 1. Relationships between CH_4-E/GEI and GEI with groups of cows of low to high genetic merits

Sub-dataset	Equations		R^2
PIN < £3	CH_4-E/GE intake =	-0.000086 GE intake + 0.09	0.53
PIN £3-15		-0.000083 GE intake + 0.09	
PIN >£15		-0.000080 GE intake + 0.09	
PLI <£23	CH_4-E/GE intake =	-0.000085 GE intake + 0.09	0.59
PLI £23-67		-0.000088 GE intake + 0.09	
PLI >£67		-0.000078 GE intake + 0.09	

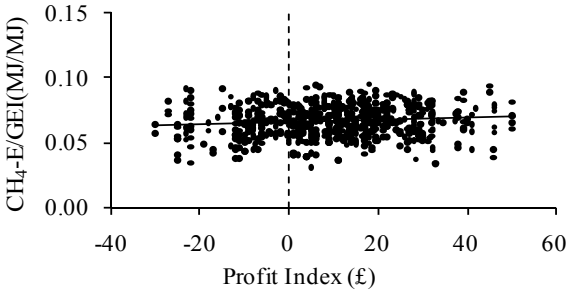

Figure 1. Relationship between CH_4-E/GEI and PIN

Conclusions The present study demonstrated that there was no relationship between genetic merit and CH_4-E/GEI, or any other proportional CH_4 emission factor in Holstein dairy cows. This result indicates that the lower CH_4-E/GEI with high yielding cows, compared with low/medium yielding cows, is most likely due to their higher feed intake and corresponding high rumen outflow rate, rather than differences in rumen methanogenesis potential. The proportion and type of rumen methanogenesis bacteria may be mainly influenced by feed intake and dietary feed composition.

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Genetic parameters for methane emissions predicted from milk mid-infrared spectra in dairy cows

P B Kandel[1], M L Vanrobays[1], A Vanlierde[2], F Dehareng[2], E Froidmont[2], P Dardenne[2], E Lewis[3], F Buckley[3], M H Deighton[3], S McParland[3], N Gengler[1], H Soyeurt[1]
[1]University of Liège, Gembloux Agro-Bio Tech, Gembloux B-5030, Belgium, [2]Walloon Agricultural Research Center, Gembloux B-5030, Belgium, [3]Teagasc, Moorepark, Co. Cork, Ireland *Email:pbkandel@ulg.ac.be*

Introduction Genetic selection of low CH_4 emitting animals is additive and permanent, unlike nutritional and other management strategies which may require continual investment. However, difficulties associated with individual CH_4 measurement result in a paucity of records to estimate the phenotypic and genetic variability of CH_4 traits. Direct quantification of CH_4 emissions by mid-infrared spectroscopy (MIR) from milk samples is possible (Dehareng *et al.* 2012). Such MIR spectrometry is currently used in the Walloon milk recording to generate a large number of phenotypes. The aim of this study was to estimate the phenotypic and genetic variation of CH_4 emissions directly predicted by MIR and its relationship with milk production traits in Holstein cows.

Material and Methods The equation used to predict the CH_4 traits was described by Vanlierde *et al.* (2013). In summary, 452 SF_6 CH_4 measurements and milk spectra were collected and, using PLS regression, the calibration equation was developed. The s.d. of predicted CH_4 was 126.39 g/day with a standard error of cross validation 68.68 g/day and a cross-validation coefficient of determination equal to 70%. Methane g/kg of fat protein corrected milk (FPCM) was obtained by dividing methane emission g/day by FPCM yield. This equation was applied to a total of 679,444 spectra obtained from milk samples collected between January 2007 and August 2012 within the Walloon milk recording of Holstein cows (parity 1-3). Single trait random regression test-day models were used to estimate the genetic variability of the MIR CH_4 indicators and other milk traits as follows:

$$y = X\beta + Q(Zp + Zu) + e$$

where y was the vector of observations for each trait, β was the vector of fixed effects, p was the vector of permanent environmental random effects, u was the vector of additive genetic effects; Q was the matrix containing the coefficients of 2nd order Legendre polynomials; e was the vector of residuals; X and Z were incidence matrices assigning observations to effects.

Results Descriptive statistics of MIR CH_4 indicators and production traits are summarized in Table 1.

Table 1 Descriptive statistics of MIR CH_4 and production traits of first three parities Holstein cows

Traits	Parity 1 (n = 338,917)	Parity 2 (n = 221,420)	Parity 3 (n = 119,107)
MIR CH_4 (g/day)	547 ± 111	559 ± 112	558 ± 114
MIR CH_4 /(g/kg of FPCM)	23.66 ± 8.21	21.51 ± 8.53	20.37 ± 8.56
FPCM (kg/day)	23.98 ± 5.64	27.58 ± 7.50	29.32 ± 8.27
Fat yield (kg/day)	0.93 ± 0.23	1.08 ± 0.31	1.16 ± 0.35
Protein yield (kg/day)	0.79 ± 0.19	0.91 ± 0.24	0.95 ± 0.26

Heritability estimates were 0.12 ± 0.005, 0.10 ± 0.005 and 0.09 ± 0.007 for CH_4 g/day; and 0.18 ± 0.007, 0.12 ± 0.008 and 0.14 ± 0.011 for CH_4 g/kg of FPCM in the first three lactations respectively. The phenotypic and genetic correlation between MIR CH_4 indicator traits and milk traits in first parity are presented in Table 2.

Table 2 Phenotypic (below diagonal) and genetic (above diagonal) correlations between MIR CH_4 and production traits

Traits	MIR CH_4 (g/day)	MIR CH_4 (g/kg of FPCM)	FPCM	Fat yield	Protein yield
MIR CH_4 (g/day)		0.42	0.03	0.19	0.04
MIR CH_4 (g/kg of FPCM)	0.59		-0.83	-0.63	-0.78
FPCM	-0.01	-0.74		0.87	0.93
Fat yield	0.03	-0.68	0.95		0.70
Protein yield	0.02	-0.70	0.94	0.82	

Conclusions Estimated heritability for CH_4 g/day and CH_4 g/kg of FPCM were relatively low compared to common production traits but could still prove useful in breeding programs. While selection for cows emitting lower amount of MIR CH_4 (g/day) would have little impact on milk production traits, selection on MIR CH_4 (g/kg of FPCM) would decrease FPCM, fat and protein yields. These genetic parameters of CH_4 indicator traits may potentially be a starting point for selection that takes mitigation of CH_4 from dairy farming into account.

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Heritability for enteric methane emission from Danish Holstein cows using a non-invasive FTIR method

J Lassen, P Løvendahl
Aarhus University, Aarhus, Denmark *Email:jan.lassen@agrsci.dk*

Introduction Enteric methane emission from ruminants contributes substantially to the greenhouse effect. Few studies have focused on the genetic variation in enteric methane emission from dairy cattle. One reason for that is the limited number of methods appropriate for large scale phenotyping to measure a sufficient number of animals available to estimation of additive genetic variance. A method to measure methane in dairy cattle using a Fourier Transformed Infrared (FTIR) approach during milking in Automatic milking systems was implemented by Lassen *et al*. (2012). Such data showed repeatability estimates around 0.40 for the ratio between methane and carbon dioxide concentrations. Using the ratio between methane and carbon dioxide as a phenotype makes it possible to quantify the amount of methane produced per cow, because the amount of carbon dioxide can be estimated from variables such as weight, milk production and feed intake (Madsen *et al*., 2010). In a study of 548 heifers a heritability estimate of 0.35 was obtained for predicted methane emission based on registrations on feed intake rather than on direct measurements (de Haas *et al*., 2011). Estimates of this magnitude justify the use of genetic tools to reduce methane emission from dairy cattle. Another study (Wall *et al*., 2010) has shown that selecting for correlated indicator traits such as productivity and efficiency would help lowering the methane emission from the cattle production. Furthermore, it is still important to have emphasis on production traits through use of a total merit indexes to avoid a decline in economically important traits when reducing methane emission. However, key genetic parameters are still inaccurate and would therefore benefit from being re-estimated on larger numbers of animals and records based on reliable direct methods. The objective of this study was to estimate the heritability for enteric methane emission from Danish Holstein cows using a non-invasive method.

Materials and methods On a total of 683 dairy cows a Fourier Transformed Infrared (FTIR) measuring unit was used to make large scale individual methane emission records (Madsen *et al*., 2010). The cows were measured in 7 herds during their visits to automatic milking systems (AMS). The FTIR unit air inlet was mounted in the front part of an AMS close to the cows head for 7 days, recording continuously every 5 seconds (Lassen *et al*., 2012). All cows were within 365 days from calving when measured. Mean number of days in milk was 158 with a s.d. of 97.9. The phenotype analysed was the mean methane to carbon dioxide ratio across visits during the measuring period, as this ratio reflects the proportion of the metabolizable energy exhaled as methane.
The linear mixed model included fixed effects of herd, month, days in milk, lactation number, and random effects of animal and residual. Variance components were estimated using a REML approach in an animal model design with a pedigree containing 9661 animals.

Results The heritability of the methane to carbon dioxide ratio was moderate (0.21) but significantly different from 0 (s.e.=0.08). All systematic effects in the model had significant effect on the methane/ carbon dioxide level. Mean methane/carbon dioxide ratio was 0.059 with a s.d. of 0.0089 ranging from 0.018 to 0.11. Mean number of visits during a week was 18.3 and ranged from 10 to 42. The accuracy of this and similar method where measuring during milking has earlier been validated by comparing results with data from chambers (Garnsworthy *et al*., 2012; Madsen and Bertelsen 2012) and estimating repeatability between visits (Lassen *et al*., 2012). The results from this study suggest that individual cow's methane emission can be accurately measured using FTIR equipment and that the trait is moderately heritable.

Conclusions Enteric methane emission from dairy cows is under genetic control. It is concluded that FTIR breath analysis is effective for measuring GHG emissions and may find further applications with a wider panel of gases including acetone and its relation to ketosis. The data can be used for both management and genetic analysis and opens for future studies on the correlation between methane emission and other traits of economic importance as well as the possibility to use methane as an indicator of feed efficiency.

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Effect of soil moisture status, animal treading and a nitrification inhibitor on ammonia oxidizers and N_2O emissions

H J Di[1], K C Cameron[1], B C Ball[2], A Podolyan[1], J Z He[3]
[1]Lincoln University, Christchurch, New Zealand, [2]SRUC, Edinburgh, UK, [3]Chinese Academy of Sciences, Beijing, China
Email:hong.di@lincoln.ac.nz

Introduction Nitrous oxide (N_2O) is a potent greenhouse gas with a long-term global warming potential about 296 times that of carbon dioxide (CO_2) (IPCC, 2007). In grazed grassland, most of the N_2O is emitted from nitrogen (N) excreted by the grazing animal, particularly in the animal urine. When the soil is wet, such as that in winter grazing conditions, animal grazing can cause soil structural damage, leading to soil compaction. The combination of a wet soil plus soil compaction is particularly conducive for N_2O production. A nitrification inhibitor technology using dicyandiamide (DCD) has been developed to reduce N_2O emissions from grazed grassland. However, the efficacy of this technology under wet and compact soil conditions has not been well studied. The objectives of this study were to determine: (1) The impact of soil moisture content on the abundance of ammonia oxidizers and N_2O emissions; (2) the impact of animal treading on N_2O emissions; and (3) The effectiveness of the nitrification inhibitor DCD in reducing N_2O emissions, as affected by soil moisture status and animal treading.

Materials and methods A laboratory incubation study was conducted to determine the effect of soil moisture status on the abundance of ammonia oxidizers and N_2O emissions using a grassland soil (Horotiu soil: Typic Udivitrand). Two sets of incubations were set up, one set for soil sampling to determine the ammonia oxidizer abundance and the other set for determining N_2O emissions using static chamber methods. Three soil moisture conditions were compared: 60%, 100% and 130% field capacity. For each moisture regime, the following treatments were applied: Control; Control + DCD (dicyandiamide nitrification inhibitor) at the equivalent rate of 10 kg ha^{-1}; Urine at 700 kg N ha^{-1} (simulating N application rate under a dairy cow urine patch in grazed grassland); Urine + DCD. The incubation vesicles were incubated at a constant 12 ^{o}C to simulate late autumn conditions in New Zealand when DCD was applied to reduce nitrate leaching and N_2O emissions. Soil samples were collected to determine mineral N concentration and the abundance of ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA) using real-time PCR (Di *et al*., 2009). N_2O emissions were determined using gas chromatography (GC). A field experiment was conducted to determine the impact of animal treading on N_2O emissions on a Wakanui sandy loam (Aquic Dystric Utrochrept) (Ball *et al*., 2012). Field plots of 0.5 m diameter were established to simulate dairy cow urine patches. Dairy cow urine at the rate of 1000 kg N ha^{-1} was applied to the plots to simulate animal urine deposition. The nitrification inhibitor DCD was applied to some of the plots at 10 kg ha^{-1}. Some plots were un-trampled, and some were trampled with a mechanical hoof delivering the same pressure as that of an adult cow hoof walking over the field. N_2O emissions were determined using static chamber methods.

Results The laboratory incubation study showed that soil moisture content was a major driver affecting the growth of ammonia oxidizing bacteria (AOB) and N_2O emissions in the soil that received animal urine. Total N_2O emissions from the soil at 130% soil moisture holding capacity (MHC) were 400 times higher than those from the soil at 60% WHC. Total N_2O emissions were significantly related to the abundance of AOB *amoA* gene copy numbers but not to that of the AOA. The field plot study showed that animal treading of a wet soil resulted in a reduction in air permeability and air-filled pore space in the top 5 cm soil layer, and led to significant increases in N_2O emissions. Trampling increased average cumulative N_2O emissions from 1.74 to 4.66 % of urine applied N. DCD was highly effective in reducing N_2O emissions, with N_2O emissions being decreased by 58-63%. Trampling did not significantly affect the effectiveness of DCD in reducing N_2O emissions. These reductions are similar to those that have been reported previously (e.g. Di *et al*., 2010).

Conclusions Soil moisture status and animal treading are critical factors affecting N_2O emissions and the total N_2O emissions were quantitatively related to the abundance of AOB but not to that of AOA. The DCD nitrification inhibitor is an effective mitigation technology for N_2O emissions under both trampled and un-trampled soil conditions, thus showing the potential of this mitigation technology for wet and heavily trampled winter grazing conditions.

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Impact of surface applications of dicyandiamide on nitrous oxide emissions from bovine urine patches in south-western Victoria, Australia

K B Kelly[1], G N Ward[1], J Hollier[1], R J Eckard[3,2]
[1]Department of Primary Industries, Victoria, Australia, [2]University of Melbourne, Victoria, Australia, [3]Primary Industries Climate Challenge Centre, Victoria, Australia *Email:kevin.kelly@dpi.vic.gov.au*

Introduction Applying the nitrification inhibitor dicyandiamide (DCD) as a surface spray to soil/pasture has been shown to reduce nitrous oxide (N_2O) emissions from urine deposited by grazing livestock (Di and Cameron 2002), with repeated applications of DCD having no effect on efficacy (de Klein *et al.* 2011). We conducted two experiments to address how long a single application of DCD was effective in reducing N_2O emissions from urine patches deposited at a series of times typical of dairy rotational grazing systems.

Materials and methods Experiments were conducted in south-western Victoria, Australia (38°14'S, 142°55'E), on a brown chromosol (Isbell 1996) derived from quaternary basalt, one of the major soil types used for dairy production in the region. Long term annual rainfall is about 780 mm, with an estimated evapotranspiration of about 915 mm, and average daily air temperature ranging from 8.6°C in July to 18.0°C in January. The gas measurement system comprised a Fourier Transform Infrared Spectrometer (Bomem™ FTLA2000-100, ABB-Bomem, Quebec, Canada) with KBr optics, multipass white cell and InSb (1 mm Indium Antimonide photovolatic element) detector, linked directly to eight automatically controlled chambers (0.8 x 0.8 x 0.45 m high). Gas was collected from the chambers in sequence through the day with 5-6 measurement points per chamber per day. Flux was calculated from the change in N_2O density in the air within the chamber during the closure period (18 min). Chambers bases were divided into 4 parts with bovine urine at a patch rate of 1000 kg N/ha applied to 25% of the chamber base area after each grazing of the surrounding paddock. Two chambers had no urine applied (Nil), two had urine only applied (Urine), two had urine + DCD (10 kg a.i./ha) surface applied after the initial urine deposition (Urine+DCD), two had urine applied +DCD (10 kg a.i./ha) after each urine application (Urine+DCD*4). Where DCD was applied it was applied to the whole chamber base. In the first experiment urine was applied in 23 Sept, 21 Oct, 10 Nov, 7 Dec 2010. In the second experiment chambers were moved to an adjacent site with urine applied on 6 May, 2 June, 25 Sept, 15 Oct 2011. Initial urine application dates correspond to day 1 of each experiment. Surface soil water content and soil temperature at 50 mm were measured continuously within each chamber base using thetaprobes and thermocouples respectively.

Results A single application of DCD in early spring (Sept) reduced N_2O emissions by about 38%, with the application reducing emissions for about 70 days. A single application of DCD in late autumn (May) reduced N_2O emissions by about 42% with the application reducing emissions for about 90 days. Application of DCD after each urine deposition reduced N_2O emissions by 78-83%.

Table 1 Average soil temperature (°C), soil (0-65 mm) water content (mm^3/mm^3) and effect of treatments on N_2O emissions (kg N_2O-N/ha) over 4 consecutive 40 day periods of each experiment. Standard deviation of total emissions in parenthesis.

	Experiment 1 (day 1, 23 Sept 2010)						Experiment 2 (day 1, 6 May 2011)					
Per.	Soil temp	Soil water	Nil	Urine	Urine +DCD	Urine +DCD*4	Soil temp	Soil water	Nil	Urine	Urine +DCD	Urine +DCD*4
1	13.8	0.47	0.05	1.68	0.80	0.48	10.7	0.49	0.15	2.11	0.68	0.60
2	17.7	0.44	0.09	3.45	2.03	0.55	9.3	0.52	0.02	1.08	0.15	0.14
3	18.9	0.38	0.15	2.24	1.75	0.77	10.8	0.51	0.14	0.66	0.63	0.16
4	19.9	0.28	0.04	0.16	0.15	0.11	12.8	0.46	0.32	1.96	2.15	0.61
Total			**0.32** ±0.022	**7.53** ±0.347	**4.73** ±0.002	**1.91** ±0.452			**0.63** ±0.259	**5.81** ±0.284	**3.61** ±0.141	**1.52** ±0.170

Conclusions Applications of DCD to pasture in south-western Victoria significantly reduced N_2O emissions from urine patches, however reductions were less than those reported in New Zealand (Di and Cameron 2002, de Klein *et al.* 2011). A single application of DCD impacted on emissions for 70-90 days. DCD reduced N_2O emissions from urine deposited within 60-70 days of the DCD application. Repeated applications of DCD after each urination event resulted in about an 80% reduction in emissions, highlighting the potential of alternative DCD delivery systems, such as direct feeding to livestock (Ledgard *et al.* 2008) to target urine patches.

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A common protocol for measuring N_2O fluxes using chamber methods

C A M de Klein[1], M J Harvey[2], M A Alfaro[3], D R Chadwick[4], T J Clough[5], P Grace[6], K M Kelliher[7], P Rochette[8], R T Venterea[9]
[1]AgResearch, Invermay Research Centre, Mosgiel, New Zealand, [2]National Institute of Water and Atmospheric Research, Wellington, New Zealand, [3]INIA Remehue, Osorno, Chile, [4]School of Environment, Natural Resources and Geography (SENRGY), Environment Centre Wales, Deiniol Road, Bangor University, Bangor, UK, [5]Department of Soil & Physical Sciences, Faculty of Agriculture & Life Sciences, Lincoln University, Lincoln, New Zealand, [6]Queensland University of Technology, Brisbane, Australia, [7]AgResearch, Lincoln Research Centre, Lincoln, New Zealand, [8]Soils and Crops Research and Development, Agriculture & Agri-Food Canada, Quebec, Canada, [9]USDA-ARS, Soil and Water Research Management Unit, St Paul, MN, USA *Email:cecile.deklein@agresearch.co.nz*

Introduction For the last 30 years static chamber methodologies have been most commonly used to measure N_2O fluxes from agricultural soils. The main advantages of this technique are that it is relatively inexpensive, versatile in the field, and the technology is very easy to adopt. Consequently, this method underpins the majority of our knowledge and understanding of N_2O emissions, including the estimation of national emission inventories from agricultural soils and efficacies of potential mitigation practices. A recent review of N_2O emissions studies using chamber methodologies from around the world highlighted that there is large variation in chamber design, deployment and data analysis resulting in a range of data quality. This could affect the reliability of N_2O emission factors that are derived from these data and is one reason that limits comparisons of data across studies. At a pre-conference workshop of the GGAA in Banff, Canada, the need for standardised guidelines was widely recognised.

Approach In 2011 and 2012 the New Zealand Government, in support of the objectives of the Global Research Alliance on Agricultural Greenhouse Gases, funded New Zealand scientists to lead and participate in an international collaboration to progress the development of standard guidelines for the use of chambers to measure N_2O fluxes. At an initial workshop in New Zealand in May 2011, leading experts from Alliance member countries reviewed the current state of understanding of N_2O chamber methodologies. Agreement was reached on the broad content and structure of a guideline document for measuring N_2O emissions using chamber methodologies. Lead- and co-authors, and editors were also assigned and between May 2011 and November 2012 the team work closely together to write the guidelines.

Guideline content The First Edition of the Guidelines includes the following chapters:
1. Introduction (de Klein & Harvey; eds.) – sets the scene and purpose of the Guidelines.
2. Chamber design (Clough *et al*.) – discusses recommendations with a focus on static chambers. Design requirements seek to maximise flux detectability and minimise measurement artefacts (chamber biases) from poor design.
3. Chamber deployment (Rochette *et al*.) – provides good practice on how to *use* the chambers to achieve the acquisition of best quality data for emission estimation. In addition to the individual chamber deployment, there are recommendations within design plot experiments for group deployment, replication and for accompanying environmental measurements that should be made.
4. Air sample collection, storage and analysis (Kelliher *et al*.) – outlines best practice for analytical lab determination of N_2O gas samples, calibration requirements for optimal accuracy and how to assess adequate analytical precision.
5. Automated systems (Grace *et a*l.) – discusses the underlying requirements for their successful deployment. As a relatively new technology, there are a variety of design solutions that have been successfully deployed to date.
6. Data analysis (Venterea *et al*.) – provides guidance to allow selection of the most appropriate flux calculation method, how to best interpolate non-continuous measurements to obtain best estimates of emissions and emission factors.
7. Data reporting (Alfaro *et al*.) – describes the desirable minimum requirements for reporting N_2O results of chamber methodologies, to ensure that the soundness/robustness of the results can be verified, and derived emission factors (EF) and/or mitigation technologies can be reliably evaluated.
8. Health and Safety (Chadwick *et al*.) – summarises risks associated with all stages of chamber measurement from field deployment through to laboratory and subsequent analyses are discussed. Issues are identified and personal protective equipment (PPE) and hazard minimisation procedures which should be considered as a minimum when complying with institutional and national legislation.

This first version of the guidelines will be freely available to download from the Alliance website from early 2013 *http://www.globalresearchalliance.org/research/livestock/activities/knowledge/*.

Key issues A major discussion point that emerged was the difficulty of having to balance limited resources between the trade-offs of carefully measuring individual flux measurements *vs.* increasing the number of chambers and/or sampling occasions to account for spatial and temporal variability. Understanding the size of the uncertainties of each step of the chamber measurement approach and their relative impact on calculating cumulative emissions and emission factors will be of critical importance for balancing (limited) resources to achieve the best possible (most accurate) results. Proposed next steps are to i) conduct a thorough assessment of relative uncertainties in N_2O chamber flux measurements across a range of soil/climate/management combinations; ii) set up a moderated "N_2O chamber measurement wiki" site to collate emerging knowledge; and iii) establish a process for updating this first version of the Guidelines as new knowledge emerges.

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Effect of grassland renovation on the greenhouse gas budget of an intensive forage production system

C Ammann, J Leifeld, M Jocher, A Neftel, J Fuhrer
Agroscope ART, Zürich, Switzerland *Email:christof.ammann@art.admin.ch*

Introduction European grasslands used for animal grazing and forage production are usually considered as a net carbon sink on average (e.g. Soussana *et al.*, 2007). This carbon sequestration effect can be counteracted by the emission of methane (by grazing ruminants) and N_2O depending on the management and fertilisation intensity. In addition it has to be taken into account that intensive forage production grasslands are often subject to periodic renovation activities, including ploughing and reseeding, in order to maintain an optimum plant composition and high harvest yields. But grassland renovation represents a major disturbance of the perennial grass ecosystem that can lead to enhanced greenhouse gas emissions (e.g. Velthof *et al.*, 2010) . In the present study we investigated the greenhouse gas (GHG) budget of an intensively managed grassland field in Central Europe several years before and after a renovation was carried out.

Material and Methods Within the framework of the European flux network projects GreenGrass, CarboEurope and NitroEurope we have measured the GHG exchange and the carbon cycle on the field scale at the intensive grassland site Oensingen during 9 years (2002-2010). The site is located on the Swiss Central Plateau. The soil is classified as Eutri-Stagnic Cambisol developed on clayey alluvial deposits with clay contents between 42% and 44%. The vegetation is a grass-clover mixture dominated by *Lollium perenne* and *Trifolium repens*. The field was subjected to intensive management over the entire observation period with an average nitrogen input of 230 kg N ha^{-1} y^{-1} as mineral and organic fertiliser and 4–5 harvests (hay or silage) per year. Within the measurement period, one grassland renovation was performed at the end of the sixth year. The field was ploughed (to ca. 20 cm depth) in December 2007 and was reseeded in the following spring (May 2008). The total carbon budget (sequestration or loss) of the grassland soil corresponds to the net biome productivity (NBP) and was assessed by continuous measurement of the ecosystem CO_2 exchange using an eddy covariance system and by analysing the carbon import by manure application and the carbon export by harvest biomass removal (see Ammann *et al.*, 2007). N_2O fluxes were measured using stainless steel static chambers (side length: 300 mm, height: 250 mm). Up to eight chambers were automatically operated in the field and provided flux measurements at a regular interval of typically two hours (Flechard *et al.*, 2005). For the present study the emission data of all available chambers were averaged.

Results In the 6 years before the renovation, the annual carbon budget resulting from the summation of the individual carbon fluxes (net CO_2 exchange, import by manure, removal by harvest) was generally positive indicating a carbon accumulation (sequestration) in the grassland soil of about 100 g C ha^{-1} y^{-1} on average (Ammann *et al.*, 2009). Yet a considerable year-to-year variability could be observed showing a correlation with the harvest yield but also with soil moisture conditions. Years with high productivity (2002, 2004, 2007) showed highest carbon accumulation, while the year 2003 with extreme temperature and drought conditions showed an almost neutral carbon budget. Before the renovation, an average N_2O emission of about 1.5 kg N ha^{-1} y^{-1} was observed with annual values up to 2.6 kg N ha^{-1} y^{-1} (Ammann *et al.*, 2009), mainly as a result of large emission pulses following fertiliser applications (see Flechard *et al.*, 2005). In the other periods (cold season or growing phases) generally small fluxes in both directions, emission or deposition, were recorded.
In the 3 years following the renovation, large differences to the previous years were found. The carbon budget changed its sign from sequestration to carbon loss (-130 g C ha^{-1} y^{-1} on average). This was partly due to considerable respiration losses during the fallow phase (January-Mai 2008) between ploughing and reseeding, but the net carbon loss, although smaller, continued in the following two years (2009, 2010). In addition to this effect, the N_2O emission was also considerably enhanced after the renovation. On average it was about 3 times larger than before the renovation.

Conclusions Renovation (ploughing and reseeding) of an intensively managed permanent grassland field had a large impact on the carbon budget (CO_2 uptake/loss) as well as on the soil N_2O emission. It changed the grassland from a net carbon sink to a carbon source (for the following three years) and approximately tripled the annual N_2O emissions. It can be concluded that grassland renovation has a considerable potential for GHG emission that may offset the net carbon sink activity of grassland in undisturbed periods.

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Intake, total-tract digestibility and methane emission of Texel and Blackbelly sheep fed C4 and C3 grasses tested simultaneously in a temperate and a tropical area

H Archimede[1], M Rira[2], M Eugene[2], D P Morgavi[2], C Anais[1], F Periacarpin[1], B Calif[1], C Martin[2], C Marie-Magdeleine[1], M Doreau[2]
[1]INRA UR143, Petit bourg, France, [2]INRA UR1213, Clermont Ferrand, France *Email:harry.archimede@antilles.inra.fr*

Introduction Enteric methane (CH_4) emission from forages may be higher for tropical forages than for temperate ones (Archimède *et al*., 2011). One possible reason is the difference in plant structure associated to the C4 or C3 metabolism of tropical and temperate plants, respectively. Animal breed and environment could also be other explanatory factors (Martin *et al.*, 2010). In order to better know the origin of these differences in CH_4 emission, a sheep breed from the Caribbean and another developed in temperate conditions were used in two parallel trials in a tropical and a temperate area.

Material and methods Two 4 x 4 Latin squares have been carried out simultaneously in two sites (France, temperate, and West Indies, tropical) with two sheep breeds (Texel (T), temperate origin, and Blackbelly (B), tropical origin) and four hays from natural grasses (two C3 grown in France and two C4 grown in West Indies). High (H) and low quality (L) hays were tested within each type of grass; NDF and crude protein content in % dry matter (DM) of C3-H, C3-L, C4-H and C4-L were 58.6 and 13.4; 62.3 and 8.3; 74.3 and 12.0; 74.2 and 6.9, respectively. Sheep body weight (BW) was 46.9 and 59.2 for B and T in France and 48.2 and 40.3 kg for B and T in West Indies. DM voluntary intake, organic matter (OM) total-tract digestibility and enteric CH_4 emission (using the SF_6 method) were measured. Statistical analyses were performed using the mixed procedure of SAS with period, site, hay, breed, and the interactions between the 3 latter factors as fixed effects and animal as random effect. Statistical differences were declared significant when $P \leq 0.05$.

Results DM intake (g/kg BW/d) was similar in the two sites. Independently of site, C4 grass in particular C4-L had lower DM intake than C3 grass. A breed×site interaction was also observed with intake higher in B compared to T in West Indies whereas the opposite was registered in France. OM total-tract digestibility was significantly higher in West Indies compared to France (62.3 vs 59.4; P=0.0044). In contrast, no breed effect or breed×site interaction were observed on digestibility. H grass digestibility was higher than that of L grass The geographic site did not affect CH_4 emissions expressed per kg DM intake or per kg digestible OM intake (DOMI) but a breed×site interaction was registered with lower emission for B compared to T in West Indies whereas the opposite was registered in France. In France, CH_4 emissions were significantly higher with C4-H compared with C4-L diet.

Table 1 Voluntary dry matter intake (DMI), organic matter digestibility and methane emission per kg DMI and kg digestible organic matter intake (DOMI) of two sheep breeds fed four different forages in two simultaneous trials in France and the West Indies

Forage	C3-H		C3-L		C4-H		C4-L			
Breed	B	T	B	T	B	T	B	T	s.e.m.	P and effect of factors
Dry matter intake , g/kg BW/d										
France	21.8	28.9	25.2	26.3	17.0	16.8	15.1	12.9	1.87	<0.001 (Forage, breed x site)
West Indies	22.8	25.8	23.7	21.9	22.8	14.8	18.7	13.8		
Organic matter digestibility, %										
France	68.8	64.6	57.4	56.6	62.9	62.3	52.8	49.6	1.97	<0.001 (Forage, site)
West Indies	66.6	65.2	58.5	55.3	67.0	67.4	56.6	62.1		
Enteric methane, g/kg DMI										
France	23.9	16.6	19.0	15.6	24.0	20.1	16.9	13.8	2.63	0.001 (Forage, breed x site)
West Indies	12.2	18.9	15.8	18.0	18.4	27.1	18.1	27.0		
Enteric methane, g/kg DOMI										
France	39.1	29.2	35.8	29.6	42.8	35.3	33.8	30.5	4.79	0.01 (Forage, breed x site)
West Indies	20.5	32.5	29.3	35.3	29.8	44.2	34.1	47.3		

Conclusions In the West Indies DM intake of Blackbelly was higher than for Texel breed probably because they are adapted to warm climate and consequently they have a better thermoregulation. This trial confirms that CH4 emissions were generally lower for C3 than for C4 forages but the differences are linked to the grass quality.

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Estimates of methane emission from the camel (Camelius dromedarius) compared to dairy cattle (Bos taurus)

A Guerouali, F Z Laabouri
Agronomic and Veterinary Institute, Rabat, Morocco *Email:a.guerouali@iav.ac.ma*

Introduction Kill a camel to stop pollution? That is precisely what Australia is considering. The suggestion came from Northwest Carbon Pty Ltd (Tim Moore, 2010) to the Australian Department of Climate Change and Energy Efficiency indicating that a camel produces methane equivalent to one ton of carbon dioxide a year, making the animal one of the country's biggest greenhouse gas emitters. The Northwest company proposed the shooting of camels from a helicopter or rounding them up and send them to a slaughterhouse. As scientists working on camel physiology and human beings dealing with animal welfare and protection, we developed a trial to measure methane emissions in the camel and compared them to dairy cattle receiving the same amount of feed.

Materials and methods Seven Holstein cows (average weight 350kg) and seven she-camels (average weight 330 kg) were used in this study. All animals were not pregnant and in the latest stage of lactation with very limited milk production. Animals were housed in boxes and fed individually the same ration composed of 3kg of barley and 2kg of Lucerne hay daily at 9 a. m. After eating, methane emissions measurements were made for 2 to 3 hr by using a face mask open circuit system. Nitrogen gas (0methane) and span gas containing 1000 ppm of methane were flushed through the system daily to calibrate the analyzer. Before the beginning of the trial, the cows and camels were adapted to the diet and feeding schedule, the face mask and the noise of the aspirating pump. The 2 to 3 hr methane measurements were extrapolated to 24hr and expressed in liters per day, liters per kg of metabolic weight, liters per kg of dry matter intake and presented as means and standard deviations. The difference in methane production between cows and camels was tested using paired t-tests and considered significant when $P < 0.05$.

Results Methane production was measured with a face mask in both species 2 hr after feeding and was recorded continuously to examine the methane emission cycles. In the camel, there was an average of 18 emission cycles per hour corresponding to eructation number and most of the methane was emitted by eructation (90%) while the rest was eliminated through the expiration. In cattle, the number of emission cycles (corresponding to the eructation number) averaged 54eructation per hour. As with the camel, most of the methane was emitted by eructation but less than in camel (85%) while the rest was eliminated through respiration. Methane emission from the camel was estimated to average 66.64 liters per day corresponding to 15.2 liters per kg of dry matter intake while dairy cattle methane emissions was estimated to average 193.76 liters per day corresponding to 42.20 liters per kg of dry matter intake (Table 1). The present study showed clearly that dairy cattle produced three times more methane than camel when the two species received the same diet.

Table 1 Methane production presented as mean and standard deviations in camel and dairy cattle

Species	Methane production (Liters/day)	Methane production (Liters/Metabolic weight)	Methane production (Liters/kg of dry matter intake)
Camel	66.63 ± 8.47	0.74 ± 0.09	15.20 ± 1.93
Dairy Cattle	193.76 ± 50.51	2.00 ± 0.40	42.20 ± 11.56

Conclusion Methane production was measured in camel and dairy cattle receiving the same diet and the data indicated that dairy cattle produce three times more methane that camel. Some digestive and metabolic particularities of each species may explain the difference. Other solutions to reduce the greenhouse gases should be proposed than the eradication of the camel population of Australia.

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Resources efficiency comparison on beef production in Germany, Argentina and Brazil

T Fock[1], C Fuchs[1], C R Feldkamp[2], D J Bungenstab[3]
[1]Neubrandenburg University of Applied Sciences, Neubrandenburg, Germany, [2]Universidad de Concenpción del Uruguay, Concepción del Uruguay - Entrerios, Argentina, [3]Brazilian Agricultural Research Corporation - EMBRAPA, Campo Grande-MS, Brazil *Email:davi.bungenstab@embrapa.br*

Introduction Global demand for meat will increase in the coming years. For this reason, more resources will be needed for the growing production, and they must be allocated in the most efficient way. This work comparatively analyzes resources efficiency on beef production in Germany, Argentina and Brazil. For such, several typical production systems are defined for each country.

Material and methods In contrast to poultry or pork production, for beef production there are different systems coexisting, which are set to make the best use of the respective production factors in shortest supply, like area, feed and animals (Schwartz *et al*. 2011). In Argentina and Brazil beef systems are based on long grazing phases with high demand for land while finishing phase utilizes different feeding intensities and duration. In Germany weaned calves from cow-calf operations and Fleckvieh and Holstein male offspring are finished on intensive systems (Brüggemann, 2011). To comprehensively encompass resources usage and their effect on the environment (as for example climatic effects) or competitive relationships with alternative uses, like area, different indicators were used. A detailed assessment was made on greenhouse gases (GHG) emissions related to animals and industrial inputs over crops and pasture areas, regarding land use and feed efficiency. Calculation methodology for estimating embodied energy, CO_2-equivalent emissions and shadow areas follows the methodology and indicators generated by the adapted ecological footprint approach for agriculture (Bungenstab, 2005). Shadow areas correspond to a forest area that would be necessary for carbon sequestration of the equivalent GHG emissions generated by the manufacturing of all production inputs, as well as the emissions from fuels and other energy sources used in the system. Total emissions for each input were obtained from assessments of the manufacturing process or derivate from the embodied energy of each input. Shadow area is therefore the area of forest that would be necessary for permanently sequestering this total amount of carbon equivalent. For this work it was considered that one hectare forest would sequester 6600 kg CO_2 per year. Economic indexes like profit per product unit and per area were also calculated.

Results The assessment shows that in Argentina and Brazil, CO_2 equivalent emissions vary from 7 to 12 kg per kg beef with the lowest values on the intensive systems. In Germany emissions are lower (7 to 8 kg CO_2 eq per kg beef), except for the systems including intensive finishing of Holstein male offspring from the dairy sector (up to 10 kg CO_2 eq per kg beef). Regarding land use, values vary from 15 m^2 per kg beef from Fleckvieh intensive finishing in Germany to 45 m^2 from plain grazing finishing in Argentina. Also when comparing feed efficiency (measured in MJ ME per kg beef), intensive systems (with use of grain feed) show higher values of efficiency. Finishing based on pastures takes much longer, resulting on higher maintenance costs. In this regard, in South-American systems pasture proportions on feedstuffs ranges between 75 and 100%, while in Germany, even in the cow-calf operations with weaning calves, it reaches 55%. Also, in German intensive finishing operations, pastures are practically not used.

Conclusions This work indicates that differences in production systems might originate due to differences in resources availability, so that the respective shortest factors are used in the best economical way. Resources usage regarding environmental effects, as well as global food security, shows a different picture. Conclusions depend on the selected indicator. On one hand, the apparently evident result showing that resources efficiency increases with higher intensification is valid basically for the indicator of GHG emissions. On the other hand, an important aspect in indicators like area per kg of beef or feed consumption per kg of beef is the alternative use of land: in Germany there are some legal restrictions and in Brazil and Argentina, local environmental conditions might restrict alternatives land use other than pastures for cattle grazing. In such cases, extensive systems show higher resource use efficiency than intensive systems when assessed through area and feed utilization indicators.

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Nitrous oxide emissions from bovine urine and dung deposited on a Brachiaria pasture in the Brazilian Cerrado region

R M Boddey[1], A C de R Lessa[1], D da S Paredes[1], B E Madari[2], S Urquiaga[1], C P Jantalia[1], B J R Alves[1]
[1]Embrapa Agrobiologia, Seropédica, Rio de Janeiro, Brazil, [2]Embrapa Rice and Bean Research Centre, Santo Antônio de Goiás, GO, Brazil *Email:robert.boddey@embrapa.br*

Introduction In the central savannah (Cerrado) region of Brazil there are over 70 million head of beef cattle which are grazed principally on 50 to 55 million ha of *Brachiaria* pastures. Most of these pastures are rarely fertilized and forage quality and rates of live weight gain are low. According to IPCC Tier 1 procedures (IPCC, 2006) nitrous oxide (N as N_2O) emissions from cattle excreta on pastures are calculated as 2 % of total N deposited (Emission factor = 0.02) regardless of whether the N is deposited as urine or dung. On this basis it is estimated that total direct N_2O emissions from grazing animals amount to approximately 45 % of all N_2O emissions from the agricultural sector in Brazil. Cattle grazing forages of low quality and N content often excrete more N in dung than in urine so that the objective of this study was to evaluate emissions from both forms of excreta on a *Brachiaria* pasture in the Cerrado region in both the rainy and dry seasons.

Materials and methods The study was conducted on a *B. brizantha* pasture (lat. 16°30′S, 49°17′W) on a Ferralsol (Oxisol) at the Embrapa Rice and Bean Centre near Goiânia. The mean annual rainfall at this site is 1,460 mm which falls mainly in the summer months of October to April, months with extremely low rainfall in the dry season. Plots of 2.3 m^2 were arranged in a randomized complete block design with 6 replicates with the following treatments: 1 L bovine urine spread over an area of 0.24 m^{-2}, 1.6 kg fresh dung in 0.05 m^{-2} and a control without excreta. Each treatment was applied in the centre of the area delimited by the base of the static chamber used for N_2O measurements. The experiment was set up on 25 November 2009 and repeated in the pasture but always establishing new plots on 8 January 2010 and on July 15 July 2010. The static chambers consisted of a base of 40 cm x 60 cm of 17 cm height, inserted 5 cm into the soil, and left in place during the whole period. A lid with the same dimensions was coupled to the base at every sampling. Gas sampling was daily during the first month and then each three to seven days when N_2O fluxes were no longer responding to increased soil water content. Gas sampling, analysis and calculations were according to Alves *et al* (2012). Fluxes of N_2O were integrated for each of the three periods studied, and emission factors (EF) were calculated by subtracting the emission of the control treatment from that calculated for the treatments with cattle excreta.

Results During the rainy season the of soil water pore space saturation was frequently at 60 % or above and the application of excreta increased soil mineral N levels, especially under the urine patches. These conditions are favorable to the denitrification process and soil N_2O fluxes after the addition of urine and dung were above the background level monitored in the control plot. However, urine application ended up with significantly higher fluxes than the dung or control treatments during the first two weeks of monitoring, reaching values close to 11 mg N m^{-2} h^{-1} at the 6th day after the first application. During the dry season, the application of urine or dung did not induce significant soil N_2O fluxes. Only after the application of water as simulated rainfall of 20 mm, almost 30 days after treatment application, were any significant N_2O fluxes in the urine treatment registered, but at a relatively low intensity. The calculated EF for urine was significantly higher for urine than for dung, except in the case of the dry season when fluxes were almost zero unless induced by artificial irrigation.

Table 1 Amount of N in each excreta used as soil treatment of each period, and respective direct N-N_2O emission factors.

	Amount of N in applied excreta (g N chamber^{-1})			Direct N_2O emission factor (g N-N_2O/g N excreta)		
Treatment	Nov/09	Jan/10	Jul/10	Nov/09	Jan/10	Jul/10
Urine	10.1	6.4	9.5	0.0255 a	0.0131 a	0.0001 a
Dung	4.1	4.2	4.5	0.0011 b	0.0016 b	0.0000 a

*Mean EF followed by a same letter did not differ in the column according to the Scott-Knott test at 5% probability.

Conclusions
These data indicate that the emission factor for urine N cannot be considered the same as for dung, and for the Cerrado region should be approximately 1.2% for urine N and 0.10 % of N for dung considering rainy and dry seasons are of 7 and 5 months duration, respectively. In extensive grazing systems rarely more than 60% of the N is excreted as urine so if these data are typical for *Brachiaria* pastures in this region, the weighted emission factor would be 0.7% for cattle excreta instead of 2 % used by the IPCC (2006).

