Methane emissions from grazing cattle – can a micrometeorological technique detect a treatment difference?

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Introduction Many potential approaches for reducing methane (CH_4) emissions from cattle are at present under development. The promising ones will require verification of their efficacy at the production scale (e.g. paddock scale). We report an experiment to test whether a difference in CH_4 emissions between two grazing groups of cattle could be detected by 1) a micrometeorological technique using line-averaged concentrations up- and downwind of the cattle, and 2) by the sulfur hexafluoride (SF_6) tracer-ratio technique.

Material and Methods Two groups, of 30 one-year old Hereford x Friesian steers each, were formed with equal mean liveweight. In a flat uniform paddock composed predominantly of ryegrass (*Lolium perenne*), 32 rectangular strips were fenced, each 40 m by 25 m in size. Paired strips were allocated to the two groups on a daily basis, such that one group was always 65 m north of the other. First, for 6 days (Period 1) no treatment was applied, in order to test whether the emissions from the two groups were indistinguishable. For the following 10 days (Period 2), the grazing strip for the Northern group was sprayed with canola oil at a rate of 120 L ha^{-1}. This was expected to cause a reduction in CH_4 emissions, compared to the Southern group which did not receive any oil.
For 3 days in Period 1 and for 4 days in Period 2, individual CH_4 emissions over 24 h from all steers were measured with the SF_6 tracer-ratio technique (Johnson *et al.*, 1994). Group dry-matter intakes (DMI) were estimated from daily plate-meter measurements before and after grazing. Individuals' DMI were estimated in Period 2, based on faecal outputs and *in-vitro* feed digestibility. Faecal output was estimated using titanium dioxide (TiO_2) as external faecal marker (Pinares-Patiño *et al.*, 2008). CH_4 emission rates were tested for group effects with ANOVA.
Concentrations of CH_4 in air were measured along lines parallel to the W and E fences of each rectangle, as 20-minute averages. For this, air was drawn into 44 m long perforated alkythene pipes mounted 0.7 m above ground, and from each of these four intake pipes via a switching manifold into a CH_4 analyser (DLT-100, Los Gatos Research, Mountain View, California, USA). The collective CH_4 emissions from each group were computed from downwind-upwind concentration differences, using a backward-Lagrangian stochastic model (WindTrax software, www.thunderbeachscientific.com; Flesch *et al.*, 2004). Periods of unsuitable wind direction (outside ±40° of due W or due E), low wind speed or systematically turning direction were excluded from the analysis. The differences in CH_4 emissions between the groups, for each valid 20-min run, were the input data for statistical analysis with a linear mixed-effects model, using days as the random effect.

Results In Period 1 (no treatment), CH_4 emissions from the two groups did not differ, according to both the micrometeorological technique (Table 1) and the SF_6 technique (Table 2). The groups' DMI did not differ, either.
By contrast, in Period 2 both techniques showed significant differences ($P < 0.01$) for the CH_4 emissions. Mean emissions from the oil-treated group were obtained as 0.92 and 0.89 of those from the control group, with the micrometeorological technique and the SF_6 technique, respectively. The oil-treated group had a significantly higher DMI than the control group, 8.29 (±1.43) kg d^{-1} $animal^{-1}$ versus 7.51 (±0.90) kg d^{-1} $animal^{-1}$, $P = 0.02$. Consequently, the mean CH_4 yield (emissions per DMI) of the oil-treated group was obtained as 0.83 and 0.80 of that of the control group with the micrometeorological and SF_6 techniques, respectively.

Table 1 Differences in CH_4 emission rates between N and S group (oil treatment – control) for the micrometeorological technique. Values in parentheses are s.e.

Period	Intercept value (g d^{-1} $animal^{-1}$)	Degrees of freedom	P
1 – no oil	−5.72 (8.57)	100	0.51
2 – oil in N	−14.26 (5.15)	207	0.006

Table 2 Group mean emission rates (±s.e.) and P for no-difference hypothesis, for the SF_6 technique.

Period	N group (g d^{-1} $animal^{-1}$)	S group (g d^{-1} $animal^{-1}$)	P
1 – no oil	137.8 (3.5)	142.5 (3.2)	0.25
2 – oil in N	138.6 (3.2)	156.1 (3.9)	0.002

Conclusions Both techniques, micrometeorological and SF_6, were capable to detect CH_4 emissions differences of order 10 % between two groups of grazing cattle. For the micrometeorological technique, this is the first rigorous analysis of this kind. Careful design, using line-averaged concentrations, and data screening for steady wind conditions are crucial for its success. Advantages of the micrometeorological technique are that it does not affect the animals, and is less labour-intensive in the field than the SF_6 technique.

Acknowledgements We thank Steve Lees, Derek Birch, Colin Faiers, Francisco Franco, Tony McSeveny, Peter Berben, Arezoo Taghizadeh-Toosi, Ross Martin and Gary LaRosa for their contributions in field and lab, and Guy Forrester for help with statistical analyses. This research was undertaken with CRI Core Funding from the Ministry of Business, Innovation and Employment.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Effect of body size on feed intake and methane emissions from ewes offered fresh forage

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Introduction Sheep production in the UK is stratified into systems that utilize smaller, hardier breeds in the hills, their crossbreds in the uplands, and heavier, more productive breeds and their crossbreds in the lowlands. This experiment tested the hypothesis that body mass and associated allometric relationships, rather than breed type, determines enteric methane production in sheep.

Materials and methods Methane emission measurements were made on mature, barren ewes of four different breed types: Welsh Mountain (WMO), Scottish Blackface (SBF), Welsh Mule (WMU) and Texel (TEX) (n = 8 per breed). Data were collected during two separate experiments in which the animals were offered fresh herbage cut from: 1) an intensively managed perennial ryegrass sward (RG), and 2) a long-term permanent pasture (PP). A zero-grazing approach was chosen to minimise potential confounding of differences in enteric methane production with differences in grazing behaviour. The experimental procedure adopted was similar for both experiments. The ewes were initially housed in a group pen and offered the experimental diet. Following an initial adaptation period of at least three weeks they were transferred to individual pens for a further three days acclimatisation. The ewes were then individually housed in one of four calibrated methane chambers and data were collected for three consecutive days for each individual animal. Throughout the experiment the ewes were fed on an *ad libitum* basis, with two equal portions of feed offered at 0900 and 1600. Stored forage was kept refrigerated at approximately 4°C. Fresh water was available continuously. All experimental animals were drenched with an anthelmintic prior to each experiment commencing. The weights of feed offered and feed refused were recorded on a daily basis. Methane production was determined by comparing methane concentrations in air entering and leaving the chambers at a measured airflow. Live weight was measured prior to the sheep entering the methane chambers and again as they were removed. Pearson's correlation coefficients were used to explore relationships between body mass, herbage intake and methane emissions within each experiment. Analysis of variance was used to investigate breed differences for each forage type.

Results A range of body weights was recorded across the four breed types (Table 1). There was a relatively poor relationship between metabolic live weight (kg) and dry matter (DM) intake (g/d; r = 0.54 for RG, 0.30 for PP), and consequently body mass was a poor indicator of methane emissions (g/d; r = 0.40 for RG, 0.39 for PP). There was a stronger correlation between DM intake and methane emitted (r = 0.74 for RG, 0.81 for PP). These findings are in agreement with results from a separate experiment carried out with replacement ewes (Zhao *et al*, 2013).

Table 1 Mean effect of breed type on the DM intake and methane emissions of ewes when zero-grazed on different pastures.

Sward type	WMO	SBF	WMU	TEX	s.e.d.	P
RG						
Live weight (kg)	45.8	61.5	70.9	77.6	2.29	<0.001
DM intake (kg DM/d)	1.08	1.02	1.30	1.55	0.102	<0.001
Methane (g/d)	21.1	20.7	24.7	26.4	3.03	ns
Methane (g/kg DM intake)	19.7	20.1	19.1	17.0	1.30	ns
PP						
Live weight (kg)	41.1	57.9	63.9	67.6	2.65	<0.001
DM intake (kg DM/d)	0.76	0.77	0.86	1.03	0.106	ns
Methane (g/d)	10.8	14.0	14.3	15.0	2.09	ns
Methane (g/kg DM intake)	14.4	17.5	16.9	14.8	1.42	0.095

Conclusions Regardless of pasture type ewe live weight was a relatively poor indicator of DM intake and therefore a poor predictor of methane emissions from sheep of different breed sizes. There was a stronger relationship between DM intake and methane emitted.

Acknowledgements This work was funded by Defra, the Scottish Government, DARD and the Welsh Government as part of the UK's Agricultural GHG Research Platform (www.ghgplatform.org.uk).

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Zhao, Y.G., Annett, R., Yan, T. and O'Connell, N.E.2013. This meeting.

https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Effect of breed and pasture type on methane emissions by growing beef steers

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Introduction Larger, faster-growing animals should partition relatively more nutrients into production, and therefore be more efficient. As a result the output of polluting excretion products on a per unit product basis would be expected to be lower for modern cattle breeds. In contrast, native cattle breeds are generally smaller and slower-maturing, but are perceived to have been bred under conditions that ensured they were hardy and able to survive in exposed conditions on nutritionally poor vegetation. Thus it is possible that physiological or behavioural differences may result in them utilising low quality rough grazing more efficiently than modern breeds. The aim of this study was to explore the extent to which breed might influence methane emissions from growing cattle grazing contrasting pasture types

Materials and methods Methane emission values were established for steers of contrasting breed types, a modern, fast-growing cross ((dairy × Belgian Blue) × Limousin) and a smaller and hardier traditional breed (Welsh Black). Separate experiments were conducted with groups of animals grazing contrasting pasture types: 1) intensively managed ryegrass, and 2) hill pasture. The 4.2 ha of monoculture perennial ryegrass that were grazed were located at 140 m a.s.l. near Aberystwyth, Wales. The hill pasture was located at 525 – 550 m a.s.l. within the Cambrian Mountains and the vegetation within the 16 ha enclosure consisted of a mosaic of several community types. Around a third of the total area was made up of large patches of semi-improved pasture interspersed to varying degrees with rushes, with the remaining vegetation dominated by *Molinia caerulea* and rushes. Steers born March – May 2011 were selected for each experiment based on uniformity of age and within-breed live weight (n=9 per breed type for each experiment). Each experiment consisted of three phases: a two-week initial adaptation period, a six-week performance measurement period, and a two-week methane measurement period. During the performance measurement periods live weights were recorded weekly in order to establish individual growth rates. Methane emissions were subsequently estimated during 2 x 4-day sampling periods using the sulphur hexafluoride (SF_6) marker dilution technique, and averaged to give one value per animal. The results were analysed using analysis of variance.

Results When grazing the ryegrass pasture the liveweight gain of the two breed types was similar, at around 1 kg/d, and there was no effect of breed type on the amount of methane emitted (Table 1). The growth rates of the two breed types were also statistically similar when they grazed the hill sward, with the growth rates on this pasture being substantially lower than those achieved on the ryegrass. When grazing the hill sward, methane emissions from the Limousin-cross steers were significantly higher than those from the Welsh Blacks.

Table 1 Effect of breed type on growth rate and methane emissions of steers grazing contrasting pasture types.

	Welsh Black	Limousin X	s.e.d.	P
Ryegrass				
Start weight (kg)	386	458	12.9	<0.001
Liveweight gain (kg/d)	1.08	1.00	0.091	ns
Methane emitted (g/d)	216	217	13.1	ns
Methane emitted (g/kg liveweight gain)	201	227	21.2	ns
Hill pasture				
Start weight (kg)	410	467	12.8	<0.001
Liveweight gain (kg/d)	0.34	0.29	0.086	ns
Methane emitted (g/d)	172	188	7.0	<0.05
Methane emitted (g/kg liveweight gain)	656	864	204.2	ns

Conclusions Methane emissions per unit of liveweight gain are substantially higher when growing cattle graze poorer quality pasture, and the results indicate that breed type has the potential to influence methane emissions when cattle graze such swards.

Acknowledgements This work was funded by Defra, the Scottish Government, DARD and the Welsh Government as part of the UK's Agricultural GHG Research Platform (www.ghgplatform.org.uk).

Effect of silage botanical composition on enteric methane emissions from dairy cows

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Introduction Enteric methane (CH_4) is one of the major greenhouse gases. The emissions of enteric CH_4 are closely related to rumen fermentation pattern. Chemical composition and ruminal degradation characteristics which differ between legumes and grasses and among common grass species may therefore influence rumen fermentation and thereby enteric CH_4 emission. The main objectives of this experiment were a) to investigate if there are differences in CH_4 emission among common used grass species and b) if legumes differ from grasses in CH_4 emissions.

Material and methods The experiment was conducted as a Latin square design with four rumen fistulated lactating multiparous Red Norwegian dairy cows (631 ± 3.3 kg body weight, 118 ± 40.9 days in milk and 22.5 ± 2.7 kg milk/d) at start of the experiment. Each period consisted of three weeks, with the last week as a measurement- and sampling period. The cows were fed four silages: primary growth and second regrowth (1:1 ratio on DM basis) from 1) an organically managed two-year old ley with timothy and red clover (39% on DM basis), 2) organically managed long-term grassland with a high proportion of unsown species (six year old), 3) conventionally managed ley with perennial ryegrass and 4) conventionally managed ley with timothy. All roughages were harvested at the same date, pre-wilted to approximately 30% DM and ensiled in round bales applying an additive (GrasAAT N Lacto) at a rate of 4-5 L/ton fresh weight. The silages were fed at 90% of *ad libitum* intake, and were supplemented with a barley concentrate, constituting about 30% of the total feed intake. Enteric CH_4 emissions were measured over 5 d in each period using the sulphur hexafluoride tracer technique. Feed intake, milk yield and composition were also determined.

Results Silage botanical composition had no effect on feed intake and milk production (Table 1). In contrast, preliminary results on CH_4 emissions indicate that silage from Timothy (Diet 4) was associated with higher CH_4 emission compared with the other silages. Diet 4 had significantly higher content of NDF than the other silages (534 vs. 422-434 g/kg DM), and this difference may have influenced the CH_4 emissions.

Table 1 Feed intake, milk yield and methane emissions for cows fed four silages.

	Diet					
	1	2	3	4	s.e.m	P
Feed intake (kg DM/d)						
Silage	11.4	11.3	11.5	11.6	0.8	> 0.05
Concentrate	5.1	5.5	5.2	5	0.35	< 0.05
BE (MJ/d)[5]	353.1	357	359.2	347.7	23.5	> 0.05
ECM (kg/d)[6]	18.4	19.1	18.9	17.4	0.6	> 0.05
Methane emissions:						
g/d	274.1[a]	407.8[a]	374.2[a]	569.3[b]	57.1	< 0.05
g/kg ECM	15.1[a]	22.1[a]	20.3[a]	32.0[b]	3.3	< 0.05
g/kg DMI	16.5[a]	22.6[a]	23.0[a]	35.3[b]	4.4	< 0.05
MJ, % of gross energy intake (Ym)	0.043[a]	0.066[a]	0.059[a]	0.093[b]	1.2	< 0.05

1 = silage from an organically managed short-term grassland with timothy and red clover
2 = silage from an organically managed long-term grassland with high proportion of unsown species
3 = silage from conventionally managed perennial ryegrass
4 = silage from conventionally managed timothy
[5] Brutto energy
[6] Energy corrected milk
[a,b] Means within a row with different superscripts differ (Tukey-Kramer test, P < 0.05)

Conclusions This experiment showed that botanical composition of silages affected enteric CH_4 emission.

Acknowledgements: The authors thank the CORE Organic Funding Body Network, the Swedish Research Council Formas, the Research Council of Norway, and the Danish Research Council for funding this research project. The authors also thank the staff at the Department of Animal and Aquacultural Sciences and at the Animal Production Experimental Centre for their contributions to this study.

https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Effect of sward species mix on methane emissions from ewes

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Introduction As part of the EU MULTISWARD programme a common experiment has been established across the partner sites to analyse the responses of multispecies swards to grazing and cutting managements regimes under a range of geographical conditions and intensities of management. The overall objective is to establish whether multispecies swards can capitalise on species diversity effects to give productive grazing pastures. In the current study the extent to which sward diversity influences methane emissions from grazing sheep was investigated using the SF6 tracer technique.

Materials and methods A series of replicated 7 m x 7 m experimental plots were prepared at Aberystwyth, Wales in 2011 based on different 'functional groups': Nf = non-N fixing, Sr = shallow rooting; Fi = N-fixing and Dr = deep rooting. Mixtures of the following four species were established: perennial ryegrass (cv AberDart) (Nf-Sr), *Festuca arundinacea* (cv Belfine) (Nf-Dr), white clover (cv AberDai)(Fi-Sr) and red clover (cv AberClaret) (Fi-Dr). A total of five treatments were set up as outlined in Table 1. Sowing rates for the individual species were 25 kg/ha, 33 kg/ha, 5 kg/ha and 12 kg/ha respectively. The management regime for the plots was based on biomass accumulation and removal, similar to that found under rotational grazing systems. The animals were mature Texel ewes selected on the basis of uniformity of live weight and body condition. The same animals were used throughout the growing season, with individuals returning to the same plots during each sampling session. The plots were grazed simultaneously at 6-week intervals (n=2 ewes per plot), with the pasture in each plot grazed down to a target surface sward height of 5 cm over a period of 3-4 days. When not grazing the plots the sheep were pastured on adjacent grass/clover paddocks. During the grazing periods on the plots in July and August 2012 methane emissions from the ewes were measured using the sulphur hexafluoride (SF_6) marker dilution technique. On each occasion samples were collected over 2 x 24 h periods from all 32 ewes grazing the experimental plots, and averaged to give one value per animal. The results were analysed using analysis of variance with a treatment structure of sampling month × sward treatment.

Table 1 Details of experimental swards established and the total amount of fertiliser applied per annum.

	Proportional composition of seed mixture					
Sward treatment	Nf-Sr	Nf-Dr	Fi-Sr	Fi-Dr	Fertiliser	Reps
PR-Lo	1	0	0	0	150 kg N/ha	3
PR-Hi	1	0	0	0	300 kg N/ha	4
PR/FES	2/3	1/3	0	0	150 kg N/ha	3
PR/LEG	2/3	0	1/6	1/6	150 kg N/ha	3
PR/FES/LEG	1/2	1/6	1/6	1/6	150 kg N/ha	3

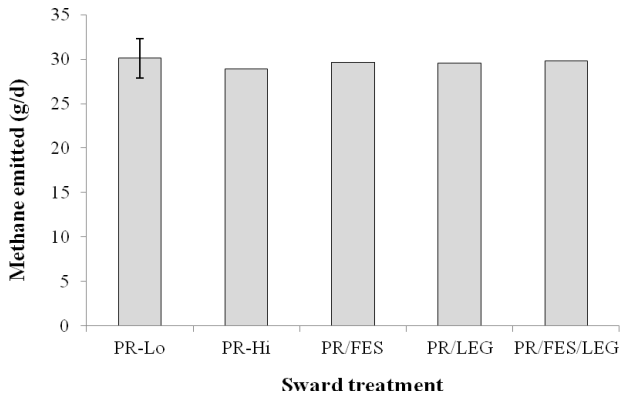

Figure 1 Effect of sward composition on enteric methane emissions from grazing ewes (error bar denotes s.e.d.)

Results Methane emissions were higher during the August grazing period (28 g/d vs 32 g/d for July and August respectively; s.e.d. 1.4 g/d; $P<0.01$), but there was no interaction between grazing period and sward treatment. There was no effect of the sward type grazed on the amount of methane produced (Fig 1).

Conclusions The different mixtures and monocultures present in this experiment did not generate differences in methane emissions from the grazing ewes. This is likely because only highly productive grasses (*L. perenne, F. arundinacea*) and legumes (*T. pratense, T. repens*) characterised by low fibre concentrations and a high digestibility were included in the mixtures. Since grazing of mixed swards incorporating legumes has been linked to increased animal performance, it is possible that methane emissions per unit of production could be lower for mixed swards, and this requires further evaluation.

Acknowledgements This work was funded by a Stapledon Travel Fellowship, Defra and the European Community's Seventh Framework Program (FP7/ 2007-2013) under grant agreement No. FP7-244983 MULTISWARD.

Prediction of enteric methane production from dairy cows

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Introduction Methane (CH_4) from enteric fermentation in ruminants is estimated to account for as much as one third of the total greenhouse gas (GHG) emissions from agriculture. Thus, strategies to reduce the emission of enteric CH_4 are being actively sought with modeling playing a key role in the identification of viable mitigation strategies. Extant models for predicting enteric CH_4 emission are based on antiquated data, and it is questionable if they are still valid given the dramatic advancements in feeding practices that have occurred over the last 20 yr. The main objective of this study was to elucidate the potential for improving the prediction of enteric CH_4 by using data from more recent experiments.

Material and methods Data from 20 experiments comprising 69 dietary treatment means published from 1997 and later on were collected. Criteria for being included in the database were besides information about enteric CH_4 emissions, data on chemical composition of the diet, and their digestibility, and animal characteristics. Half of the dataset was used to develop prediction equations for enteric CH_4 emission. Equations developed and six extern equations were tested against the second half of the dataset. The extern equations were; Mills *et al*. (2003) with DMI or metabolizable energy (ME) as input variables, Yan *et al*. (2006) with DMI or DMI and live weight as input variables and Jentsch *et al*. (2007) with intake of digested nutrients as input variables. A stoichiometric model (Volden, unpublished) was also tested. In this model, CH_4 was calculated on basis of the proportion among ruminal volatile fatty acids (VFA), and the ruminal VFA proportion were calculated from a stoichiometric model of Sveinbjörnson *et al*. (2006). Models were compared based on mean square prediction error (MSPE) and concordance correlation coefficient analysis (CCC).

Results The results of the evaluation of the best developed and selected equations for comparisons are presented in Table 1. In general, all developed equations over predicted the enteric CH_4 production. The developed equation with DMI and fat expressed as g /g NDF as input variables appears to be the best predictors of CH_4 production judged from both RMSPE (2.97) and CCC (0.883). Among the other model tested, the equations based on the stoichiometry in rumen fermentation (Volden, H., unpublished) and the equation of Jentsch *et al*. (2007) with intake of digestible nutrient as input variables showed about the same ability to predict CH_4 emission, and they were more accurate than the others.

Table 1 Comparison of model performance

	Methane emission, MJ/d		Regression		RMSPE[2]	CCC[3]
	Observed	Predicted	Intercept	Slope		
Developed equation[1]	19.4	19.7	6.30	0.69	2.97	0.883
Jentsch *et al*. (2007)	19.4	20.5	4.88	0.80	3.06	0.893
Volden, H. (unpubl)	19.4	19.5	5.60	0.71	3.57	0.844

[1] CH_4 (MJ/d) = 5.84 (±1.81) + 0.955 (±0.0873) × DMI (kg/d) – 21.2 (±6.48) × gFAT/g NDF
[2] Root MSPE (mean square prediction error)
[3] Concordance correlation coefficient

Conclusions It is concluded that the developed equation is most suitable for practical use.

Acknowledgements The authors thank the Norwegian Research Council, Norwegian Agricultural Authority, Felleskjøpet Fôrutvikling, TINE BA, Animalia and Nortura SA for funding this research project.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Effect of adaptation of rumen fluid to starch fermentation on in vitro methane production

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Introduction Production of methane in the rumen is determined by the ration the animals receive, and especially the amount of starch fermentation may negatively influence the methane synthesis. The effect of starch on methane production may be evaluated in *in vitro* tests. However, it is not known how the ration of the donor animals influences the fermentation of the substrate and subsequently the synthesis of methane, i.e. how the microbial population in the rumen adapts to the substrate and affects methane synthesis *in vitro*. The aim of the present study was to investigate the influence of adaptation of rumen fluid to different amounts and qualities of starch on the total fermentation and methane synthesis *in vitro*, using the gas production technique (Cone *et al*., 1996).

Materials and methods Rumen fistulated lactating Holstein-Friesian donor cows were fed rations with a low (27%) or a high (53%) content of starch (DM basis) in the concentrate and the starch was either slow (S; native maize starch) or fast fermentable (F; gelatinized maize starch). Starch was exchanged with beet pulp and palm kernel. The total mixed ration (TMR) consisted of 60% grass silage and 40% concentrate (DM basis). Sixteen different cows were used in a randomized block design. The substrates incubated *in vitro* in rumen fluid of the different cows were beet pulp, grass silage, native maize starch, gelatinized maize starch, the concentrates S27, S53, F27 and F53 and the TMR's S27, S53, F27 and F53. The samples were incubated in duplicate in rumen fluid from the 16 cows, adapted for 2 weeks to TMR S27, S53, F27 and F53, using an automated gas production technique, with simultaneous determination of methane synthesis (Pellikaan *et al*., 2011). This was repeated 4 times using different cows.

Results Table 1 shows that the total gas and methane production after 72 h incubation in rumen fluid depends on the substrate, with highest gas production for beet pulp (avg. 351 ml/g OM) and lowest for grass silage (avg. 265 ml/g OM). Gas production of native starch (S) was higher than of gelatinized starch (F), which was also the case for the concentrates and TMR consisting native starch, compared with gelatinized starch. The total methane synthesis after 72 h incubation varied between 55 ml/g OM for grass silage and 64 ml/g OM for beet pulp and the methane in the total gas ranged from 18.4% for beet pulp to 20.6% for grass silage. In all cases the total gas production and methane synthesis using rumen fluid from cows fed gelatinized starch (rumen fluid F27 and F53) was lower than when using rumen fluid from cows fed native starch (rumen fluid S27 and S53). Using rumen fluid from cows fed the low level of starch, both native and gelatinized starch, resulted in all cases in a higher methane synthesis than when using rumen fluid from cows fed the high level of starch. Increasing the starch content in the ration of the donor cows decreased the *in vitro* methane synthesis of all tested substrates. Also the % methane in the total gas was in all cases higher when using rumen fluid from cows fed native starch than when fed gelatinized starch and higher for the low starch level than for the high starch level, showing that the microbial population adapts to the offered rations with respect to methane synthesis, also influencing the fermentation characteristics of substrates *in vitro*.

Table 1 Total gas and methane production (ml/g OM) and % methane in the total gas after 72 h incubation in rumen fluid (RF) from cows fed rations differing in starch content and quality.