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Estimation of greenhouse gas emissions from beef production systems in the Canadian prairies using whole-farm models

A W Alemu[1], K H Ominski[1], M Tenuta[1], B Amiro[1], E Kebreab[2]
[1]University of Manitoba, Winnipeg, Manitoba, Canada, [2]University of Manitoba, Winnipeg, Manitoba, Canada, [3]University of Namitoba, Winnipeg, Manitoba, Canada, [4]University of Manitoba, Winnipeg, Manitoba, Canada, [5]University of California Davis, Davis, California, USA *Email:akliluwake@yahoo.com*

Introduction Measuring greenhouse gas (GHG) emissions from beef production is challenging as these are complex systems, composed of multiple interacting components (*e.g.,* animals, soil, and crops). A whole-farm approach of analyzing GHG emissions from a given production system, using whole-farm models, is able to capture the interrelationships among the different farm components. The objectives of this study were to: (i) estimate whole-farm GHG emissions intensity by integrating existing farm component models into an Integrated Components Model (ICM); (ii) compare estimates of GHG emissions from the ICM with other whole-farm models, and (iii) compare farm GHG emissions intensity estimates associated with changes in management strategy (*i.e.,* amount and time of hog slurry application).

Material and methods Whole-farm GHG emissions were assessed using a simulated cow-calf production system with a spring calving herd consisting of 150 cows, bulls, backgrounding steers and heifers, and calves from birth to weaning. Feedstuffs for the animals were comprised of home-grown forage, home-grown barley, imported soybean meal and minerals. The ICM and two extant whole-farm models, Integrated Farm System Model (IFSM, Rotz *et al.,* 2011) and Holos model (Little *et al.,* 2008), were used to estimate total farm GHG emissions from a cow-calf production system in the Canadian prairies. The ICM utilized components of COWPOLL (Dijkstra *et al.,* 1992), manure-DNDC (Li *et al.,* 2012) and some aspects of IPCC. Three management scenarios based on field experiment were simulated to investigate the impact of time and amount of manure application on total farm GHG emissions. The simulated management scenarios were i) baseline, no application of hog slurry on grassland; ii) split, application of imported hog slurry in fall and spring (50% of the manure is applied in fall and 50% in spring); and iii) single, application of imported hog slurry in spring (100% spring application). The imported hog slurry was surface applied to forage using a splash plate without incorporation. Emissions associated with hog slurry storage were not included. Primary (emissions produced on-farm during the production process) and secondary (emissions produced during the manufacture and transport of resources used on the farm) sources of emissions were considered in the analysis to estimate GHG intensity of the cow-calf production system. Carbon sequestration by the production system was also estimated.

Results Enteric CH_4 emissions were the primary source of total farm emissions in all scenarios for all models (43 to 74%, Table 1). Farm GHG emissions intensity per unit live weight leaving the farm gate, regardless of the models used, varied from 6 to 19, 14 to 54 and 15 to 54 kg CO_2 eq, respectively, for the baseline, split and single scenarios. Relative to the baseline scenario, application of hog slurry in split and single scenarios increased GHG emissions intensity due to the higher contribution of soil N_2O.

Table 1 Greenhouse gas emissions and farm emissions intensity for baseline, split and single scenarios estimated using Integrated Components Model (ICM), Integrated Farm System Model (IFSM) and Holos model.

Models	ICM			IFSM			Holos
Management scenarios	Baseline	Split	Single	Baseline	Split	Single	Baseline
	Emissions (Mg CO_2 eq)						
Enteric CH_4	688	655	692	664	660	688	640
Manure CH_4	81	85	91	34	68	69	36
Manure N_2O	46	43	38	13	24	27	212
Soil N_2O	80	700	516	177	598	604	91
CO_2 from farm energy use	39	54	48	71	43	63	65
Farm CO_2 flux	-251	-739	-578	-133	1621	1617	-704
Farm GHG intensity, kg CO_2 eq kg^{-1} live weight							
Including CO_2 from farm energy use	16.7	27.7	25.0	17.3	25.1	26.2	18.9
Including farm CO_2 flux	12.2	14.3	14.5	14.9	54.4	55.4	6.2

Conclusions The study demonstrates the potential to integrate extant process-based farm component models to estimate farm GHG intensity. Variation was observed among models both in estimating whole-farm GHG emissions intensity and the relative contribution of different GHG sources in the production system due to the differences in assumptions, approaches and algorithms used in the models. These differences could influence the key areas in the beef production system where management practices should be altered to improve the environmental efficiency.

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Development of a dynamic mechanistic model of dairy cattle capable of predicting milk production, dry matter intake and Body Condition Score change

E Ruelle[1,2], L Delaby[3], M Wallace[2], L Shalloo[1]
[1]Teagasc, Moorepark, Fermoy, Co.Cork, Ireland, [2]University College Dublin, Dublin, Ireland, [3]INRA, Saint Gilles, 35590, France *Email:elodie.ruelle@teagasc.ie*

Introduction Developing experiments to test the effect of various management practices at farm level are difficult and require a lot of time, labour and money in order to carry them out correctly. Modelling biological systems present significant opportunities to capture the interactions within systems while not realising all of the costs associated with conventional experiments. However there are many interacting components to biological systems which make modelling of them difficult and complex (Shalloo *et al*., 2004). The overall objective of this study was to develop a dynamic, mechanistic and stochastic simulation model of a dairy farm that can ultimately be used to model Greenhouse Gas emissions GHG from dairy systems. This paper will describe the milk production and the BCS change within the overall model framework.

Materials and methods The model described in this paper simulates the performance of each animal individually, with a daily time step. The dairy cows are represented in the model through a number of complex interactions which include intake, body condition score change, milk production, growth and ultimately death. Individual animal intake is simulated based on the French energy and intake systems (Faverdin *et al*., 2011). The model is stochastic, dynamic and mechanistic in nature allowing it to react to changing conditions at farm level. Model simulation requires an initial description of the cattle (age, body weight, body condition score, …) and management rules around the farm (drying, culling, feeding, …) as well as the length of time in which the simulation is being carried out (e.g. 1 month, 1 year, ….). The simulation of the milk production per day is calculated as an interaction between the energy intake by the cow and her genetic potential. If the energy intake by the cow allows a lower production than her potential, the cow will mobilize (loss BCS), which will allow her to produce more milk than possible through the feed alone. If the energy intake allows a higher milk production than the potential of the cow, part of this energy will go into the reserve of the cow (regain of BCS). The maximal loss of BCS for a lactation is set at calving and depends on the genetic potential of the cow (maximal potential milk production of the cow), the parity (primiparous/multiparous) and the BCS at calving. In this study the model has been evaluated and validated by simulating data from Curtin's farm over the period 2009 to 2011. The model was parameterised with the initial Curtin's data and was simulated over a three year period. The genetic index of the cattle has been set a 37kg of potential milk yield at pick in third lactation. The model was simulated a sufficiently large number of iterations (50) to ensure that the overall model was in a steady state. The Curtins experiment operated three different stocking rates (SR) SR1 3.28, SR2 2.92, SR3 2.51 (McCarthy *et al*., 2012). The outputs from the model were compared to the recorded data on a weekly basis using a number of statistical procedures to determine model accuracy which included root mean square error (RMSE) and the relative predicted error (RPE) for milk production. The model outputs and the actual data was broken into 4 periods, namely, whole year (2-40 weeks), spring (2-16 weeks), summer (17-25 weeks) and autumn (26-40 weeks), with the analysis carried out for primi and multi parous cows separately.

Results and Discussion A general guide for model usefulness centres on an RPE of less than 10% (Delagarde *et al*., 2011): In this analysis all milk production variables through the whole lactation were less than 10%. The model however overestimated the milk production of the multiparous SR2 cows in the spring and summer (RPE of 11.2 and 12.6) and underestimating the milk production of the primiparous cows in spring and autumn (RPE of 11.21 and 11.9). The overestimation of the milk for the SR2 multiparous cows is probably due to an overestimation of the genetic merit of the cattle. The underestimation for the SR1 primiparous cows is probably due to an underestimation of the ability of the primiparous cows to maintain their milk production when underfed, the maximum BCS loss for the primiparous cows may also be underestimated. The model adapted to the different stocking rates by predicting a higher milk production over the whole lactation for the lower stocking rates (22.61 kg) when compared to (20.77kg) the higher stocking rates. All BCS predictions have an RPE lower than 5%. Furthermore, every RMSE is lower than 0.25 percentage points of BCS, which is lower than the actual recorded step, which shows a very good accuracy of the model. The model adapted to the different stocking rate by predicting a lower average BCS for the higher stocking rate (2.71) when compared to the lower stocking rate (2.95). This model will be subsequently merged with the GHG emissions model developed by (O' Brien *et al*., 2010) in the next phase of this model development.

Conclusion This model accurately predicted milk production at the herd level for one study across different parities and stocking rates.

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Farm-scale modelling of greenhouse gas emissions from livestock production; bridging the gap between dynamic and static modelling

N J Hutchings, M S Jørgensen, J Vejlin
Aarhus University, Tjele, Denmark *Email:nick.hutchings@agrsci.dk*

Introduction Assessing greenhouse gas emissions at the farm scale is particularly relevant for ruminant livestock farming, since emissions occur from a number of interconnected sources (livestock, stored manure, soil). Current models can be broadly categorised as static annual budgetary models (e.g. using IPCC Tier 2) and dynamic multi-year models (e.g. process-based models). The former have the advantage of simplicity, transparency and limited data demands but their success depends crucially on the accurate parameterisation of emission factors etc. Being generally process-based, the dynamic models respond readily to changes in farm management and the biophysical environment but demand large quantities of high quality input data. As part of the EU AnimalChange project, we have developed an annual budgetary model with enhanced functionality (Tier 2+) that attempts to bridge the gap between the two.

Material and methods The model builds on the existing Farm-N model (http://www.fasset.dk/Upload/Fasset/Document/FARM-N_scientific_description.pdf), which simulates farm-scale N flows under Danish conditions. The new model (FarmAC) simulates both C and N flows. The user must input a wide range of farm management details; the number and feed ration of all livestock categories (e.g. dairy cows, calves aged 0.5-1 year), the type of animal housing used for each livestock category, the storage facilities used for manure or from each type of housing, the area and crop sequence for each rotation and the amounts and timing of application of manure and fertiliser N to each crop in each rotation. The model simulates the following:

- The input of C and N to each livestock category in animal feed.
- The partitioning of C and N in the livestock between production of milk/growth and emissions of CH_4 and CO_2.
- The flow of C and N in excreta to pasture and the appropriate animal housing, with emission of NH_3.
- The addition of C and N to manure via bedding and rejected/spilt animal feed and the flow of manure from the housing to the appropriate manure storage(s).
- The decomposition of manure C to CH_4, CO_2 and humus-like organic C.
- The decomposition of manure N to ammonium and humus-like organic N.
- The emission of CH_4, CO_2, NH_3, N_2 and N_2O from manure in storage.
- The emission of NH_3, N_2 and N_2O from fertiliser N and manure applications.
- The input of N to each crop in each rotation via atmospheric deposition and crop N fixation .
- Crop C production and N yield.
- The loss of NO_3 via leaching.
- The change in C and N stored in the soil.

The model summarises C and N flows at the farm level and calculates greenhouse gas emissions in CO_2 equivalents. The model is accompanied by a web-based graphic user interface (GUI) that stores all inputs to a database. The GUI and model exchange input data and the results of model simulations via xml files.
Two features of the model are that it describes the reduction in apparent dry matter digestibility that occurs as animal intake increases and distinguishes between readily degraded and resistant C in livestock excreta in manure. To illustrate the consequences of including these features, the model was used to simulate the CH_4 emission from enteric fermentation and from anaerobic decomposition of manure in storage. Feeding a diet based on maize silage has been proposed as a means of reducing enteric CH_4 emissions. However, recent and as yet unpublished results from empirical experiments suggest that the effect of changing diet on the subsequent CH_4 emission from manure storage should be taken into account, since these may be enhanced. Two daily diets for dairy cows were therefore simulated, one based on grass-silage (10 kg DM, 16.6% CP, 4.5% NFE, 25.3% CF, 10.2% ash, 75% DMD) and one based on maize silage (10 kg DM, 8.0% CP, 32.7% NFE, 18.2% CF, 3.8% ash, 75% DMD). Concentrate (1kg DM, 26.0% CP, 21.8% NFE, 12.0% CF, 8.0% ash, 77% DMD) was included in both diets.

Results The diet based on grass silage resulted in an emission of CH_4 from enteric fermentation equivalent to 23 g (kg milk)$^{-1}$ whereas the diet based on maize silage resulted in an equivalent emission of 20 g (kg milk)$^{-1}$. However, the CH_4 emission from manure storage for these two diets was 54 g (kg milk)$^{-1}$ and 58 g (kg milk)$^{-1}$ respectively. The total CH_4 from milk production was 77 and 78 g (kg milk)$^{-1}$ for the two diets respectively.

Conclusions The model was able to simulate the simultaneous effect on CH_4 emissions from enteric fermentation and manure decomposition of changing dairy cattle diet. The simulations are based on a preliminary parameterisation of the model, so no firm conclusions can be made concerning the effect of diet on the balance between CH_4 emissions from the two sources. However, we conclude that including the effect in assessments of the efficiency of dietary manipulation would be advisable.

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Influence of a series of inclusion rates of eleven additives with two contrasting diets on in vitro rumen methanogensis

M O'Brien[1], P O'Kiely[1], T M Boland[2]
[1]Teagasc, Grange, Dunsany, Co. Meath, Ireland, [2]University College Dublin, Belfield, Dublin 4, Ireland
Email:padraig.okiely@teagasc.ie

Introduction Rumen methane represents a loss of ingested energy for the ruminant and the emission of an important greenhouse gas. Modifying rumen fermentation can reduce methane output, but this may be diet dependent. Methanogenesis was assessed when 11 additives were incubated with perennial ryegrass or with grass silage plus barley grain using the *in vitro* gas production technique.

Materials and methods Dried (40°C), milled perennial ryegrass (PR) and grass silage + barley grain (GS+BG; 50:50, gravimetric dry matter (DM) basis) samples (0.5 g) were weighed into individual 160 ml fermentation bottles together with 50 ml of a 4:1 volumetric mixture of buffer and rumen fluid inoculum. The latter was collected from three fistulated steers. Using triplicate replication (rumen fluid source) the following additives were co-incubated (39˚C for 24 h) with each feed: oleic, linoleic, and linolenic at 0, 1.25, 2.5, 5 and 10 mL/L; lauric acid at 0, 1.25, 2.5, 5 and 10 g/L; 2-bromoethanesulfonate (BES) and bromochloromethane (BCM) at 0, 1, 5, 10, 20 and 40 µM; pyromellitic diimide (PMDI) at 0, 1, 5, 10 and 20 mg/L; mevastatin at 0, 0.25, 0.5, 1, 2, and 4 µM and lovastatin at 0, 0.25, 0.5, 1, 2, 4 and 8 µM; yeast at 0, 0.1, 0.2, 0.5, 1 and 2 g/L; and fumaric acid at 0, 1, 5, 10 and 20 mM. Data for each additive were analysed using a model that accounted for feed type, additive concentration and their interaction. They were also analysed separately for each feed type, and polynomial contrasts were used to test for linear and quadratic effects of additive concentration.

Results Fatty acids, BES and PMDI caused a dose-dependent decline in methane output when incubated with PR or GS+BG (Table 1). For PR only, yeast or fumaric acid caused a dose-dependent decline in methane output. Lauric, oleic, linoleic or linolenic acids and BES reduced methane output more when incubated with GS+BG than PR. The minimum measured concentration (per L) that reduced methane output with both feed types was 1.25 g lauric acid, 2.5 mL oleic acid, 1.25 mL linoleic or linolenic acids and 5 mg PMDI. Yeast (0.1 g/L) and fumaric acid (10 mM) reduced methane output only with PR whereas for BES the minimum requirement was 10 µM with PR and 1 µM with GS+BG. No methane output was detected for either feed with the addition of ≥5 µM BCM whereas statins were ineffective inhibitors.

Table 1 Methane output (mL/g DM incubated) for each of eleven additives with two contrasting diets

Additive	Diet	Dose rates added[1]							s.e.m.	L[2]	Q[3]
		0	I	II	III	IV	V	VI			
Lauric acid	PR[4]	36.1	15.2[9]	2.8	0.5	0.3			1.36	***	***
	GS+BG[5]	45.4	18.0[9]	4.3	1.8	0.6			0.85	***	***
Oleic acid	PR	28.4	24.1	17.7[9]	17.2	14.0			1.35	***	**
	GS+BG	36.3	30.6	29.3[9]	20.4	17.7			2.01	***	*
Linoleic acid	PR	28.4	18.5[9]	12.9	9.8	5.2			2.06	***	**
	GS+BG	36.3	25.4[9]	15.5	9.8	6.3			1.84	***	***
Linolenic acid	PR	28.4	8.4[9]	1.7	0.5	0.1			1.21	***	***
	GS+BG	36.3	14.5[9]	4.4	0.2	0.2			1.83	***	***
BES[6]	PR	27.4	29.4	20.9	18.5[9]	16.7	14.1		1.90	***	*
	GS+BG	39.3	29.5[9]	22.8	21.8	19.0	16.8		1.69	***	***
BCM[7]	PR	29.9	29.6	0	0	0	0		-	-	-
	GS+BG	33.9	36.8	0	0	0	0		-	-	-
PMDI[8]	PR	32.4	30.0	18.5[9]	8.8	0.1			1.63	***	**
	GS+BG	38.6	35.4	20.2[9]	8.7	0.1			2.24	***	**
Mevastatin	PR	31.3	28.9	29.9	29.4	27.4	28.6		1.27	n.s.	n.s.
	GS+BG	34.0	35.4	34.9	34.1	33.3	34.8		1.13	n.s.	n.s.
Lovastatin	PR	31.3	28.2	28.9	27.8	28.3	30.5	28.0	1.14	n.s.	n.s.
	GS+BG	34.0	34.8	31.2	34.3	34.2	32.9	33.7	1.25	n.s.	n.s.
Yeast	PR	31.6	25.7[9]	26.7	26.8	26.9	25.8		1.17	†	n.s.
	GS+BG	36.8	36.2	36.6	35.5	34.9	33.4		1.72	n.s.	n.s.
Fumaric acid	PR	33.2	28.4	26.8	25.0[9]	24.4			1.85	*	n.s.
	GS+BG	41.2	37.4	35.2	32.2	32.9			2.76	n.s.	n.s.

[1]Dose rates as per Materials and Methods, [2]Linear, [3]Quadratic, [4]Perennial ryegrass, [5]Grass silage + barley grain, [6]Bromoethanosulfonate, [7]Bromochloremethane, [8]Pyromellitic diimide; [9]Minimum measured concentration to reduce (P<0.05) methane output compared to the control treatment; n.s.=Not significant, †P=0.05, * P<0.05 , ** P<0.01 , *** P<0.001

Conclusions For initial *in vitro* rumen screenings, potential methane inhibitors should be assessed with different feed types.

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Role of saponin-rich sources in relation to ruminal methane mitigation at various addition levels in vitro: an evidence from meta-analysis study

A Jayanegara[1], A Sudarman[1], E Wina[2], J Takahashi[3]
[1]Bogor Agricultural University, Bogor, West Java, Indonesia, [2]Indonesian Research Institute for Animal Production, Bogor, West Java, Indonesia, [3]Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan
Email:anu_jayanegara@yahoo.com

Introduction Plant secondary compounds especially saponins have been considered as promising natural substances for mitigating methane emissions from ruminants. Some main saponin-rich sources that have been repeatedly tested in relation to methane emissions were quillaja, yucca and tea. Indeed, different saponin-rich sources determined the effectiveness of such compounds in mitigating methane (Pen *et al.* 2006) although it has to be proven across different studies. Apart from source-dependent, levels of additions apparently influenced the response as well. Graded addition levels of saponin-rich sources arrived in contrasting results, i.e. either decreased methane (e.g. Holtshausen *et al.* 2009) or no significant effect (e.g. Staerfl *et al.* 2010). Therefore, in order to mediate such disagreement, the aim of the present study is to assess ruminal methane emissions when being added with various levels of saponin-rich sources through meta-analytical approach by integrating related studies from published papers.

Material and methods A database was developed from published literatures reporting addition of saponin-rich sources at various levels and ruminal methane emissions *in vitro*. Scopus database was used as the searching tool with the keywords "saponin" and "methane". Inclusion criteria were: (1) articles were published in English, (2) treatments included additions of saponin-rich sources to certain basal feeds, (3) methane emissions were directly measured, not obtained by estimations, and (4) experiments were conducted based on *in vitro* rumen fermentation systems. Initially, based on abstract evaluations, there were 39 potential articles. After full text evaluations, a total of 21 studies from 16 articles met the respective criteria. The data obtained were subjected to a statistical meta-analysis based on mixed model methodology (Sauvant *et al.* 2008). Accordingly, different studies were treated as random effects whereas levels of saponin additions were considered as fixed effects by using SAS Statistical Software version 9.1. All data were transformed into similar units of measurements. Protozoal counts were normalized by applying logarithmic transformation.

Results Increasing levels of saponin-rich source additions decrease methane emissions, i.e. additions of 10 mg/g dry matter (DM) of saponin-rich sources lead to 0.31 ml/g DM decrease of methane emissions ($P<0.001$; Table 1). Similar response is obtained when methane is expressed in a relative unit (ml methane/100 ml total gas produced; $P<0.001$). Different from those variables, total gas production ($P<0.001$) and total short-chain fatty acids ($P<0.001$) increase with increasing levels of the saponins. There is a shift of acetate-to-propionate ratio, i.e. from higher to lower values at higher levels of saponin additions ($P<0.001$). Higher levels of saponin-rich sources tended to lower log protozoal counts ($0.05<P<0.1$). Addition of yucca saponins produces less methane than that of quillaja ($P<0.05$).

Table 1 Influence of saponin-rich source additions (mg/g DM) on methane emissions and rumen fermentation variables

Dependent variable	n	Parameter estimates					
		Intercept	s.e. Int	P Int	Slope	s.e. Slope	P Slope
CH_4 (ml/g DM)	78	37.9	3.66	<0.001	–0.031	0.0043	<0.001
CH_4 (ml/100 ml)	71	18.9	2.04	<0.001	–0.037	0.0020	<0.001
Gas (ml/g DM)	65	184	16.4	<0.001	0.065	0.0133	<0.001
Total SCFA (mmol/l)	84	72.2	6.07	<0.001	0.009	0.0022	<0.001
C_2/C_3	82	3.08	0.211	<0.001	–0.0012	0.00027	<0.001
Log protozoa (cells/ml)	56	4.73	0.207	<0.001	–0.00053	0.00028	0.059

C_2, acetate; C_3, propionate; DM, dry matter; Int, intercept; n, number of observations; SCFA, short-chain fatty acids; s.e., standard error

Conclusions The present meta-analysis study shows that, based on experiments conducted at various studies, increasing levels of saponin-rich source additions lead to decrease of ruminal methane emissions *in vitro*. Interestingly, higher levels of the saponins do not negatively influence total gas and total short-chain fatty acid productions. Part of the methane decrease with increasing levels of saponins can be explained by lower C_2/C_3 ratio and a tendency of lower protozoal counts. Although methane mitigating properties of saponins appear to be practically prospective, the effectiveness of the substances in mitigating methane emissions *in vivo* awaits further confirmation through meta-analysis.

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Effect of microbial consortia from ungulates on in vitro fermentation and greenhouse gas emission of maize stover

F N Fon[1,2], I V Nsahlai[1], P F Scogings[1,2], S P Dludla[1,2]
[1]University of KwaZulu-Natal, Pietermaritzburg, South Africa, [2]University of Zululand, KwaDlangezwa, South Africa
Email:fabiannde@yahoo.co.uk

Introduction About 12% of the total greenhouse gases (GHG) emitted into the atmosphere comes from agriculture while 0.28 of global methane emission comes from ruminant livestock enteric fermentation. The harmful effects of GHG (global warming, climate change, ozone depletion, sea level rise and adverse diversity), calls for an urgent need to protect the environment. The proposed strategies include decreasing consumption of livestock products, raising beef on grassland as opposed to feedlots and application of lipids, microbes and plant extracts as feed additives. The study was aimed at increasing the amount of energy harvested from roughages without necessarily increasing GHG. *In vitro* fermentation of maize stover by inocula (microbes) from horse (H), blue wildebeest (W) and mountain zebra (Z) were compared to that of its consortia N1 (H+W) and N2 (W+Z).

Material and methods Faecal samples were collected from H, W and Z (grazing at Tala Game reserve) within 2 min of defecation. Faecal inoculum was made by mixing 300 g of faeces with 300 mL of warm salivary buffer (Ouda & Nsahlai, 2009). Each inoculum was cultured in the laboratory for 72 h at 38.5 °C after mixing 198 mL of faecal filtrate with 402 mL of salivary buffer containing 6 g of a 1: 1 mixture of lucerne and maize stover (3mm). Microbial consortia N1 (H+W, 1:1) and N2 (W+Z, 1:1) were also cultured in the laboratory. For *in vitro* fermentation, 33 mL of each cultured inoculum was transferred into 67 mL of salivary buffer containing 1 g maize stover (3 mm) and incubated for 72 h at 38.5 °C in a computerized pressure transducer system. Incubations were replicated three times. True degradability (TD), microbial yield, total gas (GP), partitioning factor (TD/GP) and the rate of gas production were measured by fitting the raw data in the model described by Campos *et al*. (2004). For exocelullase activity, crude protein was extracted from cultured filtrate using 60% $(NH_4)_2SO_4$, dialyzed, concentrated and concentration determined by Bradford assay. Exocellulase was assayed using crystalline cellulose from ALDRICH® and reducing sugars measured by Dinitrosalicylic method. Each enzyme assay was replicated three times with four pseudo repeats. Enzyme specific activity was defined as µg of glucose/mg crude protein. Exocellulase activity, maize stover degradability and gas estimates derived from fermentation were subjected to analysis of variance (ANOVA) using the general linear model of SAS. The model accounted for the effects of the source of inoculation and treatment.

Results Exocellulase activity and TD were generally higher ($P<0.05$) for N1 and N2 than in H, W or Z (Table 1). Microbial yield was relatively the same for all ecosystems but for Z and N2 it was higher. Systems N2 and H produced the lowest ($P<0.05$) GP, followed by N1 while W and Z recorded the highest. The rate of gas production did not vary among the systems while partitioning factor was higher in H, Z and N1 than N2 and W. Total short chain fatty acids (SCFA) were higher in N1 and N2 than H, W and Z. Methane and CO_2 were lower ($P<0.05$) for N1 and Z than H, W and N2.

Table 1 Monitoring exocellulase activity and fermentation characteristics of the different microbial consortia

Inoculum source	Enzyme SP	Maize stover degradability					Fermentation products (molar proportions)					
	Exocellulase	TD (mg/g)	MY (mg/g)	GP (ml/g)	C (h^{-1})	PF	SCFA (mM)	Acet	PP	BUT	CH_4	CO_2
H	141.80^{b}	585^{a}	226^{a}	97^{a}	0.022	6.0^{b}	29.12^{b}	0.58c	0.38^{b}	0.04^{a}	0.21^{c}	0.45^{b}
W	90.05^{d}	680^{b}	221^{a}	135^{b}	0.022	5.0^{a}	46.81^{b}	0.52b	0.43^{b}	0.06^{ab}	0.18^{b}	0.45^{b}
Z	107.10^{c}	590^{a}	264^{b}	146^{c}	0.025	5.8^{b}	30.99^{a}	0.50^{ab}	0.47^{ab}	0.03^{a}	0.15^{b}	0.41^{a}
N1	163.31^{a}	715^{c}	228^{a}	132^{b}	0.021	5.6^{b}	47.42^{b}	0.45^{a}	0.53^{b}	0.02^{a}	0.10^{a}	0.39^{a}
N2	160.90^{a}	681^{b}	249^{b}	102^{a}	0.020	4.7^{a}	71.54^{c}	0.43^{a}	0.42^{b}	0.11^{b}	0.16^{b}	0.46^{b}
s.e.d.	4.151	4.2	9.0	3.7	0.001	0.23	3.343	0.001	0.001	0.001	0.001	0.001
P	0.001	0.05	0.05	0.001	n.s.	0.05	0.001	0.01	0.01	0.01	0.05	0.01

H=Horse, W=wildebeest, Z=zebra, TD=true degradability, MY=microbial yield, C=rate of fermentation, PF = partitioning factor, SCFA=short chain fatty acids, Acet=acetate, PP=propionate, BUT=butyrate, CH_4=methane, CO_2=carbon dioxide, SP=specific activity (µg glucose/mg crude protein). [a,b,c]Numbers with different superscript in the same column were different ($P<0.05$).

Conclusions Microbial consortia from N1 and N2 showed a higher potential of improving digestibility and Total SCFA with lower total gas emission. N1 was the best system in terms of production and environmental friendliness (lower CH_4 and CO_2). Application of these systems as feed additives can improve animal performance without necessarily increasing greenhouse gas emissions.

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Effect of microbial consortia from ungulates on ruminal fermentation and greenhouse gas emission of maize stover in sheep

F N Fon[1], I V Nsahlai[1,2], P F Scogings[1], S P Dludla[1]
[1]University of Zululand, KwaDlangezwa, South Africa, [2]University of KwaZulu-Natal, Pietermaritzburg, South Africa
Email:fabiannde@yahoo.co.uk

Introduction Global warming is enhanced by natural greenhouse effect caused by emissions associated with human activities of greenhouse gases (GHG) such as carbon dioxide, methane, nitrous oxide and halogenated compounds. About 18% of all GHG is produced by livestock greater than emissions from all forms of transportation. About 15% of total methane emission comes from ruminant enteric fermentation and has been estimated to be 6.5% loss of total dietary energy intake. Mitigating GHG is eminent to protect the environment and has been categorized into three groups based on the underlying mechanisms; reducing, enhancing and avoiding emission (Smith *et al.*, 2008). This study aimed at reducing emissions without decreasing the amount of energy harvested from roughages. The effect of microbial consortia on maize stover intake and fermentation characteristics in sheep was evaluated.

Methods Six fistulated male Merino sheep (mean weight 44 ±1.5 kg) were divided into two groups of three (control and treatment animals) in individual pens (230 x 203 cm). Each sheep was fed a total of 1.3 kg feed (maize stover and lucerne, 1:0.3) per day and left over collected. Water was provided *ad libitum*. Inocula were N1 (horse + blue wildebeest, 1:1) and N2 (wildebeest + plains zebra, 1:1)(animals from Tala Game Reserve, South Africa). Animals were fed for a total of 21 days including 15 days of adaptation. In experiment 1, treatment sheep were dosed (through the fistula) with 50 g of fresh faecal inocula (N1) on day 1 and 50 ml of cultured N1 after every 3.5 d (Ethics number: 083/10/Animal). Because of the limitation of fistulated animals only one treatment was tested at a time in order to have enough repeats. In experiment 2, three sheep were dosed with N2 while the other three animals received no treatment. Feeding and inoculation interval were the same as in experiment 1. *In sacco* dry matter degradability of maize stover (MS) was determined by incubating approximately 3.0 g (2 mm) in nylon bags in the rumen of fistulated sheep. All bags were withdrawn from the rumen after incubating for 96, 72, 48, 24, 12, 9, 6 and 3 h. Degradability at each time interval was calculated by taking the mean value obtained from the set of bags. The degradability (Y) of MS at time (t) was estimated by using the non-linear curve: $Y = A + B\ (1\text{-}e^{-C*(t\text{-}lt)})$, PD = A + B, EF = A + B*C/(C + 0.03) where Y= disappearance of DM at time (t), A= readily soluble fraction of MS (washing loss), B = insoluble but degradable fraction of MS, C= rate of degradation of B, lt = lag time, PD= potential degradability, EF= effective degradability and 0.03 h^{-1} was the assumed passage rate (kp). For short chain fatty acid (SCFA) analysis, rumen fluid (RF) was collected from each sheep on day 20 at the following times; 0, 3, 6, 9, 12 and 21 h. 5 ml RF was acidified in 1 ml of 25% (v/v) metaphosphoric acid solution and SCFA determined using a modified Gas Chromatograph method (Cottyn and Boucque, 1968). Theoretical calculations of CO_2 and methane (CH_4) were estimated as described by Groot *et al*. (1998) based on the stoichiometric balance of fermentation of glucose to propionic acid, acetic acid, butyric acid, iso-butyric acid, CO_2 and methane. Intake, *in sacco* degradability and SCFA were subjected to analysis of variance (ANOVA) using the general linear model of SAS. The model accounted for the effects of the source of inoculation. Student Newman- Keuls' test was used to compare means.

Results Intake increased (P<0.05) with N1 inoculation but tended to increase with N2 treatment (Fig1). No difference was observed between treatment and control for PD, B or EF in both experiments. Methane emission decreased (P<0.05) in both treatments but tended to decrease for CO_2 in both treatment. No difference was observed between control and treatment for PROP, ACET or BUTY.

Table 1 Intake, *in vivo* degradability of maize stover and greenhouse gas production

	DM Intake	B(g/kg)	PD(g/kg)	EF(g/kg)	ACET[a]	PROP[a]	BUTY[a]	CO_2[a]	CH_4[a]
Cont1	1045	559	790	563	0.74	0.19	0.06	0.52	0.35
N1	1161	556	787	570	0.71	0.21	0.07	0.50	0.33
s.e.d.	22.2	6	8	11	0.003	0.001	0.002	0.001	0.001
P	0.05	n.s.	n.s.	n.s.	0.05	n.s.	n.s.	n.s.	0.05
Cont2	966	545	776	564	0.69	0.18	0.1	0.53	0.35
N2	1064	534	775	570	0.70	0.22	0.08	0.52	0.31
s.e.d.	33.5	14.2	14.5	11.3	0.003	0.008	0.006	0.001	0.002
P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.05

Cont=control, ACET=acetate, PROP = propionate, BUTY=butyrate, Intake=g/day, [a] superscript = molar proportions

Conclusion These results showed that microbial consortia from N1 and N2 have the potential of mitigating GHG emission without decreasing intake and degradability which can play a vital role in protecting the environment from global warming.

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The rumen methane production of rice straw ammoniated with different levels of urea, evaluated using in vitro incubation technique

G Zhao, B Li, M Wang, T Zhang, W Zheng
College of Animal Science and Technology, China Agricultural University, Beijing, China *Email:zhaogy@cau.edu.cn*

Introduction Ammoniation treatment to upgrade the feeding quality of rice straw for ruminants has been used as a successful technique in ruminant production. Previous studies indicated that there existed a close positive correlation between the feed digestibility and the rumen gas production (Zhao *et al.*, 2007). Therefore the rumen gas production will be increased with the digestibility of rice straw for ruminants. Since different levels of ammonia for ammoniation treatment of straw increased the digestibility to different extents (Sundstøl *et al.*, 1978) and the rumen gas is mainly composed of methane (CH_4) and carbon dioxide, it is interesting to know if the methane production per unit of volatile fatty acids (VFA) changes when graded levels of digestibility of ammoniated straw was obtained by ammoniation treatment. The objective of the trial was to study the effects of ammoniation treatment of rice straw with different levels of urea on CH_4 production per unit of VFA.

Materials and methods Five levels of urea, i.e. 0, 2, 4, 6 and 8% (urea/rice straw, w/w) were used for the ammoniation of rice straw as experimental Treatments I, II, III, IV and V, respectively. The *in vitro* gas production technique (Menke *et al.*, 1979) was used for *in vitro* incubation. Two adult Simmental cattle, 300±2 kg in liveweight and fitted with permanent rumen fistulas, were used as the donors for rumen fluid. The cattle were fed with a mixed ration composed of 1.2 kg mixed concentrates and 5.0 kg wild rye hay in two equal meals at 07:00 and 17:00, respectively. Clean drinking water was freely available. Two hours after morning feeding, 250 ml of rumen fluid was taken from each cattle. The rumen fluid taken from the two cattle was mixed, filtered through four layers of gauze and kept in a warmed glass bottle (39°C). Then 300 ml rumen fluid and 600 ml buffer mixture were mixed, kept in a water bath of 39°C and continuously gassed with carbon dioxide as the rumen-buffer mixture for incubation. Glass syringes with 200 mm in length and 32 mm in internal diameter, with calibrated volume of 100 ml were used as incubation vessels. A water bath that could accurately control temperature (±0.1°C) was used for incubation. The air-dried rice straw was milled to pass 1 mm screen and about 0.2000 g of feed sample was put inside each syringe. Four syringes were used as replicates for each sample and three syringes without feed samples were used as the blanks for each batch of incubation. The feed samples were incubated for 48 hours and the gas and incubation residues were collected. Methane and VFA were analysed using the gas chromatography.

Results The results indicated that the total gas, CH_4 and total VFA production of rice straw were significantly increased the urea level of ammoniation ($P < 0.0001$) whereas the ratios of CH_4/total VFA and total gas/total VFA was not affected ($P > 0.05$).

Table 1 Effect of urea level for ammoniation treatment of rice straw on rumen CH_4 and VFA production

Parameters	Treatments*					s.e.m.	P
	I	II	III	IV	V		
Total gas, ml/g DM	184^{cC}	187^{cC}	199^{bB}	209^{aA}	209^{aA}	7.79	<0.0001
CH_4, ml/g DM	39^{bD}	41^{bCD}	42^{abBC}	45^{aA}	45^{aA}	3.06	0.0001
Total VFA, µmol/g DM **	1892.8^{dC}	2066.7^{bcB}	2183.3^{abAB}	2217.1^{aA}	2201.9^{abAB}	152.31	<0.0001
Total gas/total VFA, ml/µmol	9.7	9.1	9.2	9.5	9.5	0.70	0.250
CH_4/total VFA, ml/µmol	2.1	2.0	2.0	2.0	2.0	0.16	0.270

Means in the same row with different small superscripts are significantly different ($P < 0.05$) and different capital superscripts extremely significant ($P < 0.01$).
* Treatments I, II, III, IV and V refer to 0, 2%, 4%, 6% and 8% of urea for ammoniation, respectively.
**Total VFA = acetate + propionate + butyrate + *i*-butyrate + valerate + *i*-valerate.

Conclusions Different levels of urea for ammoniation treatment of rice straw did not affect the CH_4 output per unit of VFA production whereas increased the CH_4 and the total gas production in *in vitro* incubation. Techniques or products need to be investigated in the future for mitigating CH_4 production from rumen fermentation when ruminants are fed with ammoniated rice straw.

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Effect of including different proportions of untreated versus ammoniated rice straw in mixed rations on rumen methane production in in vitro incubation

M Wang, G Zhao
College of Animal Science and Technology, China Agricultural University, Beijing, China *Email:zhaogy@cau.edu.cn*

Introduction Ammoniation treatment upgraded the feeding quality, palatability and feed intake of rice straw for ruminants (Vadivelod *et al*., 2009; Wanapat *et al*., 2009). However, ammoniated rice straw produced more methane (CH_4) than the untreated rice straw in *in vitro* incubation (Zhao *et al*. 2012, unpublished data). In practice, ruminants are not solely fed with ammoniated rice straw. Instead, ammoniated rice straw is often used as part of the mixed ration. The objectives of the experiment were to study the effects of including different proportions of ammoniated rice straw in mixed rations on total gas, CH_4 and volatile fatty acids (VFA) production and also investigate the effects on the ratios of CH_4/total gas, total gas/total VFA and CH_4/total VFA.

Material and methods Untreated and ammoniated using two levels of urea, *i.e*. 4% and 6%, respectively, were used as the roughage samples and a typical concentrate mixture composed of 55% maize, 18% wheat bran, 15% soybean meal and 12% rapeseed meal was used as the concentrate sample. Each of the roughage samples was mixed with the concentrate mixture at six ratios, i.e. 0:100, 20:80, 40:60, 60:40, 80:20 and 100:0, respectively, as experimental treatments. The *in vitro* gas production technique of Menke *et al*. (1979) was used for incubation. Two Simmental cattle (liveweight 380 kg), fitted with rumen cannulas, were used as the donors of rumen fluid. The animals were fed with 6.0 kg Chinese wild rye hay and 2.0 kg concentrate mixture daily in two equal meals at 7:00 and 17:00, respectively. Fresh drinking water was freely available. Three hundred ml of rumen fluid was taken from the rumen of each Simmental cattle two hours after morning feeding. The rumen fluid from the two cattle was mixed and strained through four layers of gauze. Then 300 ml rumen fluid and 600 ml buffer were mixed as the incubation medium which was continuously gassed with carbon dioxide. Glass syringes with the calibrated volume of 100 ml were used as the incubation vessels. About 0.2000 g of air-dried feed sample was placed into each syringe, then each syringe was filled with 30 ml of incubation medium. Four syringes were used as replicates for each sample and three syringes without feed samples were used as the blanks for each batch of incubation. The syringes were placed in a water bath for incubation (39^{o}CAfter 48 hours of incubation, the total gas production was recorded, the pH of the incubation residue was immediately measured and the gas and incubation residues were sampled. The incubation residue was placed in a 50 ml centrifugal tube and centrifuged at 10000×g for 15 min. Four ml of the supernatant was mixed with 1 ml of 25% orthophosphoric acid. The mixture was kept in a refrigerator at -20^{o}C for later analysis. The CH_4 and VFA were analysed using the gas chromatography.

Results The pH of the incubation residues of all treatments ranged within 6.42-6.81. The total gas (ml/g DM), CH_4 (ml/g DM) and total VFA (μmol/g DM) as well as the ratios of CH_4/total gas, total gas/total VFA and CH_4/total VFA were significantly decreased with the increase of the proportions of rice straw either untreated or ammoniated with 4% and 6% urea ($P < 0.05$). Ammoniation treatment of rice straw significantly increased the total gas, CH_4 and total VFA production ($P < 0.05$), whereas the ratios of CH_4/total gas, total gas/total VFA and CH_4/total VFA ($P > 0.05$) were not affected. No differences were found in total gas, CH_4, total VFA and in the ratios of CH_4/total gas, total gas/total VFA and CH_4/total VFA between rice straw ammoniated with 4% or 6% urea ($P > 0.05$).

Conclusions Including untreated or ammoniated rice straw in mixed rations decreased the total gas, CH_4 and total VFA production whereas ammoniated rice straw in mixed rations produced higher levels of the total gas, CH_4 and total VFA production than the untreated rice straw. The CH_4 production per unit of VFA was not affected when the same proportions of untreated or ammoniated rice straw were included in the mixed rations.

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Nitrous oxide emissions from urine patches: APSIM model validation and sensitivity analysis

I Vogeler, R Cichota, J Beautrais
AgResearch, Palmerston North, New Zealand *Email:iris.vogeler@agresearch.co.nz*

Introduction Agricultural greenhouse gas (GHG) emissions, including nitrous oxide (N_2O), are a major contributor to New Zealand's total GHG emissions, and animal excreta deposited onto pastures are a main source of this. To reduce N_2O emissions better understanding of the factors driving emissions and evaluation of mitigation strategies is required. Computer simulation models can provide a cost effective method of estimating N_2O emissions and for evaluating the effects of heterogeneity in climate and soil. The Agricultural Production Systems Simulator APSIM model has previously been compared with data from a series of field measurements of N_2O emissions from applications of known amounts of urinary N, on four different soil types (Horotiu, Te Kowhai, Wingatui, and Otokia), in two regions of New Zealand (Waikato and Otago), and applied at different times of the year (February, May, August, October). The model predicted total N_2O emissions rather poorly over the complete datasets. However when the data were split by season the model performed well for simulations under winter conditions. This suggests that the model functions and parameters need to be improved. The objective of this study is to use a simulation modelling analysis to test the sensitivity of model functions and parameters that affect N_2O emissions from urine patches.

Material and methods APSIM runs were setup to simulate N transformations in the soil and N_2O emissions following urine application in various seasons and years (May 2000, May 2008, October 2008) to the Horotiu soil in the Waikato region of New Zealand. The sensitivity of various model parameters was examined by varying their values and comparing the model results to experimental data. Model parameters that were varied, one at a time, included the rate of nitrification as affected by soil ammonium (K_{max} and K_{NH4}), the optimum soil temperature on nitrification, the fraction of nitrified N emitted as N_2O (K_2), the denitrification rate (K_{denit}) and the effect of water content on denitrification, and the rainfall intensity (Table 1). The simulations were run for one year and simulation output included daily values of soil nitrate and ammonium, as well as N_2O emissions. The emissions were summed up at weekly intervals to analyse temporal trends. To evaluate the agreement between simulation results of APSIM and measurements the correlation coefficient (R) and the model efficiency (ME) were used.

Table 1 Decreased, default and increased values of APSIM parameters varied during the modelling exercise

			Parameter values		
Parameter name	Abbrev.	Unit	Decrease	Default	Increase
Maximum nitrification rate	K_{max}	mg/kg/d	30	40	50
NH_4 concentration for half the maximum response to $[NH_4]$	K_{NH4}	K_{NH4}	40	90	140
Optimum temperature for nitrification	T_{opt}	°C	20	32	350
Denitrification rate	K_{denit}		0.0001	0.0006	0.001
Denitrification water function shape	WFS_{denit}	-	concave	linear	convex
Rainfall intensity	RFI	mm/h	3	uniform	5
Fraction of nitrified N emitted	N_2O_{nit}	-	0.001	0.002	0.005

Results The APSIM model with default parameter settings predicted the daily pattern of N_2O emissions from the urine patches poorly with negative ME for all three datasets, Figure 1. Changing some of the default model parameters improved the agreement in some cases, especially when K_{denit} was decreased. The weekly sums of N_2O emissions showed improved agreement between measured and simulated values, resulting in positive ME values of up to 0.44 for the October 2008 dataset when N_2O_{nit} was increased to 0.005.

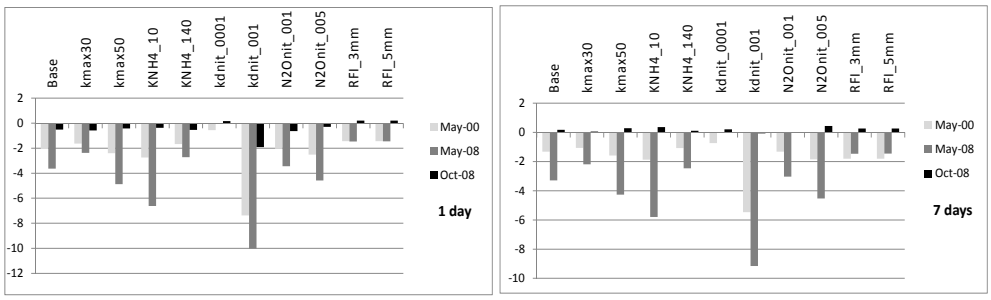

Figure 1 Model Efficiency between measured and APSIM simulated N2O emissions based on default model parameters and by changing values one at a time for three datasets measured from urine patches applied to a Horotiu soil in the Waikato

Conclusions These results show the sensitivity of several model parameters to N_2O emissions. So far none of the model parameters investigated could produce emissions that agreed reasonably with values measured under different climatic conditions. A sensitivity analysis which includes more parameters and model functions, as well as changing various parameters simultaneously is needed.

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Review of enteric methane emissions of cattle and sheep fed diets relevant to UK farming conditions

J McBride, S J Morrison, T Yan
Agri-Food and Biosciences Institute, Hillsborough, UK *Email:judith.mcbride@afbini.gov.uk*

CH_4 energy (CH_4-E) for each ruminant livestock class. The objectives of this review were: 1) to collate published CH_4 emissions from ruminant livestock recorded under dietary regimes typically used within UK agriculture and 2) to compare these reported emissions per unit of energy intake to IPCC guideline values.

Materials and methods Literature searches were performed to identify research and review papers from which enteric emission factors might be derived for all the required ruminant livestock groups. As well as terms directly covering enteric CH_4 emissions, energy metabolism data were also included as research specifically on these topics may have indirectly generated CH_4 emission data appropriate to the determination of emission factors. Treatment groups were categorised based on the animal groupings with each reviewed and the key details recorded using spreadsheet software. Accepted papers were further scrutinised to determine if the diets, breeds and housing management were applicable to that of the UK and if the experimental design was sufficiently rigorous, with adequate statistical analysis, and that the datasets were complete. On the basis of this assessment, a number of papers and/or treatment groups were rejected. Additional information was recorded if any nutritional treatments had been administered to directly reduce enteric CH_4 emission. A coded explanation for acceptance or rejection of treatments/papers was used. When each paper was reviewed, appropriate descriptions and a summary of the data for each treatment group were recorded. The complete dataset was held in a customised spreadsheet, designed to capture all relevant data and enable future meta-analysis.

Results A summary of the numbers of accepted treatments relevant to UK farming conditions is shown in Table 1. Treatments reporting emissions from dairy cows were most common followed by growing beef cattle with few treatments reporting emissions from beef cows or growing dairy herd replacements. A limited number of studies reported CH_4 emissions from grazing livestock with the majority reporting emissions from housed livestock on predominantly conserved forage diets. Mean CH_4-E/GEI values were largely in line with IPCC guidelines however the range in reported values was greater than ±1% (Table 2).

Table 1 Summary of treatments reported in the published literature relevant to UK farming conditions

Diet/System	Dairy cows		Dairy young-stock	Beef cows	Growing beef cattle	Sheep	Lambs
	Lactating	Non- Lactating					
Housed	175	19	10	2	89	41	25
Grazing	16	2	9	7	25	20	7
Total	212		19	9	114	61	32

Table 2 Methane conversion factors for each group of livestock[1, 2]

Livestock Group	CH_4-E/GEI (%)[3, 4]
Dairy cows Lactating	6.5 (3.4 - 8.8)
Non- Lactating	6.1 (4.4 - 8.4)
Dairy young-stock	7.0 (5.2 - 9.9)
Beef cows	6.4 (3.7 - 9.5)
Growing beef cattle	6.8 (2.6 - 11.3)
Sheep	6.3 (3.7 - 13.3)
Lambs	4.7 (3.0 - 8.5)

[1]Diet gross energy concentration assumed for treatments with missing values [2]Excludes treatments with mitigation strategies [3]CH_4-E/GEI (%) – methane energy output as percentage of gross energy intake [4]Range of reported values shown in parenthesis

Conclusions Based on the available literature applicable to UK farming conditions, the conversion factors (CH_4-E/GEI) for lactating cows and beef cows were generally in line with those recommended by IPCC (2006), but for other groups the factors were either higher or lower. However the range in conversion factors were much greater than the ±1% suggesting much scope for refinement exists. With treatments incorporating specific mitigation strategies excluded from the analysis, animal variation, diet type/quality and concentrate feeding levels are likely to account for much of the range in CH_4-E/GEI values. Under a Tier 3 methodology the accuracy of CH_4 national inventories should be improved as differences in diet quality/type and animal performance are inherently accounted for with the methodology.

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Factors affecting energy and nitrogen efficiency of dairy cows: a meta-analysis

H N Phuong[1,2], N C Friggens[1,2], P H Schmidely[1,2], I J M de Boer[1]
[1]INRA, UMR791 Modélisation Systémique Appliquée aux Ruminants, Paris, France, [2]AgroParisTech, UMR791 Modélisation Systémique Appliquée aux Ruminants, Paris, France, [3]Animal Production Systems Group, Wageningen University, Wageningen, The Netherlands *Email:phuong.hongoc@agroparistech.fr*

Introduction Feed efficiency (**FE**) in dairy production has received increasing attention since it influences both farm profitability and losses to the environment. However, FE has been commonly evaluated in terms of energy with nitrogen being less studied. As attention to the environmental impact of N excretion has increased, nitrogen efficiency should also be considered. A key question arises then: are nitrogen and energy efficiency affected by the same factors? Additionally, insight into the energy-nitrogen efficiency correlation and how it changes in different conditions is essential for maximizing FE in both energy and nitrogen terms. The current study explored the relationship between energy and nitrogen efficiency and examined factors affecting energy and nitrogen efficiency and their relationship.

Materials and methods A literature search using Web of Science, ScienceDirect and Google Scholar was conducted to create a database for the study. The search was built on the combination of following keywords: energy efficiency, nitrogen efficiency, dairy cows, nutrient utilization and dairy cow performance. The inclusion criteria were: a feed description in terms of ingredients (%), dry matter intake (kg/d), milk yield (kg/d), milk fat, protein and lactose (%), body weight (BW) (kg) and days in milk (DIM) (d). Energy efficiency was (1) [MY *E milk/DMI*DE diet] and nitrogen efficiency was (2) [MY * N milk/DMI* N diet]; where MY is milk yield (kg/d), E milk is the energy content of milk (Mcal/kgMilk), DMI is dry matter intake (kgDM/d), DE diet is the digestible content of the diet (Mcal/kgDM), N milk is the nitrogen content of milk (g/kgMilk) and N diet is the digestible nitrogen content of diet (g/kgDM), which is the digestible protein content of diet divided by 6.25. E milk was computed using the equation of NRC (2001), DE diet and N diet were estimated from INRA feed tables. To account for the reduced digestibility of energy due to increased DMI, an equation of Sauvant and Giger-Reverdin (2009) was applied. The reduced digestibility of protein due to increased DMI was assumed non-significant. Since both DMI and MY were used in (1) and (2), an artifactual correlation between energy efficiency and nitrogen efficiency exists, irrespective of any factor related to the diet or animals, with the extent of the correlation depending on how largely milk yield and DMI numerically contribute respectively to (1) and (2). Hence a study of the relationship between energy and nitrogen efficiency should exclude the presence of milk yield and DMI. We therefore decided to look instead at the ratio of nitrogen efficiency to energy efficiency (Neff/Eeff). When taking the ratio, the "artifact" of milk yield and DMI is removed and other factors that have an influence on the correlation between energy and nitrogen efficiency can be more clearly examined. The ratio then was [N milk*DE diet]/[N diet*E milk]. N milk and N diet were converted to Mcal using the values of 5.47 Mcal/kg protein and conversion from protein to N of 6.38 and 6.25 for milk and feed respectively. The effect of nutritional and animal variables, including neutral detergent fibre (NDF), acid detergent fibre (ADF), digestible protein (DCP), proportion of concentrate (PCO), DMI, MY, BW and DIM, on energy efficiency, nitrogen efficiency and Neff/Eeff was analysed using multiple linear regression. MY and DMI were not included in the models for predicting energy and nitrogen efficiency. DE and DCP were not included both in the model of ratio and respectively in the models of energy and nitrogen efficiency. Since the data used in this meta-analysis were gathered from multiple studies and in many different conditions, the study effect was considered to be random. The prediction variables were centred on their means. Mixed effect models were performed using the *nlme* package of the statistical software R version 2.15.1.

Results Correlation coefficients between energy and nitrogen efficiency inter- and intra-study were 0.62 and 0.30, respectively. ADF negatively affected both energy and nitrogen efficiency, DE was negatively associated to nitrogen efficiency, PCO curvilinearly affected energy efficiency (Table 1). The ratio Neff/Eeff was affected by ADF and PCO. BW and DIM were not significantly associated to either energy or nitrogen efficiency.

Table 1 The final models for predicting energy efficiency (%), nitrogen efficiency (%) and the Neff/Eeff ratio

Variables	Intercept	ADF	DE	PCO^2	AIC	R^2
Energy efficiency	33.09 (*0.49*)	-0.15 (*0.08*)		-0.002 (*0.003*)	933	0.88
Nitrogen efficiency	43.77 (*0.98*)	-0.56 (*0.18*)	-6.67 (*2.24*)	-	1254	0.79
Neff/Eeff	1.25 (*0.02*)	-0.02 (*0.004*)	-	0.0004 (0.0001)	-197	0.64

PCO^2=squared PCO; AIC=Akaike's information criterion; R^2=Coefficient of determination

Conclusions ADF, PCO and DE were the common nutritional factors affecting feed efficiency. A significant correlation between energy efficiency and nitrogen efficiency indicates a possibility to maximize both energy and nitrogen at the same time. The study also shows that this type of transverse data is not sufficient to study the effect of animal factors on feed efficiency. Longitudinal measurements per animal would probably be more appropriate.

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Can dietary Thais coronata - a local sea food waste control odour in a poultry farm?

S N Ukachukwu[1], V U Odoemelam[0,2], A U Kanu[1], I Nwogbugbo[1]
[1]Michael Okpara University of Agriculture, Umudike, Umuahia, Abia, Nigeria, [2]Federal University of Technology,, Owerri, Imo, Nigeria *Email:snukachukwu@yahoo.com*

Introduction Gas emissions are an emerging issue for all of animal agriculture. In Nigeria, increase in modern intensive animal feeding operations and the relatively large quantity of ammonia produced by the excreta have heightened environmental concerns over ammonia emissions (Ritz *et al.,* 2004). High levels of ammonia in broiler pens not only pose health risks to stockmen but also reduce the performance of the animals. Adsorption principle has found a wide range of application in pollution management including odour and toxic gas removal; and *Thais coronata,* a sea food waste that litters the riverine areas of Nigeria is a good adsorbent (Osu, 2010). This study therefore, aims at evaluating the odour reducing effect of dietary *Thais coronata* in poultry house as against the conventional *Yucca schidigera*.

Material and methods Proprietary *Yucca schidigera* powder used in the study was obtained from Adewura Farms Ltd, Ibadan, Oyo State, while *Thais coronata* shells were obtained from a local market in Calabar, Cross River State, both in Nigeria. The shells were thoroughly washed with warm water, rinsed with distilled water, air-dried for 3 days, and further dried in an oven at 110^0C for 3 days. Thereafter, they were crushed into fine particles to achieve homogeneity; then sieved using a 2mm mesh to remove the coarser particles. The sieved materials were divided into two lots. One lot received no further treatment and was termed non-activated shells. The second lot (termed activated) was soaked in 0.2M HCL for 24 hours, decanted, brought to a pH of 7.0 by continuous washing with distilled water, and dried in oven at 105^oC for three days as recommended by Osu (2010). There were 2 experimental trials. In trial 1, 60 birds were fed diets containing graded levels (0%, 0.025%, 0.050%, 0.075%, and 0.1%) of activated or non-activated *Thais coronata* for two weeks in a 2x5 factorial arrangement in completely randomized design (CRD) to reduce odour in the broiler faeces via perception scoring by 10 panellists on the 5th, 6th and 7th day and also on the 12th, 13th and the 14th day of the experimental period. In trial 2, selected treatment from trial 1 was compared with recommended proprietary inclusion level of Yucca extract and a control (containing no additive) using eighteen 12-week old broiler birds, at 6 birds per treatment and 2 birds per replicate in metabolism cages for easy collection of faecal material for faecal nitrogen analysis and odour perception scoring. Feeding trial lasted 7 days and faecal sample collection and perception scoring were done on the 3rd, 5th and 7th days of the experimental period; and the faeces were stored in a deep-freezer until the time for nitrogen analysis. Perception scoring of the degree of pungency of faecal odour was scored by 10 panellists on a 5-point scale, namely: very strongly pungent, strongly pungent, moderately pungent, weakly pungent and very weakly pungent and rated 5, 4, 3, 2, and 1, respectively. Data collected were subjected to analysis of variance (ANOVA), separating significant means by Least Significant Difference using SPSS (2006) computer application package.

Results There were significant Inclusion level x Form interactions in trial 1. Non-activated (NA) shell at 0.05 and 0.075% and Activated shell at 0.1% inclusion levels had similar (P>0.05) perception scores which were lower (P<0.05) than the scores of the control treatments (0% activated and non-activated diets), with NA at 0.05% having the lowest score. In trial 2, Perception scores of Yucca and *Thais coronata* groups were similar but lower (P<0.05) than that of the control group. Nitrogen yield of Yucca and *Thais coronata* groups were however, non-significantly lower than that of the control.

Table 1 Perception score of faecal odour of broilers fed diets containing graded levels of activated or non-activated *Thais coronata* shells

	Form (F)		
Level (L)	Activated	Non-activated	Mean
0%	14.5^{a}	14.3^{a}	14.4^{a}
0.025%	11.8^{abcd}	12.8^{abc}	12.3^{ab}
0.050%	11^{abcd}	8.3^{d}	9.7^{b}
0.075%	11.3^{abcd}	10^{bcd}	10.7^{b}
0.1%	9.5^{cd}	13.3^{abc}	11.4^{b}
Mean	11.6	11.7	
s.e.m. for F= 0.57, L= 0.91, F x L= 1.28			

Table 2 Faecal NH_3 and Perception score of faecal odour in broilers fed diets containing Yucca extracts and *Thais coronata* shells

Parameter	Control	Yucca extract^{+}	*T.cornata*$^{++}$	s.e.m.
Nitrogen yield, %	31.70	31.20	30.70	4.70$^{n.s.}$
Perception score	14.70^{a}	11.70^{b}	12.20^{b}	0.74*

$^{+}$ Included at recommended proprietary level of 125 mg /tonne of feed;
$^{++}$ Non-activated *T. cornata* included at 500 mg/tonne of feed based on the outcome of trial 1.

Conclusion The results indicate the potential of *Thais coronata* to reduce odour in the faeces of birds. The non-activated form at 0.05% level of inclusion appears to have the highest reduction effect. Dietary inclusion of yucca extracts at 125 mg and non-activated *Thais coronata* shells at 500 mg have similar effect on odour reduction. Therefore, non-activated *Thais coronata* shells, a locally available waste, can be considered as environmentally friendly feed additive for broiler production and odour reduction. However, it is recommended that standard equipment be employed for ammonia capture and measurement in subsequent studies. This was a major challenge in our experiment.

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In vitro production of CH_4 and CO_2 from diets including glycerin

V C Santos, J M B Ezequiel, A C Homem Júnior, V R Fávaro, E H Fernandes, A P D'Aurea, E M Oliveira
Faculdade de Ciências Agrárias e veterinárias -Universidade Estadual Paulista, Jaboticabal-São Paulo, Brazil
Email:vivianecorreasantos@gmail.com

Introduction Along with these fermentation processes for the production of CO_2 and CH_4, and the production of CH_4 influenced mainly by carbohydrates in the diet. The production of CH_4 represents energy losses of food consumed by ruminants and contribute to global warming. Strategies such as increasing the level of grain in the diet, including lipids and supplementation with ionophores, have high probabilities of reducing ruminal CH_4 emissions while improving production efficiency. The aim of the present study was to assess the *in vitro* production potential of the gases CO_2 and CH_4 diets with inclusion of glycerin.

Material and methods We used four Santa Inês x Dorper sheep with mean body weight of 47.3 kg, cannulated in the rumen. Diets were formulated to be isocaloric with forage:concentrate ratio of 20:80. Diets were called: G0 – control without glycerine, G10 – 10% glycerine as part of concentrate. Measurements of production of CH_4 and CO_2 gases were made using Incubater SHAKER SL 222, according to the methodology proposed by Gastaldi (2003) adapted for sheep which consists of three steps. 1 –Preparation of the sample: before feeding the animals, were collected approximately 500 mL of rumen contents, filtered on nylon fabric (100µm) and homogenized. In erlenmeyers with a capacity of 250 mL were placed 150 mL of ruminal fluid added 1.56 g DM of diets studied ground to 1 mm, in order to maintain the ratio of 1 g of DM sample:8 mL of ruminal fluid, buffer not being used. 2 – Production and storage of gases: the erlenmeyers containing the samples and rumen fluid were closed with stoppers and kept for 12 hours in a water bath at a temperature of 39°C in the dark, and the gases were led by capillary system of silicone and stored in the appropriate collectors with an internal volume of 600 mL. Such collectors were immersed in water enabling measurement of total gases by water displacement therein. 3 – Quantitative and qualitative analysis of the gas produced: an aliquot was collected directly from the erlenmeyers with a syringe with a capacity of 1mL, and immediately injected into a gas chromatograph Trace GC Ultra Scientific metanador and equipped with flame ionization detector, using argon entrainment with a flow of 25 mL per minute, and the furnace temperature of 70°C. The calibration was performed with a standard mixture of CH_4 and CO_2 gases. The peak areas were integrated using the software Chromquest 5.0. The total amount of gas produced within the collectors measured by displacement of collectors, immersed in water after 12 hours of fermentation. The experimental design was completely randomized with 15 replications per diet. The results were submitted to analysis of variance and comparison of means by Tukey test, considering a significance level of 5% using the GLM producer of Statistical Analysis System.

Results The diets differed in pH end of incubation, however, not differ in the production of CH_4 and CO_2 produced *in vitro* (Table 1). This demonstrates that the inclusion of 10% glycerine in diet does not affect the production of CH_4 and CO_2, contrary to the results of Van Cleef *et al*. (2012) when evaluated *in vitro* production of greenhouse gases using different concentrations of crude glycerin (0; 7.5; 15; 22.5 and 30%) in cattle diets Nelore and noted that crude glycerine concentration independent decreased CH_4 and CO_2, for 12 hours of incubation. Avila *et al*. (2011) evaluating the *in vitro* production of diets containing up to 21% crude glycerine instead barley, found no effect on the generation of this gas.

Table 1 *In vitro* production of methane (CH_4) and carbon dioxide (CO_2) diets include glycerin

	Diets			
Variable	G0[1]	G10[2]	CV (%)	P
pH	5.95	5.91	0.80	0.05
CH_4 (mL/g DM)	24.93	24.16	6.76	0.31
CO_2 (mL/g DM)	52.65	50.39	17.69	0.59

[1]G0: control without glycerin; [2]G10: containing 10% glycerin
Medium lines do not differ by Tukey test (p>0.05)
CV = coefficient of variation
P = probability

Conclusion The *in vitro* production of CH_4 and CO_2 are not altered by the inclusion of 10% glycerin.

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Succession of microorganisms in the developing rumen of young dairy calves

C Guzman[1], C Kentler[1], M Emmerling[2], A Franks[3], T Frankel[1]
[1]Department of Agriculture, La Trobe University, Melbourne, Victoria, Australia, [2]Department of Botany, La Trobe University, Melbourne, Victoria, Australia, [3]Department of Microbiology, La Trobe University, Melbourne, Victoria, Australia *Email:ceguzmanrodriguez@students.latrobe.edu.au*

Introduction The role microorganisms play in ruminant animals has been studied deeply for decades because they have a profound influence on the conversion of feed into end products which can impact positively or negatively on the animals and their environment. However, there is little knowledge about how they colonise the different areas of the digestive system in calves and
what their interactions are with other groups of microbes. The aim of this research was to characterize and quantify the succession of microorganisms in the developing rumen of calves at 0 to 20 days.

Materials and Methods The experiment involved 52 holstein bull calves and was conducted according to a protocol approved by the Animal Care and Use Committee, Research Animal Resource Centre, La Trobe University, Australia. Two consecutive experiments were conducted. The first involved three calves killed for collection of samples from the digestive tract (ruminal fluid, ruminal tissues, abomasums, cecum fluid, cecum tissues and faeces) at 1, 24, 36, 48 and 72 hours after birth. The second experiment consisted of four treatments given to calves from 5 to 20 days of age: 'control and unrestricted diet' (CU), 'control and restricted diet' (CR), 'nucleotides and unrestricted diet' (NU) and 'nucleotides and restricted diet' (NR). Calves given nucleotide treatments were given Ascogen® (1 g per feed, 2g/day). To establish and analyze the abundance and quantity of the genes encoding 16S, 23S and 18S ribosomal DNA of the developing rumen microbiota I used the molecular techniques polymerase chain reaction (PCR), gel electrophoresis, cloning, sequencing, quantitative polymerase chain reaction (qPCR) and automated method of ribosomal intergenic spacer analysis (ARISA). The following microorganisms were examined: Domain Archaea, Phylum Euryarchaeota, the orders Methanomicrobiales, Methanosarcinales and Methanococcales, and the genera *Methanobacterium* and *Methanobrevibacter* in the order Methanobacteriales; Phylum Bacteria, cellulolytic bacteria (*Fibrobacter succinogenes*); Phylum Firmicutes (*Ruminococcus flavefaciens* and *Selenomonas ruminantium);* Phylum bacteriodetes, the proteolytic bacteria (*Prevotella ruminicola*); Phylum Proteobacteria (*Geobacter metallireducens*); Phylum Protozoa (*Entodinium* spp) and Kingdom fungi. Details for analysis of these microorganisms can be found in Skillman *et al*. (2004, 2007), Stevenson (2007) and Stams *et al*. (2009). The data were analysed using the generalized linear model (GLM). Treatment means were compared using one-way ANOVA post hoc multiple comparisons with the Tukey's HSD test. All statistical analyses were conducted using version 8.0 SPSS (IBM). Results were considered significant at the $P < 0.05$ level.

Results First experiment: the results show that the concentrations of *G. metallireducens* and proteolytic bacteria (*P. ruminicola*) were significantly increased 2 and 3 fold respectively, and archaea significantly increased with calf age. Moreover some microorganism such as cellulolytic bacteria (*F. succinogenes*), *R. flavefaciens* and *S. ruminantium*, Protozoa (*Entodinium* spp) and fungi showed differences in concentration, and ARISA showed a difference in the relative abundance of bacterial groups. Second experiment: At day 7, the concentration of *Methanobrevibacter* and *Methanobacterium* were significantly increased by the NU and NR treatments. At day 14 and 20, *Methanobrevibacter* spp and *Methanobacterium* spp were significantly decreased and *P. ruminicola* was significantly increased by the NU and NR treatments. The bacteria, *F. succinogenes, R. flavefaciens*, *S. ruminantium,* the Protozoa, *Entodinium* spp, and fungi did not significantly differ between the treatments, but ARISA showed a difference in the relative abundance of bacterial groups.

Conclusion The novel contribution of this work is that ruminal microbiota began to colonize within 48 hours of life in calves (Methanomicrobiales, Methanosarcinales, Methanococcales, *G. metallireducens*, *P. ruminicola, F. Succinogenes*, *R. flavefaciens*, *S.ruminantium, Entodinium* spp and fungi). The total number of all these microorganisms differed between the digestive compartments. Second, two new pathways were suggested to reduce archaea which could reduce methane production. First, when the concentration of *P. ruminicola* increased 2 fold, the concentration of *Methanobrevibacter spp* decreased and the concentration of *Methanobacterium spp* decreased suggesting that their interaction could be a mechanism to reduce methane production in the rumen. Second, a 3 fold increase in concentration of *G. metallireducens* suggests a direct interspecies electron transfer, a side effect of which could be the production of methane by the resident methanogenic community. This knowledge tempts us to think that we could generate solutions to problems like green house emission and productivity in calves by understanding microbial interactions in the developing rumen.

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Review of current greenhouse gas (GHG) mitigation measures in animal agriculture and their potential impact on New Zealand's agricultural GHG inventory

C A M de Klein[1], D Pacheco[2], M D Rollo[3]
[1]AgResearch, Invermay, Mosgiel, New Zealand, [2]AgResearch, Grasslands, Palmerston North, New Zealand, [3]AgResearch, Ruakura, Hamilton, New Zealand *Email:cecile.deklein@agresearch.co.nz*

Introduction Greenhouse gas (GHG) emissions from animal agriculture contribute ca. 50% of New Zealand's total GHG emissions. Mitigation measures are being developed to reduce these emissions without reducing agricultural outputs. This paper summarises possible methane and nitrous oxide mitigation options that could be suitable for application in pastoral livestock industries. It provides the best estimate of a reduction potential for each mitigation option, based on published data, and estimates the 'potential' (assuming 100% adoption) and 'likely' (under most likely adoption rates) impacts of each mitigation option on the agricultural greenhouse gas (GHG) inventory. This analysis determines how New Zealand's agricultural inventories of enteric methane (CH_4) and soil nitrous oxide (N_2O) emissions would respond to the adoption of mitigation technologies.

Approach A review of peer-reviewed publications and reports was conducted to i) compile a list of currently available CH_4 and N_2O mitigations technologies for animal agriculture and ii) assess the potential impact of each technology on key parameters of the New Zealand CH_4 and N_2O national greenhouse gas inventory calculation methodologies. The potential reduction in total agricultural GHG emissions was then estimated for individual mitigation technologies, by running the inventory calculations with the adjusted parameters. The potential reductions were estimated for *maximum* (100% adoption) or *likely* (estimated adoption rates in ten years) adoption rates, the latter of which were assessed using an expert panel. The effects of the mitigation technologies were assessed using NZ's 2009 agricultural GHG emissions, as reported in the 2011 inventory submission (Ministry for the Environment, 2011). Results are presented as absolute reductions (in Gg CO_2-equivalents) or as relative reductions (%) compared with 2009 GHG emissions.

Results and discussion The results indicated that the estimated *maximum* and *likely* reduction in total agricultural GHG for individual mitigation options ranged between 0-19% (maximum adoption) and 0-3% (likely adoption). It should be noted that the *maximum* reductions are based solely on the current understanding of the biophysical potential of each option, and do not take account of any economic, practical, developmental or other barriers to the options. The *likely* reductions may include an assessment by the expert panel on the effects of some of these other barriers on likely adoption rates.

Some of the most effective CH_4 mitigation options, as assessed from controlled *in-vitro* and *in-vivo* experiments (e.g. increase dietary fat and rumen defaunation) had low expected adoptions rates and thus their likely impact in 10 years seems unlikely to be significant. In contrast, animal selection had only a moderate impact on the yield of methane per unit of intake, but the ability to deliver this option to a large proportion of the livestock population made this mitigation technology one of the promising options in the scenarios explored: 10% and 3% *maximum* and *likely* reductions in total agricultural GHG emissions, respectively.

To reduce N_2O emissions from agricultural soils, nitrification inhibitors (NIs) and diet supplementation (low N or tannin supplements) were the most effective N_2O mitigation options, with maximum reduction potentials of 3-11% (NIs) and 9-19% (diet supplementation). Nitrification inhibitors were one of the most promising options in terms of likely reduction potentials (2% reduction) because of their relatively high expected adoption rate in 10 years time. The likely impact of diet supplementation was lower than the maximum reductions (0-3%) due to a low expected adoption rate. Due to the lack of seasonal or monthly N_2O emission factors in the current New Zealand inventory methodology, some of the expected and observed impacts of mitigation options that target peak emissions (e.g. controlling the timing and place of fertiliser applications and avoiding N deposition) could not be not be fully explored and analysed. Furthermore, seasonal or monthly emission factors will enable full assessment of the most effective timings for applying mitigation options.

Conclusions This assessment showed that, based on our understanding of the efficacy of currently available agricultural CH_4 and N_2O mitigation strategies and their likely adoption rates, the impact of any individual measure on the New Zealand agricultural GHG emissions is very small (maximum 3% reduction). The options with the highest *potential* reduction (low N diet or diet supplementation) are difficult to implement within New Zealand's year-round grazing systems and hence have low expected adoption rates. This highlights the challenges that predominantly pastoral systems face with meeting GHG reduction targets.

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Additives have the potential to reduce methanogen colonisation in dairy calves

C Guzman[1], C Kentler[1], M Emmerling[2], A Franks[3], T Frankel[1]
[1]Department of Agriculture, La Trobe University, Melbourne, Victoria, Australia, [2]Department of Agriculture, La Trobe University, Melbourne, Victoria, Australia, [3]Department of Botany, La Trobe University, Melbourne, Victoria, Australia, [4]Department of Microbiology, La Trobe University, Melbourne, Victoria, Australia, [5]Department of Agriculture, La Trobe University, Melbourne, Victoria, Australia *Email:ceguzmanrodriguez@students.latrobe.edu.au*

Introduction During the past twenty years, additives have been extensively used in the developing rumen of calves to improve health, to decrease intestinal disorders, to enhance body condition, and to reduce feed costs. However, very few studies have examined whether feed additives can reduce methanogenic archaea, and few have examined the interaction between developing rumen microorganisms and their effect on methane production. The aim of this study was to determine the effects of a nucleotide feed additive (Ascogen®) and restricted feed intake on the microbial population in the developing rumen of calves.

Materials and Methods The animal experiment involved 40 holstein bull calves and was conducted according to a protocol approved by the Animal Care and Use Committee, Research Animal Resource Centre, La Trobe University, Australia. From 3 days of age the calves were fed twice daily according to the manufacturer's instruction at 8.00 am and 4.00 pm with the commercial calf-milk replacement (CMR) product, Venavite® full cream (Rivalea Ltd). All calves had *ad libitum* access to water and oaten hay. The factorial experiment consisted of two treatments given to calves from 5 to 20 days of age: 'control unrestricted' (CU), and 'nucleotides unrestricted' (NU). Calves given nucleotide treatment were given Ascogen® (1 g per feed, 2g/day), whereas calves receiving the control treatment were not. Ascogen® (Chemoforma, Switzerland) contains a minimum of 15% RNA/Nucleotides. Ruminal samples were taken at 7, 14, and 20 days of age. To determine and quantify the genes encoding 16S ribosomal DNA of the developing rumen microbiota I used the molecular techniques polymerase chain reaction (PCR), gel electrophoresis, cloning, sequencing, and quantitative polymerase chain reaction (qPCR). The following microorganisms were examined: Archaea, the genera *Methanobrevibacter* and *Methanobacterium* in the order Methanobacteriales and the proteolytic bacteria (*Prevotella ruminicola*); Details for analysis of these microorganisms can be found in Skillman *et al*. (2004), and Stevenson (2007). The data was analysed using the generalized linear model (GLM). Treatment means were compared using one-way ANOVA post hoc multiple comparisons with the Tukey's HSD test. All statistical analyses were conducted using version 8.0 SPSS (IBM). Results were considered significant at the $P < 0.05$ level.

Results At day 7, the ribosomal copy numbers in *Methanobrevibacter* and *Methanobacterium* were significantly increased in the NU treatments. At days 14 and 20, *Methanobrevibacter* spp and *Methanobacterium* spp were significantly decreased and *Prevotella ruminicola* was significantly increased in the NU treatments (Table 1).

Table 1 Threshold cycle (Ct) and treatment effects 7, 14 and 20 day old calve fed two different diets.

Days	7		14		20	
Treatments	CU	NU	CU	NU	CU	NU
Methanobrevibacter spp	28.72^{a} ± 2.32	25.51^{b} ± 2.09	20.29^{b} ± 2.14	23.29^{a} ± 1.26	16.80^{b} ± 1.00	21.83^{a} ± 1.71
Methanobacterium spp	31.67^{a} ± 1.70	26.29^{b} ± 1.52	24.62^{b} ± 1.18	29.19^{a} ± 1.32	25.00^{b} ± 2.56	27.21^{a} ± 1.64
Prevotella ruminicola	17.77^{a} ± 1.30	16.50^{a} ± 1.39	14.53^{a} ± 1.12	11.02^{b} ± 1.06	14.10^{a} ± 1.78	11.26^{b} ± 1.23

Treatments assigned the same letter in rows indicate no significant difference between the means ($P < 0.05$). (Mean ± s.d., n = 10).

Conclusion The results of this study show that nucleotides feed additives could have an effect on the interaction between microorganisms in the developing rumen of calves. Nucleotides in the diet increased the concentration of nucleic acids of *P. ruminicola* and decreased those of *Methanobrevibacter spp* and *Methanobacterium spp* suggesting that their interaction could be a mechanism to reduce methane production in the rumen. Further research is required to understand the interaction between microorganisms within the developing rumen of the young ruminant.

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Evaluation of methane production in high producing cows fed silages during the periparturient period

S Kume
Kyoto University, Kyoto, Japan *Email:kume@kais.kyoto-u.ac.jp*

Introduction The importance of high quality roughage has been recognized for maximal milk yield in high producing cows. Alfalfa is suitable for high producing cows because of the high protein content and high passage rate through the guts, and cows fed alfalfa silage produced more milk than did cows fed grass silage due to the high DMI during early lactation (Kume, 2002). The aim of the present study was to evaluate methane (CH_4) production in high producing cows fed silages during periparturient period, because the relationship between milk production and CH_4 production is not clear in high producing cows fed silages.

Material and methods Six and 7 multiparous Holstein cows were assigned an orchardgrass silage (GS) diet and an alfalfa silage (AS) diet from 3 weeks before the expected calving date to 4 weeks postpartum. Cows were fed to meet 120 % of TDN requirement for maintenance plus last 2 month of gestation level from 3 weeks prepartum to parturition, whereas cows were fed ad libitum after parturition. Silages and concentrates were given as a TMR in the ratio of 70:30 during prepartum and 50:50 during postpartum period. The cows were managed in individual tie stalls and a paddock. The cows were fed equal amounts at 08.00 and 16.00 h, and feed refusals were recorded every day. The cows were milked twice daily, and milk weights were recorded. Balance studies were performed for 8 cows during 7 days in the 2nd week before the expected calving date and cows were housed in the metabolic chamber. Additionally, the GS or AS diets was offered to 7 lactating cows in the switch back trials. Roughage and concentrates were given as a TMR in the ratio of 60:40 to meet the TDN requirements of cows. Cows were housed in the individual pens for 10 days during the feed adjustment period, followed by the 4 day collection period in the metabolic chamber. The metabolic chamber was maintained at 20 C and 60% relative humidity, and CH_4 production in pregnant or lactating cows was measured during the last 3 or 2 consecutive days. Data obtained from prepartum and postpartum period in cows were analyzed by least squares ANOVA using the general linear model procedure of SAS. Relationships between CH_4 production and DMI, ADF intake or NDF intake in cows were examined by correlation and regression analyses of SAS.

Results There were positive correlations ($P<0.001$) between CH_4 production and DMI (r=0.972), ADF intake (r=0.970) or NDF intake (r=0.890) in pregnant and lactating cows (Figure 1). The regression equation of DMI on CH_4 production was as follows.

$$Y = 25.9(\pm1.4)^{***}X - 100.1(\pm24.9)^{***} \quad (R^2=0.94,\ ^{***}P<0.001)$$

Prepartum DMI of cows was not affected by diets, but postpartum DMI of cows fed the AS diet was higher ($P<0.05$) than that of cows fed the GS diet. Milk yield and DMI of cows increased rapidly immediately after parturition (Figure 2), and milk yield of cows fed the AS diet was relatively high level. Prepartum and postpartum BW of cows were not affected by diets, but BW decreased drastically during 1 week after parturition. According to the regression equation of DMI on CH_4 production, the estimated CH_4 production in cows increased drastically immediately after parturition.

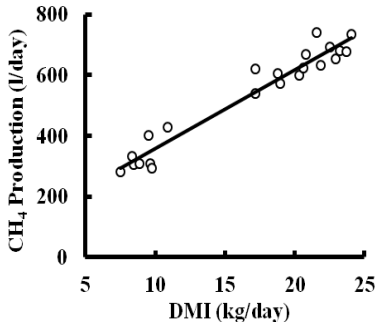

Figure 1 Relationship between DMI and CH_4 production in cows

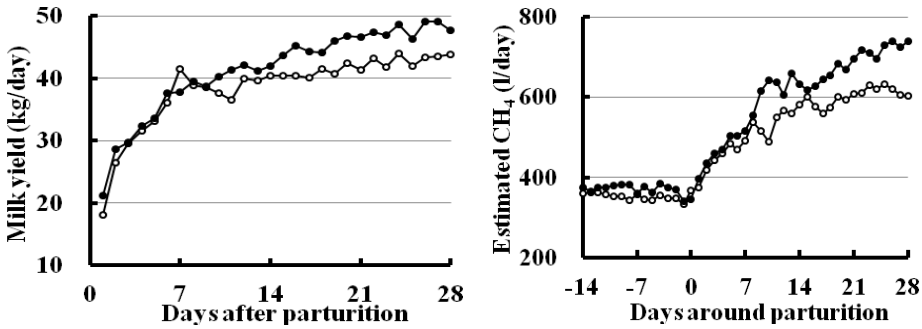

Figure 2 Milk yield and CH_4 production in cows fed different diets based on grass silage (○) and alfalfa silage (●) during periparturient period.

Conclusions These results show that DMI or ADF intake is highly correlated with CH_4 production in pregnant and lactating cows. Milk yield as well as DMI in lactating cows increased drastically during 1 week postpartum and reached at 39.7kg/day at 7 days postpartum. Feeding alfalfa silage improved DMI in lactating cows from 1 to 4 weeks postpartum. The CH_4 production in high producing cows may increase rapidly during 1 week postpartum and then increase gradually with the increased DMI.

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Effect of graded levels of tropical leaves containing- secondary metabolites on rumen fermentation pattern, protozoa population and methanogenesis in vitro

R Bhatta, M Saravanan, L Baruah, A Dhali, A Kolte, C S Prasad
National Institute of Animal Nutrition and Physiology, Bangalore, India *Email:ragha0209@yahoo.com*

Introduction Reduction of enteric methane emissions from livestock production is a high priority, since this biological process accounts for 2-12 % loss of dietary gross energy in ruminants (Johnson and Johnson, 1995). Moreover, methane is a potent greenhouse gas with a global warming potential 23 times higher than that of carbon dioxide (IPCC, 2007). Plant secondary metabolites such as tannins are particularly attractive as rumen modifiers as these compounds are natural products which are generally accepted to be environmental friendly and safe in food production systems. The objective of this study was to evaluate the potential of secondary plant metabolites to serve as anti-methanogenic additives in ruminant diets.

Material and methods After extensive screening, three tropical leaves (*Ficus bengalensis, Autocarous integrifolis* and *Azardirachta indica*) were selected to determine their optimum dose for methane suppression. 200 mg of the basal diet (containing 60 parts w/w concentrate: 40 parts w/w roughage) was incubated with graded levels (5 to 30 parts w/w) of the selected leaves individually, with 30-ml of buffered rumen inoculum at 39°C for 24 h in a shaking water bath.
Results All the leaves contained high concentration of total phenol and condensed tannin (table 1). As the level of leaves increased, the net gas volume and methane decreased. The basal diet produced 36.0 ml methane/200 mg DM. *Ficus bengalensis* leaves at 10.0 parts (w/w) inclusion in the basal diet suppressed 14.7 % methane, whereas methane suppression was 19.2 in *Autocarpus integrifolis* and 28.4 % in *Azardirachta indica,* respectively. There was no reduction ($P<0.05$) in the TVFA production except at 10 parts inclusion of *F. bengalensis*. Ammonia-N level and protozoa count reduced significantly ($P>0.05$) in *Ficus bengalensis* and *Autocarpus integrifolis*, whereas in *Azardirachta indica* leaves, ammonia-N levels reduced but protozoa count was not altered. The methane suppression recorded in *Ficus bengalensis* and *Autocarous integrifolis* was due to indirect effect (defaunation) and in *Azardirachta indica* leaves it was probably due to direct effect (reduction in the methanogens) (Bhatta *et al*., 2009).

Table 1 Nutrient content, total phenols (TP), total tannins (TT) and condensed tannin (CT) of leaves (g/kg DM)

Tree leaves	CP	NDF	ADF	ADL	TP[a]	TT[a]	CT[b]
Autocarpus integrifolis	123	362	252	93.0	76.6	66.8	186
Azardirachta indica	145	395	285	99.6	108	99.9	138
Ficus bengalensis	140	409	300	103	103	90.3	260

[a] As tannic acid equivalent [b] As leucocyanidin equivalent

Table 2 Effect of graded level of tree leaves on methane production, TVFA, NH_3-N and protozoa leaves

Sample	CH_4 ml/g	TVFA (m Mol/dl)	NH_3-N (mg/dl)	Protozoa (10^5)
Basal diet	*36.0*	*14.6*	*56.0*	*0.243*
Inclusion level parts (w/w)		*Autocarpus integrifolis*		
2.5	30.5[c]	13.7	51.8[b]	0.265[c]
5.0	30.2[b]	12.4	50.8[a]	0.230[b]
10.0	29.1[a]	12.0	50.4[a]	0.206[a]
Azardirachta indica				
2.5	28.9[c]	13.8	41.5[c]	0.250
5.0	26.4[b]	12.7	39.6[b]	0.245
10.0	25.8[a]	12.5	38.2[a]	0.229
Ficus bengalensis				
2.5	28.5[c]	16.5[b]	43.4[c]	0.264[c]
5.0	27.9[b]	16.0[b]	39.2[b]	0.257[b]
10.0	27.3[a]	12.0[a]	35.7[a]	0.221[a]

Means in a row with different superscript differ significantly ($P<0.05$)

Conclusions These results show that tree leaves containing plant secondary metabolites such as *Ficus bengalensis, Autocarpus integrifolis* and *Azardirachta indica* at 10 parts (w/w) could be strategically used in animal feeding to suppress rumen methanogenesis.

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Detectability of methane emissions from grazing cattle

S M McGinn[1], T K Flesch[2], T W Coates[1], M Bai[3], G Bishop-Hurley[3], E Charmley[3], N Tomkins[3], D Chen[4]
[1]Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada, [2]University of Alberta, Edmonton, Alberta, Canada, [3]CSIRO, Townsville, Queensland, Australia, [4]University of Melbourne, Melbourne, Victoria, Australia
Email:sean.mcginn@agr.gc.ca

Introduction It is recognized that mass balance and dispersion models can be used to estimate enteric methane (CH_4) losses from grazing cattle, when the appropriate CH_4 concentration measurements are made in the field (Harper *et al*., 2011). Critical for these techniques is having a detectable difference in the CH_4 concentration upwind and downwind of the cattle. This difference must be larger than the resolution of the CH_4 concentration sensors. The objective of our study was to evaluate the influence of stocking density on the detectable rise in CH_4 concentration when using open-path lasers to measure upwind and downwind concentrations from grazing cattle.