Substrate	RF	gas ml/g OM	s.d	CH_4 ml/g OM	s.d	% CH_4	s.d
Beet pulp	S27	369	16.8	71	4.4	19.2	1.2
Beet pulp	S53	356	15.4	66	5.5	18.4	1.4
Beet pulp	F27	350	23.9	65	3.3	18.7	1.1
Beet pulp	F53	326	8.9	56	3.0	17.1	0.7
Conc. S27	S27	331	19.2	68	5.7	20.7	1.4
Conc. S53	S53	331	23.6	64	7.9	19.4	1.5
Conc. F27	F27	310	12.4	60	3.2	19.4	0.9
Conc. F53	F53	257	36.0	48	6.5	18.9	4.1
Grass silage	S27	290	-	67	-	23.2	-
Grass silage	S53	272	7.3	58	4.1	21.4	1.1
Grass silage	F27	270	9.9	58	5.4	21.3	1.9
Grass silage	F53	226	-	38	-	16.7	-
Native starch	S27	336	35.5	67	6.3	20.0	2.8
Native starch	S53	355	15.5	63	5.9	17.6	1.5
Gelatinized st.	F27	351	11.7	61	4.3	18.4	2.0
Gelatinized st.	F53	311	9.8	50	6.0	16.1	1.5
TMR S27	S27	303	19.1	66	2.7	21.7	1.2
TMR S53	S53	322	13.5	62	6.5	19.3	2.0
TMR F27	F27	300	14.0	56	3.1	19.4	1.0
TMR F53	F53	272	1.1	46	1.2	16.8	0.4

Conclusions It can be concluded that type and level of starch fed to donor cattle results in adaptation of the rumen microbial population and influences the methane synthesis *in vitro* of all tested substrates.

Acknowledgements The authors gratefully acknowledge funding from the Dutch Ministry of Economic Affairs, the Product Board Animal Feed and the Dutch Dairy Board.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Effects of forage source and supplemental dietary fat on methane emissions from growing dairy heifers of differing body weights

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Introduction There is currently a lack of methane (CH_4) emission factors for livestock in different production states, farming systems and fed differing diets. A number of CH_4 mitigation opportunities have been identified whereby changes in diet carbohydrate amount and type, as well as the use of dietary fat supplements, can decrease CH_4 emissions. Maize and grass silage diets are applicable to typical UK rations and previous research has shown replacing grass silage with maize silage in a total mixed ration (TMR) reduced CH_4 yield (g CH_4/kg dry matter [DM] intake [DMI]) of lactating cows by 12% (Reynolds *et al.*, 2010). In addition, feeding supplemental fat typically reduces CH_4 yield by an amount dependent on the quantity fed (Beauchemin *et al.*, 2008). The objectives of this study were to measure CH_4 emissions from growing dairy heifers over two body weight (BW) ranges, and determine the effect of maize versus grass silage-based diets with or without supplemental oil from an extruded linseed product (Lintec).

Material and methods Two experiments were conducted with growing Holstein heifers. Each experiment was a 4 x 4 Latin Square design with 5 week periods and 4 Holstein dairy heifers fed either maize (MS) or grass silage (GS) diets supplemented with or without Lintec (260 g fat/kg DM) at 0.06 of ration DM. Heifers in Experiment 1 were aged 13 months at the start of the study and had a mean BW range of 395 to 506 kg from start to finish. In Experiment 2, heifers were aged 12 months at the start of the study and had a mean BW range of 317 to 416 kg from start to finish. Diets were fed as a TMR containing 750 (Experiment 1) or 733 (Experiment 2) g silage/kg on a DM basis, with cracked wheat, soybean meal and minerals added to balance Lintec and equalise diet nitrogen (N) concentration within experiment. The diets were fed once daily for 1 kg BW gain/d in Experiment 1 and 0.75 kg BW gain/d in Experiment 2 based on the estimated metabolisable energy concentration of the diets. Each 5 week period consisted of 4 weeks adaptation with measurements of DMI, CH_4 production, and energy and N balance using open-circuit respiration chambers and digestion trials in Week 5. Data were analysed using the Mixed Procedure of SAS® and a model testing the fixed effects of silage (1 df), Lintec (1 df), and their interaction (1 df), and random effects of heifer (3 df) and period (3df).

Results There was no effect of Lintec supplementation on CH_4 emissions, DMI or whole tract DM digestibility (DMD), irrespective of animal BW (Table 1). This may reflect the relatively low rate of diet oil inclusion. As observed previously in lactating dairy cows, heifers in Experiment 1 fed MS had a higher DMI and lower ($P < 0.001$) CH_4 yield (g/kg DMI) compared to GS diets, but there was no effect of diet on DMD or CH_4 production (g/d). In Experiment 2, diet had no effect on CH_4 production or yield, DMI, or DMD. Reasons for this difference are not certain, but may relate to intake level or fermentation characteristics of the specific silages fed.

Table 1 Body weight (BW), dry matter intake (DMI), dry matter digestibility (DMD), and methane (CH_4) emissions from heifers fed maize (MS) or grass silage (GS) based diets supplemented with or without Lintec

	Diet				P-value		
	MS	MS + Lintec	GS	GS + Lintec	Silage (S)	Lintec (L)	S x L
Experiment 1							
BW, kg	454	454	448	447	0.007	0.571	0.684
DMI, kg/d	9.3	9.5	7.9	7.9	0.003	0.603	0.415
DMD, g/kg	729	744	760	750	0.176	0.898	0.323
CH_4, g/d	207	194	213	207	0.579	0.315	0.404
Experiment 2							
BW, kg	362	363	360	362	0.430	0.323	0.734
DMI, kg/d	7.0	7.2	7.3	7.4	0.146	0.418	0.962
DMD, g/kg	714	727	728	742	0.117	0.135	0.969
CH_4, g/d	183	194	207	189	0.399	0.644	0.252

Conclusions Heifers fed MS at a heavier BW consumed more DM and emitted less CH_4 per unit of DMI compared to GS diets, however when repeated with heifers of a lower BW, DMI and CH_4 emissions did not differ between the two silages. Considering MS and GS are the predominant conserved forages in UK dairy rations, further work is needed to determine the specific forage characteristics that affect CH_4 yield for these silages.

Acknowledgements This work was funded by Defra, the Scottish Government, DARD, and the Welsh Government as part of the UK's Agricultural GHG Research Platform project (www.ghgplatform.org.uk).

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

In vitro production of methane and carbon dioxide from cattle fed corn grain and citrus pulp associated with glycerin

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Introduction In most studies glycerin has been evaluated as an energy source replacing the corn of the diet. In Brazil, citrus pulp represents an interesting alternative for feedlot cattle. Methane is a greenhouse gas, product of methanogenic bacteria and represents a loss of 2 to 15% of gross energy of the diet. Its synthesis affect the efficiency of conversion and nutrients utilization, and because of this, is focus of current research. Glycerol is rapidly fermented to propionate in the rumen. Due to the inverse relationship between propionate production and enteric CH_4 emissions, glycerol may reduce rumen CH_4 production (Lee *et al.*, 2011). The aim of this study was to evaluate the gases methane and carbon dioxide from *in vitro* fermentation of diets containing glycerin.

Material and methods Five rumen cannulated Nellore steers (420 ± 20 kg BW) were used in a 5x5 Latin square design. Experimental diets consisted of 30% corn silage and 70% concentrate (corn grain, citrus pulp, soybean hulls, urea and glycerin) and were labeled as (DM basis): diet with no added glycerin (CON), 30% of corn grain with 10% of glycerin (CG10), 25% of corn grain with 15% of glycerin (CG15), 25% citrus pulp with 10% of glycerin (CP10) and 20% of citrus pulp with 15% of glycerin (CP15). Glycerin replaced corn grain and citrus pulp in the total mixture of diets. Measurements of CH_4 and CO_2 gases consisted of three steps: 1- Preparation of the sample: before feeding samples of ruminal digesta were taken from rumen and strained through a 100 µm nylon filter obtaining the rumen fluid. In erlenmeyers with 250 mL of capacity were placed 125 mL of ruminal fluid and added 1.56 g of DM of the diets, no buffer was used. 2- Production and gases storage: the erlenmeyers containing the diets and rumen fluid were closed with stoppers and kept for 24 hours in a Shaker SL 222 Incubator at 39°C in dark room. The gases produced were conducted by silicone capillary system and stored in collectors (internal volume of 600 mL). These collectors were immersed in water enabling measurement of total gases by water displacement inside them. 3- Quantitative and qualitative analysis of gases produced: an aliquot was collected directly from the erlenmeyers with a syringe and injected 0,5 mL of the gas into a gas chromatograph Trace GC Ultra Thermo Scientific equipped with flame ionization detector using argon carrier with a flow of 25 mL/minute and oven 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 Chromquest 5.0 software. The total quantity of gas produced was determined by the volume occupied by the gas produced and measured by displacement of collectors immersed in water after 24 hours of fermentation. Statistical analysis was performed using the MIXED procedure of SAS. Orthogonal contrasts were used to determine the effect of 0% glycerin vs glycerin treatment, CG10 vs CG15, CP10 vs CP15 and corn grain vs citrus pulp treatment, considering a significance level of 5%

Results There were no differences (P> 0.05) on methane and carbon dioxide production between the diets evaluated (P> 0.05) whose average production was 18.3 and 63.7 mL/gDM, respectively (Table 1). The pH average after incubation was 6.6 and there was no difference between treatments (P> 0.05). O'Mara (2004) reported that the concentrate proportion in the diet has negative correlation with methane emission, in this experiment the forage: concentrate ratio was the same for all treatments, which contributed to methane production similar among diets. The degradation of ingredients with high pectin level, compared to ingredients with high starch level increase the acetic: propionic ratio (Strobel and Russel, 1986). It was hoped that treatments CP10 and CP15 had greater methane production due to increase in acetic acid concentration that favors methane production in the rumen, this was not observed. Therefore, the use of glycerin in these diets may have contributed to these results.

Table 1 *In vitro* production of methane (CH_4) and carbon dioxide (CO_2) from steers feeding corn grain and citrus pulp associated with glycerin

	Diets					CV%	Contrasts* P			
Variable	CON	CG10	CG15	CP10	CP15		1	2	3	4
CH_4 (mL/g DM)	18.4	16.4	18.7	18.1	19.7	21.8	0.935	0.386	0.540	0.469
CO_2(mL/g DM)	67.1	62.0	63.9	58.6	66.7	21.7	0.540	0.830	0.369	0.962

*Contrasts: 1 =CON *vs* glycerin treatment (CG10, CG15, CP10 and CP 15), 2 = CG10 *vs* CG15, 3 = CP10 *vs* CP15, 4 = corn grain (CG10 and CG15) *vs* citrus pulp (CP10 and CP15), CV = Coefficient of variation

Conclusions The glycerin association to corn grain or citrus pulp did not affect the production of methane neither carbon dioxide from rumen fermentation.

Acknowledgements Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Caramuru Alimentos.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Greenhouse gas emissions and mitigation options for livestock production in Europe, Africa and Latin America

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Introduction The global animal food production chain, including land use and land use changes, generates 18% of global greenhouse gas (GHG) emissions. The total contribution of livestock production to global anthropogenic greenhouse gas emissions is high, however variable across regions in the world. The largest contributors from livestock production to the global GHG budget are (i) methane (CH_4) emissions from ruminant production systems, (ii) nitrous oxide (N_2O) emissions related to manure management and feed production, and (iii) net CO_2 emissions from land use changes related to feed production. With increasing global food demand and stronger international GHG reduction targets, the pressure to reduce the GHG emissions of livestock production increases. The objective of this study was to provide quantitative estimates of GHG emissions from livestock production in European Union (EU-27), Africa and Latin America and to identify and quantify relevant mitigation options.

Methods The GHG emission estimates and mitigation potentials are based on a review of available studies and calculations using the MITERRA-Global model. MITERRA-Global is an environmental impact assessment model at global scale, based on the MITERRA-Europe model (Lesschen *et al.*, 2011). The model calculates GHG and N emissions and soil carbon stock changes on a deterministic and annual basis, using emission factors, mainly based on the IPCC 2006 guidelines. Activity data are based on three year averages for the period 2007-2009.

Results Lesschen *et al.* (2011) and Weiss and Leip (2012) assessed the GHG emissions of the European livestock sectors following a top down life cycle assessment approach. The largest livestock related GHG emissions originate from the dairy and beef sector yielding about 70% of the GHG emissions from livestock production. Large variations exist among countries; these can be explained by differences in animal production systems, feed types and nutrient use efficiencies by the animals. Based on Bellardy *et al.* (2013) the main mitigation options for livestock production in the EU-27 are (i) minimizing animal food waste, (ii) combinations of technical measures in animal production to reduce CH_4 and N_2O emissions, e.g. anaerobic digestion, dietary additions and optimized fertilizer application, with a combined mitigation potential of 15-19%, (iii) improving the efficiency of livestock production to reduce GHG emissions per kg of product, and (iv) avoiding land use changes outside the EU-27 through lower feed imports. In addition to these technical measures, a consumer driven reduction of the intake of animal derived protein by 30 to 45% can reduce emissions as much as the total of all technical measures. The mitigation potential the full suite of options is then in the order of 300±80 Mton CO_2-eq.

In Africa the main GHG sources from livestock production are enteric fermentation, savannah burning and N_2O emissions resulting from grazing animals. Due to the low fertilizer use, the direct N_2O soil emission is only a minor source. The main mitigation options for African livestock production are (i) improvement of the livestock production efficiency, which will reduce the enteric fermentation CH_4 emission intensity, (ii) restoration of soil carbon stocks in degraded pastures, and (iii) improvement of manure and fertilizer management to increase crop and feed productivity. However, only very limited quantified estimates of mitigation potentials are available for Africa.

In Latin America enteric fermentation is by far the largest source of livestock related GHG emission (668 Mton CO_2-eq), followed by N_2O emissions from grazing and CO_2 from land use change and pasture degradation. The main mitigation measures in Latin America are therefore (i) reduction of methane emissions from enteric fermentation by improving livestock productivity and diet improvements, (ii) reduction of deforestation and savannah conversion, and (iii) restoration of degraded pastures through adoption of improved pastures.

Conclusions This study provides an overview of quantitative estimates of GHG emissions from livestock production for the EU-27, Africa and Latin America. The availability of studies and data on livestock GHG emissions and mitigations potentials is very limited for the last two continents, compared to the EU-27. The GHG emission intensities differ substantially between these three continents, with Europe having the lowest per product GHG emissions and Africa the highest. Due to major differences in livestock production systems for these regions, their respective mitigation options and potentials are different, which should be accounted for in climate policies.

Acknowledgements The authors acknowledge funding from the AnimalChange project (EU FP7 Grant Agreement 266018) and the Dutch government (Ministry of Economic Affairs in program KB-12).

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SMEthane, a technological platform to develop nutritional additives to reduce methane emissions from ruminants: putting together efforts from academia and industry.

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Introduction Three main approaches to mitigate methane emissions from ruminant production have been suggested: i) improvements in efficiency through application of best practice in "on farm" management, ii) application of biotechnological solutions based on introduction of new or modified microorganisms in the animal, immunological and hormonal control of gut function and iii) dietary changes including novel forages and dietary additives that manipulate rumen function. In SMEthane we have addressed the last of these options. The project objective was to address the restrictions that SMEs face in successfully developing and marketing novel compounds, in particular plant extracts, capable of decreasing methane production from ruminant animals. Specifically we have addressed: 1) The need to standardise and report the concentration of an active component, 2) Stability of the compounds in practical conditions, 3) Persistence of the effects/adaptations of the rumen ecosystem, 4) Lack of in vivo data over a range of livestock production systems, 5) Effect of extracts on the perceived quality of milk products, 6) A lack of production data on which to base calculations of market prices.

Material and methods The consortium is comprised by 5 SMEs: Agolin SA (Switzerland), DOMCA (Spain), Neem Biotech (UK) and NorFeedSud and Phytosynthese (France) plus 5 European Research Institutes and Universities: CSIC (Spain), Aberystwyth University (UK), INRA (France) and University of Ghent and ILVO (Belgium). The project was funded by the 7th Framework Program of the European Commission through the specific call 'Research for the Benefit of SMEs' and had a duration of 2 years.
The technological platform includes : 1) baseline information on the stability of plant extracts under different environmental conditions (effect of pelleting process and effect of storing at different temperatures (4, 20, 30 ºC) over 1 and 2 months; 2) in vitro screening of different compounds that covers the use of different diets and pH; 3) in vivo measurements over short (7 days) and long term (6 weeks) treatments in sheep, goats, beef and dairy cattle and potential transfer of additives into milk.
The compounds tested cover essential oils, organosulphur compounds and saponins extracts. A total number of 29 different compounds were tested at different levels, some of them went through the whole set of tests included in the platform.
In addition to the research activities, 3 workshops with the feed industry were held in UK, Spain and France.

Results The results of the stability tests showed that the pelleting process may contribute to a loss between 8 and 74 % of the active compound, while storing temperature also accounted for variable loss (from 0 % at 4ºC to 80 % at 30ºC). Whether the compound had been protected, especially those with high volatility had a great impact on the losses.
The in vitro screening showed variable antimethanogenic activity depending on the active molecule: up to 28 % for essential oils, 8 % for saponins and 63 % for organosulphorous compounds, in a range of 200 to 600 ppm. However, the effect was largely influenced by the basal diet and pH. Those compounds with more potential were further tested in vivo in small ruminants at 3 levels of inclusion in the diet over 7-day treatments. Overall, most of the compounds confirmed their potential but the concentration needed to reach the same effect as in vitro was much higher. When the compounds were tested in cattle over 42 days, the results revealed lower effect than in small ruminants in some cases. In other cases, the reduction of methane observed (up to 32 %) did take place after 2-4 weeks of treatment. The analysis of the presence of compounds in milk from treated dairy cows showed no transfer of the essential oils used and evident presence in the case of organosulphorous compounds.
All material presented in the three workshops is available at www.smethane.eu.

Table 1 Summary of assessment of compounds tested within SMEthane platform

	Stability, %		1 month storage		Methane inhibition %		
	Pelleting	4ºC	15ºC	30ºC	*In vitro*	*In vivo* 7 d	*In vivo* 42 d
Variation range	26-92	0-72	0-84	26-88	0-84	0-35	0-32

Conclusions This project has shown that integration of tests of nutritional additives is necessary to identify those with potential for further development. The anti-methanogenic activity varies according to factors such as diet, rumen conditions and animal species. The effect persist in some cases and in other cases needs at least 2 weeks to take place, which highlights the importance of conducting medium to long term trials.

Acknowledgements SMEthane was funded by the European Commission. Reference contract FP7-SME-262270.

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www.smethane.eu Technological platform to develop nutritional additives to reduce methane emissions from ruminants. FP7-SME-262270.

https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

The addition of Ethyl-3-nitrooxy propionate and 3-Nitrooxypropanol in the diet of sheep substantially reduces methane emissions and the effect persists over a month

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Introduction A variety of active compounds have been shown to exhibit methane reduction potential in ruminants, although most of the studies have been carried out *in vitro* or over short term *in vivo* trials (McAllister and Newbold, 2008). One of the means to reduce methane emissions within the rumen is to target the activity of methanogenic Archaea, in particular the last step of saturation of CO_2 to CH_4 by the enzyme Methyl CoM reductase (Attwood and McSweeny, 2008). However, the effects in some cases revert after a few days of administration (Van Nevel and Demeyer, 1995) or compromise animal intake and overall rumen fermentation. Thus, the present study was designed with the aim of investigating the effect of two compounds (Ethyl-3-nitrooxy propionate and 3-Nitrooxypropanol), on methane emissions, rumen fermentation, microbial abundances and the rumen degradability of the diet when they are supplied to sheep over a month period.

Material and methods Nine adult non-pregnant sheep with permanent rumen cannula were used. The experimental design consisted of a 3 x 3 Latin square with 3 sheep per treatment in each period of 30 days. Experimental animals were randomly allocated in three sub-groups of 3 animals each and were assigned one of the three treatments (control C, Ethyl-3-nitrooxy propionate E3NP and 3-Nitrooxypropanol 3NP). Animals were fed a diet consisting of alfalfa hay chopped at 15-20 cm and oats in a 60:40 ratio plus mineral-vitamin supplement at 1.1 maintenance requirements in two equal meals per day. Both compounds were supplied to the animals through the rumen cannula before the feedings (100 mg/animal/day). On day 14 a mid-term methane measurements was conducted. On days 22 and 23, samples of oats and alfalfa hay, placed in nylon bags, were incubated in the rumen of sheep to determine the dry matter ruminal degradation over 24 and 48 hours, respectively. On day 30, methane emissions were measured and rumen content samples were collected two hours after the morning feeding, sub-sampled and immediately frozen prior DNA extraction, quantification of bacteria, protozoa and archaea, and analysis of volatile fatty acids concentration as described by Abecia *et al*. (2012).

Results Dry matter intake was not affected ($P > 0.05$) by the treatment and only slight reduction in intakes were observed when the animals were introduced in the methane chambers on days 14 and 30. Methane emissions were reduced ($P = 0.020$) on day 14 when both additives were incorporated in the diet (Table 1). The reduction observed against the control was 14 % and 23 %, respectively, for E3NP and 3NP. When methane emissions were recorded two weeks later, on days 29 and 30, there was still a reduction ($P = 0.062$). The study of the rumen fermentation showed a shift towards more propionate type profile in the rumen of animals receiving both additives in comparison to the control. As a consequence, in both treatments the acetate to propionate ratio was reduced ($P = 0.002$). Degradability in the rumen of both alfalfa hay and oats was not affected ($P \geq 0.167$) by any of the additives. Likewise, total and relative concentration of the analysed microbial groups in the rumen showed no difference ($P > 0.05$) among treatments.

Table 1 Effect of the addition of Ethyl-3-nitrooxy propionate and 3-Nitrooxypropanol on methane emissions, volatile fatty acids, degradability of alfalfa hay and oats and the abundance of methanogenic Archaea in the rumen of sheep.

	Control	E3NP	3NP	s.e.m.	P
CH_4 l/kg DMI (d14)	29.9	25.6	22.5	2.31	0.020
CH_4 l/kg DMI (d30)	25.8	21.7	19.6	2.12	0.061
Total VFA, mM	100	97.3	104	9.157	0.924
Acetate/propionate	4.91	4.09	3.89	0.262	0.002
Degradability alfalfa hay, %	78.6	78.3	78.8	1.22	0.725
Degradability oats, %	74.2	74.0	70.6	2.02	0.167
Archaea, log10 copy numbers	8.54	8.45	8.34	0.133	0.511

Conclusions Both tested additives showed promising potential as methane inhibitors in the rumen. The mode of action seems to be a shift in the metabolic pathways involved in H_2 transferring that do not affect the total numbers of Archaea and the degradation of feeds in the rumen. This opens the possibility of testing them in producing animals with higher intakes request.

Acknowledgements The present study was supported by DSM Nutritional Products AG, Kaiseraugst, Switzerland.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Effect of nutritional intervention with anti-methanogen compound at early life of goats on ruminal colonization by Archaea

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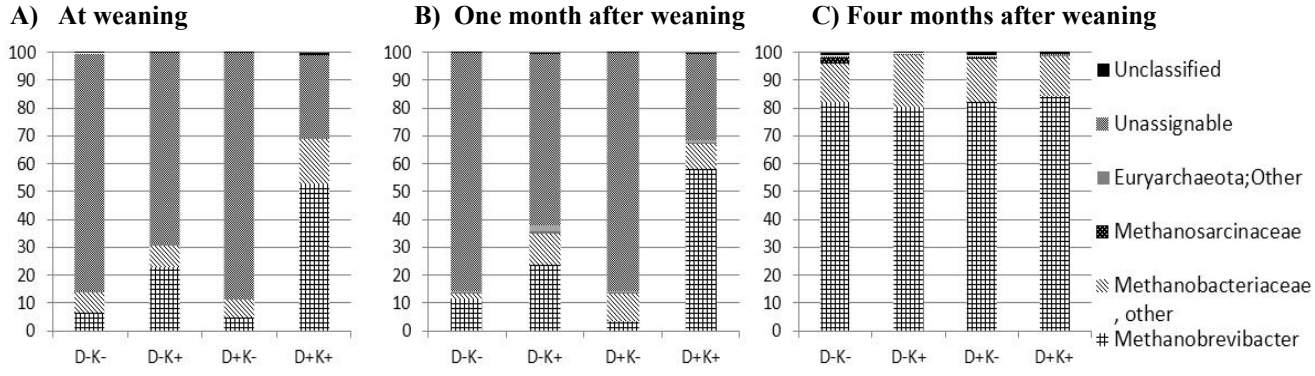
Introduction The developing rumen provides an opportunity to explore means of manipulation of the microbial ecosystem. Gagen *et al.* (2012) reported that the diversity of *mcrA* sequences in the rumen of lambs 17h after birth was not significantly dissimilar to that found in the mature rumen of conventional 2 year old sheep. This opens the possibility of methanogens that are acquired by ruminants from a very young age, are maintained throughout rumen development and life. The literature suggests that rather than the numbers, is the composition of methanogens species what drives methanogenesis in the rumen (Zhou *et al.*, 2009). The aim of this work was to study whether feeding a methanogen inhibitor at early life of kids and does has an impact on the archaeal community colonizing the rumen and to what extent the impact persists later in life.

Material and methods Sixteen goats giving birth two kids were used. Eight goats were treated (D+) with bromochloromethane (BCM) after giving birth and over 2 months when rumen samples were collected. The other 8 goats were not treated (D-). One kid per doe in both groups was treated with BCM (k+) for 3 months while the other was untreated (k-), resulting in four experimental groups: D+/k+, D+/k-, D-/k+ and D-/k-. Rumen samples were collected from kids at weaning, one month (during which time kids were grouped by treatments) and four months after weaning, when all groups were grouped together and BCM treatment ceased. The hypervariable V6 region of the *16S* rRNA gene was amplified (958F and 1048arcR-major) allowing samples to go through pyro-sequencing using Roche 454 FLX Titanium. Results were analyzed using Quantitative Insights Into Microbial Ecology (QIIME).

Results The relative abundance of Methanobrevibacter at weaning varied from 23 to 52% in treated kids (D-k+ and D+k+, respectively). A significant effect of the treatment received by the doe ($P < 0.01$) was observed. Other genus from this family also showed a significant ($P < 0.001$) higher relative abundance (16.5%) in D+k+ group. However, a group of unassignable genera significantly decreased ($P < 0.0001$) with BCM treatment, 86 for D-k- and D+k- and 68 and 29% for D-k+ and D+k+. One month after, Methanobrevibacter varied from 58 to 24% in the groups receiving the treatment (D+k+ and D-k+, respectively) showing a significant effect of the treatment applied to the doe ($P < 0.01$). Other members from this family (others) also showed ($P = 0.03$) higher relative abundance in groups D-k+ (11.2%), D+k- (9.8%) and D+k+ (8.6%) compared to D-k- (2.4%). On the contrary, there were unassignable genera that significantly decreased ($P < 0.0001$) with BCM treatment of the kids, 85% for D-k- and D+k- and 61 and 31% for D-k+ and D+k+, respectively. Four months after weaning no significant effect were found either in Methanobacteriales order or the relative abundance of Methanobrevibacter and Methanobacteriales order. Only, un-assignable and un-classified had lower abundances for treated kids (k+).

Figure 1 Relative abundance (% of total archaeal sequences) from experimental group of kids



Conclusions Results show that nutritional intervention at early life of ruminants with anti-methanogen compound results in a modified archaeal community structure colonizing the rumen. The effect seems to be influenced by the treatment applied to the mother and did not persist to a significant extent 3 months after the treatment stopped. However, some less abundant unknown archaeal groups could play a key role in the persistency of the lowered methane emissions in treated offspring. This last point would need to be further investigated.

Acknowledgements Research has been funded by the European Commission (Marie Curie Reintegration Grant 224816–METANORUMEN) and the Spanish Science and Innovation Ministry (AGL2008-04707-C02-01).

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Assessment of complete vs. incomplete manure slurry storage emptying on gaseous emissions during cold and warm seasons

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Introduction Manure storages are sources of methane (CH_4), nitrous oxide (N_2O) and ammonia (NH_3) emissions. Mitigating these emissions is important because of the environmental concerns associated with these gases. For reasons such as weather, available land base or cost effectiveness, farmers do not always completely empty their storages when spreading manure on fields. Incomplete storage emptying leaves residual manure in the system that could act as a biological or chemical inoculum, thus inducing higher CH_4 emissions when fresh manure is added (Massé *et al*. 2008). Based on simulations and laboratory experiments, Massé *et al*. (2008) recommended increasing the frequency of storage emptying and limiting the amount of residual manure remaining in the storage as a way of reducing CH_4 emissions. These findings, as well as possible inoculum effects on N_2O and NH_3 emissions need to be investigated at the pilot-scale. The objectives of this research were to compare CH_4, N_2O and NH_3 emissions from pilot-scale dairy slurry tanks with and without an inoculum during winter/spring and summer/autumn under 2 hypothetical farming situations.

Materials and Methods Research was conducted at a previously described site (VanderZaag *et al*. 2010; Wood *et al*. 2012) located at Dalhousie University's Bio-Environmental Engineering Centre in Truro Nova Scotia, Canada. Dairy slurry was stored outdoors in six concrete tanks (~10.5 m^3 each) over cold (Trial A: 2010-Dec to 2011-Jun, 192 d) and warm (Trial B: 2011-Jun to Nov, 155 d) seasons. In both trials the inoculum was derived from manure that was stored at the site during a previous experiment. Fresh manure was obtained from the Dalhousie University Dairy Farm storage. The herd consists of 40 milking cows housed in a tie-stall barn and the total solids content of slurry in the farm storage is typically ~10%. In both trials there were three tanks that were completely emptied and filled with fresh manure (non-inoculated), and three tanks that were a mixture of a manure inoculum and fresh manure. The inoculum treatments were prepared using 25% and 50% (by volume) residual manure in Trials A and B, respectively. Each tank was enclosed by a permanently fitted flow-through steady-state chamber to permit continuous monitoring of gas fluxes. Air was continuously drawn through each chamber by an exhaust fan situated in a venturi that was also equipped with a cup anemometer (Davis Instruments, Hayward CA) to enable air flow rate monitoring. During Trial B, a sprinkler inside each chamber simulated rainfall at a rate equal to the weekly normal for Truro NS. Methane and N_2O concentrations were measured using tunable diode laser trace gas analysers (Campbell Scientific Inc., Logan UT). Ammonia concentrations were measured using acid traps (Wood *et al*. 2012). Air and manure temperatures were measured using Type T thermocouples. For each trial, total emissions from the two treatments were compared using a two tailed student's t-test (TTEST, MS Excel).

Results During Trial A the surface of all the slurries froze. The entire profiles did not freeze because temperatures 10 cm above the bottom of the tanks were always >0°C. Crusts formed on the surfaces of all tanks; however, the timing of crust formation was not determined because of freezing conditions. In Trial A, CH_4 was the major contributor to the total greenhouse gas (GHG) budget (as CO_2-eq) at 63% and 89% for emptied and inoculated tanks, respectively. Complete storage emptying significantly reduced CH_4 emissions by 45%. However, completely emptying the tanks also resulted in a significant increase in N_2O emissions by 402%, which may have been due to these tanks having drier surface crusts. Despite increased N_2O emissions, total GHG emissions were significantly lower from non-inoculated tanks compared to inoculated ones. There was no difference in total NH_3 emissions. During Trial B crusts established on the surfaces of all tanks, with formation occurring more rapidly on slurries that were inoculated. Faster crust formation on inoculated tanks coincided with an increase in CH_4 fluxes from these tanks. Methane was again the dominant contributor to the total GHG budget at 78% and 91% for emptied and inoculated tanks, respectively. Total CH_4 emissions were significantly lower by 55% in tanks that had been completely emptied. There was no significant difference in total N_2O emissions in Trial B. Total NH_3 emissions were, however, significantly higher by 225% from completely emptied tanks. Significantly lower NH_3 emissions from inoculated tanks were due to the surface crusts, which formed rapidly and completely covered these slurries.

Conclusions There were significantly lower GHG emissions from storages that were completely emptied in both trials, mainly due to reductions in CH_4 emissions. However, NH_3 emissions were significantly higher from completely emptied tanks in Trial B. Despite the trade-off with NH_3 in the warm season, completely emptying manure storages offered consistent CH_4 emission reductions. The greatest potential for absolute CH_4 emission reductions will be realized if storages are completely emptied in the spring in preparation for warm season storage because CH_4 fluxes are typically higher during the warmest months. Further research is needed to determine how 'clean' a storage must be to prevent the inoculum effect and to assess the cost effectiveness of this practice on farms.

Acknowledgements Funding was provided by Agriculture Agri-Food Canada under the Agricultural Greenhouse Gases Program and the Natural Sciences and Engineering Research Council.

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The influence of echium amoneum extract on in vitro ruminal fermentation, protozoa population and methane production

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Introduction Inefficient ruminal fermentation of protein and energy is a major concern, which affects animal productivity. Plant extracts have been widely used to improve the efficiency of protein metabolism in the rumen by reducing amino acid deamination (Talebzadeh *et al*., 2012) and the reduction of the acetate to propionate ratio in the rumen (Eckard *et al*., 2010). *Echium amoenum* belongs to *Boraginaceae*, and has many uses in Iranian ethnic medicine and its antimicrobial properties have been reported by Mehrabani *et al*. (2005). The aim of the current study was to assess the effect of *Echium amoenum* extract (EAE) on *in vitro* ruminal fermentation, protozoa population and methane production.
Material and methods In this trial, the effect of different levels of EAE (0.3, 30, 300 or 3000 µl) on *in vitro* ruminal fermentation characteristics and methane production was assessed. The parameters studied were *in vitro* gas production at 54 h (GP54), methane production, ammonia-N concentration, partitioning factor (PF) (the ratio of substrate truly degraded to gas volume produced at 24 h of incubation), total volatile fatty acid (VFA) concentration and total protozoa population.

Statistical analyses The effects of five levels of EAE on the ruminal parameters were examined with nine replicates per level (*i.e.,* 3 runs were carried out; in each run 3 tubes per level were used) which were subjected to ANOVA using the following model: $Yijk = \mu + Ti + \varepsilon ijk$
Where Yijk represents the value of each individual observation, µ is the overall mean, Ti is the effect of the ith level of additive (i = five levels of EAE), and εijk is represents the residual error.
Data were analysed using the GLM procedure of SAS 9.1. For all analyses, linear (L) and quadratic (Q) effects of EAE level on parameters.

Results Gas production from insoluble fraction (*b*) (linear effect; P<0.01) was significantly (quadratic effect; P<0.01) increased with increasing level of EAE. The inclusion of EAE at either 300 or 3000µl increased GP at 54 h (linear effect; P<0.01). However, methane production was reduced (linear effect; P<0.05) by 54, 41 or 43% when EA was added at 3, 30 or 300 µl respectively, without changing the degradability of organic matter. The inclusion of EAE reduced ammonia-N concentration (P<0.01), while PF (quadratic effect; P<0.01) led to an increase. On the other hand, EAE addition decreased the concentration of VFA and acetic acid (P<0.05), but increased the propionic acid (P<0.05). Moreover, EAE had a significant antiprotozoal activity, and, there is a tendency of total protozoa population to decline.

Table 1 Effect of different levels of EAE on the ruminal fermentation parameters

	Level of Extract (Microliter)						Level of Sig	
	0	3	30	300	3000	s.e.m.	L	Q
b	59	58.8	61.5	75.4	92.4	2.6	**	**
c	0.067	0.08	0.075	0.067	0.061	0.008	ns	**
Gas production at 54 h (ml/ 200 mg DM)	67	67.5	69.7	82.5	89.9	1.9	**	ns
CH_4 (micromole/200 mg DM)	541	247	317	307	370	20.2	*	**
Ammonia-N (mg/l)	20	17.4	13.4	15.7	14.4	0.75	**	**
PF	2.8	3.5	3.3	3.2	3.1	1.4	ns	**
Total VFA (mM/200 mg DM)	54	44	43.6	53.6	46.6	4.0	ns	*
Molar proportion of VFA								
Acetate (C2)	57.6	42.3	45.0	46.9	55.1	2.07	ns	**
Propionate (C3)	23	39	31	32	26.1	1.4	ns	**
Butyrate	19	19	24	21	21	2.28	ns	ns
C2:C3	2.6	1.2	1.7	1.5	2.1	0.17	**	**
Total protozoa population (x 10^5/ml)	2.4	1.9	1.8	1.7	1.6	0.01	**	ns

*b:*gas production from the insoluble fractions (ml/g OM); *c:* rate constant of gas production during incubation (ml/h); PF: (the ratio of substrate truly degraded to gas volume produced at 24 h of incubation);

Conclusions The results finally suggest that EAE is of the potential to positively manipulate *in vitro* ruminal fermentation and reduce methane production.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Effect of diet type and feed efficiency status on rumen microbial populations in sheep

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Introduction Ruminant livestock are important contributors to the human food supply in part because of their ability to live on forages produced on land unsuited for producing plants that can be consumed directly by humans. However, there are environmental concerns associated with grazing livestock due to their contribution of methane to greenhouse gases. Novel means of assessing efficiency of grazing livestock are needed to make genetic improvements in feed efficiency, which also reduces methane emissions. Recent beef cattle studies have demonstrated taxonomic differences in rumen microbial populations associated with phenotypic differences in feed efficiency. Methanogenic and methane-reducing species may be of particular importance because of the link between improved feed efficiency and reduced methane production. Associations of rumen microbial profiles with favorable feed utilization may provide a novel means of selecting for feed efficiency while concomitantly reducing methane production. Our goal is to develop a better understanding of the link between rumen microbial populations and feed efficiency, and understand the role methanogenic and methane-reducing species may play in this association. The objective of this study was to determine differences in rumen microbial populations in sheep divergent for feed efficiency on two different diet types.

Material and methods For this study, growing wethers of Rambouillet, Hampshire, and Suffolk breed types were randomly allocated to receive a concentrate- (n = 39) or forage-based (n = 38) pelleted diet for a 49 d trial period during which individual intake data was collected using a GrowSafe automated feed intake system. Residual feed intake (RFI) was estimated for each wether as an assessment of feed efficiency. This measure of feed efficiency is independent of body weight and growth, and is a preferred assessment of efficiency in many livestock industries. Within each diet, wethers were ranked on RFI, and DNA from rumen samples collected from the most efficient (n = 4) and least efficient (n = 4) wethers were sequenced (n = 16 samples sequenced in total) at the University of Missouri's DNA Core facility using an Illumina HighSeq platform. The 16S rRNA genes were identified by using BowTie to compare reads to a database of 27K known 16S genes. Sequences with > 97% identity were linked, and operational taxanomic units (OTUs) were defined as the connected components within the resulting network of sequences. The GENMOD procedure of SAS was used to determine differences in OTU abundance due to diet, RFI classification, their interaction, and breed type.

Results Feed intake and average daily gain were greater ($P < 0.001$) in forage-fed wethers than those fed the concentrate diet. Residual feed intake ranged from -0.47 to 0.69 g/d for concentrate-fed wethers, and from -0.70 to 0.80 g/d for the forage-fed wethers. In total, 349 total OTUs were determined to be present in at least 1 of the 16 sequenced samples. Of the 20 most abundant taxa, all but one differed ($P < 0.001$) between concentrate and forage-fed wethers, with most of those taxa being of greater abundance in concentrate-fed wethers. Additionally, 12 of the 20 most abundant taxa differed ($P < 0.032$) in frequency due to RFI classification and(or) the interaction of RFI classification and diet. Species in the *Prevotella* genus represented one-half of the 20 most abundant taxa. *Prevotella ruminicola* had the greatest abundance of all OTUs, and was more ($P < 0.001$) abundant in forage-fed wethers as well as in low RFI wethers. This bacterium is primarily responsible for peptide breakdown in the rumen, leading to ammonia production and inefficient nitrogen retention in ruminant animals. The role of *Prevotella* species in methane metabolism has yet to be well understood. Both *Methanobrevibacter sp.* and *Methanobrevibacter smithii* were among the 20 most abundant OTUs. While there was no difference in *Methanobrevibacter smithii* according to RFI status, *Methanobrevibacter sp.* was unexpectedly in greater ($P <0.001$) abundance in low RFI (i.e. better feed efficiency) wethers. However, *Mitsuokella jalaludinii* was more ($P < 0.001$) abundant in low RFI wethers than high RFI wethers regardless of diet type. *M. jalaludinii* has been demonstrated as an efficient methane-reducing agent in the rumen.

Conclusions These results suggest that rumen microbial populations are influenced by diet type, and that differences in these populations are associated with phenotypic variation in feed efficiency in sheep. Further examination of individual OTU abundance differences indicated that while methanogenic species abundance was inconsistently related with feed efficiency status, the abundance of a known methane-reducing agent was strongly associated with improved feed efficiency. This may suggest that the favorable relationship between better feed efficiency and lower methane production may be due to an increased ability to reduce methane in the rumen as opposed to lower abundance of actual methanogenic species. Future work will include investigation of low abundant OTUs in this sequence data set, and (or) elucidation of rumen methanogenesis and methane reduction/oxidation pathways through network inference and gene expression analyses.

Acknowledgements The authors gratefully acknowledge funding from the University of Wyoming Agricultural Experiment Station (Laramie, Wyoming, USA).

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Computing greenhouse gas emissions from milk production in the Netherlands – a life cycle assessment

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Introduction Along the dairy chain, emissions of nitrous oxide (N_2O), carbon dioxide (CO_2) and methane (CH_4) cause global warming. Dutch policymakers and dairy sector, therefore, aim for a 30% decrease in greenhouse gas (ghg) emissions in 2020 regarding the level in 1990. In the past, many studies quantified the ghg emission resulting from milk production in the Netherlands using a life cycle assessment (LCA). The performance of a production chain can be assessed based on input-output data at sector level (top-down) or on aggregating environmental scores of individual farms (bottom-up). A bottom-up approach has the advantage that variation among farms is assessed, and it can be used to deduce mitigation options in order to meet policy objectives. The aim of this study, therefore, is to quantify the ghg emissions for 4 years from specialized dairy farms in the Netherlands using a bottom-up LCA.

Material and methods GHG emissions were derived from a cradle-to-farm-gate LCA and expressed in kg CO_2-eq. per kg of milk delivered to the milk processing industries. All farms were highly specialised dairy farms. We performed an attributional LCA. Whenever a multifunctional process occurred, mass allocation was used. IPCC (2007) characterization factors were applied. The model covers the emissions caused by the production and use of energy carriers, fuel consumption of agricultural services on-farm and the production of inputs off-farm (i.e. mineral fertiliser, feed, seeds and young plants, transport of received animal manure, pesticides, sawdust and straw and animals). Also the emissions from soil (direct and indirect), emissions of manure production and storage and enteric fermentation were taken into account. Emissions caused by land use and land use change (LULUC) and carbon sequestration were excluded from the system assessed. Emission factors were obtained from various sources such as the protocols for the National Inventory Report (NIR), Feedprint model and Vellinga *et al.* (2012). The Agricultural Economics Research Institute (LEI) continuously collects economic, technical and environmental data from a randomly selected stratified sample of Dutch dairy farms in the Farm Accountancy Data Network (FADN). These include data on, for example, quantity and type of feed used, quantity of non-renewable energy used, manure application techniques, forage yields and detailed information on housing facilities. Due to the design of FADN, aggregation of results at farm level to a sector of national level is possible, using a weighting scheme based on farm type, farm size (in Euro standard output) and region.

Results The average ghg emission varied between 1.25 and 1.28 kg of CO_2-eq. per kg milk over a period of four years (table 1). Approximately 90% of the dairy farms had an emission between 1.0 and 1.8 kg of CO_2-eq per kg milk. 70% of all emissions occurred on-farm. On-farm, methane emission from enteric fermentation was the most important contributor (0.50 kg CO_2-eq per kg milk). Off-farm, cultivation and production of concentrates had the highest impact (0.22 kg CO_2-eq per kg milk). Farms with a high production per cow and a low nitrogen input per unit of roughage produced had the lowest emission (Reijs *et al*., 2013).

Table 1 Farm descriptive and greenhouse gas emission in the Netherlands, 2008-2011 (source: Reijs *et al.*, 2013; FADN)

	2008	2009	2010	preliminary 2011
Sample farms	267	271	281	288
Farms represented	17,296	17,065	17,262	16,881
Average number of grazing animals	96.5	98.2	103.3	102.3
Average ha of utilized agricultural land (ha)	46.4	46.6	47.0	47.3
Average number of milk delivered per ha (kg)	13,009	13,347	13,972	13,655
Greenhouse gas emission (kg CO_2-eq / kg milk)	1.26	1.25	1.25	1.28

Conclusions The ghg emission of Dutch dairy farms did not decrease over the last 4 year. The computed average greenhouse gases are within the range of results of other studies. FADN enables to aggregate ghg emissions at farm level into a sector score using a life cycle assessment and therefore is a useful data source for a product, farm or national level computations. Methodology can be improved when the FADN weighing scheme included additional farm characteristics correlated with environmental performance.

Acknowledgements The authors gratefully thank the Sustainable Dairy Chain Initiative for funding this research and Wageningen UR Livestock Research for making available important data about methane emission factors (Feedprint).

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Periprandial methane production in late pregnant and early lactating German Holstein cows

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Introduction In ruminants, methane is predominantly produced in the rumen during anaerobic digestion. The amount of feed ingested is the major factor that determines the amount of methane production during digestion. However, knowledge on this relationship is primarily based on long-term measurements (1-5 days) but associations between periprandial feed intake and methane production have not been investigated in cows up to date. Therefore, the aim of the present study was to assess the rate of methane production in response to single feed intake events.

Material and methods Prior to the experiment, 24 German Holstein cows (2^{nd}-5^{th} lactation; >8.000 kg milk in the previous lactation) were halter-trained and well adapted to be housed in respiration chambers. Each 6 weeks before and 2 weeks after parturition, cows were transferred to open-circuit respiration chambers at an ambient temperature of 15°C, 60% relative humidity, and with light period between 0600 to 1900 h, as described previously (Derno *et al*., 2009). Body mass was measured directly before and after the transfer of the animals to the respiration chambers. Cows had free access to water and were milked at 0630 and 1430 h. The 24 h-experiment started on the next day at 0630 h with continuous record of gas exchange at 6-min intervals. Cows were fed ad libitum (0700 and 1500 h) a total mixed ration (TMR) according to their physiological state; during the ante partum (ap) period: 5.87 MJ NE_L and 128 g utilizable crude protein (nXP) per kg DM, during the *post partum* (pp) period: 7.06 MJ NE_L and 163 g nXP/kg DM. Feed intake was determined by feed disappearance every 6 min as measured by a scale connected to an electronic registration device. The CH_4 concentration in the chamber was analyzed by infrared-absorption (UNOR 610, Maihak, Hamburg, Germany). To determine time-dependent interrelationships between feed intake and CH_4 production, time series analyses were performed for each individual animal as described previously (Derno *et al*., 2013). To this end, cross-correlation functions were estimated using Proc Timeseries of SAS® (version 9.2; SAS Institute Inc., Cary, NC). The sample cross correlation function is an estimate of the correlation between two time series at lags k = 0, ±1, ±2, ± 3, …. The lags used for the calculation of cross-correlation functions were equidistant 6 min for feed intake and CH_4-production, respectively. The maximum or minimum of these cross-correlation functions defined the corresponding time lag $\hat{\tau}$

Results Both during the ap and pp periods the individual animal CH_4 production rate (g/min) varied up to 85% over the whole 24 h-period investigated. Cross-correlation analysis revealed that each feed intake event strongly paralleled an increase in CH_4-production. In this context, each maximum of a feed intake event was followed by a CH_4-production maximum with an offset of τ ranging between 36 and 240 min among animals (Figure 1). The extent of prandial + postprandial CH_4-production increase correlated strongly with the amount of feed ingested during the corresponding feed intake event. During between-feed intake intervals, CH_4-production decreased in an exponential manner (Figure 1), particularly during times of lying.

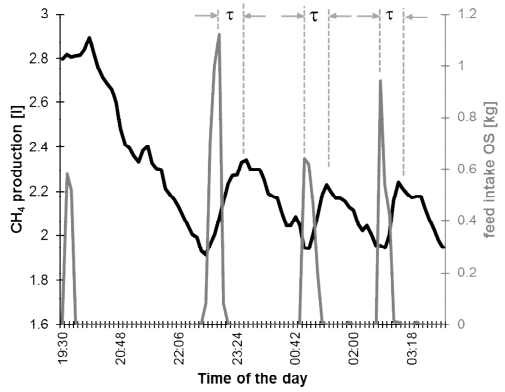

Figure 1 Time lag τ between feed intake and CH_4 production.

Conclusions Our results demonstrate that postprandial CH_4-production increase is highly variable between individuals and may last for up to 4 h. Thus, short-term CH_4-measurements during feeding only do not reflect average daily CH_4-production of an individual.

Acknowledgements The authors gratefully acknowledge funding from the German Research Foundation (DFG), and the help from the technicians of the 'Tiertechnikum' of the Institute of Nutritional Physiology "Oskar Kellner" at the FBN Dummerstorf.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Nitrous oxide emissions following cattle slurry applications to grassland on drained and undrained clay soil at different times of year

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Introduction Applications of livestock slurry to land, while returning valuable plant nutrients to the soil, can result in diffuse pollution to water and air, including emissions of nitrous oxide (N_2O) and ammonia (NH_3). Changes to the timing of slurry applications in response to the implementation of Nitrate Vulnerable Zone regulations and 'closed periods' when slurry applications are not permitted, may impact on potential losses to water and air. Soil drainage is often cited as a potentially effective N_2O mitigation method, but published studies providing empirical evidence to support this are lacking. Here we report the impact of soil drainage and cattle slurry application timing on N_2O and NH_3 emissions from a grassland clay soil. This was part of a larger study on cracking clay soils in England to assess the effect of contrasting slurry application timings on diffuse pollution to water and air.

Material and methods The experiment was conducted at North Wyke, Devon, UK, using 1 ha hydrologically isolated grassland plots on a clay loam soil overlaying an impermeable clay subsoil (the Rowden facility). Half of the plots are drained, with tile drains at 40 m spacing and 85 cm depth, with permeable backfill to within 30 cm of the surface, and with secondary mole drainage at 40 cm depth and 2 m spacing. Cattle slurry was applied at a target rate of 50 m^3 ha^{-1} using a trailing shoe slurry spreader to each of three replicate drained and undrained plots at three times of year: autumn (September 2008), spring (early May 2009) and summer (late June 2009). At each application timing three replicate control plots, receiving no slurry, were also established on both the drained and undrained treatments. Nitrous oxide emissions were measured from each plot following slurry application, using the static chamber method, with 5 replicate chambers located in a sub-plot at the centre of each 1 ha plot. Emission measurements were made on up to 40 occasions over a 12 month period following each slurry application, with a greater frequency of measurement in the first few weeks, as this is when the majority of emissions were expected to occur. An annual emission factor was derived for each slurry application timing and drainage treatment as the net (of control value) annual cumulative N_2O-N flux expressed as a percentage of the slurry N applied. Ammonia emissions from slurry-treated plots were measured for 7 days following slurry application, using the micrometeorological mass balance technique, employing passive flux samplers deployed on masts located at the centre and upwind edge of each plot. Indirect N_2O emissions from N deposition associated with the measured NH_3 emissions were estimated by applying the IPCC default emission factor of 1%.

Results Preliminary analysis of the data show that net direct N_2O-N emissions were greatest following the summer application (2.5% of applied slurry N) and lowest following the autumn application (Table 1). The influence of drainage was significant ($P<0.05$) only for the spring application, where net emission from the undrained soil was considerably greater (c. 10-fold) than from the drained soil. Following the summer application, emissions were numerically greater from the drained treatment, but not significantly so ($P>0.05$). Notably, soil moisture, as well as other environmental factors, will influence total N loss via denitrification and the ratio of the products of denitrification, N_2 and N_2O. Results from this study suggest that the hypothesis that drainage of poorly drained soils will reduce N_2O emissions is too simplistic. Ammonia emissions were related to environmental conditions at the time of application (temperature, wind speed, rainfall) and were greatest from the summer application and lowest from the autumn application. There was no influence of soil drainage status on NH_3 emissions, which accounted for 9, 12 and 15% of applied slurry N for the autumn, spring and summer application, respectively.