Material and methods Our study was carried out at the CSIRO Lansdown farm (Queensland, Australia), where we enclosed a herd of cattle (Brahman steers; average live weight 414 kg ± 51) sequentially in six 100 by 100 m paddocks (two paddocks at a time). The composition of the paddocks was similar, consisting of mostly Rhodes grass (*Chloris gayana*) and a legume seca stylo (*Stylosanthes scabra*). One set of paddocks (Control; always containing 30 cattle) was paired to a second set of nearby paddocks (Treatment) where the animal number deceased from 30 to 20 to 10 over three periods; each period was 3 d. Methane concentration was measured using open-path lasers (GasFinder, Boreal Lasers, Edmonton, Alberta, Canada). One laser was used to monitor CH_4 concentration along the north and west perimeter of the cattle enclosures (laser was mounted on a scanning motor). A second laser was 'fixed' to measure CH_4 concentration either along the south or east perimeter of each paddock. Prior to this study, the lasers were compared in a side-by-side test, and corrections were applied to account for bias in the instruments. During this time, we determined that the temporal drift in CH_4 concentration between the bias-corrected study lasers was 0.02 ppm, i.e., the minimum detectable downwind concentration rise for these lasers.

Results The difference between the downwind and upwind CH_4 concentrations was calculated for the Control and Treatment from the 10-min averaged measurements (total n=371, 89 and 124 for periods 1 to 3, respectively). Most of the missing 10-min measurements in a period (maximum n≈432) were due to instrument problems (return light levels too high on the 'fixed' laser) and unsuitable wind directions for the site configuration. The fraction of the measurement period (x/n) when the critical value of 0.02 ppm was exceeded was determined for all Control and Treatment paddocks (Fig. 1). The fraction of the time that the concentration gradient was detectable was between 0.75 and 0.83 for all paddocks except for the Treatment in Period 3 (0.32) that contained only 10 cattle/ha.

Conclusions These results indicate at least 20 cattle are needed when using open-path lasers in our 1-ha study configuration, in order to maximize the amount of CH_4 emission data from the confined cattle.

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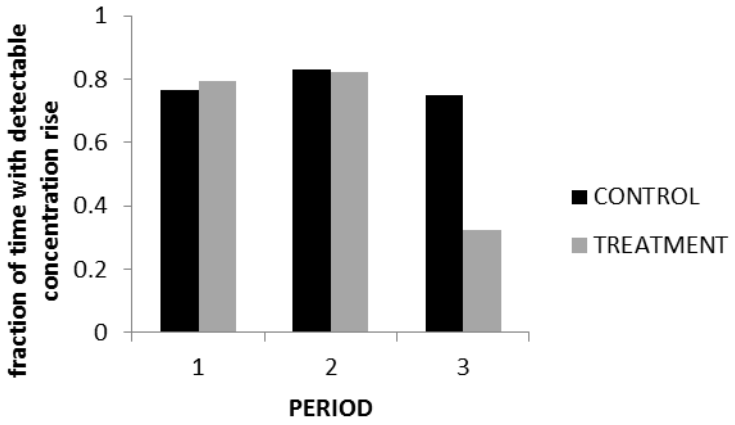

Figure 1 Fraction of the time where the methane concentration difference is detectable for three periods corresponding a Control paddock (always 30 cattle/ha) and a Treatment paddock with decreasing number of cattle (30, 20 and 10 cattle/ha for Period 1-3, respectively).

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Effects of dietary addition of cellulase/xylanase on enteric methane emissions in growing goats

Y Li[1], L Z Wang[1], B Xue[1], T Yan[2]
[1]Animal Nutrition Institute, Sichuan Agricultural University, Yaan, Sichuan 625014, China, [2]Agri-Food and Biosciences Institute, Hillsborough, Co. Down BT26 6DR, UK *Email:tianhai.yan@afbini.gov.uk*

Introduction Methane production from enteric fermentation is a major source of greenhouse gas emissions in goat production systems. There is increasing interest to develop mitigation strategies to reduce this source of emissions across the world. Dietary additives to alter rumen fermentation are widely used to explore relationships between CH_4 emission rates and feed intake or product output. The objective of the present study was to evaluate effects of dietary addition of cellulase/xylanase on enteric CH_4 emissions of growing goats.

Material and Methods Twelve castrated male growing goats (Boer x Jianchang Black) were used in a 3 treatments x 3 periods (4 weeks/period) complete changeover study. At commencement of the study, animals were at ages of around 10 months and had a live weight from 18.0 to 24.4 kg. The animals were blocked into 4 groups of 3 goats, according to live weight and age, and the 3 goats within each group were then allocated randomly to the 3 treatments. The 3 treatment diets were control, which contained 70% of forages (Lucerne and L. Chinensis at a ratio of 50%/50%) and 30% of concentrate meal (DM basis), and the control diet supplemented, respectively, with cellulase and cellulase/xylanase at a ratio of 0.2% of diets. Both cellulase and cellulase/xylanase had an enzyme activity of 10000IU and were produced by Shanghai Biochemical Co. Ltd, Shanghai, China. The control diet contained 888 g/kg of DM, 17.0 MJ/kg DM of GE, and 80, 119, 259 and 411 g/kg DM of ash, CP, ADF and NDF, respectively. Diets were offered ad libitum in 2 equal portions in the morning and afternoon. The 4 animals of each treatment were housed in a single pen for 14 days before being transferred to digestibility crates, where goats were remained for 5 days with total collection of faeces and urine during the final 3 days. Afterwards, the animals were housed individually in respiration calorimeter chambers for 3 days with gaseous exchange measured during the final 48 hours. All data were analysed using one-way ANOVA with experiment period as block.

Results The results are presented in Table 1. There was no significant difference in live weight, live weight gain or DM intake. Dietary addition of enzymes had no significant effects on total CH_4 emissions (g/d), CH_4 emission as a proportion of live weight or feed intake (DM, OM, digestible DM or digestible OM), or CH_4 energy output (CH_4-E) as a proportion of energy intake (GE, DE or ME). The CH_4-E/GE intake ranged from 0.059 to 0.061, which is within recommendations of IPCC (2006) for lamb (0.045) and mature sheep (0.065) for development of Tier 2 emission inventories. There is no recommendation of CH_4-E/GE intake for goats in IPCC (2006). There was a significant ($P < 0.001$) relationship between CH_4 and live weight ($y = 0.645x + 0.2$, $R^2 = 0.54$), CH_4 and DM intake ($y = 16.7x + 1.4$, $R^2 = 0.51$), CH_4 and OM intake ($y = 18.8x + 1.3$, $R^2 = 0.51$) and CH_4-E and GE intake (Fig 1).

Table 1. Effects of dietary addition of enzymes on enteric CH_4 emissions

	Control	Cellulase	Cellulase/ Xylanase	s.e.	P
Live weight (kg)	22.8	22.7	22.9	0.45	0.967
DM intake (g/d)	0.856	0.836	0.863	0.0159	0.473
CH_4 emissions (g/d)	15.6	15.6	15.5	0.47	0.976
CH_4/DM intake (g/kg)	18.3	18.7	18.0	0.39	0.414
CH_4/OM intake (g/kg)	20.4	20.9	20.1	0.44	0.414
CH_4/Live weight (g/kg)	0.686	0.688	0.678	0.0151	0.886
CH_4-E/GE intake	0.060	0.061	0.059	0.0013	0.435
CH_4-E/DE intake	0.093	0.090	0.090	0.0022	0.525

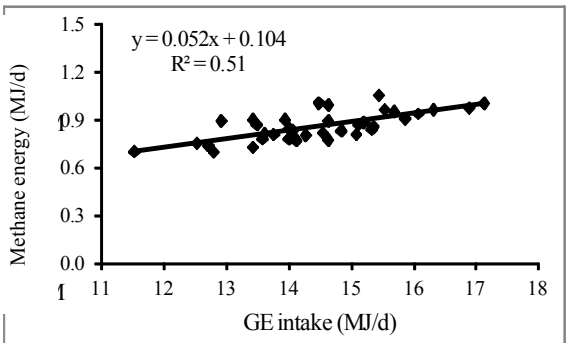

Fig 1. Relationship between CH_4-E and GE intake

Conclusions Dietary addition of cellulase or cellulase/xylanase had no effects on feed intake or enteric CH_4 emissions in growing goats. A range of equations have been developed to predict enteric CH_4 emissions from live weight and feed intake.

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Temporal variation and provincial distribution of methane emissions for dairy cattle, beef cattle, buffaloes and yaks in China

B Xue[1], L Z Wang[1], T Yan[2]
[1]Animal Nutrition Institute, Sichuan Agricultural University, Yaan, Sichuan 625014, China, [2]Agri-Food and Biosciences Institute, Hillsborough, Co. Down BT26 6DR, UK *Email:tianhai.yan@afbini.gov.uk*

Introduction The economic boom in China during the last 30 years has resulted in dramatic changes in cattle production systems (dairy, beef, buffaloes and yaks), with increasing demand in milk and meat consumption and discontinued use of beef cattle and buffaloes as draft animals. The present study aimed to evaluate the responses during this period in temporal variation and provincial distribution of CH_4 emissions from enteric fermentation and manure management for cattle in China.

Materials and methods Methane emission inventories for enteric fermentation and manure management for 4 cattle species (dairy cattle, beef cattle, buffaloes and yaks) in China from 1988 to 2009 were estimated using Tier 2 methodologies of the International Panel on Climate Change (IPCC, 2006). Data used were derived from various sources including national statistical records, publications, farm survey and advices of specialists. The CH_4 emission inventories for each species of cattle were developed according to their physiological states: milking cows, dry cows/breeding bulls and steers/heifers at various ages (0 to 1 and over 1 years old for dairy cattle; 0 to 1, 1 to 2 and over 2 years old for beef cattle; 0 to 1, 1 to 2, 2 to 3 and over 3 years old for buffalos and yaks). Enteric CH_4 emissions were calculated from collated data (live weight, milk production/growth rate, dietary energy concentration and calving rate), and default data (e.g., CH_4 energy output as a proportion of GE intake) of IPCC (2006). Methane emissions from manure management were estimated from OM output in faeces using collated data and default factors of IPCC (2006).

Results In general, CH_4 emission factors (kg/year/head) from enteric fermentation and manure management for individual cattle were in accordance with the levels of production, i.e., emission factors are higher with dairy cattle and lower with yaks, with intermediate emissions from buffaloes and beef cattle. The total national CH_4 emission inventories for enteric fermentation and manure management of all 4 species increased gradually from 4514 to 5777 Gg/year during the period of 1988 to 2009 (Table 1). This increase was mainly driven by the increase in the population and productivity of dairy and beef cattle, with which CH_4 emissions increased respectively by 43.0 and 36.8 Gg per year (8.2 and 1.3 times) during the 21 year period. However, the corresponding values for buffaloes and yaks were negative (-12.7 and -7.0 Gg per year, or reduced by 31 and 24% respectively), which reflected the decline in their population from 1988 to 2009. Beef cattle are the main emitters which accounted for 63.9% of total cattle emissions in 2009, followed by dairy cattle (17.8%), buffaloes (10.3%) and yaks (8.1%) (Fig 1). The emissions from the latter two species are regionalized, with buffaloes in Southern China and yaks in Tibet and surrounding provinces. When examining provincial distributions as a percentage of national total for all 4 species in 2007, Sichuan Province was the largest producer (10.5%), followed by Tibet (8.6%) and Henan (8.5%), while Beijing (0.2%), Zhejiang (0.2%) and Shanghai (0.1%) were the lowest producers.

Table 1. Tier 2 CH_4 emission inventories (Gg/y) for cattle in China

Year	Dair cattle	Beef cattle	Buffalo	Yak	All cattle	Increase rate (%)[1]
1988	125	2915	860	614	4514	100
1991	179	3207	909	594	4889	108
1994	230	3744	949	555	5477	121
1997	266	3617	937	489	5310	118
2000	309	3987	950	443	5689	126
2003	587	4047	932	497	6062	134
2006	805	3148	645	493	5091	113
2009	1028	3689	593	467	5777	128

[1] Based on the value of all cattle in 1988

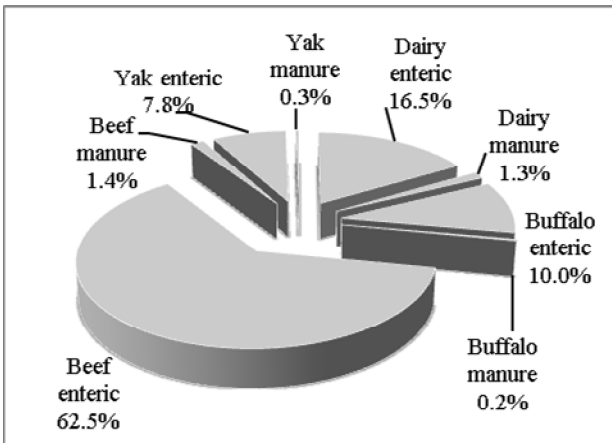

Fig 1. Tier 2 cattle CH_4 inventories in 2009

Conclusion There were considerable changes in CH_4 emissions from dairy cattle, beef cattle, buffaloes and yaks in China during the last 21 years. The results from the present study can provide benchmark information for Chinese authorities to develop appropriate mitigation strategies to reduce CH4 emissions from cattle production systems.

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IPCC. 2006. IPCC Guidelines for National Greenhouse Gas Inventories. In:www.ipcc-nggip.iges.or.jp/public/2006gl/index.html.

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Greenhouse gas emissions from pig and poultry production sectors in China during the last 50 years

L Z Wang[1], B Xue[1], T Yan[2]
[1]Animal Nutrition Institute, Sichuan Agricultural University, Yaan, Sichuan 625014, China, [2]Agri-Food and Biosciences Institute, Hillsborough, Co. Down BT26 6DR, UK *Email:tianhai.yan@afbini.gov.uk*

Introduction Pig and poultry production sectors in China have experienced considerable changes during the last 50 years in responses to the social and economic development. The economic boom in China during the last 30 years has resulted in an increasing demand in animal products to improve the food structure. The present study aimed to evaluate effects of these changes on greenhouse gas (GHG) emissions from pig and poultry (chicken, duck, goose and others) sectors in China from 1960 to 2010.

Materials and methods The present GHG emission inventories include CH_4 emissions from enteric fermentation and CH_4 and N_2O productions from manure management, and were developed using Tier 1 methodologies of the International Panel on Climate Change (IPCC, 2006) and CH_4 emission data from enteric fermentation of poultry published elsewhere. Data used in the present study were derived from various sources including national statistical records, publications, farm survey and advices of specialists. The inventories for pigs were developed as a sum of GHG emissions from 3 groups: slaughtered pigs, growing pigs and sows/boars. A similar approach was also used for each species of poultry (chicken, duck, goose and others – mainly turkey), which was categorized as 3 groups: slaughtered birds, broilers and hens/breeding birds. The populations for growing/finishing pigs and broilers (slaughtered and in stock) were adjusted according to their life span if they were less than one year in life (IPCC, 2006). The inventories for each group of pigs and poultry were developed from 3 sources of GHG emissions, i.e., CH_4 from enteric fermentation and CH_4 and N_2O from manure management. The latter sub-inventories were calculated as two separate parts, one for pigs and poultry reared in northern China and other in southern China to account for the effects of climate conditions. The CO_2 equivalent (CO_2e) was used to calculate total GHG emissions (IPCC, 2006), with the global warming potential of CH_4 and N_2O equivalent to 25 or 298 times of that of CO_2, respectively.

Results The CO_2e from enteric CH_4, manure CH_4 and manure N_2O accounted approximately for 17, 62 and 21% of total emissions in pigs and 1, 18 and 81% in poultry, respectively, during the last 50 years. Total CO_2e emissions increased gradually from 1960 to 2010 with both sectors of pigs (11582 to 55564 Gg/y) and poultry (1497 to 14873 Gg/y) (Table 1). Within the poultry sector, emissions were mainly derived from chickens, for example, emissions in 2010 were 74, 15, 11 and 0.01% from chickens, ducks, geese and others (e.g., turkey). The last 50 years also saw a significant increase in GHG emissions from slaughtered pigs as a proportion of total emissions from all pigs (29 to 53%) and a significant decrease in growing pigs (67 to 38%), with a small change in sows and boars (5 to 9%). The similar changes were also found in the poultry sector, with an increase in emissions from hens and breeding birds (34 to 57%) and a decrease from broilers (29 to 8%), with little change in slaughtered birds (37 to 36%). However, during the last 50 years, these emissions continuously reduced when related to production of a unit of pork (8.01 to 1.14 kg/kg) and poultry meat (1.19 to 0.37 kg/kg) and egg (0.47 to 0.33 kg/kg) (Fig. 1).

Table 1. GHG emissions (Gg/y) from pigs and poultry in China

	CO_2e from pig sector				CO_2e from poultry sector			
	Enteric CH_4	Manure CH_4	Manure N_2O	Total	Enteric CH_4	Manure CH_4	Manure N_2O	Total
1960	1969	7140	2473	11582	7	274	1216	1497
1970	6576	23828	8256	38660	12	372	1627	2011
1980	8957	32456	11246	52659	14	445	1995	2455
1990	8057	29237	10122	47416	30	891	4047	4969
2000	8216	29905	10336	48457	77	2228	10296	12602
2010	9408	34309	11847	55564	95	2626	12153	14873

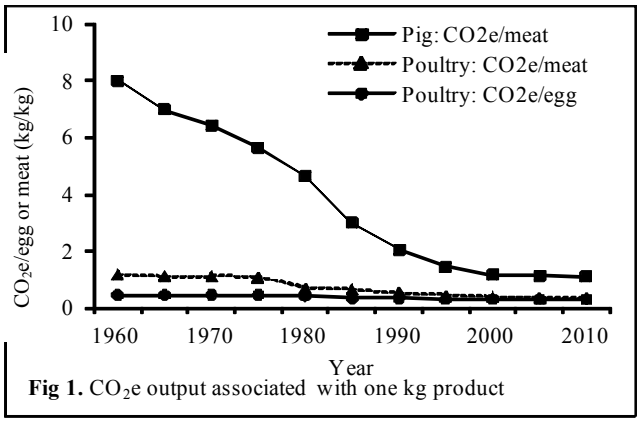

Fig 1. CO_2e output associated with one kg product

Conclusion There were considerable changes in CO_2e emissions from pig and poultry sectors in China during the last 50 years. The results from the present study can provide benchmark information for Chinese authorities to develop appropriate mitigation strategies to reduce GHG emissions from pig and poultry production systems.

Reference

IPCC. 2006. IPCC Guidelines for National Greenhouse Gas Inventories. In:www.ipcc-nggip.iges.or.jp/public/2006gl/index.html.

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Rumen microbiome profiles predict enteric methane production from dairy cattle

E M Ross[1,2], P J Moate[1], L Marett[1], B G Cocks[1,2], B J Hayes[1,2]
[1]Department of Primary Industries, Victoria, Australia, [2]La Trobe University, Victoria, Australia, [3]Dairy Futures Cooperative Research Centre, Victoria, Australia *Email:elizabeth.ross@dpi.vic.gov.au*

Introduction Ruminants such as cattle (*Bos taurus* & *Bos indicus*) are highly valued for converting forages to meat and milk. This conversion is obtained through microbial fermentation in the rumen, which is performed by thousands of symbiotic organisms. These microorganisms also produce the potent greenhouse gas methane (CH_4). Enteric CH_4 emissions from farmed ruminant livestock account for approximately 15% of global CH_4 emissions. A constraint for reducing CH_4 emissions from livestock is that the number of obtainable CH_4 production phenotypes is limited as current techniques require expensive equipment and substantial training of animals. Here we present a new method termed 'metagenomic predictions'. Metagenomic predictions use symbiotic rumen microbiome profiles to predict enteric CH_4 production from cattle.

Methods Two separate cohorts of animals were used for this experiment (Reference and Validation). CH_4 production was measured by SF_6 in the Reference cohort and respiration chambers over two days in the Validation cohort. Low CH_4 phenotypes were induced in a subset of the reference population by feeding a CH_4 mitigating feed additive (Grapemarc, a by-product of the wine industry; described in Moate *et al.* 2012). Both cohorts had rumen fluid sampled via stomach tube within one day of phenotype completion. Microbial DNA was extracted from the rumen fluid and sequenced on a HiSeq2000 as per Ross *et al.* (2012). Rumen microbial DNA sequence from a HiSeq2000 was used to generate metagenomic profiles for all animals by aligning the sequence reads to a database of rumen derived contigs (as per Ross *et al.* 2012). Several metagenomic profiles were combined form an $n \times m$ matrix $\mathbf{X}$ with elements x_{ij}, the log transformed and standardised count for sample i for contig j, with n samples and m contigs.
The phenotype used here was residual CH_4 production, calculated by obtaining the residuals of a linear regression of methane yield (g/day) on dry matter intake (kg/day). Using residual CH_4 production as the phenotype removes the effect of dry matter intake on CH_4 yield. The reference and validation cohorts of animals that were used here were maintained separately; there was no animal present in both cohorts.
The metagenomic profiles were compared to make a rumen microbiome relationship matrix (calculated as $\boldsymbol{G} = \boldsymbol{XX'} / m$). Best linear unbiased prediction (Henderson 1984) was then used to predict phenotypes for validation samples. A mixed model was fitted to the data: $\boldsymbol{y} = \boldsymbol{1}n\mu + \boldsymbol{Z}\boldsymbol{g} + \boldsymbol{e}$. Where y is the a vector of phenotypes, with one record per sample, $\boldsymbol{1}n$ is a vector of ones, μ is the overall mean, $\boldsymbol{Z}$ is a design matrix allocating records to samples, and $g \sim N(0, \boldsymbol{G}\sigma 2g)$. Using ASReml (Gilmour *et al.,* 2006), phenotype of the samples ($\hat{g}$) were predicted as:

$$\begin{bmatrix} \hat{\mu} \\ \hat{g} \end{bmatrix} = \begin{bmatrix} 1_n'1_n & 1_n'Z \\ Z'1_n & Z'Z + G^{-1}\frac{\sigma_\varepsilon^2}{\sigma_g^2} \end{bmatrix}^{-1} \begin{bmatrix} 1_n'y \\ Z'y \end{bmatrix}$$

Results Metagenomic predictions were successfully applied to predict residual CH_4 production. The prediction accuracy was r=0.472 (Figure 1), with a 95% confidence interval (from 100,000 bootstraps) of 0.155-0.743. The between day repeatability for the residual CH_4 production phenotype was 0.50. This dataset is small hence results must be regarded as preliminary.

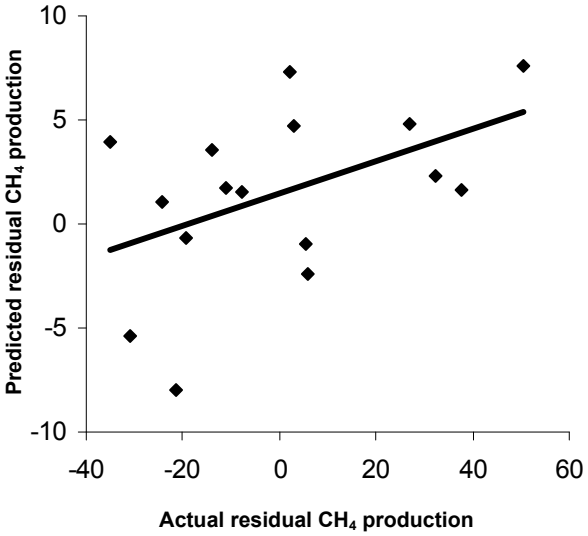

Figure 1. Accuracy of the metagenomic prediction method for CH_4 production on a small dataset (N=16). The phenotype (residual CH_4 production) is CH_4 production corrected for dry matter intake.

Conclusions Our results suggest that rumen microbiome profiling can be used to predict enteric CH_4 production from cattle, with an accuracy that exceeds the theoretical maximum accuracy of genomic prediction (0.36; based on heritability; Pinares-Patino *et al.,* 2013). These findings may allow rumen microbiome profiles to be used as low cost proxies for CH_4 production phenotypes in individual cattle. This work is an important step towards lowering the greenhouse gas footprint from cattle.

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Effect of DCD and season of application on N_2O emissions from urine patches

L M Cardenas[1], N Donovan[1], C Hodgson[1], S Norris[1], R Murray[1], E Cancer-Berroya[1], T Misselbrook[1], D Chadwick[1,2]
[1]Rothamsted Research, Okehampton, Devon, UK, [2]Bangor University, Bangor, UK
Email:laura.cardenas@rothamsted.ac.uk

Introduction The South West of the UK is characterised by grassland on soils with high clay content that is mostly grazed and so emissions of N_2O are expected to occur from urine deposition. Addition of dicyandiamide (DCD), a nitrification inhibitor, has been shown to reduce emissions in countries such as New Zealand (Di and Cameron, 2012). In this study we investigated the effect of urine with and without DCD applied in Spring, Summer or Autumn on emissions of N_2O from a grassland soil in the South West of England.

Material and methods Measurements of N_2O were carried out in 2012 on a permanent pasture in Devon, South West England. The soil was a clay loam (see characteristics in Table 1). Urine (real and artificial) was applied to small plots in three different experiments on the same field at different times of the year (spring, summer and autumn 2012). The average N loading in each application and climate data corresponding to each experiment are given in Table 2. Emissions of N_2O were measured using the static chamber method, soil mineral-N and soil moisture content were measured routinely. Here we present the data for the first 3 months after urine deposition for all three experiments.

Results The total rainfall was 674.5 mm during the entire period of measurements (15/5-18/12/2012). Mean air temperature ranged between 3.9 and 19.9◦C. Emissions of N_2O increased after application of urine in all three experiments (spring, summer and autumn) (Fig. 1). Two peaks were observed in all three experiments with the largest fluxes observed in the order spring>summer>autumn applications. Urine deposition resulted in the largest N_2O fluxes in spring and autumn, with fluxes from artificial urine and real urine being similar in all seasons. DCD appeared to be more effective in the spring and autumn than in summer.

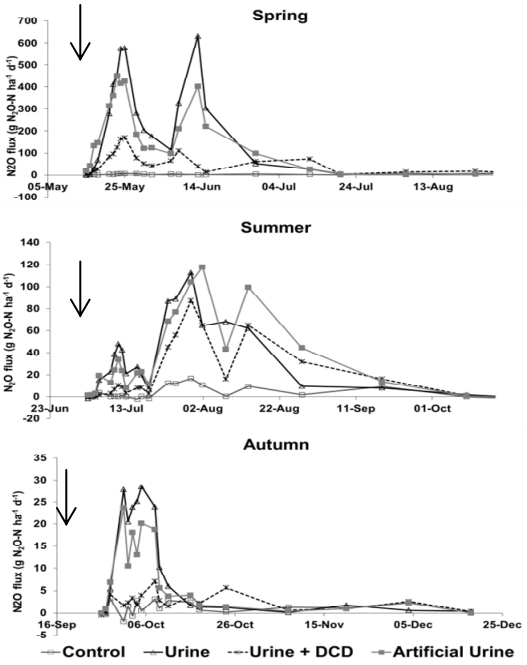
Table 1 Soil characterisation for the field site

Site	North Wyke
Field name	Beacon field
Soil type (soil series)	Clay loam
pH	6.2 (0.8)
Olsens P mg/l	12.1 (11.1)
K mg/l	102.6 (73.3)
Mg mg/l	104.5 (20.9)
%Org C	2.4 (2.2)
SO_4= mg/l	43.2 (13.1)
Total N%	0.3 (0.2)
Sand, 2.00-0.063mm	13.6 (5.6)
Silt, 0.063-0.002mm	43.2 (3.0)
Clay, <0.002mm	43.2 (6.4)
Bulk density 0-7.5cm	0.62 (0.05)

Table 2 N loadings for each urine type and climate data for each experiment

	Urine, kg N ha^{-1}	Artificial urine, kg N ha^{-1}	Rainfall, mm	Max temp °C	Min temp °C
Spring (15/5-24/8)	405	440	412.4	27.7	0.72
Summer (3/7-10/10)	429	481	388.1	27.7	2.0
Autumn (26/9-18/12)	435	423	398.2	18.5	-3.5

Figure 1 N_2O fluxes after urine application (arrows indicate date of application)



Conclusions
-There were marked differences in the magnitude of emissions from the urine treatments between season of deposition
-DCD had a marked effect in reducing N_2O fluxes in two of the three seasons
-Data are still being collected to generate 12-month IPCC compliant EFs, so results should be considered as preliminary at this stage.

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Anaerobic digestion of cattle slurry – global warming potential of stored and field-applied slurry, non-digested and digested

L K K Rodhe[1], J Ascue[1], A Willén[1,2], Å Nordberg[2,1]
[1]JTI - Swedish Institute of Agricultural and Environmental Engineering, Uppsala, Sweden, [2]Department of Energy and Technology, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden *Email:lena.rodhe@jti.se*

Introduction Anaerobic digestion of slurry can benefit agriculture as it reduces net emissions of greenhouse gases (GHG) by providing methane (CH_4) to replace fossil fuels. However, GHG from storage and after field application of slurry and digestate should also be included, in order to compare the total impact on global warming from digestion compared with non-digestion of slurry. Specific aims of this study were: to quantify the global warming potential (GWP) of non-digested and digested cattle slurry during storage in summer and winter and after spreading before sowing of cereals in autumn and spring; and to determine the effects on GHG emissions of covering stored digested slurry.

Material and methods Emissions of the GHG methane (CH_4) and nitrous oxide (N_2O) from non-digested and digested cattle slurry were measured during storage in a pilot-scale unit and after field application. In addition, emissions of ammonia (NH_3) were measured after field application in spring. Three treatments, non-digested cattle slurry without cover (CS), digested cattle slurry without cover (DCS), and digested cattle slurry with roof (DCS-R) were studied during storage in summer and winter. After storage, CS, DCS and an unfertilised control (Control) were studied when applied before sowing winter wheat in late summer and spring barley in spring. The band spread slurry was incorporated directly after spreading in autumn and in spring four hours after spreading, as additionally ammonia (NH_3) emissions was measured after spring application with an equilibrium concentration method (Salomon & Rodhe, 2013). GHG measurements were conducted using a closed chamber technique (Livingston & Hutchinson, 1995) either by closing the storage tanks with a gastight cover (Rodhe *et al*., 2012) or in the field with three chambers per small plot (Rodhe *et al*., 2006). Both storage and field experiments were organised as a randomised complete block design with three blocks. Differences between cumulative CH_4 and N_2O emissions were analysed using one-way ANOVA with blocks, followed by pair-wise comparisons with a t-test using PROC GLM in SAS. The global warming potential (GWP_{100}) was calculated from measured direct CH_4 and N_2O emissions and from indirect N_2O emissions from NH_3, measured in the field and estimated from default values in storage.

Results GHG emissions during summer storage were much higher than during winter storage. During summer, CH_4 emissions were about three-fold higher from DCS than from CS, but during winter they were higher from CS than from DCS. The roof reduced CH_4 emissions, but also stimulated formation of N_2O. Thus when recalculated to CO_2e, the roof had no effect on GWP_{100}. The N_2O emissions from field-applied CS and DCS were rather low at autumn application and very low at spring application and the soil often acted as a sink for CH_4. The fraction of applied total-N lost as N_2O-N was highest for CS, but due to the high NH_3 emissions from DCS in spring, the sum of indirect and direct N_2O emissions at spring application was higher for DCS than for CS. GWP_{100} values for the different treatments and combinations are summarised in Table 1.

Table 1 Greenhouse gas emissions (direct and indirect nitrous oxide and methane) from storage and after spreading of cattle slurry (CS), digested cattle slurry (DCS) and digested cattle slurry stored with a roof (DCS-R) during summer or winter storage followed by autumn or spring application. Emissions given in CO_2e (kg m^{-3} slurry at start of storage)

	1. Summer storage	2. Autumn spreading	3. Winter storage	4. Spring spreading	Sum of 1+2	Sum of 3+4
CS	7.90	7.62	0.51	2.00	15.52	2.51
DCS	24.19	4.50	0.04	3.88	28.69	3.92
DCS-R	23.73		-0.01			

Conclusions Digestion of cattle slurry may lead to increased GWP during handling compared with non-digested cattle slurry. Storage of digested cattle slurry should be minimised in warm seasons. Gastight covers for collecting CH_4 emissions during summer storage or cooling/acidification of the stored slurry may prevent such emissions. As NH_3 emissions were rather high from field-applied DCS in spring, it is important to use measures to minimise NH_3 emissions in order to limit indirect N_2O emissions.

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Seasonal climate influence in carbon stocks in Pampa biome natural grassland in Brazil

C B Trentin, E L Fonseca
Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil *Email:cbtrentin@yahoo.com.br*

Introduction The Pampa biome is located in Argentina, Brazil and Uruguay. In Brazil is restricted to the state of Rio Grande do Sul originally occupying 63% of the territory of the State, with herbaceous vegetation predominant. Natural grassland of the Pampa biome contain important stocks of organic carbon (C), contributing to the reduction of emission of C to the atmosphere (Pillar, Tornquist, Bayer, 2012). However, knowledge of the dynamics and carbon deposits on the Pampa biome are still partial and incomplete (Paruelo *et al.*, 2010). In order to evaluate the potential of grassland vegetation in store C, this study aims to verify the seasonal climate interference in carbon content in different storage locations (aboveground, leaf litter, roots and soil) in Pampa biome natural grassland.

Material and methods The collection of field samples (aboveground, leaf litter, roots and soil) was carried out in two periods in 2012: summer and winter (greater and lesser period of biomass accumulation in Pampa biome, respectively). The experimental design was of randomized blocks with nine replicates. Nine experimental units were defined and the samples collected in randomly 5 points per experimental unit, for each of the 4 variables: aboveground, leaf litter, roots and soil. To quantify above ground carbon samples were collected of aboveground shoots and leaf litter in a sample area of 50 x 50 cm. The shoot was collected using a pair of scissors, cutting all vegetation close to the ground within that square. All the existing leaf litter within this same area after removal of the aboveground vegetation was also collected. For soil and root carbon samples were collected in areas of 10 x 10 cm, at an average depth of 15 cm, following the methodology recommended for soil sampling for agricultural purposes. From the soil and roots were separated and analyzed separately. Analysis of organic carbon was performed by wet combustion method (Walkley Black). Seasonal variations of carbon for each of the four variables have been verified through the t-test for averages comparison, using the statistical software Statistical Package in Social Science (SPSS).

Results The t-test results showed that the largest average quantities of carbon stored is present at the plant biomass (above ground, leaf litter and roots) during the summer, as expected for this type of vegetation, since lower temperatures can decrease the productivity of vegetation, resulting in decreases in reservoir C (Kunkel *et al.*, 2011). It was also found that there are significant differences (for $\alpha = 5\%$) between the two seasons in stocks in the above ground carbon (shoots and leaf litter) and roots carbon. As expected, the Pampa biome soil had low carbon stocks in plant biomass. There were no seasonal differences in soil carbon stocks (for $\alpha = 5\%$) (Table 1).

Table 1 Significance test for the averages comparison of variables: the aboveground, leaf litter, roots and soil between the two seasons (summer and winter)

	Aboveground shoots		Leaf Litter		Roots		Soil	
	summer	winter	summer	winter	summer	winter	summer	winter
Average (%)	41.49	30.91	40.11	34.56	25.42	15.20	4.46	4.04
t measured	35.222^{**}		15.565^{**}		1.358^{*}		1.774^{ns}	

^{ns} not significant; * and ** significant at the 5% and 1% level of probability

Conclusions There are differences in plant biomass (above ground shoots, leaf litter and roots) carbon stocks between summer and winter, noting that the seasonality climate interferes in carbon content stored in natural grassland. The C content in the soil was not influenced by seasonality climate. However, further analyses should be carried out in subsequent years, since the results may have been influenced by external factors, such as temperature and precipitation conditions this year.

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Evaluating approaches to improving animal nitrogen use efficiency and reducing nitrous oxide emissions on dairy farms in south-eastern Australia

K M Christie[1], R P Rawnsley[1], M T Harrison[1], R J Eckard[2]
[1]Tasmanian Institute of Agriculture, University of Tasmania, Burnie, Australia, [2]Melbourne School of Land and Environment, University of Melbourne, Melbourne, Australia *Email:Karen.Christie@utas.edu.au*

Introduction Ruminant livestock are poor converters of dietary nitrogen (N) into animal product. Animal nitrogen use efficiency (NUE) is a measure of the relative transformation of feed N into product. In dairy systems, animal NUE is expressed as milk N per unit N intake (g milk N/100 g N intake). Improving NUE can be achieved either by increasing the amount of N exported in milk for the same level of N intake, maintaining the same level of N exported in milk from a reduced N intake, or a combination of the two. Improved NUE reduces the likelihood of high concentrations of N excreted in urine, reducing the biological conversion of soil mineral N into nitrous oxide (N_2O) and the loss of N via leaching and volatilisation. This study examined the influence of varying supplementary feed N (SN) and milk N (MN) concentrations on NUE and N_2O emissions for three dairy regions of SE Australia.

Material and methods The biophysical model DairyMod (Johnson *et al.* 2008) was used to simulate a 100 ha dairy farm, with animals grazing rain-fed perennial ryegrass (*Lolium perenne* L.) pasture sward in three temperate locations of south-eastern Australia (Elliott, -41.1°N, 145.8°E; Ellinbank, -38.3°N, 146.0°E; Terang, -38.3°N, 142.6°E). The same farm system was simulated for each site; calving 1st August (late winter) each year with a target milk production of 6,500 litres/cow per 300-day lactation. The stocking rate was intentionally set high, (3.5 cows/ha) to ensure demand for supplementary feed (i.e. > 30% diet from supplement for any given year). When daily pasture growth was insufficient for daily herd intake requirements, supplement with an energy concentration of 12.5 MJ/kg dry matter was supplied. Nitrogen fertiliser (30 kg urea N/ha) was applied to pastures when the soil N growth limiting factor fell below 0.75. To examine the influence of varying NUE on N_2O emission, 16 treatments were examined for each location; four SN concentrations of 1.0, 2.0, 3.0 and 4.0% N (representing 6.3, 12.5, 18.8 and 25.0% supplement crude protein) combined with four MN concentrations of 0.50, 0.55, 0.60 and 0.65% N, (representing 3.1, 3.4, 3.8 and 4.1% milk protein). Thirty year (years 1971 to 2000) mean annual NUEs and N_2O emissions were determined for each treatment and location. Direct N_2O emissions were estimated by DairyMod. Indirect N_2O emissions were calculated by multiplying the simulated N leached and N volatilised by the emissions factors of 0.0125 and 0.01, respectively (DCCEE 2011). A global warming potential of 310 was used to convert N_2O into CO_2e (DCCEE 2011). Linear regression analysis was used to examine the influence of NUE (via MN or SN) on total N_2O emissions.

Results Across all treatments and locations, the simulated 30 year mean NUEs ranged between 14.2 and 35.9 g milk N/100 g N intake (data not shown). Both approaches resulted in increases in NUE and corresponding reductions in N_2O emissions. However, reducing SN had a greater impact on reducing N_2O emissions compared to improving MN. When SN was reduced, for every 1% increase in NUE, there was a corresponding decline in N_2O emissions of 86.6, 93.1 and 79.4 kg CO_2e/ha.annum at Elliott, Ellinbank and Terang, respectively. When MN was increased, for every 1% increase in NUE, there was a corresponding decline in N_2O emissions of 15.4, 21.5 and 19.0 kg CO_2e/ha.annum at Elliott, Ellinbank and Terang, respectively. Nitrous oxide emissions were comparatively lower at Elliott due to climatic conditions at Ellinbank and Terang being more conducive to N_2O losses.

Table 1 Simulated thirty-year mean nitrogen use efficiencies (NUE; g N milk/100 g N intake) and total (sum of direct and indirect) nitrous oxide emissions (N_2O; kg CO_2e/ha.annum) for four supplementary feed N concentrations (1.0, 2.0, 3.0 and 4.0% N) and four milk N concentrations (0.50, 0.55, 0.60 and 0.65% N) at Elliott, Ellinbank and Terang in south-eastern Australia.

	Elliott		Ellinbank		Terang			Elliott		Ellinbank		Terang	
Supp N	NUE	N_2O	NUE	N_2O	NUE	N_2O	**Milk N**	NUE	N_2O	NUE	N_2O	NUE	N_2O
4.0%	16.4	1526	16.4	2526	16.4	2342	**0.50%**	18.4	1087	19.1	1986	19.8	1784
3.0%	18.9	1204	19.2	2129	19.4	1940	**0.55%**	20.3	1058	21.1	1937	21.9	1747
2.0%	22.3	894	23.2	1737	24.0	1533	**0.60%**	22.2	1025	23.0	1901	23.9	1707
1.0%	27.5	547	29.4	1290	31.6	1093	**0.65%**	24.1	1001	25.0	1858	25.9	1669

Conclusions Reducing SN intakes resulted in the larger reduction in N_2O, compared to increasing MN, at each location. This is a positive outcome, since reducing excess N consumed in the diet is much more practicable than increasing milk N.

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A comparative analysis of methane emission by domestic and non-domestic ruminant species with respect to body mass and feeding type

M T Dittmann[1], A Arif[2], T Bouts[2], C Hebel[2], M Kreuzer[1], A Peemöller[4], J O Zeitz[1], M Clauss[3]
[1]ETH Zurich, Zurich, Switzerland, [2]Al Wabra Wildlife Preservation, Al Shahaniyah, Qatar, [3]University of Zurich, Zurich, Switzerland, [4]Wildnispark Zürich, Langnau am Albis, Switzerland *Email:marie.dittmann@agrl.ethz.ch*

Introduction A large body of research has been dedicated to investigate methane production in domestic ruminants such as cattle, sheep and goats. However, knowledge about CH_4 production of other, non-domestic ruminant species is very limited. In order to get an insight into the factors influencing methane emission including body mass (BM) or feeding type (browser, grazer or intermediate type), this study aimed at investigating CH_4 production in a number of non-domestic ruminant species differing in these factors.

Material and methods In addition to a broad review of literature data on methane emission in (mainly domestic) ruminants fed exclusively on roughage, respiratory measurements were carried out in five non-domestic species. Species were chosen based on feeding type and body mass to fill the gap in the literature data. The species selected were Phillip's dikdik, Idmi gazelle, Speke's gazelle, Soemmering's gazelle and European moose (BM ranging from 2 to 300 kg). Two to seven individuals of each species (total n = 25 individuals) were kept in separate pens to determine feed intake. To exclude effects of the diet, all animals were fed lucerne (either fresh or dried, offered ad libitum). After two weeks of adaptation to the lucerne diet, total collection of faeces and feed refusals was performed for five to seven days to measure feed intake. Respiratory measurements were carried out using a flow-through setup with modular units of Sable Systems © devices (see Figure 1 for the setup used to measure Phillip's dikdiks). CH_4, O_2, CO_2 and water vapour were measured in comparison to ambient air while the animals remained inside a modified transport box for 24 hrs. Species were classified into feeding types (browser BR, intermediate feeder IM, grazer GR) based on Hofmann (1989) as well as Gagnon and Chew (2000). Methane emission and body mass were correlated to determine the (potentially allometric) relationship. Kruskal Wallis tests were applied to determine differences between feeding types in the amount of CH_4 emitted (both with and without control for dry matter intake).

Results Methane emission based on data of both literature and our own measurements correlated nearly linearly with body mass ($y = 0.31x^{0.95\ (95\%\ CI:\ 0.91 - 1.00)}$, with y = CH_4 in l/day, x = body mass in kg, $R^2 = 0.95$). The amount of methane emitted did not differ significantly between the different feeding types (both with and without control for dry matter intake, $P > 0.05$) (Figure 2).