Table 1 Nitrous oxide and ammonia emissions following cattle slurry applications

Application	Soil drainage status	N applied (kg ha^{-1})	Net N_2O-N emission (kg ha^{-1})	N_2O-N emission factor (% N applied)	NH_3-N emission (kg ha^{-1})	Indirect N_2O-N associated with NH_3 emission (kg ha^{-1})
Autumn 2008	Drained	148	-0.5	-0.3	11.7	1.2
	Undrained	148	-0.3	-0.2	14.2	1.4
Spring 2009	Drained	154	0.4	0.3	19.0	1.9
	Undrained	154	4.1	2.7	17.6	1.8
Summer 2009	Drained	155	4.7	3.0	25.0	2.5
	Undrained	155	3.1	2.0	22.0	2.2

Conclusions Net direct N_2O-N emissions following cattle slurry application to grassland on a clay soil at different times of year ranged from zero to 3% of the applied N. There was no consistent effect of soil drainage on direct N_2O or NH_3 emissions. Indirect N_2O emissions from N deposition associated with NH_3 emissions were as important as direct emissions.

Acknowledgements The authors gratefully acknowledge funding from Defra, UK, and technical assistance from Mark Butler, Rebecca Murray, Sarah Gilhespy, Rachel Matthews and the North Wyke laboratory team.

The association between growth and methane emissions of finishing beef cattle using an experimental trial designed to estimate breed and sire differences

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Introduction Beef cattle are associated with a large carbon footprint per kg meat produced in comparison to non-ruminant farm animals. As a consequence, reduction in methane emissions in beef cattle is a high research priority. Genetic improvement is one possible mechanism for long-term mitigation of methane production, if methanogenesis can be shown to have a genetic component. Even if there is a genetic component, direct selection for reduction in methane emissions is a challenge, because measurement of methane, e.g. in respiratory chambers, is only possible on a small number of animals in experimental trials. Therefore, indirect selection based on traits already recorded in beef breeding programmes would be the method of choice. Growth rate is recorded in all beef breeding programmes, whereas other traits such as feed conversion rate are only recorded by some breeding organisations and then only on a small sample of animals. To investigate the impact of growth rate on methane emissions, an experiment was carried out using different breed types with structured sire groups within breed. The objective of the study was to investigate differences between breeds and among sire groups within breed, as an indication for genetic control of methane emissions and growth rate. In addition, the interactions of breed (sire) effects and diets were estimated.

Material and methods The data were obtained from a 2x2 factorial experimental design using 72 steers from a two-breed rotational cross between Aberdeen Angus (AA) and Limousin (LIM) at the Beef Research Centre of Scotland's Rural College. Equal numbers of experimental animals were sired by purebred AA and LIM. Depending on the sire used, the expected additive genetic contributions were 2/3 and 1/3 of each of the two breeds. Progenies were from 5 AA and 4 LIM sires. The average number (range) of progenies per sire were 7 (2 to 12) and 9 (6 to 14) for AA and LIM, respectively. Two diets were used, a predominately concentrate-based and a mixed forage-concentrate-based diet with forage:concentrate ratios (dry matter (DM) basis) of 8:92 and 48:52, respectively. Average daily gain (ADG) was measured during the finishing period over an average of 142 ± 24d. Methane emissions were individually measured for 48h within 6 respiration chambers. The animals were allocated to the respiration chambers in a randomised block design with 3 replicates. Data on 4 animals could not be considered due to health issues and an air leak of the respiration chamber. Least Squares Means (LSM) were estimated using the GLM procedure of SAS based on a model including effects of breed (or sire within breed) diet, respiration chamber and a randomised block.

Results The LSM for daily methane emissions were different ($P<0.05$) at 184 g/d and 164 g/d for AA and LIM respectively, but not significant for methane emissions per kg ADG. The LSM for ADG were 1.310 and 1.146 kg (both s.e. 0.027 kg) for AA and LIM, respectively. There were no breed (or sire) by diet interactions ($P>0.05$). The sire group had a significant influence within breed on methane emissions, whether expressed as g CH_4/d (Figure 1) or g CH_4/d per unit of daily gain (Figure 2). In contrast to the breed comparison, the sire group LSM for methane emissions per kg growth in Figure 2 showed similar ranking to those of daily methane emissions (Figure 1). In particular for LIM crosses, the relative differences of LSM increased between significant sire groups. In contrast for AA, one sire group LSM (AA5) changed in ranking relative to others due to consideration of growth rate.

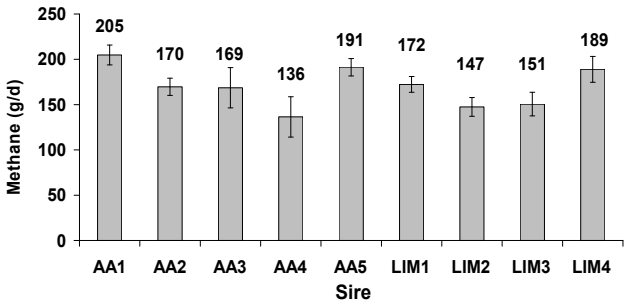

Figure 1 LSM of sire groups in methane emissions in g/d

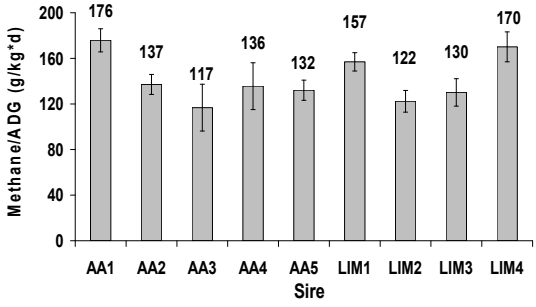

Figure 2 LSM of sire groups in methane per 1 kg growth/d

Conclusions Breed differences in daily methane emissions are due to higher ADG in AA than LIM. Differences among sire groups in methane emissions were of high magnitude, suggesting a substantial opportunity for mitigation of methane by genetic improvement. The influence of ADG on sire differences in daily methane emissions were small, indicating that there is most likely a substantial genetic regulation of the host on methane production of the microbes, which is not associated with the growth rate of the host. However, the small relative increase in sire group differences, when considering ADG, suggests that reductions in methane emissions are favourably associated with growth. There were no significant genotype by diet interactions, which would have made an implementation of selection for methane mitigation even more of a challenge.

Acknowledgements The authors gratefully acknowledge funding by the Scottish Government directly and a combination of Defra, the Scottish Government, DARD, and the Welsh Government as part of the UK's Agricultural GHG Research Platform project (www.ghgplatform.org.uk), as well as the excellent technical support at SRUC.

https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Predicting enteric methane emissions from dairy cattle using Bayesian methods

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Introduction It is generally accepted that enteric methane emissions from cattle contribute to anthropogenic greenhouse gas emissions that are responsible for climate change. National inventories calculate enteric emissions based on emission factors, however, their accuracy has been challenged (e.g., Kebreab *et al*., 2008). Moreover, several mitigation options cannot be evaluated using emission factor based predictions. Although mechanistic models are generally more accurate, national inventories and examination of methane mitigation strategies should not require very detailed information as model inputs. The aim of the present study was to identify key variables in the prediction of methane emissions, and fit methane prediction equations with associated uncertainties using a large database of dairy cattle.

Material and methods A database containing 1,111 indirect calorimetry records of dairy cattle in 62 studies conducted from 1963 to 1995 in the former USDA Energy Metabolism Unit at Beltsville, Maryland was assembled. The database is composed of individual records of total energy balance trials from Holstein and Jersey lactating cows. The data contained a wide range of dietary variables (e.g. neutral detergent fibre (NDF) ranged from 15 to 76% of diet DM). Model development was conducted in a sequential approach, with model complexity increasing at each level. The independent variables, which play a key role in predicting methane emissions, were selected through a Bayesian model selection procedure. Variables were identified through a reversible jump Markov Chain Monte Carlo method (RJMCMC) sampler. The RJMCMC is a natural extension of the Markov Chain Monte Carlo algorithm (MCMC), in which the dimension of the parameter space is allowed to vary at each iteration of the Markov Chain. The chain is allowed to "jump" between different models and the model selection is based on the competing models posterior probabilities (Lunn *et al*., 2009). In this study, variables were selected under a linear hierarchical model. At the dietary level, diet characteristics (fiber fractions, crude protein, ether extract, lignin and metabolizable energy) were allowed to be selected, as well gross energy intake (GEI). At the animal level, milk composition (fat, protein and nonfat soluble) and animal information (body weight (BW) and breed) could also be selected, as well variables from the dietary level. The RJMCMC procedure was implemented in the Jump interface of the Bayesian statistical software WinBUGS (Lunn *et al*., 2009). In the second part of the data analysis, parameters were estimated under the selected models at each complexity level. A Bayesian framework was adopted, in which parameter posterior distributions were estimated by MCMC. A linear hierarchical model was specified, in which study and animal determined a cross classified covariance structure. The Bayesian hierarchical model specifies a model for the data, conditional on model parameters, through a student-t distribution:

$$y_{ijk} \mid \boldsymbol{\beta}, \alpha_i, \delta_j, \tau, \nu \sim t\left(\mathbf{X}'_{ijk}\boldsymbol{\beta} + \alpha_i + \delta_j, \tau, \nu\right) \quad [1]$$

where y_{ijk} denotes the k^{th} ($k = 1, \ldots, n_{ij}$) observation on the i^{th} ($i = 1, \ldots, \text{I}$) animal in the j^{th} ($j = 1, \ldots, \text{J}$) study, $\boldsymbol{\beta}$ is the vector of structural parameters, α_i is the animal random effect, δ_j is study random effect, τ is the precision (i.e., $1/\sigma_e^2$), ν is the degrees of freedom parameter of the student-t distribution, $\boldsymbol{X}_{ijk}$ is the vector of covariates of the k^{th} observation on the i^{th} animal in the j^{th} study.

Results Gross energy intake was the key variable in predicting methane emissions. Dietary proportions of NDF and ether extract (EE) were present in the models selected at the dietary and animal levels of lactating cows. The probability of BW being included in the most probable model was 0.99. The high marginal probability of milk fat being included in the model (P = 1) suggests that milk fat percentage has an important role in predicting methane emissions. Breed's marginal probability was low (P < 0.13) so it was not used to build equations. The following equations were developed based on the procedure outlined above.

CH_4 (MJ/d) = 3.25 (0.42) + 0.043 (0.001) x GEI (MJ/d) [2]

CH_4 (MJ/d) = 0.2242 (0.71) + 0.042 (0.001) x GEI (MJ/d) + 0.124 (0.01) x NDF (% DM) - 0.329 (0.09) x EE (% DM) [3]

CH_4 (MJ/d) = - 9.317 (1.06) + 0.042 (0.001) x GEI (MJ/d) + 0.094 (0.01) x NDF (% DM) - 0.381 (0.09) x EE (% DM) 0.008 (0.001) x BW (kg) + 1.622 (0.11) x Milk Fat (%) [4]

A K-fold cross validation analysis was conducted and the root mean square prediction error (% of the observed mean) was 18.1, 17.9 and 15.6% for equations [2]-[4], respectively; while prediction based on IPCC tier 2 equation (IPCC, 2006) had 30.1%.

Conclusions Three prediction equations for lactating dairy cows have been developed using Bayesian methods. Dietary variables GEI, NDF and EE were selected with high probability as well as animal factors BW and milk fat percentage. As model complexity increased, model uncertainty was reduced. The models offer flexibility for the user depending on what level of input is available. For example, model [2] could easily be adopted for calculating emissions in national inventories in most developed countries.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Temporal and spatial variation of CH_4 and CO_2 concentrations in and around lying cubicles of dairy barns

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Introduction. Methane (CH_4) emissions from dairy cows accounts for a significant portion of the global CH_4 budget which is a substantial contributor to the Global Greenhouse effect. There are already several methods developed for measuring methane emission from individual cows. Still an accurate and economic method is lacking to assess methane variation between cows on a long term in commercial farm conditions. Monitoring individual methane emission of cows resting in the cubicles is a promising method without disturbing the normal life of cows in a free stall production system. However, existing information about distribution of CH_4 and CO_2 concentrations in and around cubicles is insufficient to develop a method for measurements in cubicles. The aim of the present study was to explore the level and dynamics in time and space of CH_4 and CO_2 concentration at different positions in non-occupied and occupied cubicles, and to identify relevant factors to be analysed for developing a method for measurements in cubicles.

Material and methods. This experiment was carried out from 28th July to 1st October 2012 in an experimental dairy cow house of Wageningen University. The dairy cow house was equipped with a natural ventilation system, which included large openings on the side walls and an open ridge, and slatted floors in the cow area with manure storage below (Figure 1). There were 24 cows housed in the barn (32 cubicles) during this experiment which received the same feeding management. Cows were fed once daily at 1100h, and milked twice daily at 0600 and 1630 h. Manure on the slatted floor was scraped every day. The cows could go out of the barn during the day. There were in total nine sampling points installed in a row of 6 cubicles at the same height of 0.3m and one extra sampling point at the height of about 3.5m above the central cubicle (Figure 2). All sampling tubes were connected with a pump that gases were sucked continually. And all sampling tubes were put inside the cubicle framework to prevent damage by cow contact. Two cubicles (A and B in Figure 1 and 2) were open for free access, and four adjacent and four opposite cubicles were barred. Gas concentrations of CH_4 and CO_2 were measured with a photoacoustic gas monitor. A multi-point switcher connected all ten sampling lines to the analyser. Each position was sampled and analysed during three minutes intervals, resulting in two records per point per hour.

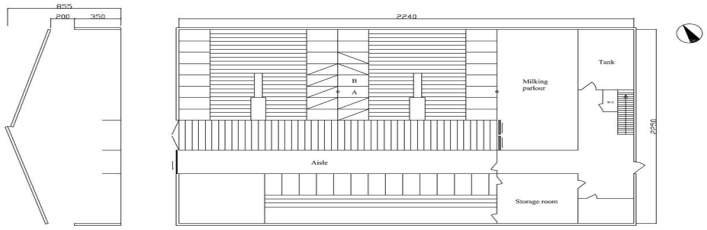

Figure 1 Layout of the experimental barn

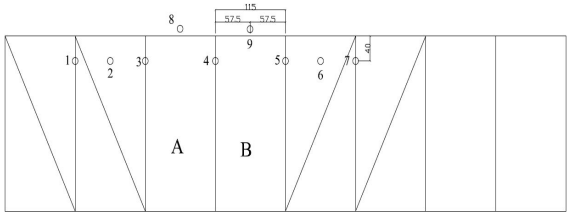

Figure 2 Distribution of sampling points around the cubicles

Results. Mean CH_4 and CO_2 concentrations at points 1-9 around the cubicles (Figure 2) varied between 30.6~38.6 ppm and 584~659 ppm, respectively. Both concentrations showed certain spatial pattern in the direction from point 1 to 7 (Table 1); decreasing from point 1 and starting to increase from position 4, reaching its peak at points 6-7. As such, concentrations were not highest at the positions close to the cows (i.e., 3, 4 or 5), but they were highest close to the wall (i.e., 6, 7). The ratios between CH_4 and CO_2 were about 0.047 and comparably consistent between different points, indicating that CH_4 and CO_2 mixed equally well. Mean gas concentrations at sampling points increased by cubicle occupancy of cows while overall spatial concentration patterns were not affected. Typically, mean CH_4 concentration at points 4 and 8 increased two and three fold, respectively, from empty to occupied cubicles. CH_4 concentration during day time (around 15 ppm) was lower than during night time (around 40 ppm), but was still about twice as high as outside atmosphere (around 6 ppm). When the cows were not in the barn, the only CH_4 emission source was manure.

Table 1 Means and standard errors of CH_4 and CO_2 concentrations at different sampling points around the cubicles

Sampling points:	1	2	3	4	5	6	7	8	9
CH_4 [ppm]	34.7±1.5	30.6±1.4	30.6±1.3	31.0±1.6	34.8±2.2	38.6±2.3	38.5±1.9	36.2±2.1	34.5±1.5
CO_2 [ppm]	627±12	584±10	584±10	613±11	607±9	644±13	659±13	659±16	627±11
Ratio (CH_4/CO_2)	0.048 ±0.001	0.046 ±0.001	0.046 ±0.001	0.044 ±0.001	0.049 ±0.001	0.050 ±0.001	0.049 ±0.001	0.045 ±0.001	0.049 ±0.001

Conclusions. Based on this preliminary study, the following conclusions were drawn: 1) Spatial distribution of CH_4 and CO_2 concentrations around the cubicles was mainly determined by the airflow pattern in the house, and only marginally by presence of cows in the cubicles; 2) CH_4 and CO_2 mixed equally well; 3) Diurnal variation of CH_4 and CO_2 concentrations existed around the cubicles because of different ventilation rates and emission sources between day and night time; 4) CH_4 emission from the pit can be an important factor to be considered when analysing and interpreting data of CH_4 and CO_2 concentrations around cubicles under practical situations.

Acknowledgements The authors gratefully acknowledge funding from Wageningen UR Livestock Research, the ministry of Economic Affairs (through KB Sustainable Agriculture) and China Scholarship Council.

https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Enteric Methane emissions estimates for dairy cows in South Africa: 2006 IPCC guidelines approach

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Introduction Methane emissions form enteric fermentation is one of the main sources of methane in South Africa. Reasonable estimates in this category would be helpful for climate change mitigation decision making and policy making. The main objective of the study was to determine emissions factors for 4 main dairy cattle breeds in South Africa and to estimate enteric methane emissions from 1999 to 2008.

Materials and methods Livestock population data was obtained from the annual animal statistics from 1999 to 2008. Annual proportions were determined according to the proportions obtained from the country milk recording scheme (ARC, 2008). Activity data to be utilized in estimating emissions factors for the dairy cattle were collected as per the 2006 IPCC guidelines (IPCC, 2006). Trends in emissions estimate were determined using the Mann-Kendall statistic.

Results Holsteins contribute over 50% of the total commercial dairy cattle followed by Jerseys (>20%), Ayrshires (around 5%) and Guernseys (around 1%). Animal weights of the 4 different breeds varied a lot with an average of 600kg, 415kg, 500kg and 475kg for Holsteins, Jerseys, Ayrshires and Guernseys respectively. There were also marked differences in the annual milk production per animal with the highest values exceeding 7000kg(Holsteins) and the lowest value of around 5000kg(Jerseys). There were small variations in other activity data like fat content. The resultant emission factors for all the breeds are 144, 107, 117 and 116kg/head/annum for the Holstein, Jersey, Ayrshire and Guernsey respectively. The results show total enteric methane emissions from commercial dairy cattle farming exceeding 80Gg with over 50% being from Holsteins. There is no significant difference between the results obtained between all the years and the results don't show any significant decreasing or increasing trend in emissions.

Conclusions Careful consideration of feeding methods, efficiency and promotion of less intensive breeds in South Africa forms part of the issues that can be addressed by mitigation policy makers owing to high emissions from some of the main dairy breeds. The country specific emissions factors for cattle were significantly higher than that of the IPCC Africa default values. This might be due to vast differences in farming methods (productivity level higher due to commercialization of the dairy farming) in South Africa as compared to most of the least developing countries in Africa.

Acknowledgements The study was done under the funding of British High Commission (South Africa) administered by the Department of Agriculture, Forest and Fisheries.

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A decision tool to evaluate greenhouse gas mitigation policies for the agriculture sector

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Introduction It is generally accepted that the rapidly increasing concentration of greenhouse gases (GHGs) in the atmosphere is contributing to climate change. In order to slow down the increase in the concentration of these gases, all sectors of the economy, including agriculture, are expected to find ways to reduce their GHG emissions. For agriculture, the challenge will be great because of the increasing demand for food, particularly for high protein diets, which require large land areas for livestock feed production, and because of the competing demand for crops for biofuel feedstock (Dyer *et al*. 2011). The aim of this presentation is to demonstrate the use of a decision tool for policy makers which estimates the impact of large-scale agricultural management changes on the sectoral GHG emissions budget and can help to identify priority areas for research to reduce emissions and increase accuracy of estimates.

Material and methods This study is based on a newly developed tool, the Unified Livestock Industry and Crop Emissions Estimation System (ULICEES), which is an agricultural land use and GHG emissions model. ULICEES is designed to estimate the GHG emissions associated with the four main livestock industries in Canada (beef cattle, dairy cattle, hogs, and poultry) and the complex of crops that feed them (Vergé *et al*. 2012). These crop complexes are based on livestock ration records and population estimates for Canada. The main GHGs emitted from the agricultural sector are methane, nitrous oxide and carbon dioxide from energy use. Methane and nitrous oxide emissions are estimated using the IPCC methodology, adjusted for conditions in Canada, whereas the carbon dioxide emission estimates from energy use are based on studies by Dyer and Desjardins (2009). Soil carbon change must be considered in any agricultural GHG mitigation strategy as it affects the atmospheric carbon dioxide concentration (Smith *et al*. 2000).

Results The potential of ULICEES is demonstrated by examining the impact on the agricultural GHG budget of reallocating 10% of protein production in Canada from a ruminant (beef) to a non-ruminant (hog) source in 2001 (Vergé *et al*. 2012). The crop areas required to support the beef and hog industries before and after this reallocation are estimated using ULICEES and the decrease in GHG emissions resulting from the reduction in beef production and the additional GHG emissions from the increase in hog production are presented in Table 1. Nationally, this 10% shift was found to reduce the GHG emissions by 2.5 Tg CO_2e yr^{-1}, and would result in 275,000 ha of surplus land. The total impact of this shift depends on what is done with the surplus land. Table 2 presents the net change in GHG emissions, over a 40 year period, for four land use scenarios when we also include the emissions associated with the surplus land. The 40 year period is assumed to be the time required for the soil carbon to reach a new equilibrium after a change in management practice and a negative value implies a reduction in GHG emissions and/or carbon sequestration.

Table 1 Comparison of annual GHG emissions from the Canadian beef and hog industries, prior to and following reallocation of 10% of beef protein production to pork protein production for 2001.

		Greenhouse gas emissions (Tg CO_2e)				Land Area
Industry	Condition	CH_4	N_2O	CO_2	All GHGs	10^3 ha
Beef	Baseline	17.4	10.4	3.3	31.0	5,944
Pork	Baseline	3.1	2.2	1.8	7.1	2,860
Beef	vs. baseline after reallocation	-1.7	-1.0	-0.3	-3.1	-594
Pork	vs. baseline after reallocation	0.3	0.2	0.2	0.6	319
All	Net Change	-1.4	-0.9	-0.2	-2.5	-275

Table 2 Changes in soil carbon and in GHG emissions in Tg CO_2e over a 40 year period under four scenarios for the residual land.

Scenario*	1	2	3	4
GHG emissions (Tg CO_2e)	-92	-16	-6	+9

* Scenario 1: residual land converted to perennial forage; Scenario 2: residual land seeded to annuals; Scenario 3: residual land converted to beef production (mixed forage/grain diet); Scenario 4: residual land converted to beef production (mainly forage diet)

Conclusions It was demonstrated that ULICEES is a tool that can be used by policy makers to objectively examine how to reduce GHG emissions from the agricultural sector. Several other applications of ULICEES will be presented including the impact of substituting 10% of dairy milk protein with soy milk protein. We will also show how this tool can be used to identify where research could help improve these estimates. Although currently developed for Canada, such a tool can easily be adapted for other countries.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Evaluation of the effect of toxin from different strains of Bacillus thuringiensis in the production of gases from in vitro rumen fermentation

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Introduction *Bacillus thuringiensis* (Bt) produces, at the time of its sporulation, crystalline protein inclusions (d-endotoxin) that has nematicide activity against some genres, Teladorsagia, Nippostrongylus, Ancylostoma and, especially, *Haemonchus contortus*, one of an endoparasite of great interest due to its high occurrence and resistance to drugs. Haemonchosis is one of the main limiting factors of sheep production worldwide. However, little is known about the activity of these toxins in the ruminal microbiota, and, consequently, on metabolism and performance of ruminant animals. The aim of this study was to evaluate the action of different strains of *Bt* toxin in the gases produced within the rumen fermentation process using the *in vitro* gas production technique.

Material and methods The *in vitro* technique was the semi-automatic system to evaluate methane production according to Bueno *et al.* (2005) and Longo *et al.* (2006). Sheep ruminal liquid inoculum was used. The inocula were prepared using liquid and solid fractions in equal proportions (50: 50) of ruminal contents of different animals. In fiber teabags (ANKOM F57), 0.5 g of substrate, composed of 30% concentrate and 70% grass hay Tifton (*Cynodon* spp.) were added and placed in 160 mL glass bottles along with 25 mL of inoculum, 50 mL of buffered solution and 100 µl of different *Bt* strains (907, 1192, 2036, 2493, 2496 and S1185), providing 54,577 spores/75 mL bottle. The control group received no strain of *Bt*. Subsequently the bottles were sealed with rubber stoppers, agitated and brought to 39° C incubator for 24 hours. The pressure of the gases produced was measured with a pressure gauge at regular intervals of 4, 8, 12 and 24 hours. During the measurement of pressure, 2.5 mL of gas from each bottle were sampled for the quantification of methane in Shimadzu gas chromatograph (column "Shincarbon ST micro packed" to 60 ºC and FID detector to 240°C). After 24 hours of incubation, the fermentation was interrupted and the bags were recovered for true degradability of organic matter (DOM) (Van Soest *et al.*, 1991). The experimental design was a randomized complete blocks with seven treatments and four replications, each inoculum a repetition. The data obtained were analyzed by analysis of variance using the PROC GLM from SAS ® 9.2, using the average of the squares with PDIFF for comparison of means ($P < 0.05$).

Results Among the strains of Bt, the values of net total gas production were similar and greater relative to the control ($P < 0.05$). The net production of methane gas was similar among treatments; however, the values of methane production in relation to the total gas production (%) of different strains of Bt were smaller relatively to the control ($P < 0.05$). Among the strains, 2493 and S1185 were statistically different, with S1185 being lower ($P<0.05$). The values of truly degraded organic matter (g/kg) of different strains of Bt were similar to each other, with Bt strain 907 being lower than control diet ($P < 0.05$).

Table 1 Net total gas production (TG), net production of methane (CH_4), methane production relative to the total gas production (CH_4/GT) and DOM (true degradability of organic matter) of the substrate containing the different strains of Bt (907, 1192, 2036, 2493, 2496 and S1185)

	Control	Bt 907	Bt 1192	Bt 2036	Bt 2493	Bt 2496	Bt S1185	s.e.m.
TG (mL/gDM)	95.5 ^{b}	130.7 ^{a}	130.4 ^{a}	130.0 ^{a}	133.3 ^{a}	129.9 ^{a}	128.4 ^{a}	30.6541
CH_4 (mL/gDM)	10.7	10.4	10.3	10.0	11.9	11.3	9.8	1.6362
CH_4/ TG (%)	9.5 ^{a}	5.0 ^{bc}	5.4 ^{bc}	4.7 ^{bc}	5.8 ^{b}	5.2 ^{bc}	4.0 ^{c}	2.8183
DOM (g/kg)	576 ^{a}	530 ^{b}	548 ^{ab}	541 ^{ab}	552 ^{ab}	547 ^{ab}	556 ^{ab}	23.1084

The least square means were compared using Tukey ($P < 0.05$) adjustment with PDIFF statement in SAS

Conclusions The presence of different strains of Bt resulted in higher net total gas production in ruminal fermentation with less methane gas concentration relative to the control.

Acknowledgements The authors thank the CNPq and CAPES funding.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Use of infrared thermography as an indicator of methane production and dry matter intake in sheep

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Introduction The current methods for direct measurement of methane production generally are laborious and/or expensive. Consequently, there is a demand for alternative assessments. The infrared thermography is an easy, fast and non-invasive method that determines skin temperature of animals. Therefore, the infrared thermography (IR) was evaluated to predict the methane production (MP) and dry matter intake (DMI) in sheep.

Material and methods Twelve 12-months ewe lambs were used. The thermographic photos were obtained using an infrared camera (FLIR® system series-i) using an emittance coefficient of 0.97 to measure the temperature (maximum, minimum and mean) at the left and right flanks. The photos were taken at -1h, -0.5h, 0h, 0.5h, 1h, 2h, 3h, 5h and 7h in relation to feeding time. The difference between temperatures at left and right flanks (left minus right) in each time of measurement was determined. The animals stayed in chambers for determination of methane production (semi-closed system) according to methodology described by Abdalla *et al.* (2012) during over two days. The regression analyses by time of IR measurement were carried out testing linear and quadratic effect of the difference between temperatures at left and right flanks on MP, DMI and MP per DMI. Pearson correlation analyses between the variables by time of IR measurement also were carried out. The minimum significance level for variables to stay in the model was 0.05 and the best fit regression equation was chosen according to higher r-square.

Results The significant regression obtained for DMI use the IR temperatures at the time of feeding (0h), as follows: DMI = 0.89 + 0.29*Point - 0.40*MaxT (R2=0.29), where Point and MaxT means the difference between point and maximum temperatures at left and right flanks respectively. The DMI had a negative and significant correlation with difference between point temperatures at left and right flanks (r=-0.52; p=0.022). The methane production had significant regressions with IR temperatures at three hours after feeding. The methane production per day showed the follow relationship with IR temperatures: MP = 12.06 + 0.74*Min2 (R2=0.19), where Min2 means the squared difference between the minimum temperatures at left and right flanks. Methane production demonstrated negative and significant correlation with Point (r=-0.47; p=0.038) and MaxT (r=-0.52; p=0.018) temperatures at three hours after feeding. The best fit regression obtained for MP per DMI was: MP/DMI = 13.71 - 2.41*Min - 0.98*Point2 + 7.54*Med2 (R2=0.51), where Min, Point and Med means the difference between minimum, point and mean temperatures at left and right flanks, respectively. And the MP per DMI had a positive and significant correlation with the difference between the average temperature at left and right flanks (r=0.55; p=0.017).

Conclusions These results showed that the best time to measure IR temperatures for predict DMI seems be at the time of feeding and, for predicting MP, three hours after feeding. Moreover, highlight the potential of IR measurements to predict methane production and dry matter intake. Further studies aiming to create a large database are needed to establish a precise regression equation for prediction of methane production from IR temperatures of animal's flank.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Methane production in sheep artificially infected with Haemonchus contortus or Trichostrongylus colubriformis

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Introduction Gastrointestinal nematodes in sheep are a major cause of economic losses in the sector. The effects of these internal parasites range from reduction in productivity until to death of the animal. Effects in digestion system such as increase in rumen fluid outflow rate, decrease in apparent digestion of organic matter and an increase in nitrogen (N) loss have been shown (Rowe *et al.* 1988), but it is not completely known how these parasites affect methane production. Methane emission by enteric fermentation is responsible for significant losses of dietary energy and is also known for its greenhouse effect. Therefore, the aim of this study was to quantify the methane produced by sheep artificially infected with *Haemonchus contortus* or *Trichostrongylus colubriformis*.

Material and methods Sixteen animals were used, of which six were experimentally infected with 10,000 L3 of *T. colubriformis*, six with 5,000 L3 of *H. contortus* and four were a non-infected control. Fecal egg counts were carried out one day before the beginning of the experiment (30 days after infection), aiming confirmation infection (2916 and 366 eggs/g to *H. contortus* group and *T. colubriformis* group, respectively). The animals received Tifton-85 hay (*Cynodon dactylon* sp.) with 7.1% of crude protein. For methane (C H4) measurement, the animals were kept in chambers (semi-closed system) according to Abdalla *et al.* (2012) during two consecutive days. The feed's offered and orts were weighed daily and daily dry matter intake (DMI) was calculated. Statistical analysis were carried out using a mixed model (MIXED procedure in SAS®) considering type of infection (*H. contortus, T. colubriformis* and control) as fixed effect and animal inside infection group as random effect. The means were obtained through least square means and means comparison were carried out using Tukey adjustment with "pdiff" procedure in SAS®.

Results The infection did not influenced DMI (P>0.05), however the methane production were higher (P<0.05) in infected animals (Table 1). The gastrointestinal infections may change the short chain fatty acids produced in rumen (Rowe *et al.* 1988 and Steel, 1972), which can induce a higher methane production.

Table 1 Dry matter intake and methane production per dry matter intake in sheep infected with *Haemonchus contortus* or *Trichostrongylus colubriformis*.

	Control	Haemonchus	Trichostrongylus	P
DMI (Kg/day)	0.68±0.03[a]	0.57±0.03[a]	0.66±0.03[a]	0.123
CH_4/KgDMI	13.91±1.06[a]	18.58±1.06[b]	20.03±1.06[b]	0.003

[a,b] Different letters in the same row means statistical difference (*P*<0,05).

Conclusions The animals infected with *Haemonchus contortus* or *Trichostrongylus colubriformis* emitted more methane, which can indicate a higher energy loss from their digestion process. Moreover, as the parasites infection reduces the animal's performance, the methane production per unit meat produced may be very high in infected herds.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Diet and milk yield effect on milk carbon footprint of dairy farms in Uruguay

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Introduction Global dairy production contributes about 3% of total anthropogenic greenhouse gas (GHG) emissions (Gerber *et al*., 2010). Consumers have increasing concern about the contribution to climate change of food products, and carbon footprint (CF) could become a trade barrier in the near future. Uruguay is the second largest global exporter of dairy products relative to its production (INALE, 2012). More than 90% of CF is explained on farm, so identifying farm management practices that minimize CF is of highest relevance. The aim of the present study was to compare CF of dairy farms with different management practices in terms of diets and milk productivity in Uruguay.

Material and methods Twenty four real dairy farms located in different soils and with a large variation in technology adoption and system management were analysed. Land use, dairy stock, diet management and milk yield were analysed using a model which integrated all animal and pasture activities that could be perform on a dairy farm. The model output was the CF and the contribution of each GHG to the CF. The methodology followed the IDF (2010) guidelines and used the IPCC equations and emission factors (IPCC 2006). The system boundaries were from cradle to farm gate. The functional unit used was one kilogram of fat and protein corrected milk (FPCM) and allocation between milk and meat was done using the physical method (IDF, 2010). In order to identify individual variables that better explained CF variation, Pearson correlation and simple regression analysis were performed between CF and: milk yield per cow, stocking rate, concentrate per cow (kg DM), concentrate on diet (%DM), grassland yield (kg DM/ha).

Results The main variables correlated with milk CF were milk yield per cow (-0.81, $P < 0.001$) and concentrate per cow (-0.71, $P < 0.001$). Regression analysis showed a decay relationship between CF and milk yield per cow (R^2=68%, $P < 0.001$) (Figure 1), consistent with results from Casey and Holden (2005). The relationship between concentrate per cow and milk CF was also a decay one (R^2=53%, $P < 0.001$) (Figure 2), which means that milk CF decreases as concentrate per cow increases.

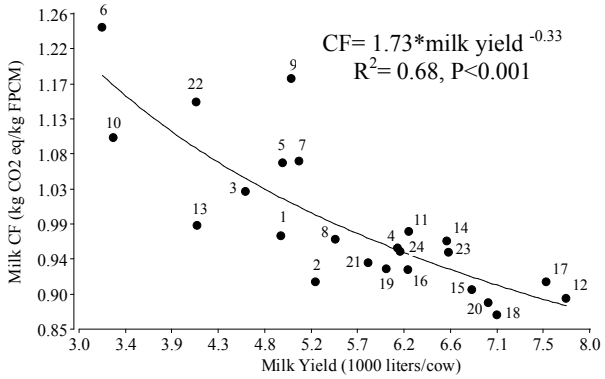

Figure 1 Milk yield (1000 kg FPCM/cow) effect on milk carbon footprint (kg CO2 e/kg FPCM)

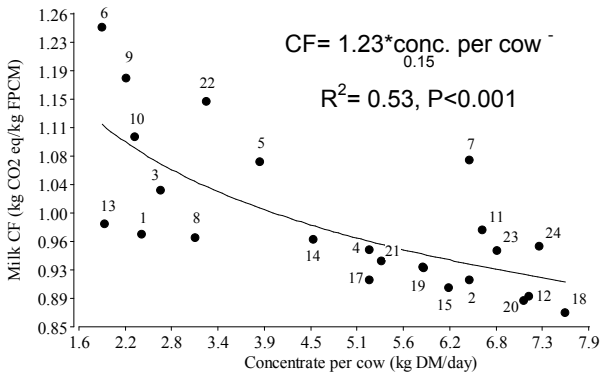

Figure 2 Concentrate intake (kg DM/cow/day) effect on milk carbon footprint (kg CO2 e/kg FPCM)

Conclusions These results shows that higher milk yield and concentrate per cow can reduce milk CF. Increasing milk yield seems to be an efficient practice for reducing GHG emissions. Although it know that supplying more concentrate increases milk production, in our study those results were not so correlated (0.79). This result might be explained by the fact that in Uruguay, the practice of supplying high levels of concentrate generally is a way of compensating for a lower forage allowance per cow.

Acknowledgements The authors gratefully acknowledge farmers from FUCREA who provided information.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Biomass production, elemental and fibre composition of Brachiaria produced under free air carbon dioxide enrichment conditions

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Introduction Potential effects of climate change on agriculture have being discussed for Brazilian conditions recently (Ghini, Bettiol, Hamada, 2011). Enrichment of atmospheric CO2 enhances the rate of growth of agricultural crops (Asseng *et al*., 2004). Almost 80 million ha of Cerrado land are planted with Brachiaria species, in which the beef cattle production in Brazil is grounded and this area supports much of the Brazilian beef industry. Little data exists on potential changes in chemical composition and nutritional quality of tropical forages for livestock production under the scenario of CO2 enriched atmosphere. The aim of this work was to determine the effects of two contrasting CO2 atmosphere: ambient and elevated (≈ 200 µmol mol-1 above ambient) CO2 concentrations upon the total biomass production, elemental and fibre composition a of *Brachiaria decumbens*.

Material and methods The free air carbon dioxide enrichment conditions (FACE) facility was established at Embrapa Environment (latitude 22°41'S, longitude 47°W, altitude of 570 m a.s.l.), Brazil, in order to generate field response data to elevated CO2 air concentration. Twelve 10-m-diameter octagonal rings located within a 7-ha field, six rings, representing the control treatment, were left under untreated conditions (current atmosphere), whereas other six rings have been treated with pure CO2 to achieve the concentration of 200 ppm above ambient concentration, supplied by a bulk CO2 container with the capacity of 20 t. Within each ring, two plots have been planted with Brachiaria decumbens cv basilic and after 10 weeks of growing (on January 2012), an initial cut for standardization was performed. Since then, forage availability has being estimated every 28 days. Samples of 0.25m2 were collected from each plot, through cutting with scissors the grazing portion of the stand (at 20 cm height). Collected samples have been split into two portions for determining the biomass availability, plant fractions and chemical determinations after dried at 55 °C for 72 h and ground to pass in a 1mm grinder. Biomass production and chemical composition was statistically analysed by Proc Mix (model = co2 (+ or -) date (from February to November 2012) plot (A and B) block (1 to 6)).

Results Elemental C, N and S composition (%) were not altered by enriched CO2 air concentration (44.2 vs 44.1 (s.e. 0.07); 3.2 vs 3.1 (s.e. 0.02) and 0.25 vs 0.24 (s.e. 0.003) respectively for enriched and ambient CO2 atmosphere). Biomass available, leaf fraction and ADF content were substantially altered by CO2 enrichment conditions (Table 1).

Table 1 Biomass available, plant and fiber fractions of Brachiaria decumbens cultivated under two free air carbon dioxide enrichment conditions

Brachiaria decumbens		FACE		s.e.	P
		+ CO2	ambient CO2		
Biomass available	g fresh / m^2	1442.15	1151.60	54.185	0.0001
	Kg DM / m^2	0.377	0.298	0.0164	0.0005
Plant fraction (%)	stem	17.92	16.85	0.577	0.1211
	leaf	80.10	81.62	0.675	0.0875
Fiber fractions (g/Kg DM)	NDF	644.21	637.92	3.300	0.1814
	ADF	313.59	306.75	2.098	0.0180
	LIG	61.42	58.80	2.705	0.5515
	CEL	252.17	247.95	3.118	0.2783
	HEMC	330.62	331.18	2.413	0.8098

Conclusions Despite the increase in pasture biomass available with CO2 enrichment atmosphere, the reduction on leaf proportion and increase ADF content of the material may lead to worries regarding to the sustainability of the beef production system in Brazil whilst ambient CO2 concentration maintain its increasing..

Acknowledgements The authors wish to thank Embrapa, CNPq and FAPESP for financial support.

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Measuring methane emissions at the herd scale in northern Australia

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Introduction Australia's north accommodates 54.5% of the national beef herd and poor quality pastures, seasonal rainfall and low animal productivity are associated with high methane (CH_4) emissions intensity/unit animal product. A number of methodologies can measure individual animal emissions; respiration chambers, sulphur hexafluoride tracer technique and may suit individual animal or feedlot measurements, but are not applicable to estimating CH_4 emissions in extensive grazing environments. The smallest unit of measure to characterise livestock greenhouse gas (GHG) emissions across pasture types and bio-agronomic regions for northern Australia will be at the herd scale with minimal intervention. An on farm methodology using open-path spectroscopy and a backward Lagrangian Stochastic (bLS) dispersion model (Loh *et al*. 2008, McGinn *et al*. 2009) is now being used across northern Australia to generate baseline data for national GHG inventory purposes and to assess the effect of mitigation activities at the herd scale.

Material and methods Five field studies were conducted across northern Queensland and the Northern Territory over 3 years to measure CH_4 emissions from beef cattle characteristic of the northern grazing herd. Mean (± sem) annual rainfall across the sites was 773 ± 112.6 mm. Animals grazed native and improved pastures; Astrebla spp., Iseilema spp., *Cenchrus ciliaris*, *Chrysopogon fallax, Choris gayana* and *Urochloa mosambiensis*. Methane flux was measured using an open-path laser (GasFinder, Boreal Lasers, Alberta Canada) mounted on a digital scanning unit (Directed Perceptions Inc. USA) which automatically aligned to retro-reflectors that terminated each path. Methane measurements were obtained for 5 h/d for up to 70 animals confined in a dedicated source area. Surface-source assumptions were used in a bLS dispersion model to derive methane flux. The surrounding area at each site was flat and considered to present no major obstacles to wind. A second stationary laser measured background CH_4 concentration along a third, upwind path. At each site a micrometeorological mast with a three-dimensional sonic anemometer (CSAT3, Campbell Scientific Inc, USA), barometric pressure sensor and temperature humidity sensors, a cup anemometer and wind sentry was used to collect wind statistic data. Wind component variances were recorded at 10 Hz and averaged over 10-min intervals. Methane flux and wind component statistics were computed to simulate herd scale CH_4 emissions using a bLS dispersion model (WindTrax dispersion model V.2.0.8.3,Thunder Beach Scientific, Halifax, NS, Canada) to generate herd scale methane emission values.

Table 1 Location, dates, class of livestock and methane emissions (mean ± s.e.m) for herd scale measurements for northern Australia

			Methane emissions	
	Start-finish	Livestock class	g/hd/d	g/kg LW
Belmont Station	27 Aug-16 Nov	Steers	136 ± 21.5	0.6
Belmont Station	02 Oct-17 Oct	Steers	231 ±16.6	0.5
Lansdown Station	23 Sept-08 Oct	Steers	226 ± 7.5	0.9
Douglas Daly Farm	21 Oct-12 Nov	Cows	212 ± 8.9	0.7
Kidman Springs Station	10 Aug -23 Aug	Heifers	162 ± 4.3	0.5

Results Methane emission estimates for over 200 cattle including steers, cows and heifers grazing northern pastures have been obtained (Table 1). The lowest emissions were associated with young steers grazing an irrigated and improved pasture fertilised with urea (150 kg/ha) and managed intensively to ensure similar phenological state across measurement periods. In comparison, the higher CH_4 emissions were associated with mature Brahman cows and heavier steers grazing either Cenchrus or Urochloa dominated pastures, respectively. High daily CH_4 emission values were generally observed within the first 3 h of measurement. This initial measurement period appears to capture the expected increase in emissions following a feeding event which is consistent with trends observed using open-circuit respiration chambers. The relationship between mean LW and methane emissions for all sites is similar to those previously described (Yan *et al*. 2009), but appears to under estimate emissions for a similar weight range.

Conclusions The results confirm that methane emissions in grazing systems across northern Australia can be benchmarked using the open-path spectroscopic methodology combined with a micrometeorological dispersion model. The ongoing generation of data sets that benchmark emissions from extensive grazing systems typical of the northern rangelands will be invaluable to beef producers adopting new and novel mitigation practices.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

The potential of 3-nitrooxipropanol to lower enteric methane emissions from beef cattle

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Introduction This study evaluated a new compound (3-nitrooxypropanol, NOP) designed to reduce enteric CH_4 emissions. Inhibition of enteric CH_4 production is of great importance because CH_4 is a potent greenhouse gas that contributes to global warming and it is also a loss of energy for ruminants. The objective was to determine whether enteric CH_4 emissions from beef cattle fed a high forage grower diet could be reduced by supplementing the diet with NOP.

Materials and methods Eight ruminally cannulated Angus heifers (549±64.3kg) were used in this study. The basal diet was a total mixed ration consisting of 60% barley silage, 35% barley grain and 5% vitamin-mineral supplement (dry matter [DM] basis). Additionally, each heifer received 600 g/d of a pellet containing 2.6 mg of melengesterol acetate to supress oestrous activity. The diet was prepared and offered ad libitum once daily. Experimental design was a duplicated 4×4 Latin square with 28-d periods. Dietary treatments were NOP fed at 0, 0.75, 2.25 and 4.5 mg/kg of body weight (BW). Heifers were adapted to their dietary treatments gradually at the beginning of each period. The NOP was mixed with a carrier comprised of 77% ground barley grain, 15% liquid molasses and 8% canola oil, and then top dressed onto the ration once daily at the time of feeding. The carrier containing the NOP was readily consumed by the animals within 5 to 10 min of allocation. After 16 days of adaptation heifers were moved to four open circuit chambers (1 heifer/chamber) and production of CH_4 was measured for 3 days according to McGinn *et al.* (2004). Rumen fermentation was monitored by collecting rumen contents at 0, 3, 6, 9, and 12 h after feeding on day 14. Ruminal pH was continuously measured from day 14 to 21 and apparent total tract digestibility was measured from day 24 to 28. Animal was the experimental unit for all variables. Data were analysed using a mixed model (SAS Institute Inc., Cary, NC) that included the fixed effect of treatment, sampling time and their interaction. Sampling time was considered a repeated measure. The PDIFF option adjusted by the Tukey method was used to separate means, and additionally the linear and quadratic effects of NOP dose were examined using orthogonal contrasts.

Results Dry matter intake (DMI) was reduced compared with the control only when 2.25 mg/kg of NOP was supplemented. No effect of NOP on digestibility was observed. Methane production (g/kg DMI) was reduced linearly with dose of NOP, with a 33% reduction compared with the control at the highest dose of NOP. Average pH was not affected, but minimum pH increased linearly with dose of NOP. Acetate (A) concentration was reduced and propionate (P) concentration increased with increasing dose of NOP, which in turn led to a reduction in the A:P ratio. Total bacteria and methanogens were not affected by NOP supplementation and protozoa populations were inconsistently affected (data not shown).

Table 1 DMI, digestibility, methane emissions and ruminal fermentation of beef cattle supplemented with 3-nitrooxipropanol

Item	Treatment (mg/kg of BW)				s.e.m.	P		
	0	0.75	2.25	4.5		Trt	Lin	Quad
Ad libitum DMI, kg/d	12.0a	11.7ab	11.3b	11.4ab	0.9	0.03	0.02	0.07
DM digestibility	0.68	0.67	0.67	0.69	0.01	0.10	0.13	0.05
CH_4, g/kg DMI	24.6a	23.5a	22.3a	16.5b	1.8	<0.001	<0.001	0.21
Ruminal pH								
Minimum	5.83b	6.00ab	6.04a	6.06a	0.10	0.04	0.02	0.14
Mean	6.46	6.54	6.57	6.55	0.05	0.28	0.19	0.17
Total VFA, mM	160.5	159.1	148.4	147.7	9.13	0.16	0.04	0.41
VFA, mol/100 mol								
Acetate (A)	61.8a	60.8a	56.3b	52.6c	0.99	<0.001	<0.001	0.57
Propionate (P)	19.3b	19.4b	21.4b	26.1a	1.16	<0.001	<0.001	0.23
A:P ratio	3.3a	3.2ab	2.7b	2.1c	0.21	<0.001	<0.001	0.76

a,b,c ($P < 0.05$)

Conclusions Results show the potential of NOP to reduce CH_4 production when supplemented daily at 4.5 mg/kg of BW (2,720 mg/animal/day). Further research is needed to confirm whether such reductions are maintained over longer feeding periods. The addition of NOP can be used as a CH_4 reduction strategy without negatively affecting diet digestibility; however, the small reduction in DMI could potentially negatively affect animal performance. These findings need to be confirmed in subsequent studies.

Acknowledgements The authors gratefully acknowledge funding from DSM.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Genetic selection for residual feed intake to reduce greenhouse gas emissions from beef production in Canada – Evaluation using farm-based life cycle assessment

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Introduction Residual feed intake (RFI) is defined as the difference between an animal's actual feed intake and its expected feed intake based on energy requirements for maintenance and production (e.g., growth in beef cattle or milk production in dairy cattle), with more efficient animals having lower RFI values. This study used a life cycle assessment (LCA) approach to explore the potential of genetic selection of beef cattle for RFI to reduce greenhouse gas (GHG) emissions from beef production.

Materials and methods A LCA was conducted to estimate whole-farm GHG emissions from beef production in Canada. The aim was to determine if a net reduction in GHG could be obtained by fully selecting a beef cattle herd for lower RFI. To do this, a baseline LCA was conducted to establish whole farm GHG emission intensity for beef production in western Canada (Beauchemin *et al*., 2010). A RFI scenario was then applied to the baseline scenario and its impact on GHG emissions was assessed. The simulated beef production operation was comprised of 120 cows, 4 bulls, and their progeny, with the progeny fattened in a feedlot and slaughtered at 18 months of age. The farm also included cropland and native prairie pasture for grazing to supply the feed requirements of the animals. The LCA was conducted over 8 years to fully account for the lifetime GHG emissions from all animals, and beef was marketed from cull cows, cull bulls, and progeny raised for market. For the fully selected RFI herd, the assumptions were based on the final outcome after 25 years of selection using the gene flow model of Alford *et al*. (2006). The proportion of feed intake of the RFI herd, relative to the unselected baseline herd, was 0.8207 for calves, replacement heifers, and backgrounding cattle, 0.8282 for finishing cattle, and 0.8544 for cows (0.8804 for pre-slaughter cows). The emissions were estimated using Holos, a whole-farm model based on the IPCC methodology, modified for Canadian conditions and farm scale. The model considers all significant CH_4, N_2O, and CO_2 emissions and removals (carbon sequestration in soils) on the farm, as well as emissions from manufacture of inputs (fertilizer, herbicides) and off-farm emissions of N_2O derived from nitrogen applied on the farm. Results are expressed as CO_2 equivalent, using the global warming potentials of the individual gases: CH_4, 25; N_2O, 298; and CO_2, 1.

Results After full selection of a beef cattle herd for RFI, the estimated GHG emissions (actual and intensity) from beef production were 14.0% lower than for the non-selected baseline herd (Table 1). Due to the lower feed intake of the RFI herd, the farm area required for grazing and feed production was 13.2% lower than for the baseline herd. The breakdown of the GHG emissions, by proportion and source, were very similar for the two scenarios (Figure 1).