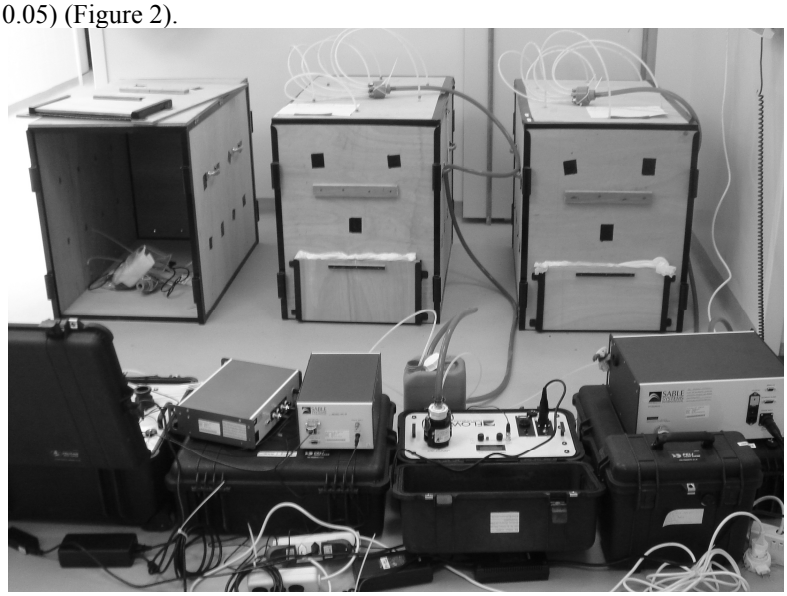

Figure 1

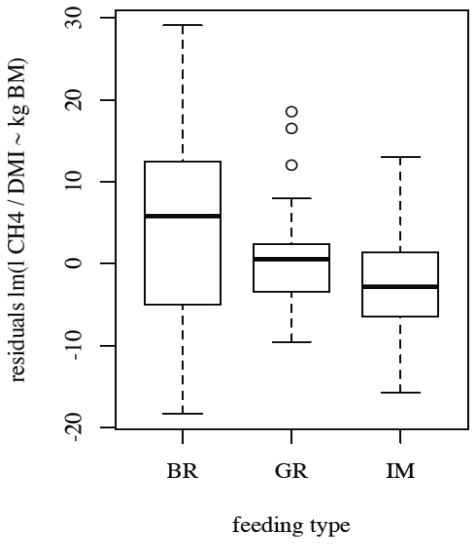

Figure 2

Conclusions Methane emission scaled almost linearly with body mass, implying that larger species have relatively higher energy losses due to methanogenesis. Albeit the digestive physiology differs between browsers and the other two feeding types, no differences seem to exist in the amount of methane emitted, indicating that there might be no fundamental influence of feeding type on CH_4 emission in ruminants.

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Greenhouse gas emissions and fossil energy consumption in suckler beef production: variability and linkage to farm economics. 2-year longitudinal analysis of results from 59 farms

P Veysset, M Lherm, D Bébin, M Roulenc, M Benoit
INRA, Clermont-Theix, France *Email:veysset@clermont.inra.fr*

Introduction Environmental performances assessment of beef production systems often leads to propose mitigation strategies, regardless of farm economics. The objective of this study was to assess both environmental impacts and economic performance of French suckler-beef production systems from commercial farms. Using data from a farms network instead of modelled or experimental data has allowed us to analyze the variability and drivers of the results for each greenhouse gas (GHG) emitted and each non renewable energy (NRE) demand source, and to study the linkage to the production system and to the farm economics.

Material and methods We've conducted a technico-economic survey on 59 charolais suckler-cattle farms over two years (2010 and 2011). This survey also included all the structural and technical data aiming to calculate the GHG emissions and NRE energy per kg of live weight (kglw) using the LCA methodology "at the farm gate" adapted to French herbivore systems. Land use change and carbon sequestration were also taken into account and allowed to calculate the carbon offsetting and the net GHG emissions. For further analysis of the mean values on environmental variables and their box plot distributions, the figures for all 59 farms for both years were pooled into a single sample of 118 observations. The nonparametric Spearman test was used to identify correlations between the values of the various sources of GHG emissions and NRE consumptions with the structural and technical variables. Across the entire 118-observation dataset, we selected two groups: the 25% (i.e. 29 farm-year pairs) that proved least net GHG-emitting (the "GHG-" group) and the 25% (i.e. 29 farm-year pairs) that proved most net GHG-emitting (the "GHG+" group). We then calculated the mean values of the full set of technical-economic variables for each of these two groups. Systemic analysis of the GHG- and GHG+ production systems was performed by comparative analysis using a nonparametric Mann-Whitney comparison of means on the main structural, technical and economic variables.

Results Mean gross GHG emissions amount to 12.79 kg CO_2e/kglw. Gross GHG emissions spread a range of 9.51 up to 21.13 kg CO_2e/kglw, i.e. a 122% variance between min-max extremes. Around 50% of farms fell in the range 11.56 to 13.60 kg CO2e, i.e. a 17% variance between first and third quartiles. Gross GHG emissions and CH_4 emissions per kglw were very strongly negatively correlated to kg live weight produced per livestock unit (LU). CO_2 emissions were positively correlated to concentrates purchased, negatively correlated to feed self-sufficiency. N_2O emissions were negatively correlated to kglw and positively correlated to amount of nitrogen fertilizer input per ha. Gross GHG emissions were partly offset by carbon storage. Although averaging out at 21%, this offset level ranges from 7% to 35% according to percent of grassland in total farm area and percent of temporary meadows under rotation and therefore tilled every year. Intensiveness of nitrogen fertilizer use and stocking rate are negatively correlated to C offset. Although we ultimately found a negative correlation between net GHG emissions and weight-gain productivity (this correlation was not as strong as for gross GHG emissions). Net GHG emissions were positively correlated to stocking rate due to the carbon offset factor. We also found that net GHG emissions per kglw were positively correlated to farm size and with number of LUs. Degree of specialization also has an impact on GHG emissions, as farms more heavily specialized in beef production tend to emit less net GHG per kglw than mixed crop-livestock farms.
The amount of NRE consumed to produce one kg of beef live weight averaged 30.4 MJ. NRE consumption levels varied strongly between farms, at 182% variance between the upper and lower extremes and 39% variance between the first and third quartiles. The main determinant driving total NRE consumption per kglw is fuel use. Intensive use of concentrate feeds and fertilizers are other determinants of NRE consumption levels.
Net GHG emissions per kglw were 51% lower on the GHG- than on the GHG+ at 8.00 versus 12.05 kg CO_2e/kglw. The GHG+ farms cumulate the highest gross emission levels (+34%), with lower carbon offset levels (-55%). GHG+ farms were significantly larger (+46% surface area and +72% herd size) than GHG- farms. GHG- farms are more specialized (beef production, their cereals are consumed by the herd) while GHG+ farms are more mixed crop-livestock farms. The GHG- group presents systematically better animal performances on all reproduction criteria (pregnancy rate, calf mortality, calving interval) and live weight production criteria. GHG- farms ultimately produce 18% more beef per LU than GHG+ farms. The profile of animals sold to market is basically the same between GHG+ and GHG- farms.
By getting higher productivity per head of livestock without higher charges, and despite being smaller structures, farms that emit the least net GHGs per kglw ultimately earn higher profits: income per worker is ultimately 29% higher at GHG- farms than GHG+ farms (+8500€/worker). Net GHG emissions were positively correlated to farm size and negatively correlated to economic performances (gross margin on cattle and farm income). The least-GHG-emitting farms are therefore midrange-sized farms that earn better profits than the bigger farms while at the same time consuming less NREs.

Conclusions It is often the same farms that outperform the others on all three fronts — technical, economic and environmental. They are the farms that optimize their factors of production and make the most efficient use of inputs. The most efficient production system was found in midrange-sized farms running just one specialized production activity (beef) while diversifying their diet resources for the benefit of the herd (grassland and cereal crops). The big farms marketing both beef and cereal crops appear to emerge as less efficient and therefore fail to capitalize on the size-driven economies of scale and diversification-driven economies of scope.

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Combining micrometeorological measurements with molecular and stable isotope techniques to understand the mechanisms producing nitrous oxide emissions in dairy manure-fertilized soils

D Snider, K Thompson, C Wagner-Riddle, K Dunfield
University of Guelph, Guelph, Ontario, Canada *Email:dasnider@uoguelph.ca*

Introduction Nitrous oxide (N_2O) is produced by nitrification, nitrifier-denitrification, and denitrification in aerobic, sub-oxic, and anaerobic environments, respectively. These processes occur simultaneously in heterogeneous soil systems, and identifying the dominant pathway of N_2O production is a difficult but important step towards mitigating emissions. The nitrogen cycle is complex and management strategies that reduce emissions from one pathway may augment emissions from another. This study characterized a high N_2O flux event after a heavy spring rainfall in a dairy manure-fertilized agricultural soil. We used microbial, stable isotope, and micrometeorological methods to: 1) quantify changes in the activity and abundance of soil denitrifiers and nitrifiers before, during, and after the rainfall using molecular methods; 2) determine the processes generating high N_2O emissions using $\delta^{15}N$-N_2O and $\delta^{18}O$-N_2O at natural abundance; and 3) determine if nitrifier and denitrifier gene abundance and expression can be used to apportion N_2O sources, and compare and contrast this information to the stable isotope biogeochemistry.

Materials and methods The study took place at an agricultural research station operated by the University of Guelph near Elora, Ontario, Canada. A flux-gradient micrometeorological approach (Wagner *et al*. 2007) was used to record half-hourly N_2O fluxes from two 4-ha fields that were cropped with corn and fertilized with liquid dairy manure in the fall or spring (150 kg N/ha). Each field had 5 replicate sampling locations where discrete point measurements of N_2O flux were taken using vented static chambers (non-steady-state, non-flow-through). N_2O concentration in the soil profile was determined at 10, 20, 30, and 50 cm below the surface at each location using pore gas samplers. Samples of the surface flux and the subsurface N_2O were collected for the analysis of nitrogen and oxygen stable isotope ratios ($\delta^{15}N$ and $\delta^{18}O$) by continuous-flow isotope ratio mass spectrometry (e.g., Snider *et al*. 2009, 2012). The nitrate (NO_3^-) and ammonium (NH_4^+) contents at each location (0-15 cm) were measured, and the $\delta^{15}N$ (NO_3^- and NH_4^+) and $\delta^{18}O$ (NO_3^- only) values were determined to characterize the isotopic endmembers for nitrification and denitrification. The $\delta^{15}N$ of the total soil nitrogen (TN) and the $\delta^{18}O$ value of the soil water (H_2O) were also characterized. Other ancillary measurements included soil profile concentrations and fluxes of carbon dioxide and methane, soil water content, soil temperature, and climate data measured at an on-site meteorological weather station operated by Environment Canada. The top 5 cm of soil at each location was subsampled over the course of the rain event for molecular analyses. Quantitative PCR (qPCR) was used to enumerate the total bacterial communities (16S), and communities of denitrifiers by targeting nitrite reductase (*nirS*) and nitrous oxide reductase (*nosZ*) genes.

Results The spring of 2012 was very dry, and during the 2 months leading up to the study only 55 mm of rain fell on our site. A day before the rain event the soil moisture in the top 12 cm was 20–25% VWC. There were very low amounts of NH_4^+-N in the soil (≤1 µg NH_4^+-N/g soil-dry weight). High levels of NO_3^- were present (37 µg NO_3^--N/g soil-dry weight), presumably due to high rates of nitrification stimulated by the dry soil conditions. Over three days (June 1–3, 2012) 44 mm of rain fell, which saturated the top soil layer (~50% VWC) and stimulated large N_2O emissions for several days. Following the onset of rain, 337 g N_2O-N/ha was emitted to the atmosphere over a 2 week period (semi-continuous micrometeorological measurements). For comparison, this was 43% of the cumulative N_2O-N lost during the entire month of March 2012 (790 g N/ha) when large snowmelt N_2O fluxes occurred. The flux-weighted isotope ratio of the N_2O emissions from the spring-fertilized field was -31‰ ($\delta^{15}N$-N_2O rel. AIR) and +25‰ ($\delta^{18}O$-N_2O rel. VSMOW), and -25‰ and +27‰ ($\delta^{15}N$-N_2O and $\delta^{18}O$-N2O, respectively) from the fall-fertilized field. These values are consistent with our expectations of a denitrification source in a well-drained soil. Subsurface N_2O concentrations were temporally and spatially variable, ranging from ambient levels (prior to the rain) to almost 100 ppm v/v (4 days after onset of rain). Isotope ratios of N_2O at depth were high prior to the rain event (enriched in ^{15}N and ^{18}O), but quickly declined to values indicative of newly-produced N_2O by denitrification. DNA and RNA analysis of the microbial communities is still on-going and results will be presented.

Conclusions Stable isotope and geochemical evidence indicates that most of the high N_2O emissions observed in this study were caused by denitrifying microorganisms that thrive in wet soils with high NO_3^- availability. Metagenomic analyses will likely confirm our hypothesis that nitrifiers were most abundant and active prior to the rainfall, but that denitrifiers responded quickly to the changing biogeochemical conditions.

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How do N_2O fluxes determined using five static closed chambers and an auto-sampler compare?

L M Cardenas[1], T H Misselbrook[1], N Donovan[1], S Norris[1], R L Murray[1], C J Hodgson[1], E Cancer-Berroya[1], D R Chadwick[2]
[1]North Wyke, Rothamsted Research, Exeter, UK, [2]SENRGY, Bangor University, Bangor, UK
Email:neil.donovan@rothamsted.ac.uk

Introduction The spatial and temporal variability associated with nitrous oxide (N_2O) emissions from agricultural soils, following nitrogen applications, make reliable determination of N_2O fluxes challenging. Manual sampling of static closed chambers is the method most widely employed. In order to capture spatial variability each experiment uses multiple chambers per plot with treatments replicated in a randomised block design. It is however far more difficult to capture temporal variability using this method. The use of an auto-sampler makes it possible to assess temporal variability in more detail, albeit without regard to spatial effects. In this paper we compare preliminary N_2O flux data obtained using these two methods following application of artificial urine to a grassland plot established on a heavy clay loam soil in SW England, as part of a larger experiment to determine N_2O.emission factors for cattle dung and urine.

Methods Five PVC static chambers (40 x 40 x 25 cm) were inserted into the soil to a depth of ca.5cm. These were closed manually with a lid bearing a neoprene seal, and the headspace sampled manually using hypodermic needles and syringe to transfer air from the closed chambers to pre-evacuated vials. The period of chamber closure prior to the final headspace sampling was 40 minutes. In contrast, the auto-sampler system which was situated on the same plot area as the 5 static chambers comprised, i) a (70 x 70 x 10cm) FOREX chamber supported within a frame enabling the chamber to be moved mechanically down onto and lifted off a metal frame inserted into the soil, ii) an aluminium, weatherproof container to house batteries, program unit, electric pump, injection assembly and carousel holding 30 pre-evacuated headspace vials. The auto-sampler was programmed to drop the chamber on the frame in the soil four times in every 24h period (05:00, 11:00, 17:00, and 23:00 GMT). On each occasion the chamber remained *in situ* for nearly 50 minutes during which separate gas samples were taken at three different time intervals following chamber closure (sampling was set at 0, 20, & 40 minutes following closure). Each sample was captured via a needle assembly penetrating a pre-evacuated headspace vial so that the air within the chamber could be circulated by the pump through the vial via the needle and back from the vial (again via the needle), into the chamber. After one minute the pump switches off and the needle withdraws from the vial which has self-sealing septa to retain the sample. The carousel rotates to allow vials to be filled in turn. The N_2O concentration of the gas samples within the vials from both chamber methods were then analysed (usually within 24h), using the same Perkin Elmer Clarus GC (Elite Q PLOT column and Electron Capture Detector). Artificial urine was applied to the area within the 5 static chambers and the area within the frame of the auto-sampler at the same loading rates (ca. 440 kg N ha^{-}1). Static chambers were then sampled according to a pre-specified sampling frequency for twelve months. The auto-sampler sampled four times per day, every day following urine application for more than six months.

Results Preliminary daily N_2O flux data were calculated by averaging the emissions from the five static chambers and by averaging the fluxes from the four separate sampling times of the auto-sampler. Data shown in Figure 1 indicate similar daily flux patterns, although cumulative N_2O emissions (area under the lines) were different (auto-sampler = 6.4kg N_2O-N; static chambers = 10.9k g N_2O-N). With care the two data sets can be combined providing a more detailed understanding of N_2O emissions following urine application to this soil. For example, the auto-sampler shows a secondary peak in emission during a period not covered by static chamber measurements, and revealed the variability in emissions in the weeks post application. Figure 2, shows a clear diurnal pattern with smaller fluxes observed during daylight hours (11:00 & 17:00 GMT), when compared with fluxes observed during the hours of darkness (23:00 & 05:00 GMT). This might reflect changes in abiotic factors such as soil temperature and moisture, and their effects on nitrifiers and denitrifiers in the soil.

Figure 1 Comparison of N_2O fluxes observed using static chambers and auto-sampler

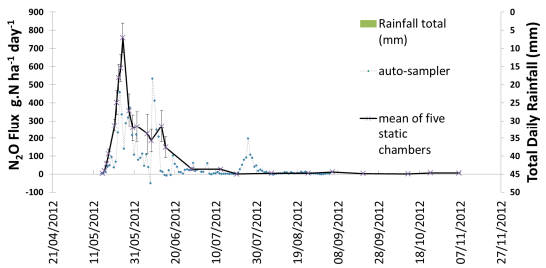
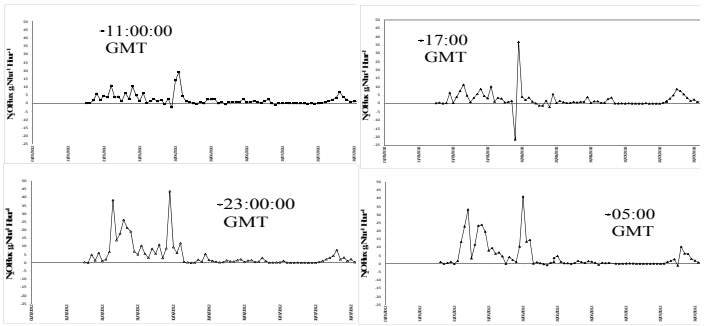


Figure 2 Temporal variation recorded using auto-sampler (X axis Date:Y axis N_2O Flux gNHa^{-1}Hour^{-1})



Conclusions. The auto-sampler deployed in this experiment has been able to augment the information obtained by means of the static chambers alone delivering new and interesting data. Further investigation is needed of the factors controlling temporal variability of N_2O fluxes, both diurnal and seasonal; to better inform sampling, prediction and mitigation strategies of these fluxes.

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Meta analysis of respiration calorimeter data for relationships between methane emission and carbon dioxide production or oxygen consumption for young, dry and lactating dairy cows

A Aubry, T Yan
Agri-Food and Biosciences Institute, Hillsborough, Co Down BT26 6DR, UK *Email:tianhai.yan@afbini.gov.uk*

Introduction Recently, there has been a growing interest in developing methods to estimate CH_4 emissions from ruminants. However, the number of animals tested is often small, resulting in a substantial level of variation unaccounted for by predictive models. Recent developments in measurement techniques to quantify gaseous exchanges for a large scale of livestock herd suggest the use of other gases such as CO_2 to estimate CH_4 emissions. However, there is little information available on the relationship between CH_4 and CO_2 productions for a wide range of animals. The objectives of the present study were to investigate relationships between CH_4 and CO_2 productions and O_2 consumption for a large number of dairy cattle of various ages, and to assess whether the predictive power of these relationships could be improved by taking into account some dietary factors, including diet forage proportion (FP) and fibre and ME concentrations.

Materials and methods Since 1992, a large number of young, dry and lactating dairy cows (n = 987, 30 studies) were subjected to gaseous exchange measurements (CH_4 and CO_2 exhaled and O_2 inhaled) using indirect open-circuit respiration chambers at this Institute. The animals were of various physiological states (young (n = 60), dry cows (n = 116) and lactating cows (n = 811)) and breeds (Holstein/Friesian cows (n = 876), Jersey x Holstein (n = 47), Norwegian (n = 50) and Norwegian cross (n = 14)). The animals were offered forage as a sole diet (n = 161) or a mixture of forage and concentrate (FP ranging from 10% to 87%). Overall, 393 different cows were used across all experiments, and each animal was used between 1 and 6 times depending on the experiment. As a result, data were analysed using a REML variance component analysis with CH_4 as the response variable, CO_2 or O_2 as a fixed factor, experiment and "cow within experiment" as random factors. A fixed factor was also included to differentiate between lactating cows and a second group of animals which included dry cows and young animals. A series of models were also obtained by adding one or two dietary factors to CO_2 (or O_2). Lastly, the variability of the CH_4:CO_2 ratio (with both gases expressed in L/d) was investigated, also using mixed models.

Results There was no effect of breed (P = 0.21) on the relationship between CH_4 and CO_2; the data were thus pooled for all subsequent analyses. There was a strong positive linear relationship between CH_4 and CO_2 (Figure 1), and observations within an experiment were very predictable (R^2 = 0.93). As expected, CH_4 production was higher for lactating cows than young and dry cows (P = 0.030, Figure 1). Adding FP, ADF or NDF slightly improved the fit of the model, whereas ME did not. Using O_2 instead of CO_2 to predict CH_4 production also provided a very good fit to the observed empirical data, but the relationship was weaker (R^2 = 0.86). There was a significant effect of animal physiological state (P = 0.024) and FP (P < 0.001) on the CH_4:CO_2 ratio. The models predict a ratio of 0.081 for young and dry cows and 0.084 for lactating cows (s.e.d. 0.0015), and a ratio of 0.085 for high FP diets (FP > 75%) and 0.079 for low FP diets (FP< 25%) (s.e.d. 0.0016). Both ADF and NDF also had marginal positive effects on the CH_4:CO_2 ratio.

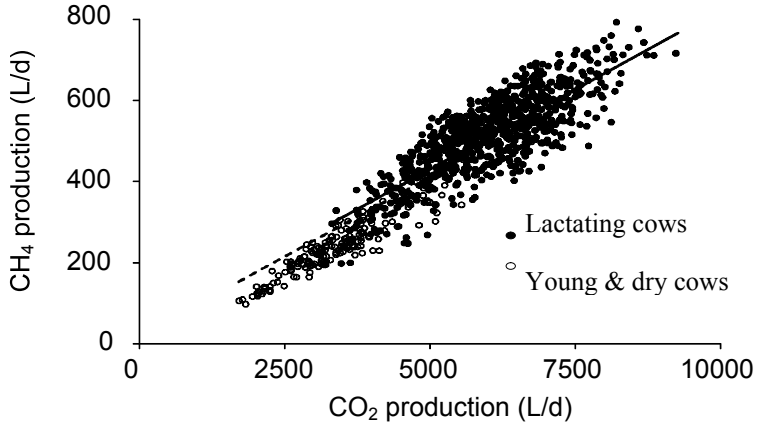

Figure 1 CH_4 (y) and CO_2 (x) production (L/d) for young cattle and dry cows (y = 17 + 0.0787x, dashed line) and lactating cows (y = 36 + 0.0787x, solid line). The R^2 value obtained using adjusted CH_4 values was 0.93 (adjusted CH_4 = predicted CH_4 values + residuals, in order to correct for the experiment

Conclusions These results indicate that there is a strong linear relationship between CH_4 and CO_2 productions with dairy cattle which applies for a wide range of animal and experimental conditions. When using CO_2 to quantify CH_4 production, inclusion of dietary factors, in particular the forage proportion, can provide a marginal improvement to the prediction of CH_4. The observed variability in the CH_4:CO_2 ratio could only marginally be explained by diet factors, and was most likely to reflect individual animal differences. The CO_2 production data can be used to accurately predict CH_4 emissions to generate large scale data for management and genetic evaluations for the dairy industry.

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Assessing the impact of variation in farm characteristics on the carbon footprint of lamb

A K Jones, P Cross, D L Jones
Bangor University, Wales, UK *Email:a.k.jones@bangor.ac.uk*

Introduction On-farm emissions represent the largest component of the lamb supply chain carbon footprint (CF). Emissions vary between farms in relation to local conditions such as quality of grazing, and management choices such as efficiency of fertiliser use. Few studies have explored how variation in farm characteristics influences the CF of lamb. The diversity of farm systems within the UK sheep industry could be expected to result in CF variation. The industry is characterised by interdependent lowland, upland and hill farm systems. Output varies significantly between average and top producers. The aim of this study was to use empirical data collected from sheep farms across England and Wales to calculate the CF of lamb produced on a range of farm types, and to use these data to assess the impact of farm characteristics on the size of the CF.

Methods Data sets from sixty sheep farms across England and Wales were compiled, using a questionnaire requesting information on farm characteristics such as: inputs (for example feed and fertiliser use); monthly stock movements (including purchases, births and housing) and outputs (including sheep numbers sold and weights). Cradle to farm gate CFs were calculated following the principles of PAS 2050. Methane (CH_4) and nitrous oxide (N_2O) emissions were estimated following the Intergovernmental Panel on Climate Change tier 1 methodology, as used in the UK greenhouse gas inventory. Emission factors used for inputs such as concentrate feeds and fertilisers were mean values from a range in the published literature as detailed in Edwards-Jones *et al*. (2009). The global warming potential of emissions were reported relative to those of carbon dioxide (CO_2) over a 100 year time horizon i.e. in CO_2 equivalents where 1 kg CH_4 = 25 kg CO_2e and 1 kg N_2O = 298 kg CO_2e. The functional unit used for reporting emissions was 1 kg of live weight (LW) finished lamb.
The relationship between the CF of finished lamb and selected farm variables was assessed using multiple linear regression models. We used Akaike's information criterion to determine how well each model was supported by the data. No one model was clearly supported, so we used model averaging to estimate the coefficients of the variables in a composite linear regression model.

Results The average CF of finished lamb produced in England and Wales increased with declining land quality from lowland to upland to hill farms (Table 1). Three variables had a significant negative relationship with CF, across the sample of 60 farms (Figure 1). These were: the average weight of sheep on farm (kg/month); weight of lamb sold or retained (kg LW lamb/kg ewe mated) and lamb growth rate from birth to finishing (kg/month). The variable weight of lamb sold or retained per ewe mated had the strongest relationship with the size of the CF of finished lamb.

Table 1 The carbon footprint of finished lamb

	Footprint (kg CO_2e / kg LW lamb)		
	Min	Max	Mean
Lowland (n=27)	5.39	21.5	10.85
Upland (n=12)	8.32	18.25	12.85
Hill (n=21)	8.79	33.27	17.86

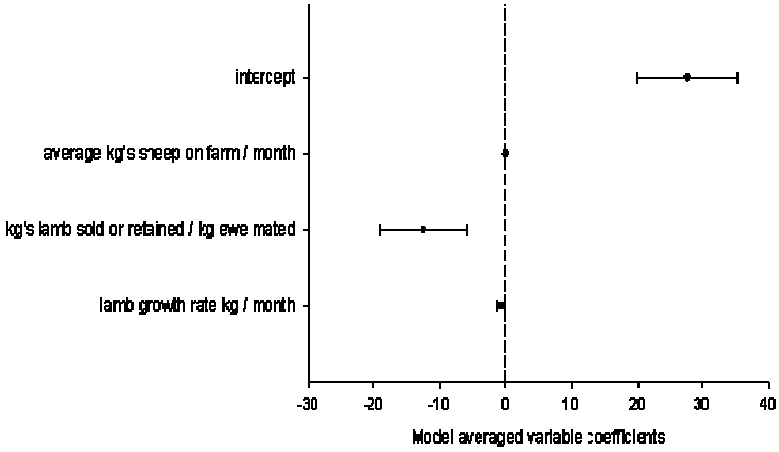

Figure 1 Regression coefficients of farm variables. Error bars are 95% confidence intervals.

Conclusions There is considerable variation in the CF of lamb within the categories of lowland, upland and hill farms, suggesting potential for emission reductions on the less efficient farms. The farm variables weight of sheep on farm; weight of lamb sold or retained per ewe mated and lamb growth rate from birth to finishing are suggested as useful indicators of CF size. Given the strength of the relationship, maximising the weight of lamb produced per ewe mated could serve as a useful industry target for reducing the CF of lamb production. At a national scale the results indicate that larger, more productive sheep farms have the lowest CFs. This implies that agricultural intensification could be adopted as a carbon reduction policy for the sheep industry. However, the wider implications of intensification for farm biodiversity, animal welfare and rural communities must also be considered.

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The potential for reducing the carbon dioxide equivalent of beef production by finishing cattle on pure Lotus corniculatus pastures compared with grain-based feedlot finishing

J W MacAdam[1], S Martini[1], T C Griggs[2]
[1]Utah State University, Logan, Utah, USA, [2]West Virginia University, Morgantown, West Virginia, USA
Email:jennifer.macadam@usu.edu

Introduction Recent life cycle assessments (LCA) that compared grain-based beef feedlot finishing with pasture-based finishing have concluded that feedlot finishing has a lower carbon dioxide equivalent (CO_2 eq.) footprint per kg of meat produced. This is due to the slow gain of cattle on grass pastures rather than higher energy use or greenhouse gas (GHG) emissions per head. In a comparison based on practices in the upper Midwest USA, cattle fed on pasture for 300 days then in a feedlot for 150 days weighed 637 kg, while cattle fed forage for 450 days weighed 505 kg (Pelletier *et al*., 2010). An Australian study that compared feedlot-finished with pasture-finished beef cattle noted that the latter may spend up to 3 years on pasture, and reported hot finished carcass weights of 363 kg for feedlot-finished cattle and 280 kg for grass-finished cattle (Peters *et al*., 2010). The environmental costs of cereal grain production were included in calculations for feedlot finishing, and the CO_2 eq. footprint was higher for feedlot-finished beef on a per-head basis, but lower per kg of finished product. The objective of the work reported here was to determine the rate of gain that beef cattle can achieve on pure *Lotus corniculatus* (birdsfoot trefoil; BFT) pastures, and to put this higher gain in the context of factors known to result in lower GHG emissions for ruminants grazing BFT compared with ruminants grazing grasses or other legumes.

Material and methods Studies were carried out in the northern Mountain West USA on irrigated pastures seeded with 'Norcen' and 'Oberhaunstadter' BFT, and with 'Monarch' *Astragalus cicer* (cicer milkvetch; CMV). Pastures were grazed in summer, from early June through late August. More recently, cooperating ranchers have established BFT pastures and carried out season-long grazing with their own cattle. A sensory panel compared consumer preferences for the ribeye steaks of BFT-finished cattle with purchased Certified Angus® ribeye steaks and purchased organic grass-finished ribeye steaks from Whole Foods®.

Results While mixed grass-legume pastures are considered to be of higher quality than grass pastures, cattle grazing pastures with a high legume content are at risk of bloat. Birdsfoot trefoil contains sufficient tannin (1-4%) that it is a non-bloating legume, and therefore pure stands of BFT can be grazed. While grass-finished beef appeals to a niche market, in part because pasture-finished beef contains lower saturated fatty acids and higher polyunsaturated fatty acids than grain-finished beef, consumer acceptance of grass-finished beef has been limited by perceived differences in flavour and juiciness. When ribeye steaks from BFT- finished cattle were compared with ribeye steaks from grain- and grass-finished cattle in a consumer sensory panel, however, BFT steaks were found to be more tender than grain-finished steaks ($P < 0.0001$), and equivalent to grain-finished steaks and superior to grass-finished steaks for flavour ($P = 0.0004$) and overall appeal ($P < 0.0001$).

Discussion Season-long gains on BFT have ranged from 1.3 to 1.5 kg d^{-1} in controlled studies and from 1.25 to 1.65 kg d^{-1} on producers' pastures, without the use of growth hormones, and was higher than beef gains on other perennial legumes, such as CMV (MacAdam *et al*., 2011) or grass pastures. The higher fibre content of grasses and slower rate of grass fibre digestion compared with legume forages is well-understood, as is the reduced rate of grass production in mid-summer due to high temperatures and rapid soil water depletion. Birdsfoot trefoil is tap-rooted, and performs well during mid-summer under irrigation in the Mountain West. The 3-year average annual yield of 16 BFT cultivars was 13.6 metric tons ha^{-1}, and the carrying capacity of our producers' irrigated BFT pastures ranged between 1100 and 1700 kg ha^{-1}. Other factors relevant to GHG emissions associated with BFT are reduced enteric methane per unit of production compared with perennial ryegrass (*Lolium perenne*) pastures (Woodward *et al*., 2004) and decreased urinary nitrogen concentration as the proportion of BFT to perennial ryegrass in the diet increased (Woodward *et al*., 2009).

Conclusions Rate of cattle gain is positively correlated with rate of forage digestion and forage nutritive value. The high rate of cattle gain on non-bloating BFT pastures addresses the disparity in finished weights between pasture and feedlot systems reported in published LCA. Biological nitrogen fixation that is regulated by the availability of soil nitrogen also favours legume-based pasture systems over the use of nitrogen-fertilized grass pastures or cereal grains. Life cycle assessments of beef production on pasture should be refined to include the implications of variation in forage nutritive value and rate of cattle gain that is dependent on pasture species and management, and specifically to factor in the higher gain and reduced methane and nitrogen emissions found with BFT grazing.

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Development and use of a comprehensive database to evaluate enteric methane prediction equations for dairy cows

P Escobar[1], K Beauchemin[2], M Oba[1]
[1]University of Alberta, Edmonton,Alberta, Canada, [2]Agriculture and Agri-Food Canada, Lethbridge,Alberta, Canada
Email:cescobar@ualberta.ca

Introduction Methane (CH_4) is the major contributor to greenhouse gas (GHG) emissions from dairy farms. For that reason enteric CH_4 production is an area that has received much attention recently, and it is well understood that emissions can vary depending upon feeding strategy and diet composition (Olesen *et al*., 2006). Empirical equations that predict enteric CH_4 production have been published; however, these have a low accuracy due to significant bias and deviation of the regression slope (Mills *et al*., 2003; Ellis *et al*., 2007). The aims of our study were to: 1) construct a database of enteric CH_4 emissions for dairy cows based on published literature, and 2) evaluate extant equations that predict enteric CH_4 emissions from dry matter intake (DMI) and diet composition.

Material and methods The database was comprised of treatment means (TRT) from research published from 2000 to 2012. These data come from a range of countries, and therefore represent different milk production systems. Methane measurements were made using respiration chambers or the tracer gas technique. Criteria for selecting data to include in the database were: measurement of enteric CH_4 production, DMI, description of the animals (body weight, milk production and breed), diet chemical composition and list of dietary ingredients. Missing values for diet composition were generated using default values from feed composition tables or by calculation from the diet and ingredient description provided. The data summarized in Table 1 show the mean ± standard deviation (s.d.) for some variables within the database. Methane was predicted using various published equations and the information on diet composition from the database: Mills *et al*. (2003) linear equation [1] and nonlinear equation [1] (both use DMI); Ellis *et al*. (2007) equation [2d] (uses DMI); and Ellis *et al*. (2007) equation [8d] (uses DMI, acid detergent fibre [ADF] and neutral detergent fibre [NDF]). These equations had an R^2 above 0.6 when developed. In addition, the IPCC (2006) equation was added. Comparisons between predicted (equation) and observed (database) CH_4 were made using HSD Tukey test (JMP 10.0 SAS(c)).

Table 1 Summary of the dairy database used to compare extant equations (n = 172 records from 48 studies)

	CH_4 g d^{-1}	CH_4 g DMI $^{-1}$	DMI kg d^{-1}	NDF g kg^{-1}	NDF kg d^{-1}	ADF g kg^{-1}	ADF kg d^{-1}	NFC g kg^{-1}	NFC kg d^{-1}	Fat g kg^{-1}	Fat kg d^{-1}	Forage* kg d^{-1}
Mean	326.5	21.1	15.9	407	6.4	243	3.8	290	4.8	42	0.69	12.6
s.d.	121.79	5.01	4.81	82.3	2.13	53.1	1.27	118	2.47	15.2	0.301	7.95

NFC = non fiber carbohydrates. * Forage intake

Table 2 Comparison of predicted and observed enteric methane production (g d^{-1})

Equations	Significance				Mean	δ**	s.e.m.	Pearson Correlation	R^2	Concordance correlation	R^2
Mills *et al.*, 2003 Linear [1]	a				370.0	12.5	6.09	0.723	0.523	0.615	0.378
Mills *et al*., 2003 Nonlinear [1]	a	b			357.8	8.8	6.99	0.727	0.529	0.670	0.449
Ellis 2007 from DMI,ADF,NDF [8d]	a	b	c		343.6	4.5	9.08	0.648	0.420	0.642	0.412
Observed value		b	c		328.9	-	9.54	-	-	-	-
IPCC 2006			c	d	317.4	-3.5	7.34	0.723	0.523	0.695	0.483
Ellis 2007 from DMI [2d]				d	289.6	-11.9	5.35	0.723	0.523	0.574	0.329

Same letter means that equations are not different ($P < 0.05$). ** Difference between observed value and each equation.

Results Table 2 indicates that prediction of CH_4 using Mills *et al*. (2003) Linear [1], Mills *et al*. (2003) Nonlinear [1], and Ellis *et al*. (2007) [8d] equations overestimated emissions. IPCC (2006) and Ellis *et al*. (2007) [2d] underestimated emissions. Predicted CH_4 using Mills *et al*. (2003) nonlinear [1], Ellis *et al*. (2007) [8d] and IPCC (2006) were not different from observed values ($P > 0.05$). However, values predicted by Mills *et al*. (2003) Linear [1] and Ellis *et al*. (2007) [2d] equations differed from observed values ($P < 0.05$). The Pearson and concordance correlations show moderate association and reproducibility between observed and predicted values for all equations evaluated, respectively.

Conclusion Of the equations considered, the IPCC 2006 and Ellis *et al*. (2007) DMI, ADF, NDF equation were the most accurate predictors of enteric CH_4, whereas the Mills *et al*. (2003) linear equation and Ellis *et al*. (2007) DMI equation were the least accurate.

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The influence of parity and sire on nitrogen use efficiency and nitrogen isotope fractionation in dairy cows on pasture

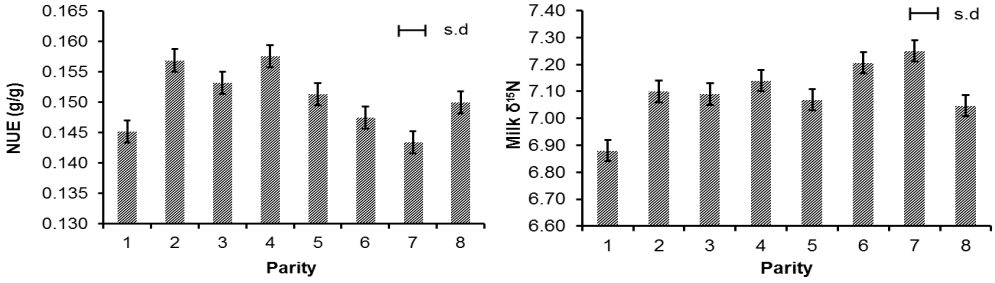
N M Wheadon[1,2], G R Edwards[2], R J Dewhurst[1]
[1]Teagasc, Animal & Grassland Research and Innovation Centre, Grange, Dunsany, Co. Meath, Ireland, [2]Lincoln University, John Burton Building, PO Box 84, Agriculture and Life Sciences, Lincoln University, Lincoln 7647, Christchurch, New Zealand *Email:nicole.wheadon@teagasc.ie*

Introduction The ruminant livestock industries face the challenging goal of increasing production whilst reducing environmental impact. The selection of animals based on their feed efficiency would be extremely beneficial, but requires the development of appropriate markers or proxies to allow work with large numbers of animals. In this study we evaluated a potential marker for nitrogen use efficiency (NUE) based on the process of isotope fractionation, whereby the partitioning of nitrogen between milk and urine may cause differences in the ratio of heavy to light isotopes nitrogen-15 and 14 ($\delta^{15}N$) in milk. We also assessed the genetic effects of sire, breed and parity on NUE and $\delta^{15}N$.

Materials and Methods Duplicate afternoon milk samples were collected from 500 Holstein Friesian × Jersey cows at the Lincoln University Dairy Farm (LUDF). Cows were in late lactation (February) and were rotationally grazed on perennial ryegrass/white clover paddocks. One milk sample was analysed for fat, protein and lactose content by infra-red analysis (LIC, Hornby, New Zealand), and the other was prepared for Isotope Ratio Mass Spectrometry (IRMS) analysis of N isotopes; ^{15}N was reported in delta units ($\delta^{15}N$; ‰). Pasture samples were taken on the day prior to milk sampling for composition analysis (Analytical Research Laboratories Ltd; Napier, New Zealand). Sub-samples of pasture were also freeze-dried, ground and prepared for IRMS. Enrichment of milk ^{15}N relative to diet ^{15}N ($\Delta^{15}N$) was calculated as milk $\delta^{15}N$ ─ diet $\delta^{15}N$. Animal live weight and milk yield was also recorded. Sire, parity, breed and BW values were obtained from the farm database. Thirty-seven sires accounted for 80% of daughters recorded. Parity ranged from 1 to 8, and BW ranged from -24 to 226. Metabolisable energy (ME) requirements were estimated using requirements for maintenance (0.56 MJ ME/kg $W^{0.75}$/day) and lactation (1.1 × milk yield (kg/day) × NE_l/ K_l, MJ/day), where NE_l = (0.376 × fat %) + (0.209 × protein %) + 0.976) MJ NE/litre, and K_l = (feed ME MJ/kg DM × 0.02) + 0.4). DM intake (kg/day) was then calculated as total ME requirement (MJ/day) divided by feed ME (MJ/kg DM). Calculations of NUE (g/g) were made by dividing milk N (g/day) by N intake (g/day). Statistical analysis was conducted in Genstat (13th Ed) using linear regression to assess the relationship between NUE and $\delta^{15}N$ and an ANOVA to evaluate effects of parity, sire and breed on both NUE and $\delta^{15}N$.

Results Pasture $\delta^{15}N$ was 6.42‰ and average milk $\delta^{15}N$ was 7.06‰ (s.e.=0.44), so that average enrichment between diet and milk ($\Delta^{15}N$) was very low (0.64‰). Average calculated NUE (g/g) was 0.15 (s.e.=0.06). There was a weak negative (but n.s.) relationship between NUE and $\delta^{15}N$. Breed of cow had no significant effect on NUE or $\delta^{15}N$. Parity had a larger influence on both NUE ($P<0.001$) and $\delta^{15}N$ ($P<0.001$; Fig 1) and sire also had a significant effect on both measurements ($P<0.05$). Mean NUE and $\delta^{15}N$ for the different sire groups was 0.15 (s.e.=0.01) and 7.09 (s.e.=0.31), and ranged from 0.10 to 0.18 g/g and 6.23 to 8.18 ‰ respectively. Sire breed had a significant effect on $\delta^{15}N$ ($P<0.05$) but not NUE, and dam breed had a significant effect on NUE ($P<0.05$) but not $\delta^{15}N$.

Figure 1 Nitrogen-use efficiency and milk $\delta^{15}N$ for cows in parity 1 to 8



Conclusions There was very little enrichment of milk ^{15}N relative to diet ^{15}N in this study ($\Delta^{15}N$=0.64) in comparison with previous research with lactating dairy cows ($\Delta^{15}N$=2.37; Sutoh *et al*, 1987) and growing beef cattle ($\Delta^{15}N$=4.2; Steele and Daniel, 1978). This was initially surprising given the low NUE, but suggests that $\Delta^{15}N$ may be driven by efficiency in the animal tissues, whilst NUE was driven by rumen efficiency. Low NUE probably resulted from excess rumen degradable protein supply that was not incorporated into microbial protein, and so was not associated with isotopic discrimination. The lack of relationship between NUE and $\delta^{15}N$ may be related to apparent inefficiency related to the use of absorbed amino acids for synthesis of body protein – particularly for parity 1-3 animals, which are still growing.

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Effects of feeding 3-nitrooxypropanol, at varying levels, on methane emissions and rumen fermentation in lactating dairy cows

J Haisan[1], Y Sun[1], K Beauchemin[2], L Guan[1], S Duval[3], D R Barreda[1], M Oba[1]
[1]University of Alberta, Edmonton, Alberta, Canada, [2]Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada, [3]DSM Nutritional Products Ltd., Kaiseraugst, Switzerland *Email:haisan@ualberta.ca*

Introduction A previous study found that feeding 3-nitrooxypropanol (NOP) at 2.5 g/d reduced enteric methane (CH_4) emissions by approximately 60% in lactating cows fed a high concentrate diet (Haisan *et al.,* 2013). The objective of this study was to determine the effect of feeding varying levels of NOP on ruminal CH_4 production, ruminal fermentation and animal productivity.

Materials and methods Fifteen ruminally cannulated lactating Holstein cows were used in a 3×3 Latin square design with 28-d periods. Cows were fed 0 (CON), 12.5 (LOW) or 25.0 (HIGH) g/d of NOP compound (10% 3-nitrooxypropanol on SiO_2, DSM Nutritional Products Ltd., Switzerland). SiO_2 was fed at 25.0, 12.5 or 0 g/d for each treatment, respectively. The NOP compound or SiO_2 was first mixed with 80 g ground barley grain, 50 g molasses and 45 g canola oil, and then hand-mixed into the ration immediately after feeding to allow animals to consume treatment mixtures continuously throughout the day. All cows were fed a 60%-forage diet that was formulated to meet nutrient requirements for a 650-kg cow producing 38 kg milk per day (NRC, 2001). After a 20 d adaptation period, rumen fluid was collected to determine the volatile fatty acid profile and total rumen bacteria present (d 21 and 28). Rumen pH was measured continuously from d 22-28 using LRC pH loggers. Enteric CH_4 production was measured for 5 days (d 23-27) using the sulphur hexafluoride tracer gas technique (Johnson *et al.,* 1994). Dry matter intake (DMI) and milk yield were recorded daily from d 25-27. Data were analyzed by the fit model procedure of JMP (version 10, SAS Institute, Inc., Cary, NC) using a model with fixed effects of period and treatment, and random effect of cow.