Table 1 Greenhouse gas (GHG) emissions and farm area needed to supply feed for the Baseline and RFI herds

	Baseline Herd	RFI Herd	% Reduction
Total GHG emissions (yearly average over 8 year cycle), Mg CO_2e	722.3	621.0	14.0
GHG Intensity, CO_2e (kg beef carcass)$^{-1}$	23.1	19.8	14.0
Total farm area required for feed, ha	2333.5	2026.4	13.2

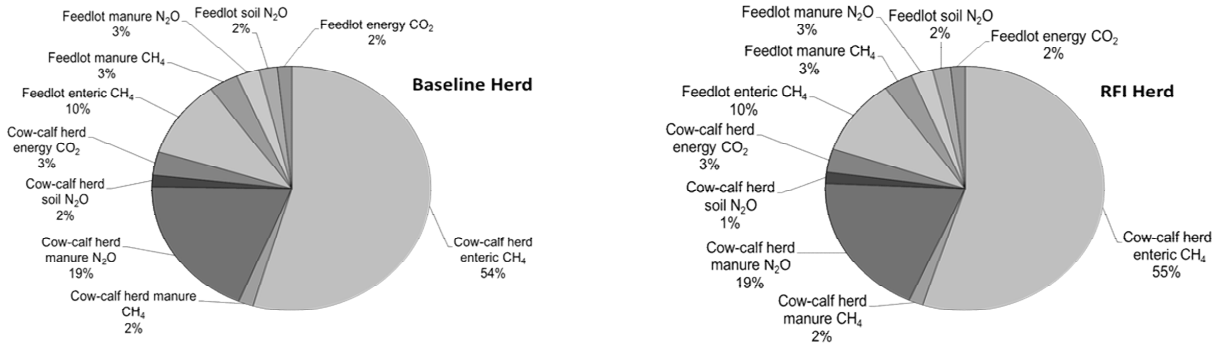

Figure 1 Breakdown of GHG intensity (CO_2e kg beef carcass)$^{-1}$ by source for the Baseline and RFI herds.

Conclusion Selection of a beef cattle herd for RFI has the potential to reduce GHG emissions (actual and intensity) from beef production by reducing feed intake and required land area. Effects of genetic selection for RFI and dietary mitigation strategies may be additive, thus their combined effects warrant further investigation as a means of lowering GHG emissions from beef production.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Effects of 3-nitrooxipropanol on methane production using the rumen simulation technique (Rusitec)

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Introduction Many strategies have been evaluated to mitigate enteric methane (CH_4) emissions from ruminants; however, to date only a few viable options have been identified. Finding strategies to decrease enteric CH_4 emissions from ruminants is necessary to reduce the accumulation of greenhouse gases in the atmosphere and to allow for a more efficient use of feed energy by ruminants. A novel compound (3-nitrooxipropanol, NOP) that has been shown to reduce enteric CH_4 emissions by up to 33% in beef cattle (unpublished data) was evaluated using the rumen simulation technique (Rusitec). Inclusion level was evaluated to identify the most effective dose for future in vitro studies.

Materials and methods Two Rusitec apparatuses (Czerkawski and Breckenridge, 1977), each equipped with eight 920 mL volume anaerobic fermenters, were used in this study. Treatments were different doses of NOP: 0, 5, 10 and 20 mg/vessel/d. Inoculum was obtained from 4 ruminally fistulated cattle fed a high-forage diet. The diet substrate evaluated consisted of barley silage (60%), barley grain (35%) and vitamin/mineral supplement (5%). Each fermenter was filled with 180 mL of artificial saliva, 720 mL of strained rumen fluid, 10 g of the corresponding diet (substrate plus NOP dose) and fresh solid digesta (10 g) contained in sealed polyester bags. The fermenters were immersed in a water bath maintained at a constant 39ºC. After 24 h, the bag containing solid rumen digesta was removed and replaced by a single bag containing 10 g of diet substrate. Thereafter, one bag was replaced daily so that each bag remained in the fermenter for 48 h except the last day when one bag in each vessel was removed after 24 h. Artificial saliva (pH 8.2) was infused into the fermenter continuously at a rate of 2.9% per hour. Effluent was collected in 1000 mL volumetric flasks and gas was collected in a 2.0 L bag. Daily, at the time of feed bag exchange, rumen fluid pH, total gas production and effluent volume from each fermenter was measured. Starting at day 8 until the end of experiment on day 15, different treatments were administered mixed with the corresponding diet contained in the feed bags. During feed bag exchange, the fermenters were flushed with O_2-free CO_2 to maintain an anaerobic environment. On days 9, 10, 11, 12 and 13, samples of gas (20 mL) were taken from the collection bags and transferred to evacuated 6.8 mL gas sample vials. The CH_4 concentration in the gas samples was determined by gas chromatography. Data were analysed using a mixed model procedure (SAS Institute Inc., Cary, NC) that included the fixed effect of the treatment, day and their interaction. The PDIFF option adjusted by the Tukey method was used to separate means, and additionally the linear and quadratic effects of NOP dose were examined using orthogonal contrasts.

Results Daily gas production was not affected by NOP; however, the proportion of CH_4 in gas was reduced with increasing dose of NOP where 10 and 20 mg/d induced the biggest reductions. There was a reduction in CH_4 produced per day when NOP was administered, with no differences among levels of NOP (5, 10 or 20 mg/vessel/d).

Table 1 Effect of different inclusion levels of 3-nitrooxipropanol on methane production

	Treatment (mg/vessel/day)					P		
Item	0	5	10	20	s.e.m.	Trt	Lin	Quad
Gas production, mL/d	1334	1318	1339	1326	86.7	0.997	0.980	0.983
CH_4 proportion in gas, mL/100 mL	2.32a	0.61b	0.39c	0.34c	0.049	<0.001	<0.001	<0.001
CH_4 production, mL/d	31.09a	7.74b	5.04b	4.21b	1.396	<0.001	<0.001	<0.001

a,b,c ($P < 0.05$)

Conclusions Results show the potential of NOP to reduce in vitro CH_4 production when supplemented at different doses. The lowest dose (5 mg/d) reduced CH_4 production by 75%, which is considerably higher than the 33% reduction observed in a companion in vivo study when the compound was fed at 4.5 mg/day/kg of animal body weight. Further evaluation of NOP using the Rusitec technique should be conducted using a dose of < 5 mg/vessel/day so that the results are applicable to animal studies.

Acknowledgements The authors gratefully acknowledge funding from DSM.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Impacts of extreme drought on livestock inventory, greenhouse gas emissions, and organic nutrient availability in Texas

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Introduction The Texas drought of 2011 is documented to be the worst one-year drought since the State's instrumental record began in 1895. Average precipitation during the period from October 2010 through September 2011 measured 279 millimeters, 206 millimeters lower than the twentieth century average for Texas. The summer of 2011 also marked the hottest summer on record. The June-July-August average daily temperature was 1.1 degrees Celsius warmer than any previous Texas summer and over 2.7 degrees Celsius above the long term average. On October 4, 2011 97 percent of Texas was categorized as in "extreme drought" by the US Drought Monitor. Agricultural losses for 2011 exceeded 7.6 billion dollars and 252 of 254 counties were declared federal disaster areas. On average, Texas produces approximately 14 million head of cattle and calves, including those on pasture, rangeland, and confined feeding operations. As of December 2011, 660,000 beef cows were either relocated out of state, sent to slaughter, or shipped to confined feeding operations due to withered pastures, limited availability and high prices for forage and other feed supplements, and water scarcity. This drop in beef cow inventory represented the largest one-year decline since 1920. The drop in cattle inventory reduced the total amount of manure produced, resulted in less total greenhouse gas emissions produced by cattle through enteric fermentation and manure excretion, and reduced the total amount of organic nutrients deposited on pastures in Texas. Relocation of cows out of the State in effect exported greenhouse gas emissions from Texas pasture and rangeland to other states. The aim of this study was to quantify the reduction in greenhouse gas emissions, manure, and associated organic nutrients as a consequence of the drought and depopulation of the beef cow herd inventory in Texas.

Material and methods Quantification of the reduction in total CH_4 emissions (expressed in CO_2 eq.) was determined using IPCC Tier 1 Method procedures for enteric fermentation and manure based upon a loss of 660,000 beef cows over a 1 year period. Change in beef cow population was determined from annual USDA National Agriculture Statistics Service reports. Emission factors for enteric fermentation ranged from 53 kg CH_4/hd-yr to 71 kg CH_4/hd-yr according to IPCC and US EPA reference values. CO_2 emissions were considered, however CO_2 emissions from livestock are not estimated because annual net CO_2 emissions are assumed to be zero – the CO_2 photosynthesized by plants is returned to the atmosphere as respired CO_2. Total reduction in manure that would have been added back to pasture was estimated based upon an excretion rate of 37.2 kg/hd-d (wet basis). The amount of organic nutrients (TKN, P_2O_5, and K_2O) lost due to inventory reduction were estimated from manure characteristics listed in ASAE Standard D384.2.2005.

Results As a result of the drought and subsequent drop in beef cow inventory over 2011, total reduction in greenhouse gas emissions from enteric fermentation ranged 735,000 MT CO_2 eq/yr to 987,000 MT CO_2 eq/yr based IPCC and US EPA referenced emission factors, respectively. Approximately 8.9 MMT less manure (or 1.04 MMT fewer total solids) were produced. This resulted in a reduction of CH_4 emissions from manure of 27,720 tonnes CO_2 eq/yr. Organic nutrients associated with manure excreted back on pastures decreased as follows: TKN (4,993 MT), P_2O_5 (1,530 MT), and K_2O (3,890 MT).

Conclusions These results show how severe drought and subsequent reduction of beef cattle inventory can influence the amount of greenhouse emissions emitted from enteric fermentation and manure, and how organic nutrients from this manure are decreased. While fewer anthropogenic greenhouse emissions are a positive outcome in regards to climate change, the impact for many farmers and ranchers has been devastating. Approximately one-quarter of cattle ranchers are no longer in business, complete herds have been liquidated, and high cattle prices and poor pasture quality will lead to a long recovery to rebuild cattle inventory in Texas.

Acknowledgements The authors gratefully acknowledge funding from Agriculture and Food Research Initiative Competitive Grant No. 2011-67003-30206 from the USDA National Institute of Food and Agriculture.

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Methane emissions from growing dairy heifers estimated using an automated head chamber (GreenFeed) compared to respiration chambers or SF_6 techniques

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Introduction Accurate techniques are required for the measurement of methane (CH_4) emissions from livestock under normal production conditions to monitor CH_4 inventory and mitigation. The sulphur hexafluoride (SF_6) tracer technique can be used to estimate CH_4 emissions under 'field' conditions, but is labour intensive. Respiration chambers are unable to make CH_4 measurements in conventional environments. The GreenFeed system (C-Lock Inc., Rapid City, SD, USA) is an automated head chamber method for estimating CH_4 emissions from free-ranging livestock. Our objective was to compare CH_4 emissions from growing dairy heifers obtained using a GreenFeed unit with corresponding values from either respiration chambers or using the SF_6 technique.

Material and methods Three experiments were conducted using growing Holstein dairy heifers fed a variety of dietary treatments. Methane emissions were determined using either GreenFeed and open-circuit respiration chambers or GreenFeed and SF_6. Experiment 1 was a 4x4 Latin Square design with five-week periods and 4 heifers fed two silages (maize vs. ryegrass) with or without added fat. Daily dry matter intakes (DMI) were measured and CH_4 emissions determined indoors using a GreenFeed unit for 7 days prior to entering respiration chambers for 4 days. GreenFeed measurements for periods 1 and 2 of Experiment 1 were preliminary and not statistically analysed. Experiment 2 was also a 4x4 Latin Square design with five-week periods. Four heifers were fed 4 forage mixtures and CH_4 emissions were measured as in Experiment 1. In Experiment 3, CH_4 emissions were from 12 growing Holstein heifers for five-day periods whilst rotationally grazing three forage mixtures using SF_6 and GreenFeed techniques simultaneously (4 measurement periods per forage mixture). Forage DMI was estimated based on forage DM removal. The GreenFeed system operated continuously for the duration of each experiment in a stationary position. Calf rearing pellets were used for enticement and the GreenFeed was programmed to deliver approximately 270 g of pellets over 6 minutes up to a maximum of 6 times per day with a minimum of 4 hours between feeds. Open-circuit respiration chambers were used in Experiments 1 and 2, providing a daily average of 345 measurements of CH_4 emissions per heifer. In Experiment 3, daily CH_4 emissions were estimated by placing a SF_6 permeation tube in the rumen of each heifer and obtaining a sample of expired air over 24 hours. Estimates of CH_4 emission for each technique were averaged according to minute of the day (except for SF_6) and daily rate for individual animals. Mean rate of CH_4 emission for each animal and treatment period (n = 8, 16, and 136 for Experiments 1, 2, and 3, respectively) for GreenFeed and either chambers or SF_6 were compared within experiment. Differences between techniques were tested using the univariate procedure of SAS®. Within experiment mixed model procedures of SAS® were used to test for effects of diet and repeated effects of period within heifer.

Results Respiration chamber and GreenFeed techniques gave similar ($P > 0.10$) mean CH_4 emission (g/d) estimates (Figure 1) for Experiments 1 (215 vs. 198, respectively) and 2 (209 vs. 208, respectively), but SF_6 estimates were higher ($P < 0.001$) than GreenFeed values in Experiment 3 (186 ± 2.6 vs. 163 ± 2.4, respectively). This may have been due to fewer visits to the GreenFeed unit under grazing conditions (1.9 ± 0.35 per heifer per day) compared to indoors (2.6 ± 0.25 per heifer per day), the timing of the visits, or the accuracy of the SF_6 data. Dry matter intakes were similar between techniques for each experiment, averaging 7.4, 7.6, and 9.2 kg DM/d for Experiments 1, 2 and 3, respectively. Methane emission patterns determined by chambers and GreenFeed were comparable, but within experiment 2, diet effects were significant for respiration chamber measurements, but not GreenFeed.

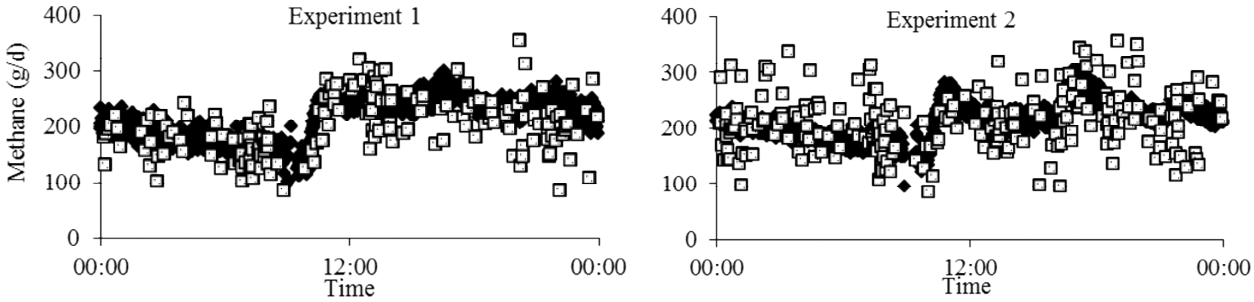

Figure 1 Comparison of CH_4 emission rate measured using respiration chambers (◆ and GreenFeed (⊡

Conclusions Overall estimates of CH_4 emission made by the GreenFeed system were comparable to values obtained by respiration chambers, but only 0.89 of the SF_6 technique. This may have been attributable in part to a lower number of visits to GreenFeed during grazing measurements, or the timing of the visits. The GreenFeed system is capable of estimating CH_4 emissions from livestock, but deployment and replication must be considered carefully to ensure adequate numbers of measurements are obtained.

Acknowledgments This work was funded by Defra, the Scottish Government, DARD, and the Welsh Government as part of the UK's Agricultural GHG Research Platform project (www.ghgplatform.org.uk).

Arterial, rumen, and milk concentration and net absorption of methane into the portal vein of lactating dairy cattle

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Introduction Previous measurements of methane eructation and expiration using dairy cows with tracheal cannula (Hoernicke *et al*., 1965) indicated that substantial amounts of expired methane reached the lungs via absorption into blood as compared to eructation. To our knowledge, there are no direct measurements published of methane absorption into the hepatic portal vein of any species, and only limited measurements of blood methane concentration (e.g. Ramirez-Restrepo *et al*., 2010). Variations in blood methane concentration could affect milk concentrations, and either has potential as a proxy for total methane production. Therefore our objective was to determine if there is measureable absorption and/or liver metabolism of methane in lactating dairy cows, and to measure the relative concentrations of methane in rumen fluid, milk, and arterial blood of dairy cows.

Methods One dry, one early lactation and one late lactation Holstein dairy cow surgically prepared with a rumen fistula and splanchnic blood sampling catheters were fed once daily for *ad-libitum* dry matter intake (DMI) total mixed rations containing maize silage or grass silage/straw as primary forage sources for the lactating and dry cows, respectively. Milk yield and DMI were measured daily. Beginning 2 h before feeding, simultaneous arterial and portal and hepatic vein blood samples were obtained every 30 minutes for a total of 12 samplings, whilst rumen samples were obtained hourly. Portal-drained visceral (PDV) and liver blood flow was measured by dilution of ρ-aminohippurate infused into a mesenteric vein. Samples (2 ml) of blood, centrifuged rumen fluid, and milk (sampled before and after blood sampling) were kept anaerobic and chilled and added to an equal volume of lactic acid (13 M) in an evacuated vial and thoroughly mixed as soon as possible. The vials were then returned to atmospheric pressure with N_2 and the headspace analyzed for CH_4 concentration by gas chromatography. Only rumen and arterial blood samples were obtained from the dry cow. Measurements for lactating cows were repeated 8 weeks later. Net flux of methane across the PDV and liver was calculated based on blood flow and venous-arterial concentration difference. Data were analyzed using univariate and mixed models procedures of SAS® to test for random effects of cow and repeated effects of sampling time and sampling day within cow.

Results Feed DMI (kg/d) was 6.8 for the dry cow and for lactating cows averaged 19.8 and 21.7 for the first and second samplings, respectively. Milk yield (kg/d) for the early lactation cow was 67.8 and 59.2 and for the late lactation cow was 16.8 and 14.8 for the first and second samplings, respectively. Milk methane concentration was lower in the morning than the afternoon ($P < 0.05$; 3.5 vs 12.1 ng/ml, respectively). Rumen fluid concentration of methane averaged 4.1 ± 0.24 µg/ml and was not affected ($P = 0.240$) by sampling time. Arterial concentration (ng/ml) of methane (63.7 ± 5.1) varied with sampling time ($P < 0.042$) and was highest (113.8 ± 18.4) 1.5 hours after feeding. Portal vein methane concentration was 987.6 ± 45.9 ng/ml higher than arterial concentration. Neither portal vein concentration or portal-arterial concentration difference for methane was significantly affected by sampling time ($P > 0.345$). Hepatic-portal vein concentration difference for methane was not different from zero ($P = 0.572$), indicating no effect of the liver on blood methane concentration. Net PDV absorption of methane (2.08 ± 0.10 g/h) was not affected by sampling time ($P = 0.660$).

Conclusions Portal vein concentration of methane in two lactating dairy cows was nearly 10 fold higher than arterial concentration, reflecting a significant absorption of methane from the PDV. Assuming the period of sampling was representative of a 24 hour period, net PDV absorption in these lactating dairy cows would account for 50 g/d of methane, or 12% of methane emission estimated from DMI. We did not observe a significant increase in net PDV absorption of methane after feeding, which agrees with previous estimates of methane absorption obtained using tracheal cannulas (Hoernicke *et al*., 1965). Similarly, we did not observe an effect of feeding on rumen fluid methane concentration. Although relatively small, we did observe a significant increase in arterial methane concentration after feeding. However, the concentration of methane in the rumen and arterial blood of the dry cow sampled was similar to the concentrations observed in the lactating cows (data not shown), in spite of the lower level of intake and estimated methane production for the dry cow. There was no significant effect of the liver on blood methane concentration. The substantially lower concentration of methane in arterial blood, and milk, compared to the portal vein may be a consequence of methane expiration by the lungs, as well as dilution by blood from the rest of the body. The higher concentration of methane in afternoon milk compared to morning milk may reflect differences in milk yield, but milk concentration of methane was similar for the two cows (8.6 ± 2.6 vs 7.0 ± 4.0 ng/ml for the late and early lactation cow, respectively). Alternatively, the higher concentration of methane in afternoon milk may reflect the higher arterial concentration of methane observed after feeding. The lack of difference in milk concentration of methane between the two cows in the present study, which were consuming similar amounts of the same diet and producing very different amounts of milk, suggests that milk methane production may have limitations as a proxy measurement of methane emission.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Exploring the production frontier 1. A multi-objective optimization of diet composition to simultaneously minimize cost and environmental impact of beef production in the United States

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Introduction A socially-driven demand for greater sustainability exists in food production systems. For a food production system to be sustainable it must balance environmental responsibility, economic viability and social acceptability. Within the United States (US) beef production system, improved efficiency reduces environmental impact (Capper, 2011); and enhances both economic viability and social acceptability (Capper and Hayes, 2012; White and Capper, 2012). One method to improve efficiency on-farm is to adjust cattle diets. Few studies have identified how to modify cattle dietary composition to concurrently reduce environmental impact and improve profitability. Our objective was to use a multi-objective optimization of diet composition to identify the opportunities that exist to concurrently minimize cost and greenhouse gas (GHG) emissions, land use and water use in the US beef production system.

Material and methods An optimization model was constructed using the Generic Algebraic Modelling System (Rosenthal, 2012). The model simulated 21 different cattle groups representing animals in the cow-calf, stocker and feedlot sectors categorized by gender and production stage. Population sizes were calculated based on a target amount (100,000 kg) of hot carcass beef produced from both feedlot and culled breeding cattle. Diets were formulated based on the nutrient requirements of each group to ensure adequate nutrient supplies for maintenance, production and growth. The optimizer was constrained to generate diets with reasonable forage-to-concentrate ratios that reflect US cow-calf, stocker and feedlot systems. Objective metrics in the model included total diet cost, land use, water use and greenhouse gas emissions (GHG) for the entire production system. A set of reference optimizations were conducted to assess the opportunity for altering diets to minimize diet cost and environmental impact. Reference optimizations consisted of four single-objective optimizations, one minimizing each output metric. A least-cost reference scenario was chosen as a comparative point for the multi-objective optimizations. Multi-objective optimizations were then conducted by minimizing the percentage deviation of each output metric from the least-cost management output. Scenarios were conducted minimizing pairs, triplets and all four output metrics. Cost was included as a metric in all scenarios. Scenarios were grouped by objective as least-land, least-water, or least-carbon management, and analysed for trends in dietary adjustment.

Results Output metrics were minimized individually in the single-objective reference optimizations. By altering dietary ingredients, GHG emissions, land use and water use could be reduced by 8%, 17% and 23%, respectively, compared to the least-cost management scenario. The dietary adjustments responsible for generating these differences were due to changes in the concentrate-based system (feedlot). In the least-GHG scenarios, diets were altered to reduce enteric CH_4 and crop system CO_2 by increasing the digestibility of the forage source and supplementing with by-product feeds such as soybean meal and distillers grains. Least-water scenarios altered diets to reduce irrigation water use by supplementing with lower-quality forages and by-products that require minimal water use for production. Least-land scenarios adjusted to higher input/output forage sources (alfalfa hay) and high yield cereals (corn grain). In the forage-based systems, the dietary changes exhibited little influence on model output. In least-land management, the diet was supplemented with high input/output stored forage. In least-water scenario, the diet was adjusted to a low input/output rangeland. The GHG-minimizing scenarios used moderate input/output forage (pasture) as the base of the diet and supplemented with high quality stored forage (alfalfa hay). Multi-objective optimizations of different combinations of output metrics revealed several patterns. Moderate increases in diet cost (0.1%-4%) were required to achieve significant reductions in GHG emissions (6%), water use (7%) or land use (5%). When diet cost, GHG emissions and land use were minimized together, simultaneous reductions in GHG emissions (6%) and land use (8%) could be achieved with a 3% increase in diet cost. A 4% reduction in GHG emissions and a 4% reduction in water use could be achieved with a 6% increase in diet cost. When land use and water use were optimized simultaneously, a 1% increase in diet cost helped reduce land use and water use by 2% each. The multi-objective optimization of all output metrics revealed that a 1% increase in cost could bring about a simultaneous 2% reduction in each environmental output metric. The minimal response of the outputs to dietary changes in the forage-based systems may be due to the feedstuffs available for use in this comparison.

Conclusions Substantial opportunity exists to reduce environmental impact by altering diets while maintaining productivity. Changes in diet formulation can reduce environmental impact while incurring slight increases in cost. It is possible to alter diet composition to reduce land use by 2%, water use by 2%, and GHG emissions by 2% while incurring only a 1% increase in diet cost. Future research should explore the opportunities for forage management practices in the cow-calf and stocker systems to reduce the environmental impact of the US beef production system.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Synergistic effect of Alcaligenes faecalis and nitrate to reduce in vitro rumen methanogenesis

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Introduction The greenhouse gas methane (CH_4) is produced during rumen fermentation as a result of the reduction of carbon dioxide (CO_2) with hydrogen (H_2) by methanogenic *Archaea*. Nitrate (NO_3) reduction is energetically more favourable than CO_2 reduction, and the presence of NO_3 in the rumen redirects H_2 from methanogenesis to NO_3 reduction, thereby decreasing CH_4 production. However, the practical use of NO_3 is precluded by the toxicity associated with its reduced intermediate, nitrite (NO_2). Nitrite accumulates in the rumen due to the more rapid reduction of NO_3 to NO_2 than the reduction of NO_2 to ammonia. It would be desirable to be able to enhance the rate of nitrite reduction or alternatively, reduce the quantity of NO_3 added. There is some evidence that *Alcaligenes faecalis* is capable of anaerobic denitrification (M. Shoda, pers. comm.), and thus may reduce NO_2 accumulation if introduced into the rumen. When this bacterium was initially introduced into the artificial rumen system with NO_3, there was no difference in the rate of NO_2 reduction compared to the control treatment. However, it became evident that a synergistic mechanism to reduce CH_4 output was occurring with this bacterium and low concentrations of NO_3. Therefore, the objectives of this study were to demonstrate the synergistic effect of *A. faecalis* and NO_3 to reduce *in vitro* CH_4 output.

Materials and methods To demonstrate the effect of *A. faecalis* cells and NO_3 on *in vitro* CH_4 output and other rumen fermentation variables, the following four treatments were used: (1) control (sterile H_2O), (2) *A. faecalis* cells, (3) Sterile H_2O + 2 mM NO_3 and (4) *A. faecalis* cells + 2 mM NO_3. *Alcaligenes faecalis* cells were suspended in sterile H_2O and contained approximately 1.0 x 10^9 colony forming units per ml. The *in vitro* gas quantification system was used to monitor gas output. Briefly, 10 g of dried milled hay and concentrate (1:1, w/w) was weighted into individual 1 litre fermentation vessels together with 760 ml of a 4:1 volumetric mixture of CO_2-saturated sterile artificial saliva and fresh rumen fluid inoculum from two fistulated Holstein cows. To start the experiment, 40 ml of each specific treatment was added to an individual vessel and the vessel was sealed to ensure anaerobic conditions. Fermentation was allowed to continue for 24 h at 39 °C. Gas output from each fermentation vessel was measured at 40 min intervals by the auto infrared CH_4 and CO_2 analysers. At the end of each 24 h fermentation period, the contents of the fermentation vessels were discharged, and the vessels were thoroughly washed and autoclaved prior to the next fermentation. The fermentation vessels were then re-charged with fresh artificial saliva, rumen fluid inoculum and treatment to begin the next 24 h fermentation period. The experiment was repeated four times on different days, with treatments assigned randomly to fermentation vessels each day. For each treatment, an aliquot from the fermentation medium at 24 h was analysed for individual volatile fatty acids (VFA) concentrations. For the statistical analysis of the data, the effects included in the GLM for each variable were replication (day) and treatment effects. Differences among means were identified using Tukey`s multiple comparisons with $P<0.05$ being considered significant.

Results The *in vitro* CH_4 output and other fermentation variables are presented in Table 1. *Alcaligenes faecalis* + NO_3 treatment had a lower ($P<0.05$) cumulative CH_4 output (ml CH_4/24 h) and a higher ($P<0.05$) propionic acid concentration than all other treatments. Acetic acid concentration in the *A. faecalis* + NO_3 treatment was similar ($P>0.05$) to the control and NO_3 only treatments. Total VFA concentration was lower in the control than in the *A. faecalis* and *A. faecalis* + NO_3 treatments. Cumulative CO_2 output was not affected by treatment ($P>0.05$).

Table 1 Effect of *Alcaligenus faecalis* and nitrate on *in vitro* rumen methane and other fermentation variables

	Treatments					
	Control	*A. faecalis*	Nitrate (2 mM)	*A. faecalis* + Nitrate (2 mM)	s.e.m.	P
Cumulative CH_4 (ml)	51^{a}	51.7^{a}	37.8^{b}	11.5^{c}	1.17	<0.001
Cumulative CO_2 (ml)	774	856	750	718	43.7	0.217
Acetic acid (mM)	32.0^{a}	36.4^{b}	34.5^{ab}	33.1^{a}	0.71	0.010
Propionic acid (mM)	14.5^{a}	17.3^{b}	16.4^{b}	19.3^{c}	0.42	<0.001
Butyric acid (mM)	4.7^{a}	6.3^{b}	4.9^{a}	4.9^{a}	0.31	0.016
Valeric acid (mM)	0.8^{ac}	1.1^{ab}	0.8^{c}	0.6^{c}	0.07	0.010
Total volatile fatty acids (mM)	52.0^{a}	61.1^{b}	56.6^{ab}	57.9^{b}	1.34	0.006

[a-c]Means within a row with common superscripts do not differ ($P>0.05$).

Conclusion A combination of *A. faecalis* + NO_3 can reduce CH_4 output to a greater extent than 2 mM NO_3 alone, without having an adverse affect on fermentation. With lower concentrations of NO_3 required when combined with *A. faecalis*, the risk of NO_2 accumulation to toxic levels in the rumen would be greatly reduced. Further experiments will be required to ascertain the mode of action of *A. faecalis* and NO_3 to reduce methanogenesis.

Acknowledgements The authors acknowledge funding from the Japanese Society for the Promotion of Science.

https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Nitrous oxide (N_2O) emissions from sheep excreta as affected by feeding forage rape compared to ryegrass pasture

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Introduction Animal excreta have been identified as a major source of nitrous oxide (N_2O) emissions from grazing systems. Forage brassicas have been increasingly used due to their faster growing ability, higher dry matter yield and higher nutritional value than perennial ryegrass (Sun *et al*. 2012). The lower crude protein content and the greater readily fermentable carbohydrate content of brassicas compared to ryegrass is expected to potentially improve the efficiency of N utilisation in the rumen and consequently reduce N losses. The aim of this study was to determine the partitioning of dietary N between urine and dung in the excreta from sheep fed forage brassica rape (*Brassica napus* subsp. *Oleifera* L.) and to measure N_2O emissions when the excreta are applied to the soil.

Materials and methods A sheep metabolism study at the AgResearch Grasslands in Palmerston North, New Zealand, was used to collect urine and dung from sheep fed forage rape or perennial ryegrass (*Lolium perenne L.*). Twelve Romney male lambs at the age of 9-months were randomly allocated to two groups fed either forage rape or ryegrass. After 7 weeks acclimation to the diets, sheep were fed fresh cut forage twice a day with equal portions provided at 09:00 and 16:00 h. The sheep were fed at 1.5 times of their metabolisable energy requirements for maintenance and kept in metabolism crates for 7 days with N intake and excretion in urine and dung determined for the calculation of N partitioning. Urine and dung collected from this study were then used in a field plot trial at the AgResearch Ruakura Farm in Hamilton. The experimental site contained white clover (*Trifolium repens* L.) and perennial ryegrass pasture on a poorly-drained silt-loam soil (classified as a Typic Orthic Granular soil). The treatments, with four replicates each, included urine from sheep fed forage rape, urine from sheep fed ryegrass, dung from sheep fed forage rape, dung from sheep fed ryegrass, and a control (without urine or dung). Fresh sheep urine was applied at a rate equivalent to 4 L m^{-2} and dung applied at a rate equivalent to 5 kg m^{-2}. Following the treatment application in early September 2011, N_2O emission measurements were carried out using the soil chamber technique (Luo *et al*. 2008). Gas sampling from the urine treatments was completed in December 2011 when emissions from the treatments returned to the control level, and from the dung treatments was completed in May 2012. Gas samples were analysed using a gas chromatograph equipped with a ^{63}Ni-electron capture detector. Total N_2O emissions and the emission factors (EF3) for each excreta type were calculated. An analysis of variance was conducted to determine differences between the treatments. Excreta N transformations in the soil were also regularly measured.

Results The results (Table 1) indicated that urine N output per unit of N intake was similar for both forages. Dung N output from sheep fed forage rape was slightly less than that from those fed ryegrass, but the difference was not statistically different ($P > 0.05$). This suggests that N use efficiency by sheep was equivalent for both forage rape and ryegrass. Urine from sheep fed ryegrass showed slower N transformation rates in soil from organic N to ammonium-N and to nitrate-N, compared with that from those fed forage rape. As a consequence, the duration of N_2O peaks was longer and the EF3 was significantly ($P < 0.01$) higher for urine from sheep fed ryegrass compared to forage rape (Table 1). However, dung for sheep fed ryegrass showed higher N transformation rates from organic N to ammonium-N and to nitrate-N and as a result, the duration of N_2O peaks was shorter and EF3 was lower, although the difference was not significant ($P > 0.05$). EF3 for sheep dung was substantially lower than that for sheep urine.

Table 1 Excreta N partitioning and EF3 values of urine and dung excreted from sheep fed either perennial ryegrass or forage rape

Feed type	Excreta type	Excreta N partitioning (g/kg N intake)	EF3 (%)
Ryegrass	Urine	476	0.27
Rape	Urine	493	0.11
Ryegrass	Dung	321	0.03
Rape	Dung	228	0.08
LSD ($P < 0.05$)			0.01

Conclusions The efficiency of N use by sheep was equivalent for both forage rape and ryegrass. The use of forage rape reduced EF3 for sheep urine by about 60%, compared to the use of ryegrass. The findings from this study are important to assess the effect of diet on N_2O emissions. Further field trials under different climate and soil conditions need to be conducted to confirm these findings. Additionally, the reason for such effects needs to be explored.

Acknowledgements This study was supported by the Ministry for Primary Industries of New Zealand under the Sustainable Land Management and Climate Change research programme and the Pastoral Greenhouse Gas Research Consortium (NZ).

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Exploring the production frontier 2. A multi-objective optimization of pasture management techniques to simultaneously minimize cost and environmental impact of beef production in the United States

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Introduction Pasture management is frequently identified as a way to improve the economic and environmental efficiency of forage-based cattle production systems. Forage is a unique resource in the United States (US) beef production system because it is typically the only feedstuff that is managed directly by the cattle producer, and it therefore provides an opportunity for producers to control a greater portion of their operation's environmental impact. The objective of this study was to use a multi-objective optimization model to identify how different pasture management techniques can be used to simultaneously minimize diet cost, greenhouse gas (GHG) emissions, land use and water use in the US beef production system.

Material and methods The McCall pasture model (McCall and Bishop-Hurley, 2003), as updated by Romera (2009), was adapted to simulate different pasture management strategies (irrigating and/or fertilizing continuously- or rotationally-grazed pasture). The pasture model was parameterized to fit growth curves sourced from USDA-ERS for the ten US states producing the largest annual beef calf crop based on data from 2008 through 2011. Irrigation and fertilization simulations were validated against a group of 8 datasets from the Oak Ridge National Laboratory: Distributed Active Archive Center. The model was used to generate yield and pasture quality data for all available growth curves in the ten states of interest under eight different pasture management strategies. Strategies included rotationally- or continuously-grazed pastures that were unmanaged, fertilized-only, irrigated-only or fertilized and irrigated. Weighted averages of the yield and quality data simulated from each growth curve were calculated and used as the inputs into an optimizer for each pasture management scenario to generate diets. The optimization simulated cradle-to-farm-gate GHG emissions, land use and water use required to produce a target amount of hot carcass-weight beef (100,0000 kg). Cattle within the optimization were categorized into different populations by gender and production stage. Cattle populations included cow-calf, stocker and feedlot systems, but diets were only altered for cattle in the cow-calf and stocker systems. Each optimization sought to minimize the objective metrics selected (diet cost, land use, water use and/or GHG emissions) by altering the type of pasture used as a feedstuff in the diets. Objective metrics for the optimization included total diet cost, land use, water use and greenhouse emissions. Single-objective reference optimizations were conducted to individually minimize each output metric. Multi-objective optimizations were then conducted by minimizing the percentage deviation of each output metric from the least-cost management scenario. Multi-objective optimizations were grouped based on objective as least-land, least-water, or least-carbon management and analysed for trends in the dietary adjustment.

Results Single-objective optimizations of each output metric indicated that pastures could be managed to reduce land use by 7%, water use by 33% or GHG emissions by 5% compared to least-cost management. The primary management strategy used in least-cost management was to irrigate and fertilize continuously-grazed pasture. Minimizing water use and diet cost concurrently indicated that water use could be decreased by 7% without impacting diet cost. Least-water scenarios altered the diet to a basic rotational grazing system with slightly higher yield than unmanaged pasture and no additional irrigation water input. When land use and diet cost were minimized together, a 3% increase in diet cost could reduce land use by 5%. This reduction was achieved by adjusting the base diet to intensively-managed, rotationally-grazed pasture and supplementing with high quality stored forages (alfalfa hay). Rotationally-grazed pastures and alfalfa hay had higher yields than the intensively-managed forage system used in the least-cost scenario. Carbon emissions could be reduced by 3% with only a 3% increase in diet cost. The adjustment to least-GHG scenarios was achieved by reducing enteric CH_4 emissions by switching to younger, more digestible forages. Multi-objective optimizations indicated that a 4% increase in diet cost could reduce both land use and GHG emissions by 2%. Additionally, when GHG emissions and water use were simultaneously minimized, a 3% increase in diet cost decreased land use by 12% and GHG emissions by 3%. When land and water use were minimized, concurrent reductions of 5% (land use) and 15% (water use) were achieved. Similarly, a 4% increase in diet cost could simultaneously minimize water use by 14%, land use by 2% and GHG emissions by 1%. Simultaneous reductions in all metrics were achieved by using both intensively-managed, continuously-grazed pastures and intensively-managed, rotationally-grazed pastures. This feedstuff combination reduced enteric CH_4, manure N_2O, cropping CO_2 and irrigation water used.

Conclusions Improving pasture management in the cow-calf and stocker systems is instrumental to improving the environmental impact of the entire beef production system. Specifically, altering pasture management can reduce land use by 7%, water use by 33% or GHG emissions by 5% compared to least-cost management. Simultaneous reductions in all metrics of environmental impact were achievable by altering the management of forage fed in the cow-calf and stocker systems. Before these adjustments are advocated as sustainable, an assessment of the consumer willingness-to-pay for beef produced with low environmental impact must be conducted to ensure that the increase in diet cost will be rewarded with a higher product price.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

US consortium for the genetic improvement of feed efficiency in beef cattle

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Introduction. Selection is the only technology proven to unabatedly increase the food produced per animal. However, the cost and difficulty of measurement of feed intake (FI) in cattle has limited improvement of feed efficiency (FE) by constraining genetic improvement to primarily output traits such as growth, meat yield and meat quality. Our goal is to sustainably reduce the feed resources required to produce beef via the rapid development and deployment of novel nutritional, genomic and genetic improvement technologies. Our research objectives are: To assemble DNA samples, individual animal FI, growth and carcass composition data for 8,000 animals representing 8 major US beef cattle breeds; genotype 2,400 animals from 6 breeds and develop and validate across-breed molecular expected progeny differences (MEPDs) for FI, growth and carcass traits; sample animals from the tails of the FE distribution from large groups (≥300) of cattle with individual FI data for basic studies of GHG emissions, gut microbiome composition and tissue-specific gene expression/network studies; identify FE causal mutations; develop and maintain DNA, RNA and phenotype repositories for project samples and publicly distribute these to the international research community. Extension objectives include: development and delivery to a national industry-wide stakeholder audience the resources, tools, information and educational activities that will enhance their understanding of the: a) importance of FE to farm economics and resource use, b) emerging technologies for genetic improvement in FE and component traits (FI, growth, carcass composition), and c) options to use marker assisted management (MAM) systems to sustainably improve profitability; to conduct a field demonstration project to demonstrate the utility of MEPDs for FE and component traits and "push" the technology into the beef industry; and to deploy GS based on MEPDs for FE and component traits to the beef industry.

Materials and methods.
We have collected Illumina HD or BovineSNP50 genotypes on 6,151 individually fed animals from 9 breeds (852 Hereford, 37 Limousin, 1,603 Angus, 155 Red Angus x Angus, 2,840 Simmental x Angus, 369 Gelbvieh, 24 Charolais, 35 Wagyu, 236 Piedmontese crossbreds) for analysis under Bayesian models to identify quantitative trait loci (QTLs) and generate MEBV prediction equations. An additional 2,515 animals will be collected in 2013. Because MEBV accuracies will progressively decrease in advanced generations if additional phenotypes are not collected and the MEBV prediction models retrained, we will attempt to find causal mutations underlying QTL by combining RNA-seq, Gene Set Enrichment and whole genome re-sequencing analysis using animals with extreme RFI phenotypes. A genome-wide association analysis (GWAS) was conducted using GBLUP to identify the additive genetic variance explained by the 777,000 SNPs in the Illumina Bovine HDBeadChip for RFI (DMI) in Angus and Hereford cattle. SNPs were evaluated for quality by testing for Hardy-Weinberg Equilibrium and those failing (#) were removed. Uncalled SNPs were imputed using Beagle (version 3.3). Methane emissions measurements and nutritional experiments are underway to evaluate the relationships between GHG and FE and the influence of diet energy concentration on efficiency.

Results.
The results of GWAS analyses indicate: heritabilities of RFI (DMI) in Angus and Hereford are 0.21 (0.35) and 0.45 (0.41) and the 10 largest QTL explain 9.1 (21.0) and 8.0% (12.6%) of additive genetic variance; additive genetic variance is similar between Angus and Hereford cattle; there is larger residual variance for Angus (and lower heritability) perhaps reflecting feeding at two different locations using Calan Gates versus GrowSafe systems; the QTLs with largest effects differ between Angus and Hereford; either different genes/mutations may underlie variation in these closely related breeds or allele frequencies differ greatly affecting QTL allele substitution effects and ability to detect them via linkage disequilibrium; and, identification of causal mutations must be performed within breeds. Examination of the locus in Angus cattle that explained 10% of the variation in DMI revealed that *FNIP1* is located in this region and may be important in the regulation of feed intake. Animals that more efficiently convert feedstuffs into growth do so with higher levels of methane emission than do less efficient animals. Published studies in which reduced methane production was found in high efficiency animals may be explained by the reduced feed intakes of efficient relative to inefficient animals at the same body weight and levels of growth. To address our extension objective, a website (www.beefefficiency.org) has been created and populated with information for scientists and producers. Video and handout materials are available explaining the potential role of MAM in beef production and the progress of the producer demonstration project.

Conclusions. By developing sample and data repositories and leveraging existing industry commercialization relationships, we will ensure rapid technology deployment and the sustainability of accomplishments. Genetic improvement of FE will increase farm participation in sustainable practices, increase animal production efficiency, improve US beef competitiveness and ensure a reliable and cost effective supply of high quality protein to consumers.

Acknowledgement. Project is supported by the NRI Initiative Competitive Grant no. 2011-68004-30214 from USDA NIFA.

Screening of Chinese herbal plants for reducing rumen methane production in vitro

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Introduction The structure of more than 200,000 plant secondary metabolites (PSM) has been defined. Some plants or their extracts contain high concentrations of bioactive PSMs, such as saponins and essential oils, have potential to inhibit rumen methane production (Patra and Saxena, 2010). Chinese herbal medicine has a long history and magical effect. In this study Chinese herbal plants were screened to identify the species containing active PSM that could be used to develop new feed additives to modify rumen fermentation.

Material and methods One hundred Chinese herbal plants containing volatile oil or saponin were collected and extracted by 70 % ethanol. The active ingredients were then evaporated under nitrogen and freeze-dried. The extract was dissolved to 40 mg/ml with dimethyl sulfoxide (DMSO) before assay. Incubations were carried out using Batch tube following the method of Theodorou *et al*. (1994). A total of 0.1 gram of Chinese wild rye meal and corn silage (7:3) was incubated in triplicate with 1 ml of rumen fluid collected from three donor cattle before morning feeding and 9 ml buffer medium prepared as described by Menke and Steingass (1988). A hundred µl of DMSO dissolved extract was added as treatment, and DMSO without extract was used as control. In each incubation run, three blanks were included simultaneously to correct the gas production for gas release from endogenous substrates. After 24 h incubation, gas pressure was recorded, and methane production was measured using gas chromatography.

Results Eighty percents of the tested plant extracts could increase gas production ($P < 0.01$). Twenty-seven percents could reduce methane production by more than 10% ($P < 0.01$). Eighteen extracts had reduced methane production more thane 10% without influence on gas production ($P > 0.05$). Six extracts had both reduced methane production and increased gas production by more than 10%. These six herb plants were *Geranium*, *Citrus aurantium*, *Viticis*, *Basil leaves*, *Ginger* and *Cinnamon* (Table 1).

Table 1 Effects of *Geranium*, *Citrus aurantium*, *Viticis*, *Basil leaves*, *Ginger* and *Cinnamon* on rumen fermentation *in vitro*

	Control	*Geranium*	*Citrus anrantium*	*Viticis*	*Basil leaves*	*Ginger*	*Cinnamon*	s.e.m
Gas production (ml/g)	105.1^{d}	119.3^{bc}	116.9^{c}	129.2^{ab}	134.0^{a}	128.2^{ab}	115.8^{c}	2.39
Methane (mmol/g)	0.612^{a}	0.519^{bc}	0.522^{bc}	0.499^{bcd}	0.537^{b}	0.481^{cd}	0.461^{d}	0.017

^{abcd} Means in the same column with different superscripts differ ($P<0.01$).

Conclusions Differnet Chinese herbal plants have varying influence on rumen fermentation. Some extracts improve rumen fermentation and lower methane production simutaneously. Chinese herbal plant may be good candidates to use for rumen manipulation and methane mitigation.

Supported by the Fundamental Research Funds for the central Universities (2011XZZX006) is acknowledged

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Technologies for the production of livestock feeds from cattle slaughterhouse wastes in sub-Sahara Africa

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Introduction The disposal of wastes (blood and rumen content) from cattle slaughter slabs into drains, streams, and rivers have constituted serious environmental problems in sub-Sahara Africa. These slaughterhouse wastes when processed have been identified as alternative source of protein feedstuff for livestock such as poultry, fish, rabbit and pig. Previous research has focused on the development of a technology/process-line for the production of fortified livestock feed from cattle blood and rumen content. Major limitations identified are in the collection, pressing, pulverization of the rumen content and mixing it efficiently with blood. These were hitherto carried out manually, which makes the entire process slow, laborious and inefficient thereby making commercial production difficult. This work focuses on the design of an integrated system for small/medium scale processing of cattle rumen content and blood into vegetable-carried blood meal for livestock.

Material and methods The integrated system was conceived as a simple, easy to operate set of machines capable of being used for small/medium scale commercial production with the benefit of increased capacity when the occasion demands. The integrated system comprises of an on-site rumen content collector and strainer; an improved screw press for dewatering the rumen content; a combined pulverizer/mixer to pulverize and mix the pressed rumen content/vegetable carrier with deoxygenated blood. Materials selection was based on locally available materials.

Results It promises to make in-situ collection and processing of the slaughterhouse "wastes" into protein fortified livestock feed an additional source of income for those involved in slaughterhouse business. The system has good potentials in abating environmental pollution problems which has characterized an average slaughter house in Nigeria and other countries in sub-Saharan region. The rumen collector was designed as a false bottom plastic container sunk inside a stainless-steel bucket with compartmentalized draining trough. The trough has a tap at its base, through which the rumen fluid can be collected without spillage. The rotary motion of the power screw unit of the double cage screw press causes a vertical displacement of the ram/plunger inside a stainless cylinder which in turn exerts pressure on the rumen contents. Mechanical mixing of liquid with powdery granules can be achieved by a horizontal drum mixer with a screw auger mechanism rotating inside a cylinder. This makes mixing to the required ratio and desired consistency possible with minimum drudgery.

Conclusions An integrated system for small/medium scale processing of cattle rumen content and blood into vegetable-carried blood meal for livestock was developed. The integrated system comprises of an on-site rumen content collector and strainer; an improved screw press for dewatering the rumen content; a combined pulverizer/mixer to pulverize and mix the pressed rumen content/vegetable carrier with deoxygenated blood. Performance evaluation of the system is on-going based on comparison of product quality with that of the manually processed. This design makes in-situ collection and processing of the "wastes" into protein fortified livestock feed an additional source of income for those involved in slaughterhouse business. Overall, it is a step in abating environmental pollution problems associated with slaughter houses in Nigeria.

Acknowledgements Authors acknowledge Dr O.A. Makinde of Animal Science Department for providing baseline information for this work.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Emissions of direct and indirect greenhouse gases from Australian cattle feedlots

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Introduction Loh *et al*. (2008) gave a brief report of the first measurements of greenhouse gas emissions from Australian cattle feedlots. They employed open-path lasers and Fourier transform infrared (FTIR) spectroscopy to make line-averaged measurements of CO_2, CH_4, N_2O and NH_3 concentrations within 2 feedlots, one in the country's South and one in its North, in 2-week campaigns in summer. Coupled with measurements of atmospheric turbulence, the concentration measurements allowed calculation of gas fluxes from the feedlot through the micrometeorological technique of backward Lagrangian stochastic (bLs) dispersion. Continuation of the measurement program for a second year allowed firmer estimates of the emissions to be made, while new equipment permitted continuous measurement of emissions of the indirect greenhouse gases NH_3 and NO_x over several weeks. This paper reports the new emission estimates and gives special consideration to emissions of the nitrogen gases, which in terms of CO_2 equivalents, appear to contribute significantly to greenhouse gas emissions from feedlots.

Materials and methods The methodologies for measurements of emissions of CO_2, CH_4, N_2O and NH_3 were the same throughout the 2-year study as those described by Loh *et al*. (2008). In initial field comparisons of gas sensors, controlled gas releases from point and grid sources were used to compare open-path lasers (Gasfinder 2, Boreal Laser Inc.) and open-path FTIR spectroscopic units (provided by the University of Wollongong). The instruments provided 15-min line-averages of concentrations of the gases over parallel path lengths which varied between 100 and 400 m. Turbulence measurements were made with a 3-D sonic anemometer (CSAT3, Campbell Scientific Inc.). Implementation of the bLs analysis was achieved through the software package WindTrax (Thunder Beach Scientific). After the last campaign, point measurements of concentrations of NH_3 and NOx were made for more extended periods by employing a closed-path chemiluminescence analyser (Ecotech Model 9840) capable of unattended running.

Results The initial field tests showed that both open-path lasers and FTIR have more than adequate precision and sensitivity for measuring CH_4 and NH_3 emissions from cattle feedlots. The measurements provided side-by-side FTIR-laser comparisons and demonstrated excellent correlations between line-averaged concentrations, generally within 2%. Typical feedlot concentrations of greenhouse gases and the precision of open-path FTIR for measurements over a path of 100-200 m are: for CH_4, concentration ~ 4000 ppbv and detection limit (3σ) < 6ppbv; for CO_2, ~ 400 ppmv and < 1.5 ppmv; for N_2O, ~ 320 ppbv and < 1 ppbv; for NH_3, ~ 1000 ppbv and < 3 ppbv. Detection limits for the open-path lasers were around twice those for FTIR, but still adequate for detecting the large gas concentrations in the feedlots. The long-term stability of FTIR was far superior to that of the lasers.The average emission rate of CH_4 over the 80 days of measurement at North and South feedlots was 113 ± 4(s.e.) g $head^{-1}d^{-1}$. This is substantially lower than the 201 g $head^{-1}d^{-1}$ predicted by the current Australian methodology (Moe and Tyrell, 1979) and closer to the IPCC Tier II estimate of104 g $head^{-1}d^{-1}$. There was some diurnal variation in emission rate with maximum emissions occurring in early morning and late afternoon, apparently associated with increases in animal eruction and general activity (Loh *et al*., 2008). The average CO_2 emission rate for all 8 campaigns was 12.9 ± 0.8 kg $head^{-1}d^{-1}$ with a similar small diurnal variation.he average N_2O emission rate for all 8 campaigns was 3.3 ± 0.8 g $head^{-1}d^{-1}$, about half that modeled by IPCC (6.5 g $head^{-1}d^{-1}$), while in contrast, the average NH_3 emission rate was 176 ± 8 g $head^{-1}d^{-1}$ which is around 3 times the IPCC Tier II estimate. The high air temperatures in Australia could be expected to increase NH_3 volatilisation in comparison with northern hemisphere rates, which, in turn, would reduce the substrate available for nitrification and N_2O production. Both gases exhibited marked diurnal variation.
The measurements of NH_3 and NO_x reveal features of N gas emissions from feedlots not previously explored. The mean of the N_2O emissions over 4 campaigns was equivalent to 1.30 kg N ha^{-1} d^{-1} and those of the combined campaign and long-term measurements of NH_3, 95 kg N ha^{-1} d^{-1}, and those of NO_x, 1.2 kg N ha^{-1} d^{-1}. Assuming that 1% of the NH_3 and NO_x released to the atmosphere is converted to N_2O after deposition back on the land surface (Mosier *et al*., 1998), we estimate a net annual contribution to the atmosphere from Australian cattle feedlots of 0.42 t CO_2-e, 75% of which comes from conversion of deposited NH_3 and NO_x to N_2O. In terms of CO2-e, this is as large as 60% of the greenhouse contribution of CH_4.