Results Feeding the NOP compound had no effect on DMI, mean rumen pH, total volatile fatty acid concentration, total bacteria or methanogenic bacteria counts (Table 1). Methane production was reduced for the HIGH treatment when compared to CON (12.4 vs. 21.2 g/kg DMI) while no treatment effect was seen between LOW and CON. Feeding the NOP compound increased ruminal acetate concentration, but decreased butyrate concentration in a dose dependent manner, while it increased ruminal propionate concentration and decreased the acetate-to-propionate ratio regardless of the dosage.

Table 1 The effects of feeding NOP compound on animal productivity and rumen fermentation profile.

	CON	LOW	HIGH	s.e.	P
Dry matter intake (DMI), kg/d	19.0	17.4	18.7	0.74	0.13
CH_4 g/d	379^{a}	269^{b}	210^{b}	26.7	< 0.001
CH_4/DMI, g/kg	21.2^{a}	15.4^{a,b}	12.4^{b}	1.84	0.02
Milk, kg/d	28.2	25.8	26.8	1.22	0.22
Milk fat, g/kg	34.4	35.6	34.7	1.10	0.59
Milk crude protein, g/kg	31.8	31.6	31.9	0.51	0.82
Milk lactose, g/kg	45.2	45.5	45.4	0.65	0.59
Mean rumen pH	6.46	6.50	6.50	0.044	0.77
Volatile fatty acids					
Total, mM	96.9	95.9	91.7	3.09	0.40
Acetate, mol/100mol	58.5^{a}	54.6^{b}	52.7^{c}	0.65	< 0.0001
Propionate, mol/100mol	22.1^{b}	24.0^{a}	24.4^{a}	0.52	< 0.001
Butyrate, mol/100mol	13.8^{c}	14.9^{b}	15.8^{a}	0.29	< 0.001
Acetate-to-propionate ratio	2.67^{a}	2.30^{b}	2.18^{b}	0.077	< 0.0001
Total bacteria, $\times 10^{10}$	8.98	9.53	7.48	1.181	0.46
Methanogen, $\times 10^{8}$	2.90	2.83	2.59	0.319	0.76

[a, b] Treatment means within a row with different superscripts differ ($P < 0.05$).

Conclusion Feeding the NOP compound at 25 g/d significantly reduced CH_4 emissions per kilogram of DMI in lactating dairy cows. Cows fed the NOP compound had decreased ruminal acetate concentration and increased propionate concentration; however, rumen fermentation and animal productivity were not negatively affected.

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Predicting methane emissions from growing beef cattle fed tropical forages

E Charmley[1], G Bishop-Hurley[2], C Ramirez-Restrepo[3], J Baldock[4]
[1]CSIRO, Townsville, Australia, [2]CSIRO, Brisbane, Australia, [3]CSIRO, Townsville, Australia, [4]CSIRO, Adelaide, Australia
Email:ed.charmley@csiro.au

Introduction The rangelands of northern Australia encompass vast and diverse grazing environments and the methane emissions from livestock in the rangelands are poorly understood but it is believed that approximately half of Australian methane emissions from cattle are attributed to the northern beef herd (Henry *et al*. 2012).

Materials and methods A preliminary model described by Charmley *et al*. (2008) was used as the basis for a modelling exercise designed to include northern cattle emissions into the National Carbon Accounting System (NCAS) developed by the Department of Climate Change and Energy Efficiency (DCCEE). This work tested the model using input data collected at the Lansdown Research Station, Townsville, Australia (19°39' S 146°50' E). Results were compared with observed data for live weight (LW) gain and methane emissions. The objective was to develop a model that accurately estimates the methane (CH_4) production of beef cattle in northern Australia for accounting purposes. Lansdown data were used to populate a mechanistic excel model based on the metabolisable energy (ME) system (SCA 2007). These data comprised of repeated measures of animal LW and estimates of diet quality, intake and proportion of non-grass in the diet, based on faecal near infra-red spectroscopy (NIRS) measurements taken over a grazing season. The model ran on a daily time step. Dry matter intake (DMI) was estimated from diet quality (Figure 1a), availability and grazing pressure and predicted LW and LW gain. Consumed energy was partitioned to maintenance and production, with the level of production (LW; Figure 1b) related to the surplus/deficit of energy after accounting for essential processes (maintenance, pregnancy, etc.).

Results Total daily or annual methane production was estimated from DMI for each animal in a class and the classes summed to provide herd methane emissions. The mean methane emissions across the year were 127 g/d which equates to 0.97 tonnes CO_2 –equiv/yr (Figure 1c). The figure also shows the sensitivity of the model to changes in diet quality. In this scenario, diet quality was arbitrarily increased by 5% in the wet season and by 8% in the dry season as a result of introducing *Stylosanthes* (seca stylo). Although there was an increase in LW and LW gain in response to improved diet quality, methane emissions over the year were not affected. This can be attributed to DM intake not being markedly altered due to the stocking rate and biomass on offer limiting intake.

Conclusions It is concluded that the model successfully predicted methane emissions, but underestimated LW gain. This is of concern as it implies the model under-predicted DM intake, the main biological driver for methane emissions.

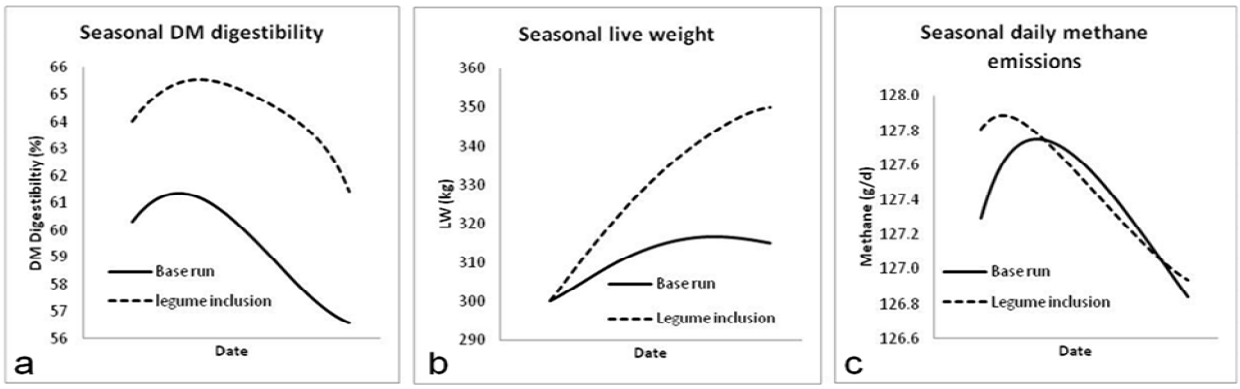

Figure 1 Model results for the Lansdown data and the sensitivity of the model to increasing diet quality through an arbitrary introduction of a legume

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Nitrous oxide emissions from dairy sludge used as an organic fertiliser on resown pasture in south-western Victoria, Australia

G N Ward, J L Jacobs, K B Kelly, S K Muir
Department of Primary Industries, Victoria, Australia *Email:graeme.ward@dpi.vic.gov.au*

Introduction Application of dairy waste (solid and liquid phases) from dairy effluent ponds onto pastures and crops is increasing in southern Australia. The practice is encouraged to reduce nutrient losses to the environment and to provide more efficient use of nutrients through recycling onto the farm. While the application of these materials have been shown to enhance agronomic performance, little is known on subsequent effects on nitrous oxide (N_2O) emissions of applying them to land in southern Australia. It was hypothesised that compared to using inorganic N fertilisers, the high organic matter content of sludge would result in slower mineral N release making them less prone to N_2O losses.

Materials and methods An experiment was conducted near Terang in south-western Victoria, Australia (38°14'S, 142°55'E) on a brown chromosol (Isbell 1996) derived from quaternary basalt. The site has a long term annual rainfall of about 780 mm, and average daily air temperatures ranging from 8.6°C in July to 18.0°C in January. In late summer-autumn 2011 an existing pasture was sprayed out with a knockdown herbicide, cultivated and sown (2 May) to a perennial ryegrass (*Lolium perenne L.*) based mix. A 4 replicate, randomized block design trial with 2 N sources, urea and dilute dairy first pond sludge and a nil control were established. Urea was applied 3 times during the growing season (31 May at 40 kg N/ha; 12 Sept and 28 Oct at 50 kg N/ha) while sludge was applied once on 21 April by vacuum tanker (30 mm, 3% dry matter, 112 kg Total N/ha with 49 kg NH_4-N/ha) and soil incorporated prior to sowing the pasture. Gas sampling to determine N_2O flux from treatments was conducted using manual chambers with a basal area of 0.25 m^2 fitted with a mixing fan, weekly where possible until November 2011 and daily for 4 days following urea applications. Sampling was not possible for much of July and August due to extremely wet soil conditions.

Results and discussion There were large variations in nitrous oxide emissions (g N_2O-N.ha^{-1}.d^{-1}) in all treatments between sampling times (Table 1). Very low emissions of less than 10 g N_2O-N.ha^{-1}.d^{-1}, such as those recorded on 26 Jul and 16 Aug occurred when the soil was saturated (data not presented); while emissions were considerably higher when volumetric soil water contents were close to or below field capacity. This is consistent with denitrification occurring under wet soil conditions, but under complete anaerobiosis there is the reduction of N_2O to N_2 (Aguilera *et al.* 2013). There were indications at some sampling times during winter and early spring that the sludge treatment had higher emissions than the urea treatment. This effect for slurries ('liquid organic fertilisers') was also noted by Aguilera *et al.* (2013) and may be a result of the addition of organic matter to the soil in the applied sludge enhancing denitrification through (i) providing more labile C substrates and (ii) the creation of more anaerobic microsites, even at lower soil water contents (Garcia-Ruiz and Baggs 2007). Application of inorganic N as urea did not lead to an increase or spike in N_2O emissions in the following days at any of the application times (Table 1). This is despite urea raising soil NH_4-N levels for at least several weeks after application (data not presented). It is also apparent that there were comparatively high background levels of N_2O emissions that may be masking some of the treatment effects.

Table 1 Nitrous oxide emissions (g N_2O-N.ha^{-1}.d^{-1}) from Cont (no N applied), N (urea applied during growing season) and sludge (applied once on 21 April) treated resown pasture plots at a range of dates during the 2011 growing season (l.s.d P=0.05)

A. Urea applied 31 May					B. Winter – no urea applied					C. Urea applied 12 Sep				
Date	Cont	N	Sludge	l.s.d	Date	Cont	N	Sludge	l.s.d	Date	Cont	N	Sludge	l.s.d
1Jun	426	266	289	196.6	30Jun	55	23	110	84.2	14Sep	66	37	78	39.8
2Jun	302	170	251	182.8	26Jul	5	9	6	8.4	15Sep	53	38	80	39.6
9Jun	83	64	131	94.6	3Aug	51	17	110	92.7	16Sep	63	43	76	40.6
15Jun	308	156	358	219.1	16Aug	3	5	5	3.5	19Sep	40	46	83	42.3

Conclusions The application of dairy sludge as an N source in place of urea to resown pasture did not lead to a reduction in N_2O emissions and at some sampling times resulted in higher emissions. While current state regulations require such material to remain within the farm boundary, its use in place of inorganic sources may increase N_2O emissions.

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Supplementation effect of myristic acid and fumaric acid on nutrient utilization, methanogenesis and milk yield in dairy cattle fed wheat straw containing total mixed diet

S Sirohi, M Mohini, N Goel
National Dairy Research Institute, Karnal,Haryana, India *Email:sirohisk@gmail.com*

Introduction

The recent goal of ruminant nutritionists is to manipulate the rumen microbial ecosystem to improve the feed conversion efficiency and reduce methane production. Much of the research in the past decades has focused on the effects of antimicrobial compounds on ruminal fermentation mainly on ionophores and antibiotics. Some of the previous studies indicated that organic acids and medium chain fatty acids are able to provide an alternative hydrogen sink to currently used antimicrobial compounds. Moreover, the antimicrobials and antibiotics are banned in several countries. Therefore, the present study was undertaken to evaluate the supplementation effect of fumaric acid and myristic acid mixture in dairy cattle.

Material Methods

Ten crossbred (Bos indicus×Bos taurus) multiparous cows were selected from the herd maintained at National Dairy Rerseach Institute, Karnal and randomly divided into two groups of five each on the basis of milk yield and lactation stage. Initial body weight of control and treatment groups cows is 348.40±13.21 and 333.20±14.39 respectively. Cows in both group is in their third month of lactation and yielding around 10 litre of milk. All the animals were maintained under individual experimental feeding for 98 days on a roughage and concentrate mixture. Roughage part consists of wheat straw and maize fodder while, concentrate mixture consists of maize, 29%; groundnut cake, 20%; wheat bran, 20%; barley, 5%; mustard cake, 12%; de oiled rice bran, 11%; mineral mixture, 2%; and common salt, 1%, on dry matter basis. Animals of experimental group were additionally supplemented with 4 percent mixture of myristic acid and fumaric acid (50:50) on dry matter basis. Daily milk yield and fortnightly milk composition estimated as per standard methods. A conventional digestion trial of 7 days duration was conducted on both groups of animals in the mid-experiment period(after 49days), keeping the 24-h record of intake of feeds, feces voided out, and orts if any. Digestibility coefficients were calculated for proximate principles and cell wall components.After digestion trial for methane estimation, cattle were kept individually in controlled chamber for three days. All of the air of chamber was exhausted by a pump. The air flow rate from exhaust pump and hourly methane concentration (%) in exhausted air was measured by methane analyzer (The Analytical Development Co. LTD., Hoddesdon, England). Methane production (litre/hr) was calculated by multiplying methane percentage to air flow rate and a calibration factor The data were subject to test of significance between diets using one way analysis of variance (Snedecor and Cochran, 1989) by SYSTAT 7.0 software and statistical significance was expressed at $P<0.05$.

Results

Results of the study indicated that the overall mean DM intake was 8.97 kg and 9.53 kg d^{-1} in control and treatment group, respectively. The DMI was non-significantly higher in the treatment group over the control group. The roughage to concentrate ratio of the diets in the two groups was also similar, i.e. 72.92:27.08 and 72.51:27.49 in control and treatment fed groups, respectively.DM intake in terms of kilograms per day and gram per kilogram $BW^{0.75}$ were also remained similar ($P>0.05$) in both groups. Similarly digestibility coefficients of nutrients also did not vary significantly ($P>0.05$) between the groups. Average milk yield (kg/d/animal) during entire period in treatment group apparently remained on higher side i.e 9.45 vs 8.33 but the statistically values were found remained similar in both groups.Total methane emission in terms of gram/day/animal or gram/kg DMI or litre/day were significantly reduced in treatment group than control.Methane emission were 150.80 and 107.03 gram/day/animal respectively and reduced in tune of about 29 percent in treatment group with comparison to control group.

Conclusion

Combination of fumaric acid and myristic acid have potential to reduce methanogenesis without affecting production performance of lactating cattle.

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Supplementation effect of garlic oil and garlic powder on methane production and milk production performance in buffaloes

R Zafarian, S Sirohi, M Mohini
National Dairy Research Institute, Karnal,Haryana, India *Email:sirohisk@gmail.com*

Introduction Garlic (*Allium sativum*) is an herb or spice plant that has been used by humans as a source of antimicrobial agents for the gastrointestinal tract. It has a complex mixture of many secondary plant products including allicin, diallylsulfide, dialyldisulfide and allyl mercaptan among others (Lawson, 1996). These active compounds could manipulate rumen fermentation such as decreasing the proportion of acetate and increasing the proportion of propionate and butyrate, inhibition of methanogenesis and decreased in the CH_4:VFA ratio (Busquet *et al*., 2005). Therefore, a study was planned to see the effect of garlic powder and garlic oil supplementation in lactating buffaloes.

Material Methods An *in vivo* trial on fifteen lactating buffaloes was conducted to see the effect of garlic powder and garlic oil supplementation in wheat straw based diet on nutrient utilization, milk production and methane production. Garlic powder(GP) and garlic oil(GO) were supplemented at the rate 20gram/kg DMI and 150mg / litre of rumen volume respectively in treatment groups. Animals were divided into three groups i.e control, treatment group I and II having five animal in each group. In mid of the experimental period a digestibility trial was conducted to see the nutrient utilization in different groups. Daily Milk yield and fortnightly milk composition also recorded during the whole experimental period up to 100 days. Methane production in animals was estimated using the SF_6 method for five consecutive days. The data were subject to test of significance between diets using one way analysis of variance (Snedecor and Cochran, 1989) by SYSTAT 7.0 software and statistical significance was expressed at $P<0.05$.

Results Results of study presented in table which shown that GP and GO increased milk production significantly during 1st to 3rd, 4th to 7th and 8th to 11th wks than control group. Due to treatment the milk composition remained same in both groups Nutrients digestibility of DM, OM, CP, EE, NDF, ADF, CHO and HC was also not affected by supplementation of garlic oil and garlic powder in lactating buffaloes. Results of *in vivo* study indicated that the total methane emission (g) and methane emission g/kg DM intake in experimental buffaloes reduced significantly ($P<0.05$) by 31% and 70% in GP and GO supplemented groups in comparison to control groups.

Table 1 Effect of Garlic powder and Garlic oil on milk production and Methane Production

Treatment Parameters	Control	Garlic Powder	Garlic Oil	LSD*
Body Weight Average	528.3	538	524.7	NS
Milk Yield(kg/d/a)	5.3	6.0	5.8	NS
DMI (kg/d/a)	10.98 ± 0.49	12.24 ± 0.59	11.90 ± 0.62	NS
CPI (kg/d/a)	1.36 ± 0.05	1.48 ± 0.09	1.47 ± 0.07	NS
TDNI (kg/d/a)	8.38 ± 0.36	9.08 ± 0.41	9.20 ± 0.50	NS
Methane(g/kg DMI)	40.70 ± 2.91	27.0 ± 01.99	10.83 ± 0.81	7.36
Methane(g/kg DDM)	54.03 ± 3.75	36.37 ± 2.11	14.23 ± 0.90	8.95
Methane(mM/kg DMI)	2.53 ± 0.19	1.67 ± 0.15	0.67 ± 0.07	0.50

*Least Significant Difference

Conclusion It was concluded from results that both garlic oil and garlic powder able to decrease the methane significantly without affecting the nutrient utilization, milk composition and milk yield. The reduction of methane was higher in case of oil than powder in lactating buffaloes fed wheat straw based diets.

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Production performance and methanogensis as affected by supplementation of rumensin in wheat straw based diets in lactating crossbred cows and buffaloes

S Sirohi[1], M L Mahabalarao[2], M Mohini[1], B Singh[1]
[1]National Dairy Research Institute, Karnal, Haryana, India, [2]Eli Lilly Asia Inc, Bangalore, India
Email:sirohisk@gmail.com

Introduction Ionophores such as monensin, lasalocid, laidlomycin, salinomycin and narasin are antimicrobial compounds that are routinely fed to ruminant animals to improve feed efficiency. These antimicrobials specifically target the ruminal bacterial population and alter the microbial ecology of the intestinal microbial consortium, resulting in increased carbon and nitrogen retention by the animal, increasing production efficiency**.** The most commonly used ionophores is Monensin Sodium in ruminants diets.Monensin is an ionophore approved for use in lactating dairy cows in several countries, including Australia, Argentina, Brazil, Canada, New Zealand, South Africa, and recently the United States. Ionophores have been shown to decrease CH_4 emissions also from cattle, although effects may be transient. The decrease in methanogenesis results from a shift in bacterial population from gram positive to gram negative organisms with a concurrent shift in the fermentation from acetate to propionateTherefore, the present study was undertaken to evaluate the effect of rumensin in wheat straw based diets fed to lactating cattle and buffaloes.

Material and methods Ten adult dairy cattle and buffaloes were used and animals were divided into two groups containing five animal in group as replicates. The groups are identified as control group and treatment group. Each animal was selected on the basis of lactation length, stage of lactation milk yield and body weight. Animals were housed in sheds for conducting feeding trials and methane measurement for a period of 98 days. Roughage and concentrates were fed to the animals as per NRC requirements. Rumensin® Premix was fed @200mg/kg DMI in treatment group for both cattle and buffaloes. In between the experimental period, digestibility trial was also conducted after 40days for a period of 7 days. Feeds and dung samples were collected for digestibility studies. Methane estimation was undertaken using SF_6 method. The data were subject to test of significance between diets using one way analysis of variance (Snedecor and Cochran, 1989) by SYSTAT 7.0 software and statistical significance was expressed at $P<0.05$.

Results It was revealed from the results of cattle and buffalo trial that actual dry matter intake per day per animal in both groups remained statistically similar i.e. 11.86 and 12.14 kg in cattle and 14.27 and 14.17 kg in buffaloes in control and treatment group respectively. The results of digestibility of nutrients indicated that all the nutrients digestibility like dry matter, organic matter, crude protein, ether extract, NDF, ADF and hemi cellulose remained similar and the differences were remained non significant in both groups. DCP and TDN intake values were higher in treatment groups however, differences were found non- significant between both groups in lactating cattle and buffaloes. The results of methane production in lactating buffaloes after 50 days of Rumensin premix feeding showed in table which significantly reduced after addition of rumensin in wheat straw based diets.

Table 1 Effect of monensin sodium supplementation on dry matter intake and methane production

Item	Cattle		Buffaloes		Significance
	Control	treatment	control	treatment	
Body wt. (kg/animal)	374.64	334.48	536.90	544.30	NS
DMI (kg/day/animal)	11.86	12.14	14.22	14.17	NS
Methane(L/kg DMI)	15.77[a]	13.48[b]	21.00[a]	17.25[b]	*
Methane (g/day/animal)	117.44[a]	102.65[b]	187.18[a]	153.49[b]	*

Conclusions It was observed on the basis of in-vivo trials in cattle and buffloes that digestability of nutrients remained unchanged with the addition of Rumensin in the diet. The similar effect was also noticed on the dry matter intake. Futher it was observed that monensin was effective to reduce the methane production in diet which contains about 60% roughage and 40% concentrate both in cattle and buffloes. It is futher concluded that when Rumensin was fed with wheat straw diet @20mg per kg of dry matter in dairy cattle and buffaloes, redcued methane production up to 12.59% and 17.99% in dairy cattle and buffaloes respectively.

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Effect of scraping frequency on amount, dry matter and nutrient content of liquid manure and gaseous emissions from a naturally ventilated dairy house with concrete floors

I Schiefler, A Schmithausen, W Büscher
Institute of Agricultural Engineering, Bonn University, Bonn, Germany *Email:ischiefler@uni-bonn.de*

Introduction Manure removal on concrete walking alleys in dairy housings is mostly performed by chain or cable pulled scrapers. A high cleaning frequency intends to keep walking surfaces clean and safe and thus supports claw health and cow comfort. This study is dealing with the effect of three scraping frequency variations on the amount, the dry matter and nutrient content of the liquid manure. Furthermore, CH_4 and NH_3 emissions were recorded.

Material and methods Measurements were performed in a naturally cross ventilated dairy barn on the agricultural centre Haus Riswick in Germany. The free stall barn was equipped with cubicles for 48 Holstein cows. The cows were fed with a maize/grass silage based ration and additional concentrate according to their performance. There were two double rows with cubicles and four walking alleys with solid surface and rubber coverage. Each walking alley was equipped with one separate cable pulled scraper moving to an external manure discharge shaft. The gaseous emissions from the external slurry pits and shafts were not covered by the emission measurements. Emission measurements were focused on the emissions from the barn covering emissions from animals and from manure on floors and surfaces. The experiment was performed from October to November 2012 with daily average temperatures from 5-17°C. Three variations of scraping frequency (high-20, medium-10 and low-4 times scraping a day) were tested for 7 days with two repetitions. The filling level of the slurry was measured once a day by using a measuring stick in order to calculate the amount of liquid manure. Prior to each measurement the liquid manure within the pit was stirred carefully in order to ensure a smooth surface for measuring. After each 7-day measurement period, a sample of the mixed liquid manure was sent to a specialized laboratory for chemical analyses in agriculture (VDLUFA). Afterwards, the liquid manure was transferred to the long term storage. Gas concentrations were measured quasi continuously using a photo acoustic field gas monitor (Schiefler *et al*., 2011). The air exchange rate was determined by CO_2 mass balance method according to CIGR 2002.

Results There were no significant differences in amount, dry matter content and nutrient content of liquid manure. Only the total Nitrogen content of the high scraping variation was significantly lower compared to medium and low frequency (Table 1**).**
However, the NH_4-N did not differ between variations. No significant differences in CH_4 and NH_3 emissions between scraping variations were found. The effect of temperature on NH_3 emissions was more relevant than the effect of scraping frequency.

Table 1 Amount, dry matter and nutrient content of liquid manure and emissions of methane and ammonia referring to different scraping frequencies

Variation		Temperature	Amount of liquid manure	DM-Content	N_{total}	NH_4-N	CH_4	NH_3
Scraping	frequency per day	°C daily mean	l cow^{-1} day^{-1}	%	% of fresh matter	% of fresh matter	g cow^{-1} day^{-1}	g cow^{-1} day^{-1}
High	20	11.8	64.5	9.8	0.38	0.18	810.4	57.1
Medium	10	10.6	58.1	10.1	0.40	0.18	836.4	68.7
Low	4	11.7	65.4	10.0	0.40	0.18	719.3*	55.9*

*within this period only few days were considered for emission calculation

Conclusions This preliminary test showed no significant differences in the amount and nutrient content of liquid manure (except total Nitrogen). However, it has to be proven, if the results are reproducible under differing climatic conditions and measurement periods.

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Greenhouse gas emissions from beef production systems in Denmark and Sweden

L Mogensen[1], T Kristensen[1], N I Nielsen[2], M Henriksson[3], C Swensson[3], M Vestergaard[4], P Spleth[5], A Hessle[6], C Lindahl[7]
[1]Aarhus University, Dept. of Agroecology, Foulum, 8830 Tjele, Denmark, [2]AgroTech A/S, Dept. of Food Innovation, Skejby, 8200 Århus N, Denmark, [3]Swedish University of Agricultural Sciences, Dept. of Biosystems and Technology, Alnarp, Sweden, [4]Aarhus University, Dept. of Animal Science, Foulum, 8830 Tjele, Denmark, [5]Knowledge Centre for Agriculture, Dept. of Cattle, Skejby, 8200 Århus N, Denmark, [6]Swedish University of Agricultural Sciences, Dept. of Animal Environment and Health, Skara, Sweden, [7]Taurus Köttrådgivning AB, Kalmar, Sweden
Email:lisbeth.mogensen@agrsci.dk

Introduction Beef is in general the livestock product with the highest greenhouse gas (GHG) emission per kg of meat produced. However, beef is produced in different systems ranging from intensively milk fed bull calves of dairy breeds to extensive suckler cow system. The aim of the present study was to assess the effects of typical production systems used in Denmark (DK) and Sweden (S) on GHG emissions from beef by life cycle assessments (LCA), and to identify hotspot emissions.

Material and methods Existing types of beef production systems in DK and S was initially identified and defined, resulting in 5 Danish and 4 Swedish systems (see Table 1). For each system, typical feed rations and figures for daily gain, feed conversion and carcass yield that represent average productivity of the given system was collected. GHG emissions from the systems were calculated in a life cycle perspective, which means that all emissions in the chain until the animals leave the farm was included. The total emission of GHG was calculated for each system over a year and presented per kg carcass weight. Carbon footprints (CF) for feedstuffs were calculated based on typical cultivation systems and national yields in DK and S. Methane from enteric fermentation was estimated according to new equations developed from trials where typical Nordic diets have been fed (Nielsen *et al*., 2013). A CF was calculated for the input of 'a dairy calf' to the beef system. The manure collected was handled as an output in the way that each beef system got credit for this production corresponding to the fertilizer value of the manure. GHG emissions from indirect land use change (LUC) was included as suggested by Audsley *et al*. (2009) with a LUC emission factor of 143 g CO_2 per m^2 land used for feed production. We assumed that production of feed on permanent pastures does not put pressure on land use and thereby does not cause deforestation anywhere in the world. Contribution from carbon sequestration was included as suggested by Vlesshouwers and Verhagen (2002), where productive pastures works as a sink and other crops cause release of carbon. Other emissions, for example that of nitrous oxide gas (N_2O), was calculated as described by Kristensen *et al*. (2011).

Results Carbon footprint (CF) from production of 1 kg meat showed a variation from 8.7 kg CO_2 in the most intensive system with fattening of bull calves coming from dairy herds to 30.7 kg CO_2 in an extensive suckler cattle system. Taking contribution from LUC and soil carbon sequestration into account did not change the ranging of the systems but reduced the difference in CF between the most extreme systems. Production of and enteric fermentation of feed were the major hotspots, responsible for 28–58% and 28-56% of total emission, respectively. In the extensive suckler cow system, the highest single contribution was enteric methane, whereas in the intensive bull fattening system it was GHG from feed production.

Table 1 Carbon footprint (CF) from production of 1 kg meat (carcass weight) in different beef production systems

System	Suckle Exten.	Suckle Inten.	Suckle Inten.	Dairy Steer	Dairy Steer	Dairy Bull	Dairy Bull	Dairy Bull	Dairy Bull
Age slaughter, months [1)]	22	14	15	24	26	19	11.3	9.3	9.5
LW at slaughter, kg	431	589	633	574	610	631	450	383	307
Country	DK	DK	S	DK	S	S	DK	DK	S
Feed, kg CO_2/kg meat	8.7	7.6	8.1	6.4	5.1	4.3	5.0	4.7	3.0
CH_4, kg CO_2/kg meat	17.4	11.4	12.9	7.2	8.2	3.8	2.2	2.0	1.8
CF, kg CO_2/kg meat	30.7	22.9	25.0	16.8	16.2	10.8	9.0	8.9	8.7
LUC, kg CO_2/kg meat	2.1	2.9	2.8	2.5	1.6	2.6	1.7	1.5	1.3
Soil C, kg CO_2/kg meat	-1.0	-0.1	-2.0	-1.6	-0.7	3.1	3.6	3.2	1.7

1) For the suckler system only age and months at slaughter is given for the bull calves

Conclusions There is large variation in CF between production systems when producing 1 kg meat. Feed production and the enteric fermentation of it, are the hotspot emissions. However, important contributions also come from LUC and soil C sequestration.

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An industry approach to measuring greenhouse gas emissions from Irish beef cattle production systems

P Crosson[1], P Brennan[2], P O'Kiely[1]
[1]Teagasc, Grange, Dunsany, Co. Meath, Ireland, [2]Bord Bia, Clanwilliam Court, Lower Mount Street, Dublin 2, Ireland
Email:paul.crosson@teagasc.ie

Introduction The carbon footprint of a product or service is the quantity of greenhouse gas (GHG) emissions generated in the production of that product or service. In the case of the food industry, the challenge to reduce GHG emissions is compounded by the increasing demand for food arising from the burgeoning global population and increasing affluence in developing countries. The GHG emissions generated from Irish beef production has been reported previously by Casey and Holden (2006) and Foley *et al*. (2011), however these studies were based on research farm conditions. The objective of this study was to quantify the GHG emissions from commercial beef farms in Ireland. This was a collaborative study between Teagasc and Bord Bia (the Irish Food Board) and involved the development of a carbon audit programme and the consequent application of this programme to a sample of commercial beef farms.

Materials and methods The GHG model was based on the framework developed by Foley *et al*. (2011). 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 were simulated. No land use or land use change GHG emissions or sinks were assumed. A key requirement was to use an internationally accepted accreditation process and, therefore, the model was modified to be compatible with the requirements of the British Standards Institute (BSI) Publically Available Specification (PAS) 2050 which builds on the life cycle methods of ISO 14040 and ISO 14044. These modifications primarily involved changing the emission factors used in the model of Foley *et al*. (2011). The farms selected for participation in the study were participants in the Beef Quality Assurance Scheme operated by Bord Bia. The BQAS is an independently accredited national scheme for beef production in Ireland. This scheme currently has approximately 32,000 participants, which are audited at least once every 18 months. For the purposes of this project, 200 BQAS certified farms were selected which were deemed to provide a cross-section of the various production systems that prevailed.

Results Suckler beef cow production systems had higher GHG emissions than dairy beef systems (Figure 1). Although numerically different, there were no significant differences between systems within the suckler beef or dairy beef categories. The store to finish category, incorporating both suckler and dairy bred progeny, had higher GHG emissions than dairy beef systems but similar GHG emissions to suckler beef systems. In terms of the relative importance of the animal groups on farms (Figure 2), suckler cows were responsible for the greatest amount of GHG emissions on suckler beef farms, accounting for 36% of total farm GHG emissions. This ranged from 42% for suckler to weanling systems (SRW) to 32% for suckler to finish systems (SRF). Heifer followers, i.e. all heifers on the farm between 12 and 36 months of age, were the next most important animal group. For the dairy beef systems, there was a higher number of followers (74% *vs*. 63%) and a greater number of male cattle (63% *vs*. 41%) for DYF when compared to DYS. The store to finish system was dominated by followers with males the main category represented.

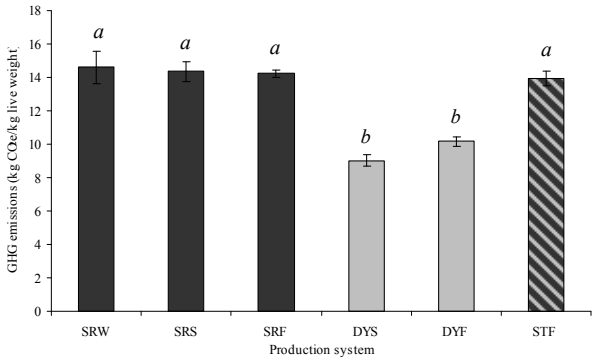

Figure 1. Greenhouse gas emissions from Irish beef production systems. SRW = Suckler to weanling; SRS = Suckler to store/finish; SRF = Suckler to finish; DYS = dairy calf to store/finish; DYF = dairy calf to finish; STF = store to finish.

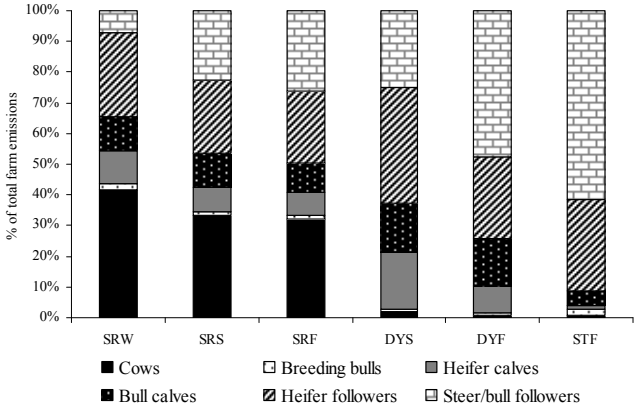

Figure 2. Greenhouse gas emissions by animal group for Irish beef farms. For key to systems see Figure 1.

Conclusions Significant differences between beef production systems were apparent in this study with suckler beef systems having higher GHG emissions than dairy beef systems. This is largely due to the allocation of cow emissions to milk in the dairy beef systems and to beef in the suckler system.

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Dietary nitrate but not linseed oil decreases methane emissions in lactating dairy cows fed a maize silage based diet

J B Veneman[1,2], S Muetzel[3], H B Perdok[1], C J Newbold[2]
[1]Cargill Animal Nutrition Innovation Centre Velddriel, Velddriel, The Netherlands, [2]IBERS, Aberystwyth University, Aberystwyth, UK, [3]AgResearch Limited, Grasslands Research Centre, Palmerston North, New Zealand
Email:jbv@aber.ac.uk

Introduction Linseed oil and nitrate have potential to mitigate methane production in ruminants. The aim of this experiment was to investigate the effect of dietary linseed oil and nitrate on methane production and rumen fermentation in lactating dairy cows.

Material and methods Eighteen multiparous lactating Holstein (n=12) and Holstein-Jersey cross (n=6) dairy cows (533 ±65 kg BW, 180 DIM, 20.5 ±2.6 L of milk) were blocked according to milk yield and BW and randomly assigned to one of three treatments. Treatments consisted of a control, dietary supplementation of linseed oil (4% of feed DM) and supplementation of nitrate (2% of feed DM). The basal ration contained maize silage, alfalfa hay, soybean meal and a mineral supplement. Diets were fed twice daily and formulated to be iso-nitrogenous and similar in non-protein nitrogen through the addition of urea to the control and linseed oil diet. After a minimum adaptation period of 14 days animals were moved from group pens to individual pens to determine *ad libitum* feed intake, where-after animals were restricted to 95% of the animal consuming the least within a block. Rumen samples were taken via stomach tube 3 hours post morning feeding on two consecutive days before animals were moved to open circuit respiration chambers. Gaseous emissions and feed intake were recorded for a 48h period directly after animals entered the chambers. Animals were adapted to the restrained environment of the chamber by restraining them in the individual pens for short periods for the last two days. Chambers were a larger version of the small ruminant chambers described by Pinares-Patiño *et al*. (2008).

Results Dietary treatment did not affect DMI or milk yield (P>0.05) during the gaseous emissions measurement period. Nitrate, but not linseed oil decreased (P<0.03) total methane emission per day, per kg DMI and per kg milk compared with the control treatment (19.5%, 18.3% and 31.0%, respectively). Cows receiving nitrate had increased hydrogen emissions compared with cows on the control and linseed oil treatment (P<0.03). Total VFA concentration was similar between treatments (P>0.353), however nitrate supplementation increased the acetate:propionate ratio (P<0.02).

Table 1 Effect of dietary linseed oil and nitrate supplementation on DMI, milk yield and gaseous emissions and rumen fermentation.

	Control	Linseed oil	Nitrate	s.e.d.	P
DMI (kg/d)	18.4	17.7	18.3	0.53	0.352
Milk yield (kg/d)	16.4	16.9	19.5	1.11	0.054
Methane (g/d)	394	378	317	23.5	0.020
Methane (g/kg DMI)	21.3	21.5	17.4	1.19	0.010
Methane (g/kg milk)	24.2	22.6	16.7	1.63	0.002
Hydrogen (g/d)	0.7	1.1	3.0	0.74	0.026
Total VFA (mM)	100.3	91.0	94.5	6.17	0.353
Acetate: Propionate ratio	3.16	3.55	4.51	0.373	0.013

Conclusions Nitrate but not linseed oil decreased methane emissions per day, per kg DMI and kg milk in lactating dairy cows without affecting animal performance. Although total VFA concentration did not differ, acetate:propionate ratio was increased when feeding nitrate. Together with the increased hydrogen production this suggests changes in the distribution of metabolic hydrogen among methanogenesis, nitrate reduction and the production of individual VFAs when feeding nitrate. These results are in line with previous *in vitro* results (Veneman *et al.* 2012). Further research is needed to establish whether the benefit of lowering methane emissions when feeding nitrate is not undone by changes in nitrogen excretions.

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Exogenous fibrolytic enzymes applied to forage grasses affected the ruminal degradability, fermentation and methanogenesis in vitro

Y A Soltan[1], A L Abdalla[2], A S Morsy[3], S M A H Sallam[1], H Louvandini[2], R C Lucas[2], L R F Silva[2]
[1]Faculty of Agriculture, Alexandria University, Alexandria, Egypt, [2]Centre for Nuclear Energy in Agriculture, University of Sao Paulo, Sao Paulo, Brazil, [3]Animal Production Research Institute, Cairo, Egypt *Email:uosra_eng@yahoo.com*

Introduction Livestock production plays an important role in the Brazilian economy where the predominant livestock production systems are based mostly on low digestibility forage grass pastures (Abdalla *et al*., 2012). Thus exogenous feed enzyme products that contain xylanases and cellulases may enhance fiber digestion in the rumen consequentially reducing methane (CH_4) produced per unit of product from grazing animals. This hypothesis was investigated *in vitro* by determining the enzymatic activity of two different fibrolytic enzyme products and evaluating their effect on common Brazilian pasture grasses for ruminal degradability, fermentation and methanogenesis.

Material and methods Forage grass pasture samples (0.25m^2 plots and 20 cm height) [Aruana n=20 (*Panicum maximum*), Napier grass n=4 (*Pennisetum purpureum*), Brachiaria n=14 (*Brachiaria decumbens*), Buffel grass n=4 (*Pennisetum ciliare*), sugarcane bagasse n=4 and chopped sugarcane n=4 (*Saccharum officinarum)*] were harvested according to pasture availability from each production site within a year. Proximate analyses were carried out according to AOAC, (2006) and Van Soest *et al*., (1991). Samples were treated or not with exogenous fibrolytic enzymes and evaluated *in vitro* using semi-automatic gas production (GP) system for 24 h within 3 runs (Bueno *et al*., 2005). Two different commercial fibrolytic enzyme products were evaluated for their endoglucanase, exoglucanase and xylanase activity at rumen conditions prior to the *in vitro* incubation. One ml of the cellulase product liberated 277 µmol of xylose/min from oat spelt xylan, and 720 and 1.17 µmol of glucose from carboxymethylcellulose and Sigmacell Cellulose, respectively while, one ml of the xylanase product liberated 6760 µmol/min of xylose from oat spelt xylan and 732 µmol of glucose from carboxymethylcellulose, but no exoglucanase activities were detected for both products. Each product was supplemented individually at zero (without addition) and 7.5 or 0.46 units/500 mg DM substrate for endoglucanase and xylanase products, respectively. The enzymatic effects were statistically analyzed separately for each activity and incubation time and analyzed with a model that included substrate, enzyme level (with or without additive), their interaction as fixed effects, and experimental run (replication in time, n=4) as a random effect.

Results Aruana, Nappier and Brachiaria presented the highest (P<0.01) CP content (162± 35 g/kg DM), while chopped sugarcane and bagasse showed the lowest (P<0.01) values (34 and 31 ± 3.5 g/kg DM, respectively). Buffel and sugarcane bagasse had the highest (P<0.01) concentrations of NDF, ADF and cellulose compared with the other substrates. Xylanase addition had no carry over effect (P<0.05) on CH_4, rumen degradability and fermentation parameters (Table 1). The improvement in the forage grasses pasture fermentation and fiber degradability by endoglucanase supplementation while the CH_4 production remained unchanged might to be promising approach to reduce CH_4 produced per unit of product by grazing animals.

Table 1 Effect of endoglucanase and xylanase products applied to six grass substrates in the rumen fermentation and degradability

	Endoglucanase			P			Xylanase			P		
Item	+	-	s.e.m.	E	S	S × E	+	-	s.e.m.	E	S	S × E
GP (ml/g DM)	144	126	2.9	< 0.001	< 0.001	< 0.001	122	121	2.6	NS	< 0.001	0.0161
CH_4 (ml/g TDOM)	12.8	11.7	1.12	NS	< 0.001	0.0044	12.6	11.9	1.03	NS	< 0.001	NS
CH_4(ml/g DNDF)	27.1	30.8	5.10	NS	< 0.001	NS	32.7	42.0	12.11	NS	< 0.001	NS
TDOM	451	400	16.3	< 0.001	< 0.001	NS	397	397	11.2	NS	< 0.001	< 0.001
DNDF	274	187	21	< 0.001	< 0.001	NS	201	203	14.5	NS	< 0.001	< 0.001
C2/C3	4.13	4.26	0.401	NS	0.001	NS	4.61	4.80	0.62	NS	0.047	NS
Total VFA	46.3	45.1	3.23	NS	0.061	NS	54.2	51.0	5.08	NS	0.034	NS

GP= gas production, CH_4 = methane, TDOM= truly degraded organic mater, DNDF= degraded neutral detergent fibre, C2/C3 acetate: propionate ratio, VFA= volatile fatty acids, s.e.m. = standard error of mean, S× E= substrate × enzyme, NS= not significant.

Conclusions Our results highlighted of the importance of matching the enzyme product to the forage substrate to achieve maximal benefit of using exogenous enzyme products in ruminant diets. Thus good quality forages would reduce overall lifetime animal CH_4 emissions**.**

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Greenhouse gas emissions of Uruguayan beef cow-calf systems

G Becoña[2], L Astigarraga[1], J Franco[1], V Picasso[1]
[1]Universidad de la Republica, Montevideo, Uruguay, [2]Instituto Plan Agropecuario, Montevideo, Uruguay
Email:gbecona@planagropecuario.org.uy

Introduction Livestock accounts for 80% of greenhouse gas (GHG) emissions in Uruguay. Cow-calf systems spread over half of the agricultural land, with historically low weaning rates (60 to 68%), low stock efficiency (heifers first served at 2.5 years) and low weaning weights (150 kg). Adequate estimation and analyses of carbon footprint (CF) from real livestock systems, accounting for the complex interactions between forage management and animal performance are crucial for identifying opportunities to reduce GHG emissions. The objective of the present study is to identify technological variables associated with GHG emissions and describe animal management practices in farms with lower carbon footprint in Uruguay.

Material and methods We used data from 23 livestock farms collected by the Uruguayan extension service (Instituto Plan Agropecuario) as case studies based on the reliability of farm records, and diversity of forage and animal management practices. These farms were not selected randomly from the population of farms in Uruguay, so our results are not necessarily representative of the whole country. Extension officers verified and summarized the information from each farm. On-farm information included animal stocks by category and animal weights at the beginning and end of the fiscal year. We assumed that the animals were fed exclusively pastures (concentrate use was less than 1% of dry matter intake). Feed intake quality was predicted considering land-use in each farm by the forage dry matter production (DMP) from each pasture resource, assuming 50% utilization. Forage production or quality was not measured directly. Digestibility and crude protein of each pasture type was estimated from Mieres *et al*., 2004. DMP was estimated from satellite monitoring of pastures (LART-UBA-IPA). To avoid variability in results due to climate only one year was used (2010-11), which had rainfall similar to the long-term average. CF was estimated at farm level, according to IPCC (2006) guidelines using coefficients and equations from tier 2 protocols. Functional unit used in cow-calf systems was kg CO_2-e/ kg of weaned calf. Cull cows were not taken into account. CH_4 emissions from cattle management (enteric fermentation and excreta), N_2O direct emissions from manure and urine in soils and N inputs (fertilizer), N_2O indirect emissions from N leaching, run-off and volatilization, and CO_2 emissions from energy used in sowing, fertilization and other activities and off-farm energy used for inputs manufacturing and transporting were considered in this study. We used IPCC default values for all variables except for the conversion factor for methane emissions (Y_m) which was estimated as a function of digestibility of diet using Cambra–López *et al*. (2008) equation and the coefficient for fossil fuel burned (3.0 kg CO_2-e/L of diesel). To identify which variables better explained the differences in CF we estimated correlations and simple linear regressions between CF and possible explanatory variables. A Cluster technique was used to identify groups of farms with different forage and animal performance variables and GHG emissions intensity.
Results The results indicated that the emissions to produce one calf were on average 5213 kg CO_2-e, and GHG emissions intensity was 33.2 kg CO_2-e/kg weaning calf with a range of 20.7 to 52.0 kg CO_2-e/kg (VC= 24%). The main GHG emitted was CH_4 generated in the enteric fermentation, which accounted for 74% of the total emissions. Animal performance and feed were the main determinants of the intensity of GHG emissions in the cow-calf systems in our study. We found high correlations between CF and weaning weight per stock efficiency, % Weaning, Weaning weight per served cow, % diet digestibility and % crude protein (0.77, 0.60, 0.64, 0.62, 0.60 respectively with $P \leq 0.001$). Nevertheless, multivariate analyses showed that systems that optimized forage production per hectare, improved animal production and produced lower GHG emissions per unit of output at the same time.

Table 1 Number of farms (N) and average values for percent area of grasslands oversown with legumes (GOL), weaning rate (WR), calf weaning weight (WW), weaned weight per served cow (WWSC), stock efficiency-weaned weight per stock (SE), diet digestibility (DD), and dry matter production (DMP) for 3 clusters of livestock farms in Uruguay.

Variables	N	CF	GOL (%)	WR (%)	WW (kg)	WWSC (kg)	SE (kg)	DD (%)	DMP (kg/ha)
Group 1	6	25.2	41.1	80	175	122	101	58	5168
Group 2	10	33.3	8.6	80	146	120	90	56	4141
Group 3	7	39.7	8.1	59	152	90	67	56	4191

Conclusions Our results showed that cow-calf systems in Uruguay have a wide range of CF estimates, so using a simplified average country values is not recommended. This study suggests that GHG intensity can be reduced through changes in management practices to increase animal productivity and improve quality and quantity of forage which are currently available in Uruguay.

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Prediction of methane production in the rumen by stoichiometric techniques in hair sheep fed low quality tropical grass supplemented with foliage of Brosimum alicastrum

V Valdivia-Salgado[1], E G Briceño-Poot[2], J R Canul-Solis[2], L Ramírez-Avilés[2], J C Ku-Vera[2]
[1]SAGSA-DISAGRO, Managua, Nicaragua, [2]University of Yucatan, Merida, Yucatan, Mexico *Email:kvera@uady.mx*

Introduction There is increasing concern regarding the environmental impact of methane emissions arising from ruminant production systems. Methane production from enteric fermentation in ruminant livestock accounts for 28% of total global anthropogenic methane emissions per year. Low quality tropical grasses contribute significantly to methane emissions in ruminant production systems (Kurihara *et al.*, 1999). There is scope to mitigate methane emissions from ruminants through the incorporation of foliage of tropical plants (Kennedy and Charmley, 2012) in practical rations. The aim of the present work was to estimate methane production by stoichiometric techniques in hair sheep fed a basal ration of low quality *Panicum maximum* grass supplemented with increasing levels of foliage of *Brosimum alicastrum.*

Material and methods The experiment was carried out at the University of Yucatan in South Mexico. Four rumen cannulated Pelibuey lambs with an average live weight of 37.4±4.9 were used. Sheep were housed in metabolic crates. Foliage of *Brosimum alicastrum* and *Panicum maximum* were ground with a hammer mill to pass a 0.5 cm sieve. Experimental treatments consisted of mixtures of *B. alicastrum* and *P. maximum* in the proportions 0:100, 15:85, 30:70 and 45:65 on dry matter basis. Cane molasses (9.4 % DM) were incorporated in all treatments. Sheep were fed *ad libitum* at 8:00 h. DM intake was measured daily as the difference between the feed offered and that refused. Apparent digestibilities of DM and NDF were measured by total collection of feces. Samples of rumen liquor were taken through the rumen cannula for VFA analysis by gas chromatography. Estimates of methane production were carried out from the amount of digestible carbohydrate consumed and VFA molar proportions with the stoichiometric model as developed by Ørskov *et al*. (1968). The experiment design was a Latin square 4 x 4 with twenty days per period, thirteen days for adaptation and seven days for measurements. Data were analyzed with the GLM procedure of SAS and orthogonal contrasts were used to assess the linear, quadratic and cubic terms of the response to the increasing levels of *B. alicastrum* in sheep rations. Data of energy lost as CH_4 were not analyzed statistically and only treatments means are presented.

Results DM and NDF intakes were increased linearly ($P<0.01$) as the level of *B. alicastrum* in the ration was augmented. Apparent digestibilities of DM and NDF were linearly increased as the level of tree foliage was increased. There were no significant differences ($P>0.05$) in the molar proportions of VFA in rumen liquor. There were no apparent differences in the estimated heat of fermentation and efficiency of conversion of glucose to VFA. Energy lost as methane was increased as the amount of digestible carbohydrate consumed was increased (Table 1).

Table 1 DM intake, apparent DM digestibility, molar proportions of volatile fatty acids and prediction of energy lost as methane in hair sheep fed low quality *Panicum maximum* grass supplemented with foliage of *Brosimum alicastrum*

	Levels of *Brosimum alicastrum* in ration (% DM)					
Item	0	15	30	45	s.e.m.	Response[a]
DM intake (g/d)	511	848	1106	1315	106	L **
NDF intake (g/d)	364	522	675	711	55	L **
Digestible carbohydrate intake (g/d)	101.6	194.5	265.5	294.5	-	-
DM digestibility (kg/kg DM)	0.36	0.45	0.47	0.50	1.7	L**
NDF digestibility (kg/kg DM)	0.33	0.44	0.46	0.48	3.1	L*
Acetic acid (mmol/100 mmol)	74.8	73.1	73.4	72.2	1.4	n.s.
Propionic acid (mmol/100 mmol)	16.4	17.7	16.6	17.3	0.5	n.s.
Butyric acid (mmol/100 mmol)	7.0	7.4	7.9	8.6	0.6	n.s.
Conversion efficiency of hexose to VFA	0.72	0.72	0.72	0.72	-	-
Estimated heat of fermentation (kJ)	0.064	0.064	0.064	0.064	-	-
Energy loss as CH_4 (kJ/mol)	387.4	719.2	998.3	1085.2	-	-

[a] L = Linear response; * $P < 0.05$; ** $P < 0.01$

Conclusions Energy loss as CH_4 from the rumen of sheep fed foliage of a tropical tree was increased as the intake of digestible carbohydrate by the sheep was increased. It is possible that the absence of secondary metabolites (tannins, saponins), in foliage of *B. alicastrum* precluded any effect on energy loss as methane from the rumen of hair lambs.

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Assessing soil carbon stocks and accumulation rates in Brazilian livestock production systems

A Segnini[1], P P A Oliveira[2], P L Otaviani Junior[1,3], A A P Xavier[1,4], E J Ferreira[1], M A Sperança[2,3], J R M Pezzopane[2], D M B P Milori[1], L Martin-Neto[1]
[1]Brazilian Agricultural Research Corporation (Embrapa)- Instrumentation Research Center, São Carlos, SP, Brazil, [2]Brazilian Agricultural Research Corporation (Embrapa)- Southeast Livestock Research Center, São Carlos, SP, Brazil, [3]Federal University of São Carlos (UFSCar) - Department of Chemistry, São Carlos, SP, Brazil, [4]Institute of Chemistry of Sao Carlos - University of São Paulo, São Carlos, SP, Brazil *Email:alinesegnini@hotmail.com*

Introduction The adoption of intensive pasture management has shown potential for mitigation of greenhouse gases (GHG) due to high production of forage of tropical grasses with efficient nitrogen fertilizer use and the accumulation of soil organic matter. The aim of this study was to evaluate the impact of pasture management on the basis of carbon (C) stocks, focusing mainly on the sustainability of livestock farming in Brazil. Carbon stocks and C accumulation rates were compared in surface (0-30 cm) and deeper layers (30-100 cm), having a native forest as reference. These results were obtained by PECUS Research Network, a multi-institutional project conceived by Brazilian Agricultural Research Corporation (Embrapa) with the objective of obtaining comprehensive data, using internationally accepted research protocols, in order to subsidize governmental policies and to contribute to the development of mitigation alternatives for GHG emissions.

Material and Methods The study was performed at Southeast Livestock Research Center of Embrapa, located in São Carlos, state of São Paulo, Brazil. The grazing areas cover 4 livestock systems for beef cattle production, with two blocks, in dystrophic Red Latosol (Oxisol): Intensive irrigated with high stocking rate (IHS) and Intensive dryland with high stocking (DHS) (1.75 hectares / block), have been covered by *Panicum maximum* since 2002; Dryland with moderate stocking rate (DMS) and Degraded pasture (DP) (3.3 hectares / block) have been covered by *Brachiaria decumbens* since 1996. The native forest soil, Atlantic forest (two blocks), near the experimental area, was sampled as representing the original soil conditions of this site. Soil samples were collected in different depths: 0-5, 5-10, 10-20, 20-30, 30-40, 40-60, 60-80 and 80-100 cm, with six field replicates (three replicates/block), two cores from each field replicate. Samples from each depth intervals were collected through an aluminum ring of known volume and pooled for the subsequent evaluation of dry soil weight (105 ºC). Soil samples were air-dried and crushed and ground in a mortar to pass a 0.150 mm sieve. Carbon concentration was analyzed using CHN equipment. Carbon stock (Mg ha^{-1}) of each sample was calculated and corrected to an equivalent mass depth (Ellert and Bettany, 1996), using the native forest soil as reference. In addition, C accumulation rates in the 0-30 cm and 0-100 cm soil layers of each pastureland condition were calculated.

Results Management systems affected the total soil C stocks between 0 to 30 cm and 0 to 100 cm layers (Table 1). The greatest soil C stocks were observed for the DHS and DMS systems and the lowest ones for the DP system in both layers. In DHS and DMS systems C accumulation rate was 1.93 and 1.80 Mg ha^{-1} $year^{-1}$, respectively, showing a favorable accumulation of C.

Table 1 Stocks and accumulation rates of carbon in livestock systems and native area in 0-30 cm and 0-100 cm soil layers.

Management systems	Soil C stocks (Mg ha^{-1})	C accumulation rate (Mg $ha^{-1}year^{-1}$)
	----------0-30 cm----------	
IHS	44.0(±3.4)^{a,b}	-0.71
DHS	56.9(±10.2)^{c,d}	+0.73
DMS	63.8(±8.8)^{d}	+0.90
DP	38.2(±4.4)^{a}	-0.81
forest	50.3(±5.6)^{b,c}	-
	----------0-100 cm----------	
IHS	109.2(±15.2)^{a,b}	-0.69
DHS	132.8(±20.3)^{b,c}	+1.93
DMS	142.4(±14.4)^{c}	+1.80
DP	99.3(±8.6)^{a}	-1.08
forest	115.5(±10.1)^{a,b}	-

IHS= Intensive irrigated with high stocking rate; DHS= Intensive dryland with high stocking; DMS= Dryland with moderate stocking rate; DP= Degraded pasture. Means followed by the same letter do not differ (Tukey test, $P > 0.05$).

Conclusions These findings indicate that a large proportion (from 55 to 60%) of the C accumulated in the soil is stored in deeper layers (below 30 cm). These results indicated that this tropical pasture soil site was suitable as long-term C sink for two management systems (DHS and DMS) probably due to management conditions and similarities in soil textures; however new system management assessments will be done after some adjustments for better efficiency. This is a very valuable result showing the capacity of well-managed tropical grasses to mitigate GHG emissions from livestock systems.

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Ammonia emissions from cattle urine and dung deposited on pasture

A Taghizadeh-Toosi[1,3], J Laubach[2], R Sherlock[3], J Gibbs[3], F Kelliher[4], T Clough[3], S Grover[2]
[1]Aarhus University, Tjele, Denmark, [2]Landcare Research, Lincoln, New Zealand, [3]Lincoln University, Lincoln, New Zealand, [4]Ag Research, Lincoln, New Zealand *Email:arezoo.taghizadeh-toosi@agrsci.dk*

Introduction Ammonia (NH_3) is generated at the soil surface in abundant quantities shortly following the surface application of nitrogenous fertilisers, various ammoniacal wastes to soil, and ruminant urination. Volatilisation of NH_3 is of concern because it contributes to aerosol formation and because of its environmental impacts upon redeposition to natural and agricultural systems. The latter include its role as a source of nitrous oxide emissions. The objective of the present study was to provide a direct in-field measurement of the likely maximum extent of NH_3 emissions following urine application to pasture and also the combined effect of NH_3 emissions from urine and dung, in conditions similar to a real-world rotational-grazing practice.

Material and methods Two experiments were conducted in summer 2010 (Experiment 1, Laubach *et al.* 2012) and 2011 (Experiment 2, Laubach *et al.* 2013) on paddocks near Lincoln University, New Zealand. NH_3 emission rates were determined with the micrometeorological mass-budget method. A circle with a 15 m radius was marked as the experimental plot each year, and the vertical profiles of the horizontal NH_3 flux were measured in the center of the circular plot with samplers as designed by Leuning *et al.* (1985). The wind profile was determined outside the circle. In Experiment 1, the circular plot was filled with a rectangular grid of 132 urine patches to simulate the urine load from a dozen dairy cattle grazing an area of this size for 24 h. The NH_3 measurements were conducted for 6 d, and soil samples from selected urine patches were repeatedly taken for analysis of pH and mineral-N contents. A three-resistance approach (aerodynamic, laminar boundary-layer and soil resistance) was formulated to model the emission rates dependent on these parameters, soil temperature and moisture, as well as wind speed. In Experiment 2, twelve cattle were kept for 3 d in the experimental plot and fed with freshly-cut pasture. The NH_3 emissions were determined for 12 d. Soil temperature and moisture and dung properties (pH, moisture content and mineral-N) were measured. Using these, it was tested whether the resistance of dung to NH_3 transport could be modelled in a similar fashion as for urine-treated soil.

Results Cumulative fractional NH_3 losses were 25.7 (±0.5) % from the urine-patch pattern, and 22.4 (±1.3) % from the in-situ deposited cattle excreta. The simultaneous observations of the pH evolution in urine patches and dung pats were used to infer separate volatilisation rates for urine and dung in Experiment 2 (Fig. 1).The results from the soil resistance model in Experiment 1 were physically realistic, so in principle repeated soil sampling together with wind speed measurements should suffice to estimate NH_3 emissions within the urine-affected area. However, the results also show that the accuracy of this approach depends on accurate knowledge of the horizontal and vertical N distribution in the soil. In Experiment 2, volatilisation from dung was controlled primarily by the dung crust resistance. The magnitude of this resistance and its moisture dependence are comparable to that of a soil resistance.

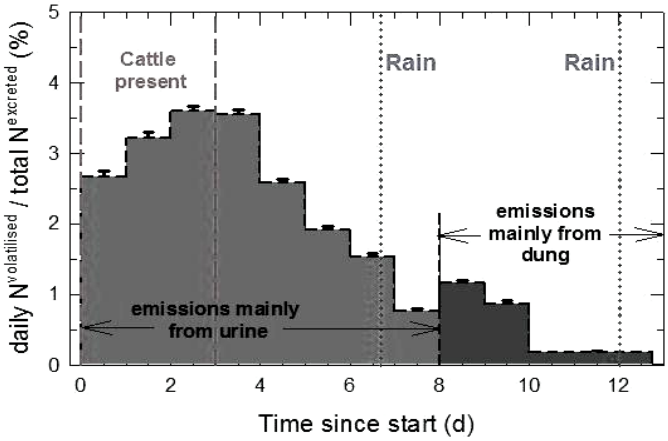

Figure 1 Evolution of N loss fraction due to volatilisation, relative to the amount of N excreted by 12 cattle over the first three days

Conclusions In two experiments, the observed NH_3 emission rates were consistent with each other. Since both experiments were conducted at the warmest time of the year, the emission rates were at the upper end of the range likely to occur in New Zealand. The fractional loss of N from dung is less than half that from urine. Emission rates both from urine-treated soil and from dung with a hard crust are more strongly controlled by the respective resistances of these media than by the atmospheric resistances.

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Ruminal methanogen population is enhanced by tropical environment

M Rira[1], D P Morgavi[1], M Popova[1], C Marie-Magdeleine[2], T Silou-Etienne[2], H Archimède[2], M Doreau[1]
[1]INRA, Saint-Genès Champanelle, France, [2]INRA, Petit-Bourg, Guadeloupe *Email:michel.doreau@clermont.inra.fr*

Introduction Ruminants fed tropical forages would produce more enteric methane (CH_4) than those fed temperate forages because of structural and physiological differences between C4 plants, native of tropical areas, and C3 plants, native of temperate areas. Methane production is closely linked to rumen microorganisms such as cellulolytic bacteria and protozoa that produce hydrogen and methanogens that use this hydrogen to produce CH_4. The environment, the diet and the animal breed can influence the composition and hence the function of the rumen microbial ecosystem. A companion abstract showed that CH_4 emissions were higher with C4 forages than with C3 forages but that the environment or breed had no effect (Archimède *et al*., 2013). This study aimed to understand these differences in CH_4 emissions between temperate and tropical sites by monitoring selected rumen microbial populations.

Material and methods Two parallel experiments were carried out in temperate (France) and tropical (West Indies) environments with the same forages in a double 4×4 Latin Square design (one per site). We used two breeds, Texel (T), temperate origin, and Blackbelly (B), tropical origin. Animal of both breeds were born and grown in the environment where they were used for the experiment. In both sites, sheep were fed forages from permanent grasslands grown in temperate or tropical areas. For each forage, there were two maturity stages which determined forage quality (high H and low L quality). Rumen contents were sampled before morning feeding. Microbial groups (total bacteria, *Fibrobacter succinogenes, Ruminococcus albus, Ruminococcus flavefaciens* and total methanogens) were enumerated by quantitative PCR (qPCR) using group-specific primers targeting the *rrs* gene for bacteria and the *mcrA* gene for methanogens. Protozoa were counted by microscopy. Statistical analyses were performed using the mixed procedure of SAS with period, site, forage, breed, and their interactions as fixed effects and animal as random effect. Statistical differences were declared significant at $P \leq 0.05$.

Results Concentrations of total rumen bacteria, *F succinogenes* and methanogens were higher in the tropical environment (Table 1). The number of methanogens was higher in sheep fed C4 grasses than in those fed C3 grasses. There was not site effect on *R albus* but a breed×site interaction was observed. There was no difference in *R. flavefaciens* numbers between sites, breeds and type of forage (average 6.56 *rrs* copies/g DM $\log_{10}$). Protozoal numbers were similar among sites, type of forage and breeds but a site× forage interaction was observed.

Table 1 Rumen microbes of Texel and Blackbelly sheep fed C3 and C4 forages in 2 sites

Forage	C3-H		C3-L		C4-H		C4-L			
Breed	B	T	B	T	B	T	B	T	s.e.m.	P
Total bacteria (*rrs* copy number/g DM ($\log_{10}$))										
France	11.64	11.64	11.46	11.41	11.49	11.47	11.26	11.17	0.089	<0.001 (Site), 0.019 (Forage)
West Indies	11.51	11.59	11.61	11.80	11.71	11.74	11.62	11.65		0.001 (Site*Forage)
Fibrobacter succinogenes (*rrs* copy number/g DM ($\log_{10}$))										
France	9.67	9.78	9.57	9.59	9.60	9.54	9.42	9.30	0.143	<0.001 (Site)
West Indies	9.06	9.00	9.23	9.41	9.13	9.27	9.10	9.03		
Ruminococcus albus (*rrs* copy number/g DM ($\log_{10}$))										
France	8.76	8.76	7.19	8.43	8.33	7.81	7.86	7.67	0.302	0.009 (Site*Forage)
West Indies	7.57	7.62	8.07	8.48	8.45	8.47	8.00	8.17		
Total methanogens (mcrA copy number/g DM ($\log_{10}$))										
France	8.92	8.93	8.91	8.80	8.83	8.89	8.71	8.65	0.072	<0.001 (Site), 0.013 (Forage)
West Indies	8.93	9.22	9.20	9.31	9.24	9.26	9.23	9.09		0.004 (Site* Forage)
Total protozoa ($\log_{10}$ cells / ml)										
France	6.21	6.26	6.36	6.36	6.12	6.22	6.16	5.96	0.10	0.008 (Site* Forage)
West Indies	6.94	5.45	6.13	6.31	6.15	6.33	6.34	6.45		

Conclusions Methanogens along with total bacterial numbers were higher in sheep raised in the tropical environment. However, this factor does not seem to concur with actual methane emissions from the same animals that were more affected by other factors such as forage type and quality.

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Climate change (greenhouse gas emissions) and other environmental impacts of dairy systems based either on maize silage and grass or on grass only

T T H Nguyen[1,2], M Doreau[1], M S Corson[2], M Eugène[1], L Delaby[3], G Chesneau[4], Y Gallard[5], H M G van der Werf[2]
[1]INRA, Saint-Genès Champanelle, France, [2]INRA, Rennes, France, [3]INRA, Saint-Gilles, France, [4]Valorex, Combourtillé, France, [5]INRA, Exmes, France *Email:michel.doreau@clermont.inra.fr*

Introduction There is a large variability in dairy systems in temperate countries, depending on animal management strategies and farmer goals. This results in differences in environmental impacts, for which greenhouse gas (GHG) emissions are a major issue. Feeding strategies, especially the nature of forages, may influence impacts. We compared systems based either on maize silage and grass (MS) or on grass alone (G). Life cycle assessments (LCAs) were performed to evaluate the environmental impacts climate change (due to GHG emissions), eutrophication and land occupation.

Material and methods Two dairy systems, composed of a dairy sub-system plus a cash-crop sub-system, with the same on-farm area (55 ha) and milk quota were compared. The MS dairy sub-system had 33% of silage maize in the forage area with highly productive cows (8.7 t fat and protein-corrected milk (FPCM)/cow/yr), whereas the G dairy sub-system was based on grassland only (i.e. no silage maize in the forage area) with cows producing 6.7 t FPCM/yr. More details about system description are given in Nguyen *et al.* (2013). The LCAs of these dairy-production systems were conducted from cradle-to-farm gate for a one-year period, i.e. including the production and delivery of inputs used for grassland and cereals produced on-farm and for feed produced off-farm, herd management and associated upstream processes, emissions from the animals and manure storage. Environmental burdens from the application of manure to cereal crops and pasture were included, as were those from buildings. Veterinary medicines were excluded due to lack of data. Allocation based on the protein contents of commercialised milk and beef was used to attribute the impacts to milk and meat co-products of dairy sub-systems. The impacts, i.e. climate change (GHG emissions with the effects of land use and land-use change (LULUC)), eutrophication and land occupation, were expressed per 1 kg FPCM.

Results GHG emissions per kg FPCM of G was higher than that of the MS dairy farm by 12% (Table 1). The highest contribution to GHG emissions per kg of FPCM was enteric fermentation (43 and 50% for MS and G, respectively), followed by feed production (40 and 48% for MS and G, respectively, including grassland, maize silage and concentrate feed) (Figure 1). N_2O emission from feed production per kg FPCM of G was higher than that of MS by 56% (Table 1). CO_2 emission due to LULUC per kg FPCM of G was lower than that of MS by 18% because C sequestration was higher and CO_2 emissions related to deforestation due to soybean meal were lower in G than in MS. There was no significant difference per kg FPCM of G and MS for eutrophication, but land occupation of G was higher than that of MS by 36%. Differences in impacts of milk of MS and G can be explained by (1) G having a lower milk yield/cow (more cows were needed to produce the same milk quota, even though it produced more meat), (2) cows in G having first calving at age 3 whereas MS cows calved at age 2, and (3) grass requiring more N fertiliser, but producing lower yields than silage maize, even though grassland sequestered C in the soil.

Table 1 Environmental impacts per kg FPCM of dairy systems based on maize silage and grass (MS) or on grassland only (G)

Emissions/impacts	MS	G
GHG emissions (kg CO_2 eq.)	1.28	1.44
CH_4 (kg CO_2 eq.)	0.69	0.72
N_2O (kg CO_2 eq.)	0.37	0.58
CO_2 (kg CO_2 eq.)	0.17	0.20
CO_2 LULUC (kg CO_2 eq.)	0.05	-0.06
Eutrophication (g PO_4^{3-} eq.)	4.98	4.92
Land occupation (m^2*yr)	1.17	1.59

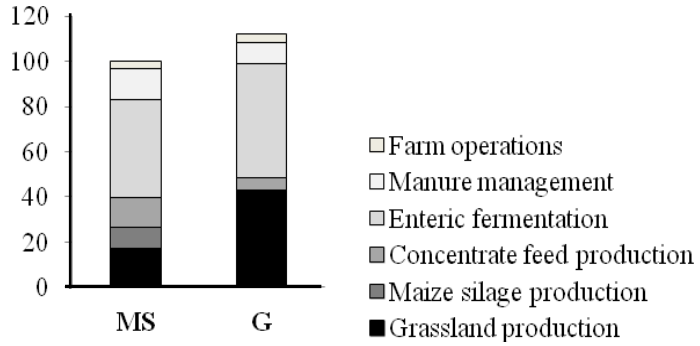

Figure 1 Contribution (in %) of main processes to GHG emissions per kg FPCM of dairy systems based on maize silage and grass (MS) or on grassland only (G) (MS represents 100%, G is relative to MS)

Conclusions Differences in impacts of milk of MS and G dairy systems were due not only to ration (maize silage and grass vs. grass only) but also to whole-system management (animal, feed and cash-crop production). The large differences in land occupation of systems producing MS and G milk could influence land-use change and GHG emissions if demand for grass-based milk increased.

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Methane emissions and diet digestibility for sheep offered diets varying in nutritive value and fat content

E J McGeough[1], Y H Chung[1], K A Beauchemin[1], S M McGinn[1], O M Harstad[2], T A McAllister[1]
[1]Agriculture and Agri-Food Canada, Lethbridge, AB, Canada, [2]Norwegian University of Life Sciences, Ås, Norway
Email:Emma.Mcgeough@ad.umanitoba.ca

Introduction It is well established that methane (CH_4) arising from anaerobic fermentation of feed in the gastrointestinal tract of ruminants is a significant contributor to agriculture's greenhouse gas footprint. Fat supplementation and altering the nutritive value of the diet potentially offer mitigation benefits; however, there is a paucity of studies that assess these approaches simultaneously. Thus, the objective of this study was to determine diet digestibility and CH_4 emissions of sheep offered diets varying in nutritive value and fat content.

Materials and methods This study was conducted as a replicated (4 square) Latin Square design with 4 treatments. Each period consisted of 17 d adaptation followed by 4 d of simultaneous diet digestibility and CH_4 measurement. Twenty four Canadian Arcott sheep (16 male and 8 female), initial BW 60.4 (s.d. 4.80) kg, were assigned to 1 of 4 treatments based on differing nutritive value and fat inclusion. Within treatment, sheep were assigned to 1 of 2 groups based on liveweight and sex to facilitate staggered measurements. During adaptation, animals were offered ad libitum access to feed, with intake during CH_4 and diet digestibility measurement restricted to 0.90 of ad libitum based on the previous 5 d intake. The 4 diets were: 1) low fat, low quality, 2) low fat, high quality, 3) high fat, low quality and 4) high fat, high quality. For the low quality diets, oat hulls were the predominant basal ingredient (400 g/kg dry matter [DM]), with beet pulp the predominant ingredient for the high quality diets (500 g/kg DM). The dietary neutral detergent fibre (NDF) content for the high and low quality diets were formulated to be similar (~ 320 g/kg DM), with differences in quality achieved through the known differences in NDF digestibility of the basal feed ingredients. The low fat diets were formulated to contain 21.5 g fat/kg DM, and the high fat diets formulated to contain 60.0 g/kg DM. Fat levels in the diet were altered using oilseed ingredients sourced from canola (*Brassica napus* L.), including meals and oil. Production of CH_4 was measured using 4 climate-controlled, open circuit chambers according to Beauchemin and McGinn (2005). Each chamber housed 3 sheep, accommodated individually in metabolism crates. Apparent total tract digestibility was determined for Group 1 during chamber measurements by collecting the total output of feces and urine from each sheep for 4 d. All data were analysed using the MIXED procedure of SAS (SAS Inst., 1999). For DM intake (DMI) and diet digestibility data, animal was considered the experimental unit, with chamber the experimental unit for the CH_4 data as a single value was generated for each chamber. Sampling day was considered a repeated effect in all models.

Results Improving the nutritive value of sheep diets reduced ($P < 0.001$) DMI, but DMI was not affected by fat content (Table 1). As intended, diet digestibility was higher ($P < 0.001$) for the diets where beet pulp was the basal ingredient. Increasing the fat content of the low quality diet resulted in higher NDF digestibility ($P < 0.05$) for the low quality diet; however, this effect was not observed for the high quality diet. Improving diet quality increased ($P < 0.01$) CH_4 emissions when expressed relative to intake. Increasing fat content increased ($P < 0.05$) CH_4 emissions when expressed relative to intake. A fat by quality interaction ($P < 0.05$) was observed for CH_4 per kilogram of NDF digested.

Table 1 DMI, diet digestibility and CH_4 emissions of sheep offered diets varying in nutritive value and fat content

	Low Quality		High Quality			P value		
	Low fat	High fat	Low fat	High fat	s.e.m.	Quality	Fat	Quality × Fat
DM intake, kg/d	2.5	2.4	2.0	2.1	0.12	< 0.001	ns	ns
DM digestibility, %	60.6	61.8	72.6	73.1	0.85	< 0.001	ns	ns
NDF digestibility, %	23.6	29.8	55.8	57.6	2.09	< 0.001	0.013	ns
CH_4, g/kg DM intake	13.8	14.7	15.8	17.1	0.61	0.003	0.047	ns
CH_4, g/kg NDF intake	40.2	43.2	50.1	55.7	1.93	< 0.001	0.025	ns
CH_4, % GE[1] intake	4.2	4.3	4.9	5.2	0.20	0.003	ns	ns
CH_4, g/kg DMD[2]	19.8	19.4	19.8	21.5	1.32	ns	ns	ns
CH_4, g/kg NDFD[3]	139.3[a]	111.9[b]	81.2[c]	88.9[c]	4.76	< 0.001	0.018	0.003

[1] GE = gross energy [2] DMD = DM digested, [3] NDFD = NDF digested. ns = non-significant $P > 0.05$

Conclusions When expressed relative to intake, improving diet quality increased CH_4 emissions, with such improvements in diet quality expected to improve animal performance. Increased diet quality may be an effective means of reducing emissions per unit of animal product, but can result in a greater release of CH_4 into the atmosphere. For low quality diets, increasing dietary fat content reduced CH_4 emissions adjusted for NDF digested; however, this response was not observed when a high quality diet was offered thus limiting the potential of fat supplementation as a means of enteric CH_4 mitigation.

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The effects of different Propionibacterium strains on ruminal fermentation, nutrient digestibility and methane emissions in beef heifers fed a high forage diet

D Vyas[1], E J McGeough[1], S M McGinn[1], T A McAllister[1], A H Smith[2], K A Beauchemin[1]
[1]Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada, [2]Dupont Nutrition and Health, Waukesha, Wisconsin, USA *Email:diwakarvyas@gmail.com*

Introduction Previous studies have underscored the importance of increasing molar proportion of ruminal propionate in mitigating enteric methane (CH_4) emissions from ruminants (McGinn *et al*., 2004). The selection of *Propionibacterium* strains for dietary supplementation may offer an effective means of increasing propionate production in ruminants fed forage based diets. This approach would not only decrease enteric CH_4 production, which is beneficial to the environment, but may also increase feed efficiency as propionate is the only major gluconeogenic volatile fatty acid (VFA) in ruminants (Bergman, 1990).

Material and Methods Twenty ruminally cannulated beef heifers were used in a randomized block design with 28-d periods. The heifers were blocked in 5 groups on the basis of body weight (BW). Dietary treatments included 1) Control (10 g/head/d maltodextrin), 2) *Propionibacterium* strain P169 (10 g/head/d maltodextrin containing P169 at 5×10^9 CFU), 3) *Propionibacterium* strain P5 (10 g/head/d maltodextrin containing P5 at 5×10^9 CFU), and 4) *Propionibacterium* strain P54 (10 g/head/d maltodextrin containing P54 at 5×10^9 CFU), and were randomly allotted to each group. Each treatment was dosed into the rumen in a gel capsule. All heifers were fed the basal diet (70:30 forage to concentrate, dry matter [DM] basis) formulated to provide adequate metabolizable energy and protein for a 400 kg animal gaining 2 kg/d (NRC, 2000). During each experimental period, d 1 to 17 were used to adapt heifers to their treatments. Rumen contents were collected on d 15 and d 18, ruminal pH was measured continuously from d 15 to 22, enteric CH_4 was measured from d 19 to 22 and diet digestibility was measured from d 25 to 28. Methane emissions were measured from individual heifers housed in stalls within 4 environmental chambers. Following CH_4 measurement, feed intake was restricted to 0.95 of ad libitum consumption and total fecal collection was employed for determining total tract digestibility.

Results No significant treatment effects were observed for ad libitum dry matter intake (DMI; $P = 0.81$) or DMI in the chambers ($P = 0.27$; Table 1). Similarly, no treatment effects were observed for the average BW ($P = 0.43$) or the final BW ($P = 0.38$). Mean ruminal pH averaged 6.47 and was not affected by treatments ($P = 0.37$). In addition, no treatment differences were observed for other ruminal pH variables, including minimum pH and pH range. However, maximum pH was numerically lower with P5 ($P = 0.17$). No significant treatment differences were observed for total VFA, ammonia-N (NH_3) or molar proportion of individual VFA including acetate, propionate, butyrate, valerate and caproate ($P > 0.10$). However, the molar proportion of isobutyrate was reduced ($P < 0.03$) by P5 and P54. Total enteric CH_4 production (g/d) was not affected by *Propionibacterium* strains (P169, P5, and P54) as compared to control and averaged 178 g/d ($P = 0.67$). However, enteric CH_4 emission intensity, indicated as ratio of grams of CH_4/kg of DMI, was reduced ($P = 0.02$) by 12, 8 and 13% with P169, P5 and P54 respectively. A similar effect was observed for CH_4 emission expressed on the basis of neutral detergent fiber (NDF) intake. No major treatment effects were observed for total tract digestibility of nutrients.

Table 1 Enteric methane emissions from beef heifers fed high a forage diet with control, P169, P54 and P5 treatments

Variable	Control	P169	P5	P54	s.e.m.	P
Overall DMI, kg/d	9.15	9.59	9.59	9.35	0.44	0.81
Chamber DMI, kg/d	5.75	7.48	6.90	6.84	0.60	0.27
CH_4, g/animal per day	167	190	181	172	14.7	0.66
CH_4, g/kg of DM intake	29.1[a]	25.6[b]	26.6[b]	25.3[b]	0.90	0.02
CH_4, g/kg of NDF intake	79.7[a]	69.3[b]	71.9[b]	69.2[b]	2.32	0.02
Total VFA, m*M*	121	116	129	124	4.93	0.35
Acetate, mol/100 mol	62.8	62.1	62.4	62.9	0.69	0.78
Propionate, mol/100 mol	20.3	21.3	21.0	20.4	0.76	0.72
Butyrate, mol/100 mol	11.1	11.1	11.2	10.9	0.44	0.99

[a,b] Values within a row with different letters differ ($P \leq 0.05$).

Conclusions *Propionibacterium* strains P169, P54 and P5 had no major effects on DMI, ruminal pH, VFA profiles or nutrient digestibility. However, the strains were effective in reducing enteric CH_4 production adjusted for intake of DM and NDF. With no changes in molar proportion of propionate, the reduction in CH_4 emissions might be explained by the tendency for increased intake with all *Propionibacterium* strains thereby reducing the residence time of feed in the rumen and subsequently CH_4 production per unit of feed intake.

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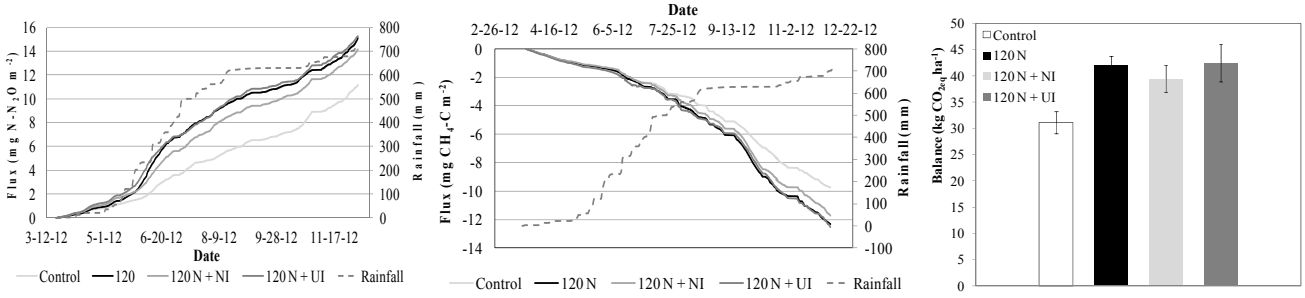
Can carbon sequestration account for nitrous oxide emissions in a volcanic ash soil?

M A Alfaro[1], S Hube[1], P Grace[2], C Scheer[2], C Brunk[2], D Rowlings[2]
[1]INIA Remehue, Osorno, Los Lagos, Chile, [2]Queensland University of Technology, Brisbane, Australia
Email:malfaro@inia.cl

Introduction Carbon (C) sequestration in agricultural soils represents a mitigation option to reduce greenhouse gas (GHG) emissions from this activity. Volcanic ash soils from southern Chile have high organic matter concentration (14-25%), so that C sequestration in these soils is considered negligible, especially in areas dedicated to livestock production. The objective of this work was to evaluate the mitigation of nitrous oxide emissions in a volcanic ash soil through enhancing C sequestration.

Material and methods To determine GHG emissions, oat (*Avena sativa* cv. Nehuén) was seeded on a volcanic ash soil (serie Osorno, Typic Hapludands) located at INIA Remehue (40° S, 73° O). In this andisoil, the crop was seeded (20th of March 2012) considering four treatments: 0 kg N ha^{-1}, 120 kg N ha^{-1}, 120 kg N ha^{-1} + nitrification inhibitor (DCD, NI) and 120 kg N ha^{-1} + urease inhibitor (Agrotain, UI). All treatments received at seeding a basal nutrient application consisting in 150 kg P_2O_5 ha^{-1} (TSP, 46% P_2O_5), 150 kg K_2O ha^{-1} (KCl, 62% K_2O), 40 kg S ha^{-1} ($CaSO_4$, 18% SO_4) and 40 kg Mg ha^{-1} (MgO, 85% Mg). Nitrogen was applied in two splits with an initial application at seeding equivalent to 30 kg N ha^{-1} (urea, 46% N), and the remaining 90 kg ha^{-1} and the respective N inhibitors were applied 45 days after seeding (urea, 46% N). Treatments were organized on a randomized block design with 3 replicates, in 4x3 m^2 plots. The plot was divided long wise in two, so that one half was used for destructive sampling (soil moisture, soil available nitrogen, crop harvest) and the other half was used for GHG quantification. Greenhouse gas emissions were measured using an automated chamber system (Breuer *et al*., 2000; Kiese *et al*., 2003). One chamber per plot was used for GHG measurements, although two frames were placed within each plot so that the chamber was moved between both positions every 2 weeks during the cropping season. Each gas determination cycle run for three hours for N_2O and CH_4 determinations, while accumulated emissions were measured over a 45 min period. Integration of concentrations was carried out in relation to a standard gas, used every four samples. Because CO_2 captured by plants is considered biogenic and would account for C sequestered in the upper soil layer (IPCC, 2006), to estimate soil C balance only N-N_2O and C-CH_4 emissions were considered. Emissions of CH_4 may include CO_2 coming from heterotrophic respiration in soils. Values measured were transformed to CO_{2eq} using the global warming potential estimated for these gases (IPCC, 2006). GHG emissions and C balance results were analyzed by ANOVA using Genstat 12.0 as statistical package.

Results GHG emissions were low varying between 0.10-0.16 kg N-N_2O ha^{-1} kg N_2O-N ha^{-1} and -0.08 and -0.16 kg CH_4-C ha^{-1}. Nitrogen fertilized treatments had greater emissions than the control treatment (P<0.01), with no reduction in emissions because of N cycle inhibitors addition (P>0.05), in agreement with previous results (Vistoso *et al*., 2012). Increases in N-N_2O emissions were associated to rainfall events, increasing over winter months (June-July) (fig 1). There was no difference among treatments for C sequestration, thus, results suggest that the andisoil can sequestrate C as CH_4 over a cropping period, and that the amount sequestered is related to the soil and no to fertilizer management (P>0.05). Carbon sequestration could not account for the total of N-N_2O emissions, so that overall C balance was positive in all treatments, and higher in response to N addition (P<0.05) (fig 1).



(a) (b) (c)

Figure 1 (a) Cumulative N_2O fluxes (mg N_2O-N m^{-2}) and rainfall (mm); (b) Cumulative CH_4 fluxes (mg CH_4-C m^{-2}) and rainfall (mm); (c) C balance (kg CO_{2eq} ha^{-1}) per treatment.

Conclusions Volcanic soils can sequestrate C as CH_4, although this capacity cannot account for the total N_2O emissions in areas receiving N fertilizer. Nitrogen cycle inhibitors do not reduce emissions as N_2O in these soils.

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Additive effect of nitrate and cashew nut shell liquid in an encapsulated product fed to lambs on enteric methane emission and growth performance

H M El-Zaiat[1,2], H O Patiño[1], Y A Soltan[2,3], A S Morsy[3,4], R C Araujo[5], H Louvandini[3], A L Abdalla[3]
[1]Animal Science Department, Faculty of Agronomy, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil, [2]Animal Production Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt, [3]Laboratory of Animal Nutrition, Centre for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, São Paulo, Brazil, [4]Animal Production Research Institute, Dokki, Giza, Egypt, [5]GRASP Ind. e Com. LTDA, Curitiba, Paraná, Brazil
Email:abdalla@cena.usp.br

Introduction Research data have recently confirmed the potential of nitrate salts as an enteric methane mitigation option. In the rumen, some microbes effectively reduce nitrate to nitrite and then to ammonia, thereby sinking hydrogen originally used in methanogenesis. Ammonia as a final product of nitrate reduction is a clear advantage, allowing its use as non-protein nitrogen (NPN) source. Scientific information is still lacking about the possibility of nitrate or nitrite accumulation in the meat of ruminants fed nitrate. Cashew nut shell liquid (CNSL) has also proved to effectively reduce methane production, having a mode of action different than nitrate. The aim of this study was to evaluate the effects of two slow-release encapsulated nitrate products as urea replacers on methane emission, growth, and nitrate/nitrite residues in the meat of lambs.

Material and methods Eighteen Santa Inês male lambs (27 ± 4.9 kg) were blocked by initial BW and age in three treatments: 1) Control: 1.5% urea in the dietary DM; 2) Nitrate: 4.51% encapsulated calcium nitrate product in the dietary DM; 3) Nitrate + CNSL: 4.51% encapsulated calcium nitrate product containing CNSL (2.96% in the product DM) as an attempt to further reduce methane. Both products contained 61.15% NO_3^- in the product DM. Lambs were kept indoors and fed *ad libitum*. Diets were isonitrogenous with 60:40 concentrate:forage (Tifton-85 hay) ratio. The experiment lasted for 92 days and it consisted of 28 days for adaptation and 64 days for data collection. Feed intake was recorded daily and animals were weighed every two weeks after 16-h fast. Methane production was measured in 2 consecutive days using six open-circuit respiration chambers as described by Abdalla *et al*. (2012) during the beginning, middle, and end of experimental period. Animals entered in chambers in a staggered manner according to blocks. At the end of experiment, animals were slaughtered and *Longissimus dorsi* samples were analyzed spectrophotometrically for sodium nitrate and sodium nitrite residues (Instituto Adolfo Lutz, 2005).

Results Final body weight (BW), dry matter intake (DMI), average daily gain (ADG), and feed efficiency were not affected ($P > 0.05$) by both encapsulated nitrate products. Methane production, expressed as L/kg DMI, was reduced ($P < 0.05$) by Nitrate and Nitrate + CNSL, emitting in average 32.3% less methane than Control. The Nitrate + CNSL did not result ($P > 0.05$) in additional reduction of methane production when compared to Nitrate. Residues of sodium nitrate in meat did not differ ($P > 0.05$) among treatments. The nitrate meat residues can be considered very low, contrasting for example to the average NO_3^- ingestion by humans which is approximately 40 to 100 mg/day (USEPA, 1987). Nitrite residues in meat were not detected.

Table 1 Effects of encapsulated nitrate products on growth, methane emission, and nitrate/nitrite residues in meat of lambs

Item	Treatments			s.e.m.	*P*		
	Control	Nitrate	Nitrate + CNSL		Treat.	Time	Treat. x Time
Initial BW, kg	26.75	27.25	27.17	-	-	-	-
Final BW, kg	37.80	36.83	37.03	0.882	0.80	< 0.01	0.17
DMI, g/d	1112	1029	1037	44.7	0.39	< 0.01	0.05
ADG, g	173	156	153	18.3	0.71	0.15	0.06
Feed efficiency	0.159	0.150	0.147	0.0144	0.84	0.97	0.21
CH_4, L/d	27.50[a]	18.27[b]	20.54[ab]	2.363	0.05	0.24	0.05
CH_4, L/kg DMI	28.57[a]	19.14[b]	19.53[b]	2.178	0.02	0.03	0.72
$NaNO_3$, mg/kg fresh meat	0.283	0.494	0.215	0.177	0.63	-	-
$NaNO_2$, mg/kg fresh meat	n.d	n.d	n.d.	n.d.	-	-	-

Conclusions Encapsulated nitrate products as a NPN source fed to growing lambs persistently reduced CH_4 production without affecting animal performance. The inclusion of CNSL in the product formulation showed no additional benefits on methane mitigation. Dietary nitrate inclusion did not result in nitrate or nitrite accumulation in lamb meat.

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Additive effect of nitrate and cashew nut shell liquid in an encapsulated product fed to lambs on ruminal and blood constituents

H M El-Zaiat[1,2], H O Patiño[1], Y A Soltan[2,3], A S Morsy[3,4], R C Araujo[5], H Louvandini[3], A V Pires[6], A L Abdalla[3]
[1]Animal Science Department, Faculty of Agronomy, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil, [2]Animal Production Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt, [3]Laboratory of Animal Nutrition, Centre for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, São Paulo, Brazil, [4]Animal Production Research Institute, Dokki, Giza, Egypt, [5]GRASP Ind. e Com. LTDA, Curitiba, Paraná, Brazil, [6]Department of Animal Science, College of Agriculture "Luiz de Queiroz", University of São Paulo, Piracicaba, São Paulo, Brazil *Email:abdalla@cena.usp.br*

Introduction Research data have recently confirmed the potential of nitrate salts as an enteric methane mitigation option. The reduction of nitrate to ammonia sinks hydrogen used for methanogenesis, at the same time changing the concentration rumen fermentation end-products. Sudden intake of nitrate by non-adapted animals may result in methaemoglobinemia, a disease characterized by oxidation of the ferric ion in haemoglobin, hampering its ability to transport oxygen. Cashew nut shell liquid (CNSL) has also proved to reduce gram-positive bacteria, thereby increasing propionate production and, consequently, mitigating methane. The aim of this study was to evaluate the effects of two slow-release encapsulated nitrate products as urea replacers on ruminal and blood constituents when fed to lambs.

Material and methods Eighteen Santa Inês male lambs (27 ± 4.9 kg) were blocked by initial BW and age in three treatments: 1) Control: 1.5% urea in the dietary DM; 2) Nitrate: 4.51% encapsulated calcium nitrate product in the dietary DM; 3) Nitrate + CNSL: 4.51% encapsulated calcium nitrate product containing CNSL (2.96% in the product DM). Both products contained 61.15% NO_3^- in the product DM. Lambs were kept indoors and fed *ad libitum*. Diets were isonitrogenous with 60:40 concentrate:forage (Tifton-85 hay) ratio. The experiment lasted for 92 days and it consisted of 28 days for adaptation and 64 days for data collection. Rumen fluid samples (30 mL) were collected every two weeks (4 collecting days during experimental period) using an oesophageal probe always 3 h after morning feeding. Rumen pH was measured immediately and short-chain fatty acids (SCFA) concentrations were determined by gas chromatography. Ruminal ammonia was determined by steam distillation and protozoa numbers were counted microscopically using Neubauer chambers. Blood samples were collected from the jugular vein every two weeks 6 h after morning feeding. Haemoglobin (Hb) was analysed using colorimetric kits, methahemoglobin (MetHb) determined within 30 min after sampling as described by Sato (2005), and red blood cells (RBC) were counted by light microscopy using a hemocytomer.

Results Rumen pH was not affected ($P > 0.05$) by treatments. Nitrate increased ($P < 0.01$) total SCFA and acetate concentrations, whereas Nitrate + CNSL additionally increased ($P < 0.01$) both variables compared with Nitrate. Nitrate + CNSL showed greater ($P < 0.05$) propionate and butyrate concentrations than Control, with Nitrate showing intermediary results. The C_2:C_3 ratio did not differ ($P > 0.05$) among treatments. A decreased acetate:propionate ratio is a common effect of CNSL in the rumen. The NH_3 concentration and protozoa counts were decreased ($P < 0.01$) for both Nitrate treatments. Blood Hb and MetHb did not differ ($P > 0.05$) among treatments, whereas both nitrate treatments showed greater ($P < 0.01$) RBC concentration than Control.

Table 1 Effects of encapsulated nitrate products on ruminal and blood constituents of lambs

Item	Treatments			s.e.m.	P		
	Control	Nitrate	Nitrate + CNSL		Treat.	Time	Treat. x Time
Rumen pH	6.76	6.78	6.74	0.063	0.92	< 0.01	0.36
Total SCFA, mM	90.5^{c}	99.5^{b}	110.1^{a}	2.09	< 0.01	0.18	0.62
Acetate, mM	48.9^{c}	55.2^{b}	64.0^{a}	1.31	< 0.01	< 0.01	0.43
Propionate, mM	14.8^{b}	16.0^{ab}	17.9^{a}	0.81	0.04	0.64	0.97
Butyrate, mM	9.3^{b}	11.3^{ab}	13.0^{a}	0.60	< 0.01	0.89	0.22
C_2:C_3	3.57	3.61	3.67	0.204	0.94	0.05	0.89
NH_3, mg/100 mL	34.9^{a}	26.4^{b}	22.3^{c}	0.28	< 0.01	0.10	0.39
Protozoa, x 10^5/mL	22.6^{a}	19.9^{b}	19.9^{b}	0.41	< 0.01	< 0.01	0.29
Blood Hb, g/100 mL	11.81	12.29	11.65	0.416	0.54	< 0.01	0.05
Blood MetHb, %	0.62	1.08	0.92	0.13	0.08	0.23	0.30
RBC, x 10^6/µL	10.36^{b}	12.75^{a}	12.01^{a}	0.246	< 0.01	< 0.01	0.71

Conclusions Encapsulated nitrate changed ruminal fermentation, increasing total SCFA concentration with no effects on C_2:C_3 ratio. The CNSL added to encapsulated nitrate further increased SCFA. More SCFA leads to the speculation of a greater microbial fermentation, but data must be considered carefully because samples were collected only 3 h after feeding. Blood methahemoglobin did not change with nitrate and its basal values indicated that animals were not at risk for methaemoglobinemia development.

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Methane production is not associated with sire groups or residual feed intake in feedlot Angus cattle

K L Weber, J A Rendon, C Antwi, A L Van Eenennaam, E Kebreab
University of California, Davis, Davis, CA, USA *Email:ekebreab@ucdavis.edu*

Introduction The relationship of greenhouse gas emissions with global warming has sparked immense interest in better understanding human activities which impact greenhouse gas production. Enteric methane (CH_4), one of the major GHG produced by ruminant livestock, is associated with feed intake; therefore, we might expect that low residual or net feed intake (RFI) animals, those which consume less feed for a given size and rate of gain, would produce less CH_4 than high RFI animals. This has been demonstrated in selected cattle lines (Hegarty *et al.,* 2007) and several populations of feedlot cattle (Okine *et al.*, 2003; Nkrumah *et al.*, 2006). The objective of this work was to determine whether CH_4 production is associated with sire RFI breeding values or progeny RFI phenotypes in feedlot Angus cattle.

Material and methods We selected two highly used Angus bulls (HIGH, LOW) whose breeding values for RFI have been predicted to differ by 0.32 kg/d based on the HD 50K MVP genetic test (Pfizer Animal Genetics, Kalamazoo, MI), released in 2010, to use as sires at the Sierra Foothill Research and Extension Center, Browns Valley, CA, USA. Of steer progeny produced, eight from each sire were selected based on body weight for participation in the trial. Between 10-11 months of age, steers were shipped to the feedlot unit of UC Davis. Following an adaptation period of 14 d, steers were individually housed to allow individual feed intake measurements for 70-105 d (mean 91 d), during which they were fed a finishing ration of predominantly corn, distillers' grain, alfalfa hay, and oat or rye grass hay, with 0.01% rumensin. Feed was provided either as a total mixed ration or a fully pelleted ration. During this period we measured CH_4 production using the GreenFeed^{TM} system (C-Lock Inc., Rapid City, SD), which uses a baiting system (either a pelleted feed or water) to entice animals into a head chamber from which emissions may be measured. The GreenFeed was provided to each steer for at least six 24h periods randomly selected from trial period, during which all feed consumed was from the GreenFeed. Actual measurement time per animal after quality control averaged 9 h. Methane production was analysed as a daily weighted average or in 10 min increments. Results were analysed using the GLM procedure of SAS.

Results Sire effect (-0.81 ±0.32 kg DM/d) was found to be significant ($P<0.05$) for dry matter intake adjusted for metabolic mid-test body weight and average daily gain (Table 1). Methane production (g/d) was associated with dry matter intake on the day of measurement ($P<0.0001$), and individual methane flux measurements (<10 min each) were associated with time since feeding ($P<0.0001$). However, average CH_4 production (g/d) alone and adjusted for dry matter intake and/or body weight were not associated with sire ($P>0.05$), average dry matter intake (kg/d), or estimated residual feed intake (kg/d) (Table 2).

Table 1 Comparison of feed intake, residual feed intake and enteric methane production in two sires of Angus beef cattle (Mean ± s.e.)

	Sires		
	HIGH	LOW	P
DMI, kg/d	8.51±0.28	8.38±0.33	0.977
RFI, kg/d	0.26±0.18	-0.30±0.18	0.027
CH_4, g/d	157±15	166±12	0.663
CH_4, g/kg of DMI	36±8	31±5	0.618
CH_4, g/kg of BW	0.336±0.031	0.345±0.022	0.826

Table 2 Correlations between feed intake, metabolic body weight, residual feed intake, and enteric methane production.

	DMI, kg/d	MBW, $kg^{0.75}$	RFI, kg/d	CH_4, g/d
DMI, kg/d	1.0			
MBW, $kg^{0.75}$	0.65	1.0		
RFI, kg/d	0.72	0.20	1.0	
CH_4, g/d	0.35	0.47	0.03	1.0

Conclusions. In this study, no statistically significant association was found between CH_4 and sire genomic breeding value for either RFI or progeny RFI phenotype. However, it should be acknowledged that this study was small in scope, in number of sire groups and number of progeny measured per sire. It is advised that larger scale studies be performed in the future.

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Effect of bromochloromethane and fumarate on microbial community structure of acetogenic bacteria in the bovine rumen

M Mitsumori[1], H Matsui[2], K Tajima[1], T Shinkai[1], A Takenaka[1], S E Denman[3], C S McSweeney[3]
[1]National Institute of Livestock and Grassland Science, Tsukuba, Ibaraki, Japan, [2]Mie University, Tsu, Mie, Japan, [3]CSIRO, St Lucia, Qld, Australia *Email:mitumori@affrc.go.jp*

Introduction Acetate-producing bacteria (acetogenes) employ the reductive acetogenesis metabolic pathway for consuming hydrogen and are present in anaerobic environments including the rumen. The diversity of acetogens has been confirmed by using formyltetrahydrofolate synthetase (FTHFS) gene (*fhs*) as a functional marker, which encodes a key enzyme of the reductive acetogenesis pathway. FTHFS sequences obtained from the rumen indicate that many of the ruminal acetogens are composed of unknown and uncultured species. But there is very little known about the dynamics of homoacetogens in the rumen. In this study, changes of homoacetogenic community in the bovine rumen, in which methanogenesis was inhibited by an antimethanogenic compound, bromochloromethane (BCM) or microbial populations were stimulated with the substrate fumarate, were analysed by using nucleotide sequences of the *fhs* genes.

Material and methods Rumen contents from 4 Holstein steers (mean bodyweight 572 kg) treated with BCM complexed in cyclodextrin (CD; BCM-CD) or fumarate (Itabashi *et al*. 1999; Bayaru *et al*. 2001), were kindly provided by Dr. H. Itabashi. DNA extraction and construction of clone libraries for *fhs* genes were carried out according to previously described methods (Matsui *et al*. 2008). Nucleotide sequences of *fhs* genes obtained from each library were deposited in the DNA Data Bank of Japan database under accession numbers AB085236-AB085320, AB085321-AB085403, AB085404-AB085488, and AB085489-AB085579, respectively. A phylogenetic tree was generated from the nucleotide sequence alignment of the *fhs* genes by CLUSTALW. The nucleotide sequences of the *fhs* genes were also analyzed by SOM as described previously (Mitsumori *et al*. 2010). An open source software package QIIME was used to compute the weighted UniFrac metric and for beta-diversity principal component analysis (PCA). The 40 residues used to calculate the HS scores for FTHFS sequences were extracted from the alignment file using a lane mask and calculated using the method described by Henderson *et al*. (2010). Sequences showing high HS scores (≥80%) were assumed as possible homoacetogens.

Results Phylogenetic tree analysis indicated that most of the *fhs* sequences categorized into homoacetogens were divided into clusters (A to I), which were in close agreement with a result shown in a self-organizing map (SOM). These analyses suggested that BCM affected diversity of actogens in the bovine rumen. The PCA also showed that addition of BCM to the rumen altered the population structure of acetogenic bacteria significantly but the effect of fumarate was comparatively minor.

Table 1 Composition of clusters presumed to be homoacetogens

	Total number[a]	Homoacetogens[b]	Cluster									Significant difference[d]	*H'*
			A[c]	B	C	D	E	F	G	H	I		
BC	85	49	21	2	5	0	0	1	1	2	0	a	1.63
BT	83	52	9	0	2	8	7	4	0	5	3	b	2.66
FC	85	46	19	7	2	1	0	1	4	0	0	a	1.84
FT	91	50	22	4	3	1	1	2	3	0	1	a	2.03

BC, BCM-untreated group; BT, BCM-treated group; FC, fumarate-untreated group; FT, fumarate-treated group. *H'* means the Shannon-Wiener diversity index. [a]Number of *fhs* gene sequences in each group. [b]Number of sequences presumed to be homoacetogens.[c]Number of *fhs* gene sequences in each cluster. [d]Cluster compositions in each group were compared by Mann-Whitney U-Test. Different letters are significantly different ($P < 0.05$).

Conclusions The community structure of the acetogen population in the bovine rumen was influenced by BCM, while not being significantly affected by fumarate. Because the clusters showed by the SOM analysis corresponded to the clusters on the phylogenetic tree, this study reveals that the SOM analysis is useful for clustering gene sequences. Further study is required to clarify roles of acetogens in the rumen by both molecular and culture-based investigations.

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Low ambient temperature elevates plasma Triiodothyronine concentrations while reducing digesta mean retention time and methane yield in sheep

M C Barnett[1,2], J R McFarlane[1], I R Godwin[1], J V Nolan[1], R S Hegarty[1]
[1]University of New England, Armidale, NSW, Australia, [2]CRC for Sheep Industry Innovation and the University of New England, Armidale, NSW, Australia *Email:mbarnet2@une.edu.au*

Introduction Developing processes which reduce enteric methane production without compromising animal productivity has become critical for sustainable livestock production. Mean retention time (MRT) of rumen contents positively correlates with the methane yield (MY) of rumen fermentation (Pinares-Patiño *et al* 2003; Barnett *et al*. 2012). There is an increasing understanding of the role hormonal and neural systems plays in the control of digesta kinetics in ruminants (Onaga *et al*. 2011). Significant correlations have been observed between plasma concentrations of the endocrine regulator, Triiodothyronine (T_3), within physiological levels, and the MRT of digesta in ruminants (Barnett *et al*. 2012). Cold ambient temperature in known to increase plasma concentrations of T_3 (Todini 2007) but ambient temperature's effect on methane yield is less certain. It is hypothesised that exposing sheep to low ambient temperatures would increase plasma T_3 concentration and decrease MRT of digesta within the rumen resulting in a reduction of MY.

Material and methods A cross-over study was undertaken to determine the effect of ambient temperature on MRT and MY. Six freshly-shorn mature Merino wethers were given sustained exposure to either 9±1°C (cold treatment; n=3) or 26±1°C (warm control; n=3) and fed a lucerne and wheaten chaff diet (ratio 50:50). The ration was offered continuously ($1/12^{th}$ every two hours) at 1.35 times maintenance by overhead feeding apparatuses. After two weeks adaption, methane yield for each animal was measured (twice), followed immediately by sampling of rumen fluid then a 6d collection of faecal and urine output, and another period of methane measurements. Blood samples (10mL) were taken from each sheep twice a week and stored at -20°C while digesta kinetics was measured by log extrapolation of faecal excretion concentration of dual markers administered (Aharoni *et al*. 1999). A two sample Student t-test was used to assess the effect of temperature with the Hills-Armitage approach applied to account for any period effect.

Results Animals exposed to cold ambient temperature experienced increased plasma T_3 levels, microbial protein outflow, and total ruminal VFA concentrations while total ruminal protozoa numbers decreased. Daily wool growth increased due to cold treatment by almost 30% as MY decreased. Mean retention times of digesta was found to be significantly reduced in both the rumen ($P<0.01$) and post-ruminally ($P<0.01$). Dry matter digestibility did not change.

Table 1 The effect of exposure to cold or warm ambient temperature (9±1°C v 26°±1C) on plasma T_3 concentrations, methane yield, microbial protein outflow, ruminal VFA concentrations, protozoa abundance, wool growth and digesta kinetic parameters of sheep fed every two hours (mean ± s.e.)

	Cold	Warm	s.e.
Free Triiodothyronine (pg/mL)	4.4^a	3.0^b	0.5
Total Triiodothyronine (ng/mL)	2.6^a	1.4^b	0.4
Methane Yield (g CH_4/kg DMI)	17.7^a	19.3^b	0.6
Microbial Protein Outflow (g/day)	8.6^a	7.6^b	0.3
Total VFA Concentration (mM/L)	96.6^a	85.0^b	4.7
Protozoa Abundance (count/mL) x 10^5	16.9^a	20.8^b	1.5
Wool Growth (mm/day)	286^a	221^b	26
Rumen MRT (h)	25.2^a	26.4^b	0.5
Hindgut MRT (h)	12.0^a	14.2^b	0.7

^{a,b} Means with different subscripts within a row are significantly different

Conclusion This study indicates that exposing sheep to cold ambient temperatures causes a physiological modification of digesta kinetics resulting in a reduction in digesta MRT and decreased MY. The cold exposure also elevated plasma T_3 concentrations, a known endocrine involved in MRT modification associated with metabolism. While it is unclear whether T_3 is a controlling hormone in digesta MRT, this experiment suggests T_3 has a strong influence in regulating MRT and, therefore, MY.

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Development of micrometeorological techniques for assessing nitrous oxide emission and mitigation from dairy pastures

M J Harvey[1], A M S McMillan[1], R J Martin[1], A M Bromley[1], M J Evans[1], J Laubach[2], S Mukherjee[3]
[1]NIWA, Wellington, New Zealand, [2]Landcare Research, Lincoln, New Zealand, [3]University of Canterbury, Christchurch, New Zealand *Email:mike.harvey@niwa.co.nz*

Introduction Just over half of the total New Zealand agricultural sector emissions of nitrous oxide ~32Gg(N_2O) are estimated to originate from urine nitrogen deposited in pastoral grazing systems. Verifying these emissions in situ at paddock (field) scale requires a careful approach, given the substantial spatial and temporal variability in emission. Following a series of field measurements, we have developed methodology recommendations for assessing emissions and emission reductions that are possible to achieve on farm through improved management practise and/or use of inhibitors. Micrometeorological techniques have the benefit of being non-intrusive and providing continuous assessment with spatial integration over length scales of hundreds of metres. Whilst chamber measurements are small scale and intrusive, their advantages include limiting emission to the defined area of cover. A combination of these two techniques has combined benefits.

Methods The flux-gradient technique has the advantage of presenting a single analyser with gas samples from a range of inlet locations and heights. The surface emission flux F_{N2O} is the product of eddy diffusivity k_g and the vertical gradient of N_2O ($F_g = k_g \, \partial C_g/\partial z$) and can be expressed as the product of a transfer coefficient C_{Tr} (mol_{Air} m^{-2} s^{-1}) which incorporates both the molar density of air and the stability-corrected diffusivity integrated over the height interval of interest; ΔN_2O, the N_2O difference (nmol/mol) over the vertical distance between two sample inlets ($z_2 - z_{1)}$, at ~ 1 and 0.5 m; and a units conversion constant 100.8 so that: F_{N2O} (µg N_2O-N m^{-2} h^{-1}) = 100.8 × C_{Tr} × ΔN_2O
Nitrous oxide measurements were made at 10Hz by tuneable diode laser TGA-100A (Campbell Scientific Inc) alternating between 2 sample inlets on a single measurement mast every 6 seconds as described by Wagner-Riddle *et al* (2005). We have found good agreement between sampling multiple heights into accumulators (Martin *et al*, 2011) and switching between 2 heights (Harvey *et al*, 2008). Up to 4 measurement mast sites have been sequentially sampled via a gas manifold in a 20 min cycle in order to compare side-by-side fluxes from up to four treatment plots.

Results The transfer coefficient C_{Tr} ranges up to 20 mol_{Air} m^{-2} s^{-1} and ΔN_2O up to 4 µmol/mol. The two are inversely related (high ΔN_2O at low C_{Tr} and vice versa). On pasture without recent grazing, emission is of order 1 – 10 µg N_2O-N m^{-2} h^{-1}. Measured fluxes can rise to ~ 250 µg N_2O-N m^{-2} h^{-1} in the week following grazing, and depending on conditions remain elevated for 3 – 4 weeks decaying further to background over several months. The minimum (significant) resolvable gradient (ΔN_2O) was estimated as 0.04 nmol/mol and corresponds to a significant resolvable flux of 16 µg N_2O-N m^{-2} h^{-1} at a single site and significantly resolvable flux difference of ~ 25 µg N_2O-N m^{-2} h^{-1} between two sites in an instantaneous comparison of consecutive 20 min periods. With repeated observations, small differences (18%) in cumulative N_2O flux were detectable after 23 days with modal flux of order of 27 µg N_2O-N m^{-2} h^{-1}. Comparison of micrometeorological fluxes and static chamber fluxes requires care. Good agreement can be achieved provided there is adequate spatial and temporal coverage, especially for capturing high emission episodes. Giltrap *et al* (2012) found that representative sampling of urine patches (requiring knowledge of their locations) was important for accuracy of spatial and temporal integration.

Conclusions/Recommendations Emissions from highly non-uniform urine patch distributions are difficult to assess accurately. Arising from the measurement series, some recommendations for continuous field-scale emission measurements have been developed to include: (1) assessment that the method has adequate resolution for expected fluxes (and flux differences in comparison experiments); (2) depending on objectives, measurements should be of sufficient duration for accurate integration of the emission envelope with measurement over full grazing cycles, and over the expected range of environmental and seasonal conditions; (3) supporting environmental information including irrigation, rain, soil moisture, temperature, soil physical properties, and knowledge of N inputs to allow for model application and development; (4) a flux footprint method to ensure that the measured flux originates from the defined treatment area; (5) assessing that micrometeorological relationships are valid- e.g. for flux-gradient method friction velocity is above threshold (~0.1 m s^{-1}), and boundary layer not highly stable, (i.e. excluding still clear nights where emission (gap filling) methods may be needed); (6) combined micrometeorological/chamber measurements to allow scaling assessment, where chamber estimates of total flux Ft are estimated from the urine patch emission chambers (Fu), the proportion of field area covered by urine (Pu) and the non-patch emission chambers (Fb), i.e. Ft = FuPu+Fb(1 - Pu) as recommended in deKlein and Harvey (2013) rather than random chamber placement.

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The effect of combining garlic oil with hydrogen sinks on methane production and in vitro ruminal fermentation

N J Choi[1], S Seo[2], D T Mbiriri[1], C H Ryu[1], C H Kim[3]
[1]Chonbuk National University, Jeonju, Jeonbuk, Republic of Korea, [2]Chungnam National University, Daejeon, Chungnam, Republic of Korea, [3]Hankyong National University, Ansung, Kyonggi, Republic of Korea *Email:nagjin@jbnu.ac.kr*

Introduction Commercial feed additive products are usually a combination of various compounds, yet generally less investigative studies on understanding possible synergistic, additive or antagonistic effects of combining compounds are done. Theoretically, combining an antimicrobial compound with a H_2 sink is expected to enhance their individual efficacy in the reduction of CH_4 emissions and also improve rumen function. Antimethanogens will affect the microbes that utilize H_2 in the conversion of CO_2 to methane. Adding a hydrogen sink will avail an optional metabolic pathway for the utilization of H_2 to the methanogenesis pathway. The objective of this experiment was to determine the effects of combining garlic oil, an antimicrobial agent, with fumarate or nitrate, which are rumen hydrogen sinks, on methane (CH_4) production and rumen fermentation parameters.

Materials and methods An *in vitro* study following the procedures of Tilley and Terry (1963) was carried out. Six treatments were formulated; control (no additive), GO (garlic oil at 300 mg/L), F (fumarate at 10 mM/L), N (nitrate at 24 µM/L), GOF (garlic oil (300 mg/L) + fumarate (10 mM/L)) and GON (garlic oil (300 mg/L) + nitrate (24 µM/L)). The sources of garlic oil, fumarate and nitrate were a commercial garlic oil blend, fumaric acid and sodium nitrate respectively. All the additives were dissolved in concentrated ethanol (99.5 %) to make stock solutions. Three serum bottles (250 mL capacity) containing 0.5 g of ground orchard grass (0.9 mm) were randomly assigned to each treatment. After the anaerobic addition of buffered strained rumen fluid, 100 µL of each treatment were anerobically transferred into their allocated bottles, sealed and incubated at 39°C for 24 h. The control serum bottles received 100 µL of ethanol. The following fermentative parameters were determined at the end of 24 h, total gas, pH, NH_3-N, dry matter (DM) digestibility, CH_4 and H_2.

Results

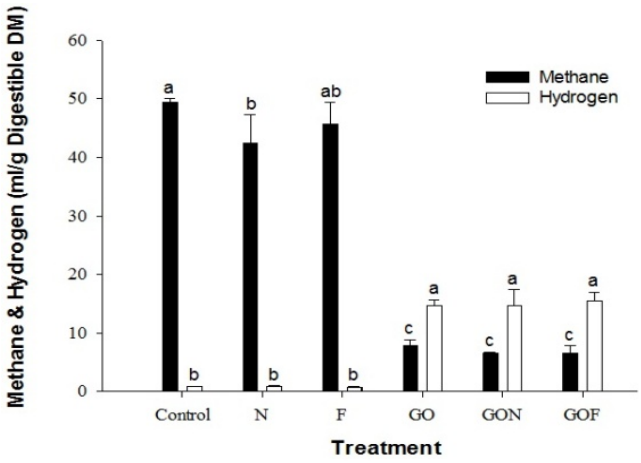

Figure 1 Effect of treatments on CH_4 and H_2 output

Garlic oil, either alone or in combination with fumarate or nitrate significantly reduced CH_4 production ($p<0.01$). When included alone, it reduced CH_4 output by 84 %, in comparison with the control. Nitrate also reduced CH_4 production when compared to the control, although its effect was lower than that of garlic oil. The significant reduction in CH_4 output was associated with higher H_2 recovery. Garlic oil suppressed total gas production. The addition of fumarate alone or in combination with GO, significantly increased propionate production. GO suppressed total VFA. Dry matter digestibility was not affected by the treatments.

Conclusions The combinations of sodium nitrate or fumaric acid at 24 µM/L and 10 mM/L respectively with 300 mg/L of garlic oil were the same as garlic oil alone in inhibiting methane production *in vitro*. These combinations were more effective than nitrate or fumarate in reducing CH_4 output. Garlic oil reduced acetate, iso-butyrate, iso-valerate and total VFA production.

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Effects of digested and non-digested cattle slurry on ammonia emissions and nitrogen field balance in spring barley

E Salomon, M Sundberg, L K K Rodhe
JTI - Institute of Agricultural and Environmental Engineering, Uppsala, Sweden *Email:eva.salomon@jti.se*

Introduction Anaerobic digestion of slurry is expected to increase in Sweden due to the benefits of using methane to replace fossil fuels and to reduce net emissions of greenhouse gases (GHG). An appropriate spreading strategy for the digestate can decrease ammonia (NH_3) emissions and create a nitrogen (N) yield effect, which can further reduce net GHG emissions. This study quantified the NH_3 emissions generated by spreading digested and non-digested cattle slurry before sowing spring barley and determined the proportion of applied N recovered in harvested grain and lost as NH_3 and nitrous oxide (N_2O).

Materials and Methods A field experiment was organised as a randomised complete block design with three blocks. The treatments were: 1) Control (C) with no N application. 2) Non-digested cattle slurry (CS) 25 tonnes ha^{-1}. 3) Digested cattle slurry (DCS) 25 tonnes ha^{-1}. 4) CS 25 tonnes ha^{-1} + chemical N fertiliser (CF). 5) DCS 25 tonnes ha^{-1} + CF. Treatments 4 and 5 were supplemented with N to meet the crop's N requirements. Cattle slurry and digestate were band-spread on a harrowed silt loam soil. Ammonia emissions were measured twice in CS and at 3 times in DCS in all three blocks during four hours before harrowing and sowing of spring barley. After sowing, one further measurement period of ammonia emissions was included. Ammonia emissions were measured with an equilibrium concentration method (Svensson, 1993), using two chambers and one ambient sampler unit randomly placed in each plot. For the time between two measuring periods, NH_3 emissions were interpolated (Malgeryd, 1998). Yield and grain N content were determined plot-wise and differences between treatments were analysed using ANOVA with GLM in Minitab. The N field balance for each treatment was calculated from Eq. (1). Data from measurements of N_2O emissions in this field experiment (Rodhe *et al*., 2013) were used in the N field balance calculations.

$$N_{\text{field balance}} = N_{\text{input}} - N_{\text{output}} = (N_{CS} + N_{DCS} + N_{CF}) - (N_{\text{grain}} + N_{NH3\text{-}N} + N_{N2O\text{-}N}) \quad \text{Eq. (1)}$$

Results Total NH_3 emissions were higher from DCS (13.0 kg N ha^{-1}) than CS (1.7 kg N ha^{-1}) probably due to higher pH (7.9 and 7.5, respectively). Ammonia emissions from DCS represented 33% of applied NH_4-N and those from CS 6% of applied NH_4-N. After sowing and harrowing, NH_3 emissions declined markedly in both treatments. Nitrogen emissions represented a considerable proportion of N output in DCS (Table 1), with NH_3 comprising the majority of N emissions. Due to long winter and dry summer conditions, the potential effect of applied N was not reached. The CS and DCS treatments had the same proportion of applied N recovered in grain and further supplementation with CF (treatments 4 and 5) decreased this proportion (Table 1).

Table 1 Summary of nitrogen inputs and outputs for the five treatments.

	C	CS	DCS	CS + CF	DCS + CF
N input (kg ha^{-1})	0	42.5	67.5	115.4	113.4
N output (kg ha^{-1})	21.7	27.5	53.5	42.5	57.3
N field balance (kg ha^{-1})	-21.7	15.0	14.0	72.9	56.1
Fraction of applied N recovered in grain (%)	-	60	60	35	39
Fraction of applied N lost as NH_3 and N_20 (%)	-	4	19	2	12

Conclusions Digested cattle slurry lost 33% of its NH_4-N content as NH_3 emissions during the four hours after spreading, while undigested cattle slurry lost 6%. This highlights the importance of direct incorporation of digestate into soil after spreading in order to minimise NH_3 emissions. Minimising NH_3 emissions is important for reducing indirect GHG emissions and for increasing the N fertiliser value of the digestate.

Acknowledgements The authors gratefully acknowledge funding from the Swedish Farmers' Foundation for Agricultural Research.

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454 Pyrosequencing reveals the difference in bacteria and archaea diversity between rabbit and dairy cattle

J K Wang, Z Y Sun, J X Liu
College of Animal Sciences, Zhejiang University, Hangzhou, China *Email:jiakunwang@zju.edu.cn*

Introduction Methane production is much less in monogastric herbivores than in ruminant (Franz *et al*., 2011). Pyrosequencing was applied in the current study to compare the bacteria and methanogen communities of dairy cattle with those of rabbits to investigate the possible mechanisms for the differences in methane production.

Material and methods Rumen contents were collected from six rumen fistulated Holstein dairy cattle during late lactation. Cecal contents were collected from five Japanese White Rabbits at age of three months. The samples were put into the anaerobic glycerol storage solution immediately with 100 µl Titanium III nitrilotriacetate. The glycerol stocks were kept on ice for transporting and then frozen at -80^{o}C for store. Microbial DNA from the glycerol stocks was extracted using the CTAB method (Gagen *et al*., 2010). Genomic DNA was purified by the QIAamp DNA Stool Mini Kit. Bacterial and archaeal 16S rRNA genomic sequences were amplified by PCR using a pair of combination tagged primers 519F/907R and 86F/486R, respectively (Wang and Qian, 2009). The amplification was purified by Magnetic silica particles. ROCHE 454 GS FLX Systems were used for bacterial and archaeal pyrosequencing. The quality criteria were employed using the trimseq script from the MOTHURA. After quality trimming, sequences shorter than 100 and 200 bp were not considered for bacteria and archaea analysis.

Results and discussion A total of 53993 bacterial reads passed the quality control, in which 31549 and 22444 were collected from dairy cattle and rabbit samples, respectively. A total of 4486 and 2868 OTUs were identified for cattle and rabbits. The OTU coverage was higher than 93 % (Table 1). *Fimicutes* accounted for 63.5 % of total bacteria in cecal samples, much higher than in rumen (Figure 1). A total of 85494 archaeal sequences passed the quality control, and the number of reads per sample ranged from 7033 to 8946 for dairy cattle and 6050 to 8662 for rabbits. A average of 3644 OTUs were identified in the rumen with a 67.9 % OTU Coverage. 1732 OTUs representing 84.2 % of the predicted total reads were identified in cecal samples (Table 1). At the phylum level, the archaeal community composition revealed different taxa between dairy cattle and rabbits (Figure 2). *Methanobrevibacter* was the most abundant archaea in the rumen contents of dairy cattle, while *Methanosphaera* was the most abundant in cecal contents of rabbits.

Table 1 Bacterial and archaeal diversity calculated by MOTHURA at 97% cutoff

	No of OTUs	Chao 1	Shannon	% OTU Coverage
Bacteria				
Dairy cattle	4486	8003	6.93	93.1
Rabbits	2868	5750	6.59	93.6
Archaea				
Dairy cattle1	3757	12723	7.30	66.3
Dairy cattle2	2813	7942	6.96	73.8
Dairy cattle3	3561	10827	7.24	69.6
Dairy cattle4	3273	9391	7.42	66.9
Dairy cattle5	4059	12326	7.45	66.6
Dairy cattle6	4402	12878	7.68	64.2
Rabbit1	1980	5345	5.19	84.3
Rabbit2	1362	3061	5.69	86.3
Rabbit3	1376	3445	5.72	86.1
Rabbit4	2062	5813	5.65	82.7
Rabbit5	1884	5204	5.42	81.5

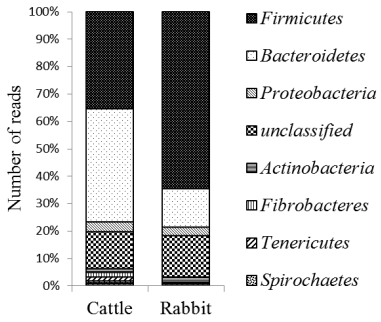

Figure 1 Abundance of bacterial groups at the phylum level

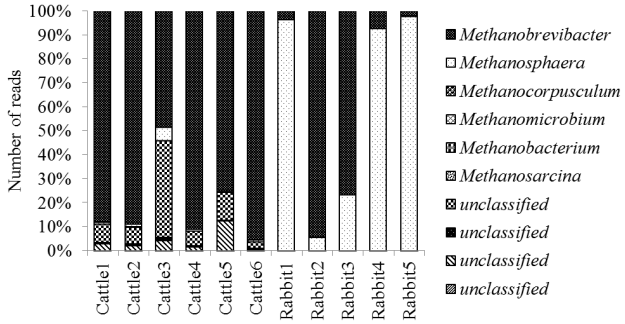

Figure 2 Abundance of archaeal groups at the phylum level

Conclusions *Fimicutes* and *Methanosphaera* are the main bacteria and archaea in the rabbit cecal contents, respectively. *Bacteroidetes* and *Fimicutes*, and *Methanobrevibacter* are the main bacteria and archaea in the rumen of dairy cattle, respectively.

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Quantification of enteric methane emissions from young Holstein heifers and steers fed grass silage diets

H P Jiao[1,2], T Yan[1], S J Morrison[1], C P Ferris[1], A F Carson[1], D A McDowell[2]
[1]Agri-Food and Biosciences Institute, Hillsborough, UK, [2]University of Ulster, Newtownabbey, UK
Email:haopeng.jiao@gmail.com

Introduction Livestock farming is a major contributor to atmospheric methane (CH_4) accumulation. Although CH_4 emission data for adult cattle have been reported extensively across the world, there is little information available regarding enteric CH_4 emissions from young cattle. The lack of such information can impact the accuracy of quantification of CH_4 emission for dairy and beef industries. The objective was to quantify enteric CH_4 emissions from young Holstein cattle managed under a typical UK feeding condition.

Material and methods Twenty Holstein cattle (10 steers vs. 10 heifers) at age of 5 months were selected for a 4-periods study (28 d/period) with measurements taken at age of 6, 12, 18 and 22 months, respectively. The animals were blocked into 10 pairs (steers vs. heifers) according to birth date, birth and weaning weights, growth rate and body condition score (BCS). In each period, they were housed in a cubicle accommodation for the first 20 days, and then transferred to metabolism units where they stayed for further 3 days. Afterwards, they were housed in indirect open-circuit respiration calorimeters chamber for 5 days with measurement of gaseous exchange (CH_4, CO_2 and O_2) over the final 96-h period. During each period, all cattle were allowed free access to water and offered a single diet for *ad libitum* intake once daily at 09.00 h. The diet offered was a typical mixed ration of grass silage and concentrates used on UK commercial farms. A single grass silage was used in all 4 periods with the silage offered during the final 8 d boxed in evacuated conditions at the beginning of each period to maintain its quality. The concentrates were based on barley, maize, sugar beet pulp and soybean meal. The accumulated CH_4 emissions (kg) from birth to 6 months were calculated from CH_4-E (CH_4 energy)/ME intake and ME intake from milk and milk replacers, and from silage and concentrates, from 6 to 24 months were estimated from measured CH_4-E/ME intake and ME intake from silage and concentrates. The ME intake was calculated from maintenance energy requirement measured in the present study and measured live weight gain using equations of AFRC (1993).

Results There was no significant difference between heifers and steers in any variable of animal performance, energy utilisation efficiency, or CH_4 emission data in any period. The average CH_4-E/GE intake obtained in the present study were 0.068, 0.067, 0.064 and 0.064 for cattle at age of 6, 12, 18 and 22 months, respectively, which were close to 0.065 of IPCC (2006), but lower than 0.070 of lactating dairy cows (Yan *et al*., 2010), and 0.080 of growing-finishing beef cattle (Yan *et al*., 2009). The statistical analysis of all data found that there were strong relationships ($P < 0.001$) between CH_4-E (MJ/d, y) and GE intake (MJ/d, x) ($y = 0.057\ x + 1.09$, $R^2 = 0.90$), and between CH_4 and DM intake (Fig. 1), and between accumulated CH_4 emission (kg, y) from birth to 12 months and LW (kg) ($y = 0.061\ LW_{6month} + 0.040\ LW_{12month} + 12.1$, $R^2 = 0.95$), and between accumulated CH_4 emission (kg, y) from 12 to 24 months and LW ($y = 0.039\ LW_{12month} + 0.058\ LW_{22month} + 18.1$, $R^2 = 0.75$). The average accumulated CH_4 emissions for all cattle from birth to 6, 12, 18 and 24 months were calculated to be 12.6, 36.2, 66.4 and 100.4 kg, respectively.

Table 1. Mean data on animal performace and methane output

	6 month	12 month	18 month	22 month
Age (days)	185	368	548	675
LW (kg)	175.5	320.3	493.4	570.2
LW gain (kg/d)	0.71	0.75	0.82	0.78
DM intake (kg/d)	4.0	6.7	7.2	8.2
Forage ratio (g/kg DM)	462	764	794	814
CH_4 (g/d)	93	159	175	190
CH_4 / DM intake (g/kg)	23.5	23.9	24.3	23.3
CH_4-E / GE intake	0.068	0.067	0.064	0.064

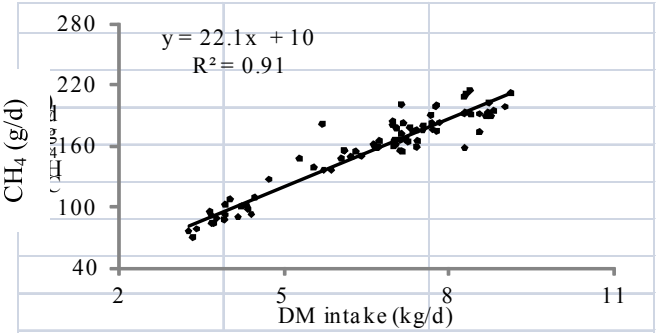

Figure 1. Relationship between CH_4 emission and DM intake

Conclusions There was no difference in any variable of CH_4 emissions between Holstein heifers and steers. However, there were strong relationships between CH_4 emissions and LW, GE intake and DM intake. These relationships can be used to predict CH_4 production from young cattle for development of CH_4 emission inventories for dairy and beef cattle industries.

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