Conclusions The micrometeorological approach, using open- and closed-path measurement systems and atmospheric dispersion modeling has proved to be a suitable methodology for quantifying emissions from cattle feedlots. It has the desired precision and while quantifying emission rates, it has identified diurnal cycles in the emissions of CH_4, N_2O, NH_3 and NO_x. Emissions of CH_4 (113 g $head^{-1}$ d^{-1}) and N_2O (3.3 g $head^{-1}$ d^{-1}) were generally lower than model estimates and emissions from northern hemisphere studies while emissions from NH_3 (176 g $head^{-1}$ d^{-1}) are much higher. Higher atmospheric temperatures may play a role in the N_2O- NH_3 discrepancies. Our studies of emissions of the latter gases have revealed a possibly very large contribution to greenhouse gas emissions from feedlots that increases N_2O emission by 75% and in CO_2-e, puts the net N_2O emissions at 60% of CH_4 emission.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Can soil carbon accumulation offset methane and nitrous oxide emissions when transitioning from cropping to livestock production?

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Introduction Methane (CH_4) and nitrous oxide (N_2O) are significant greenhouse gases generated from livestock production systems (IPCC, 1997). However, grasslands also have the potential to accumulate carbon (C) in the soil, if perennial pasture is established following an extended cropping phase (Guo and Gifford, 2002). The aim of this study was to model net farm emissions, with 'net farm emissions' defined as CH_4 plus N_2O emissions minus soil C accumulation, from a grazing system in south-eastern Australia.

Materials and Method CH_4, N_2O and soil C were simulated in a pasture-based grazing system at Hamilton (37.83°S, 142.06°E) in south-eastern Australia, using the Sustainable Grazing Systems Pasture model (Johnson *et al.* 2003). The simulation used a brown chromosol soil type, and a pasture based on perennial ryegrass and white clover stocked at 15 ewes/ha (mature liveweight 60 kg). Simulations were run for 111 years using local climate data from 1901-2011. CH_4 emissions were predicted as 6% and 4% gross energy intake from pasture and grain (fed when required to maintain bodyweight >40 kg) respectively. N_2O was predicted dynamically based on available soil N, temperature and water filled pore space. Soil C was modelled in three pools (fast and slow turnover, inert) with half lives of 1.05 and 34.50 years for the fast and slow pools. Firstly, the soil C simulation was tested against measured data from the site using a long term simulation (1901-2011) starting with an initial soil C measured at the site (104.6 t C/ha, 0-30 cm) and comparing with measured mean values of 104 and 111 t C/ha in 1994 and 2004 (Graham *et al.* 2008). Secondly, soil C accumulation was simulated after an extended cropping phase, assuming an initial value of 71 t C/ha. Changes in soil C were converted to CO_2-e by multiplying by 3.7. Net farm emissions (CH_4 emissions + N_2O emissions – soil C change, t CO_2-e/ha) were calculated on an annual basis. CH_4 and N_2O emissions were converted to carbon dioxide equivalents (CO_2-e) using global warming potentials of 21 and 310 respectively.

Results The total soil C over 111 years starting from the measured or post-cropping conditions are shown in Figure 1a. Using the measured initial conditions total soil C was stable over time, consistent with measured data. There was an accumulation of 18.8 t C/ha over 111 years when starting from the post-cropping soil C (71 t C/ha). Annual emissions of CH_4 were similar across years (range of 1.8-2.4 t CO_2-e/ha) but N_2O emissions varied according to seasonal conditions (range of 0.5-2.1 t CO_2-e/ha). There was high variation in annual net farm emissions (Figure 1b), reflecting the effects of climatic conditions on N_2O emissions and soil C storage. In only 10 of the 111 years simulated did soil C accumulation fully compensate for CH_4 and N_2O emissions resulting in negative net farm emissions. Over time a trend was observed with net farm emissions increasing as soil C reached a new steady state (Figure 1b).

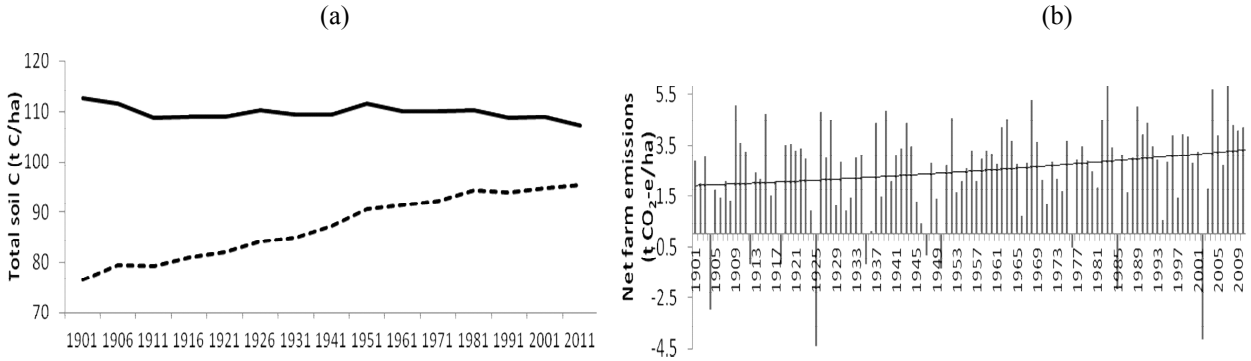

Figure 1 a) Total soil C (t C/ha, 0-30 cm soil depth) simulated over 111 years from the 'measured' (solid line) and 'crop' (dashed line) initial soil C levels, and **b)** Net farm emissions (t CO_2-e/ha) simulated for the 'crop' initial soil C level.

Conclusions These modelled results show that if moving from cropping to permanent pasture, soil C levels will increase, offsetting net farm emissions. Over the 111 years simulated soil C offset 25% of the CH_4 and N_2O emissions. However soil C accumulation reduces over time, as a new steady state is reached, leaving CH_4 and N_2O emissions the predominant driver of net farm emissions.

Acknowledgements The authors would like to gratefully acknowledge funding from the Australian Government Department of Agriculture, Fisheries and Forestry, Meat and Livestock Australia, Dairy Australia and the Australian Wool Innovation.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Quantify the distribution of bacterial and methanogens colonization associated with particulate phases of developing rumen ingesta in calves

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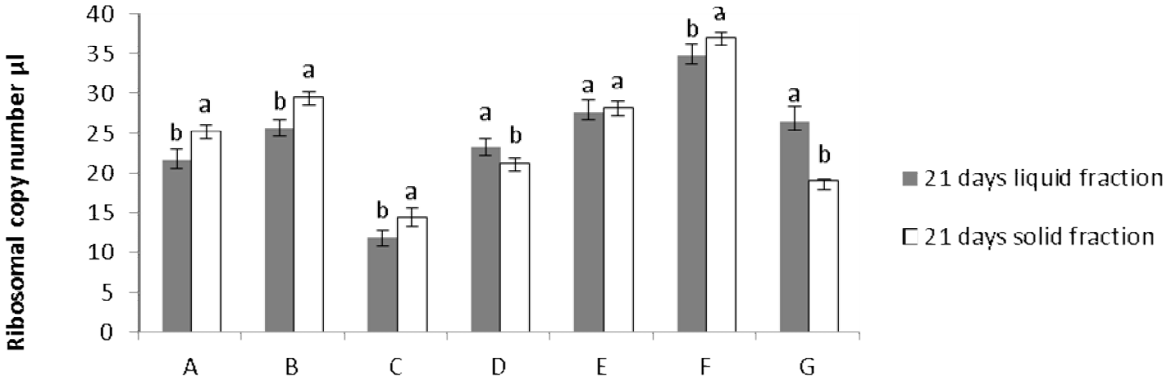
Introduction Forage is exposed to a unique population of microbes that begin to ferment and digest the plant cell wall components and break these components down into carbohydrates and sugars. Rumen microbes use carbohydrates along with ammonia and amino acids to grow. The microbes ferment sugars to produce VFAs (acetate, propionate, butyrate), methane, hydrogen sulfide, and carbon dioxide but immature ruminants, such as young growing calves from birth to about 2 to 3 months of age, are functionally non-ruminants. The aim of this study was to compare the distribution and quantification of microbial populations in the liquid and solid fractions in the rumen of three week old calves.

Materials and Methods The experiment involved 20 friesian 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. The experiment consisted of two treatments were given to calves from 5 to 21 days of age. From 3 days of age they were hand-fed with calf-milk replacement (CMR) twice a day. The first treatment called control (C) was fed CMR twice a day and was allowed unrestricted access to solid feed (hay and calf meal). The second group was called 'control restricted' (CR) in which the calves were fed CMR twice a day plus hay and allowed restricted access to calf meal daily at 60% of the unrestricted ration. At 21 days of age the calves were killed and samples were taken post mortem and separate samples were taken from both the liquid and solid fractions of the rumen contents. For solid extraction of DNA used methods Masoni *et al*. (2011). Quantity of the genes encoding 16S and 18S ribosomal DNA of the developing rumen microbiota I used the molecular techniques gel electrophoresis, cloning, sequencing, and quantitative polymerase chain reaction (qPCR). The following microorganisms were examined: Domain Archaea, the genera *Methanobacterium* and *Methanobrevibacter* in the order Methanobacteriales; Phylum Bacteria, cellulolytic bacteria (*Fibrobacter succinogenes*); Phylum Firmicutes (*Ruminococcus flavefaciens);* Phylum bacteriodetes, the proteolytic bacteria (*Prevotella ruminicola*); Phylum Protozoa (*Entodinium* spp) and Kingdom fungi. Details for analysis of these microorganisms can be found in Stevenson (2007).The data were analysed using the generalized linear model (GLM). Treatment means were compared using one-way ANOVA post hoc multiple comparisons with the Duncan HSD test. All statistical analyses were conducted using version 8.0 SPSS (IBM). Results were considered significant at the $P < 0.05$ level.

Results



Control treatment (A) *Methanobrevibacter spp,* (B) *Methanobacterium spp,* (C) *P. ruminicola, (D) F. succinogenes* spp, (E) *R. flavefaciens,* (F) *Entodinium* spp and (G) fungi. The trend was the same for the restricted treatments. Treatment means assigned the same letter indicate no significant difference ($P < 0.05$).

Conclusion No significant difference was found between unrestricted and restricted diets. The novel of this study show for the first time how are the distribution of the microorganisms in solid and liquid fraction. The distribution of microorganisms on the substrate suggest that at this early stage they do not use specific resources because the activities and different processes in the developing rumen is changing all the time until the rumen has fully developed. Further research is required to understand the interaction between microorganisms within the developing rumen of the young ruminant.

Acknowledgements This study was financially support by La Trobe University, Department of Agricultural Science. Victoria, Australia.

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The effect of earlier mating and improving fertility on emissions intensity of beef production in a northern Australian herd

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Introduction Enteric methane (CH_4) generated from livestock production systems accounts for approximately 39% of global anthropogenic CH_4 emissions (Lassey, 2007). In beef production systems earlier mating, improving weaning rates and increasing daily weight gain have potential to reduce greenhouse gas (GHG) emissions from the herd (Eckard *et al*, 2010). The aim of this study was to investigate the changes in GHG emissions and emissions intensity from a typical beef system in northern Australia, and compare it to herds managed to achieve higher reproductive performance.

Materials and Method A case study farm which produced high quality steers was modelled using three herd structures. The farm is 23,000 ha and located in the Mitchell Grass Downs bioregion, 65 km south of Longreach (23.44°S, 142.25°E), in central Queensland, Australia with an annual average rainfall of 380 mm. The three herd structures that differed in approaches to management of reproduction were: (1) 'typical' herd was based on average management for the region with heifers mated at 2 years and low weaning rates; (2) 'early joining' had heifers mated at 1 year with low weaning rates; and (3) 'early joining and high fertility' with heifers mated at 1 year and selection to achieve higher weaning rates. The third herd also used cross-breeding which contributed to higher steer liveweights at sale. The herd structures were simulated in Breedcowplus (Holmes, 2012). Table 1 lists the key herd data. Total stocking pressure (adult equivalents) was kept the same across all three herds.

Table 1 Key herd characteristics for the 'typical', 'early joining' and 'early joining and high fertility' herd structures.

Herd	No. mated	Breeder weight (kg)[A]	Age at first joining	Weaning %[B]	Adult equivalents
Typical	984	481	2 years	62	1748
Early joining	1256	436	1 year	54	1752
Early joining and high fertility	1007	435	1 year	79	1748

[A] weighted mean of 1 and 2 year old heifers and mature cows mated.
[B] weaners as percentage of all cows mated.

To estimate farm GHG emissions (t CO_2 equivalents (CO_2e)) the animal numbers, liveweights and growth rates were entered into the Greenhouse Accounting Framework for beef (GAF-beef; Browne *et al,* 2010). Farm emissions were estimated from methane (enteric and manure), nitrous oxide (dung, urine and other indirect sources) and farm energy use. Liveweight turnoff (t) was calculated for each animal class, and used to estimate the emissions intensity of beef production (t CO_2-e/t liveweight). Farm gross margin was estimated based on regional average costs and prices.

Results Beef turnoff was highest in the 'early joining with high fertility' herd, with 33% higher turnoff compared to the 'typical' herd (Table 2). Total GHG emissions were similar across the herds. Emissions intensity was reduced by 24% for the 'early joining with high fertility' herd compared to the 'typical' herd. The gross margin for the 'early joining with high fertility' herd was more than double that of the 'typical' herd.

Table 2 Beef turn-off, GHG emissions, emissions intensity and gross margin for the three herd structures.

Herd	Beef turn-off (t lwt)	GHG emissions (t CO_2-e)	Emissions intensity (t CO_2-e / t lwt)	Gross margin after interest ($)
Typical	236.3	3,593	15.2	157,153
Early joining	255.4	3,571	14.0	176,537
Early joining and high fertility	314.2	3,598	11.5	339,765

Conclusions In this study, total stocking pressure (adult equivalents) was kept constant so there was only a small difference in total GHG emissions between the herds. Earlier mating and improved weaning rates increased turnoff and reduced the GHG emissions intensity of beef production in northern Australia. The earlier mating, improved steer growth rates and improvements in weaning rates were also highly profitable.

Acknowledgements The authors gratefully acknowledge funding from the Australian Government Department of Agriculture, Fisheries and Forestry, Meat and Livestock Australia, Dairy Australia, Australian Wool Innovation, and the Queensland Government.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

The choice of rumen sampling and DNA extraction methods affects the apparent microbial community composition: Implications for the Global Census of Rumen Microbial Diversity Project

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Introduction Ruminants such as cattle, sheep, deer, yak, buffalo and goats are of great importance for the production of meat and dairy products, wool and leather worldwide. Ruminant digestion of feed mainly takes place in the rumen where microbes play a key role in the breakdown of feed components such as cellulose and produce short chain fatty acids that provide an energy source for the host. These microbes are thus essential providers of energy and nutrition, and play a key role in the productivity and health of ruminants. Rumen microbes known as archaea produce the greenhouse gas methane as a metabolic end product. The rumen microbial community can be influenced by many parameters such as diet, ruminant species, treatment with antibiotics or methane mitigation agents, experimental design and data analysis. Knowledge and understanding of rumen microbial communities is essential to elucidate complex microbe-driven processes such as ruminant feed digestion and to find means to reduce greenhouse gas emissions. AgResearch has initiated an international collaborative "Global Census of Rumen Microbial Diversity Project" affiliated with the Rumen Microbial Genomics Network and funded by the New Zealand Government in support of the Global Research Alliance on Agricultural Greenhouse Gases. The Census will survey DNA markers for different microbial groups and so relies on suitable rumen sampling and DNA extraction methods. The project aims to obtain rumen samples covering different locations, breeds and diets. The aim of the study reported here was to systematically compare the suitability of different sampling and DNA extraction methods for downstream analysis of rumen microbial communities with molecular microbial ecological methods.

Materials and Methods To compare extraction methods, DNA was extracted in triplicate from total rumen contents from a ruminally-fistulated hay-fed cow and a pasture-fed sheep following 15 different protocols. DNA yield and quality were compared. Bacterial, archaeal, fungal and protozoal marker gene copy numbers and community compositions were determined by quantitative PCR and titanium pyrosequencing, respectively. To compare sampling techniques, rumen samples were collected from 14 cows by sampling via either a stomach tube or the rumen fistula and the microbial community composition determined as described above.

Results Absolute microbial marker gene copy numbers per gram of sample differed over 100-fold, depending on the DNA extraction method used. The choice of DNA extraction method significantly affected the prevalence of bacterial, archaeal, fungal and protozoal groups at various taxonomic levels. In the sheep sample, the relative abundances of certain microbial taxa such as the phylum *Fibrobacteres* (1.5 to 7.2%) and the archaeal Rumen Cluster C group (2 to 13.2%) differed more than 4-fold, resulting in shifts in the apparent microbial community composition. Increasing the duration of mechanical lysis in DNA extraction resulted in greater concentrations of DNA being extracted and appeared to enhance the extraction of DNA from representatives of the phylum *Firmicutes*. The choice of sampling method had an impact on the prevalence of some microbial groups in that sampling via stomach tube appeared to bias towards the *Prevotellaceae* family (1.3-fold increase, 24.8 to 31.3%) but against members of the family *Lachnospiraceae* (1.4-fold decrease, 20.1 to 14.5%).

Conclusions Some DNA extraction methods are more suitable and comparable than others to analyse rumen microbial communities and should preferably be used. Also, caution must be exercised when comparing data from studies in which different DNA extraction methods were employed. We will use comparable DNA extraction methods for the Global Census of Rumen Microbial Diversity Project, in which bacterial, archaeal, protozoal, and fungal marker genes will be sequenced and analysed in context with sample background information such as species, diet and location. This information will allow several questions to be addressed: Is there a core rumen microbial community? What is the extent of diversity in each microbial group? How much variation is there in rumen microbial communities? What novel groups are present? Are there any indications of factors controlling variation (from metadata) for future follow-up work? The Global Census of Rumen Microbial Diversity Project can only be accomplished with a global network of researchers willing to collaborate and provide input. To date, researchers from over 20 countries have committed to the project. Researchers are asked to contact Dr Gemma Henderson (global.rumen.census@agresearch.co.nz) to obtain further information and participate in the project.

Acknowledgements We thank Michelle Kirk for technical assistance. Research was funded by and carried out under contract to the Pastoral Greenhouse Gas Research Consortium (PGgRc) and the New Zealand Government to support the objectives of the Livestock Research Group of the Global Research Alliance on Agricultural Greenhouse Gases. The information contained within this presentation should not be taken to represent the views of the Alliance as a whole or its Partners.

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Global Census of Rumen Microbial Diversity Project – www.globalrumencensus.org.nz
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Global Research Alliance on Agricultural Greenhouse Gases - www.globalresearchalliance.org

https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Milk fatty acids can assist in the prediction of methane from dairy cows fed a range of diets

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Introduction It has been proposed that methane emissions from dairy cows could be predicted from the concentrations of specific milk fatty acids (Chilliard *et al.* 2009). The proposed relationships were developed from a corn silage and concentrate diet with an addition of one of whole linseed, extruded linseed or linseed oil. The relationships have been tested by others but only for cows fed total mixed rations incorporating a limited range of feed additives (Mohammed *et al.*, 2011; Dijkstra *et al.*, 2011). The aim of this work was to determine if fatty acid concentrations in milk could assist in the prediction of methane emission from dairy cows fed a range of diets.

Material and methods Data (151 individual cow records) were collated from five experiments conducted at Department of Primary Industries, Ellinbank, Victoria, Australia (38°14'S, 145°56'E). Diets included alfalfa forage supplemented with docosahexaenoic acid, cottonseed oil, grape marc, corn grain and wheat grain, and perennial ryegrass pasture supplemented with tannin. Methane emissions (g/day), yield (g/kg DMI) and intensity (g/kg milk) were modelled using a range of variables. For the dependent variable, a search of all possible regression models was conducted in the independent variables DMI, milk yield (kg/d), milk fat (kg/d), days in milk, DeNovo fatty acids (total C4 to C15, proportion of total fatty acids in milk), C16 fatty acid (proportion of total fatty acids in milk), C17 fatty acid (proportion of total fatty acids in milk), pre-formed fatty acids (total C18 +, proportion of total fatty acids in milk), and odd chain fatty acids (C13, C15, C17, proportion of total fatty acids in milk), using the RSEARCH procedure in GenStat 15 (VSN International, Hemel Hempsted, United Kingdom). The model with smallest Mallows Cp statistic (Thompson 1978) was identified and re-fitted, including random effects for experiment and cow, using ReML in GenStat 15.

Results

Table 1 Best-fit models for predicting methane emission, yield and intensity (coefficient ±standard error)

Predicted	Constant	DMI (kg/d)	Milk (kg/d)	DIM	DeNovo (% total FA)	C16 (% total FA)	C17 (% total FA)	Preform (% total FA)	adjusted R^2	Residual standard deviation
CH_4 (g/d)	441 ±12.0	12.8 ±1.98	n.s.	n.s.	4.0 ±1.43	4.1 ±0.94	-137 ±13.0	n.s.	78.0	54.0
CH_4(g/kg DM)	21.0 ±0.55	-0.39 ±0.097	n.s.	n.s.	0.19 ±0.071	0.21 ±0.047	-6.4 ±0.65	n.s.	68.2	2.64
CH_4(g/kg milk)	19.4 ±0.24	n.s.	-0.50 ±0.051	0.03887 ±0.0059	1.6 ±0.39	1.7 ±0.35	-4.8 ±0.85	1.44 ±0.355	89.9	2.75

DMI = dry matter intake, DIM = days in milk, DeNovo = total C4 to C15 fatty acids in milk, C16 and C17 = fatty acid in milk, Preform = total C18+ fatty acids in milk, n.s. = not significant in best-fit model $P \leq 0.005$.

Conclusions Milk fatty acids, when combined with other production parameters, can assist in the prediction of methane intensity (g/kg milk) and methane yield (g/kg DMI) when cows are fed a wide range of diets. However, there is still a large error in the prediction of methane emission (g/d).

Acknowledgements This research was funded by the Department of Primary Industries - Victoria, Dairy Australia, the Gardiner Foundation, Meat and Livestock Australia, and the Australian Government Department of Agriculture, Fisheries and Forestry under its Australia's Farming Future Climate Change Research Program.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

The Hungate1000. A catalogue of reference genomes from the rumen microbiome

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Introduction The Hungate1000 project aims to produce a reference set of rumen microbial genome sequences from cultivated rumen bacteria and methanogenic archaea, together with representative cultures of rumen anaerobic fungi and ciliate protozoa. The reference genome information gathered will be used for two main purposes 1) to support international efforts to develop methane mitigation and rumen adaptation technologies, 2) to initiate genome-enabled research aimed at understanding rumen function in order to find a balance between food production and greenhouse gas emissions. The project is funded by the New Zealand Government in support of the Livestock Research Group of the Global Research Alliance on Agricultural Greenhouse Gases. The sequencing effort has obtained support from the US Department of Energy Joint Genome Institute Community Sequencing Program, and the overall project is a global collaboration between members of the Rumen Microbial Genomics Network, a network established to accelerate knowledge development and mitigation solutions in the rumen microbial genomics research area.

Rumen microbial resources A variety of bacteria and methanogenic archaea have been isolated from the rumen using techniques for cultivating strictly anaerobic organisms, and these have been characterised on the basis of their morphology and phenotypic properties. To determine if cultured bacterial isolates capture the phylogenetic diversity found in the rumen, 16S rRNA gene sequence data from rumen metagenome datasets were analysed and overlaid with sequences from known rumen bacterial isolates. This indicated that there are cultured representatives for most of the bacterial groups present in the rumen, and that bacteria belonging to the families Lachnospiraceae, Ruminococcaceae (phylum Firmicutes), and Prevotellaceae (phylum Bacteroidetes) make up a significant proportion of the rumen microbiome. Many of the genera within these families have yet to be described taxonomically. While in recent years there have been periodic reports of novel bacteria isolated from the rumen of domesticated and wild ruminants, there have been few attempts to systematically bring additional organisms into cultivation. Recent studies in Japan (Koike *et al*., 2010, Nyonyo *et al*., 2013) and NZ (Kenters *et al*., 2011, Noel *et al*., unpublished) have successfully increased the number of taxa of rumen bacteria that have cultured representatives. The rumen methanogenic archaea are poorly represented in culture collections, and work is in progress to isolate and genome sequence additional rumen methanogen cultures.
Anaerobic fungi and ciliate protozoa make up a significant proportion of the rumen microbial biomass and form important interactions with the methanogenic archaea. The rumen anaerobic fungi are early colonisers of ingested plant material and use their hyphae to invade and degrade recalcitrant fibre in the rumen. There is a high degree of genetic diversity within the rumen fungal communities (Kittelmann *et al*., 2012). Ciliate protozoa are represented by comparatively small numbers in the rumen, but a large number of different species have been described based on morphological features (Williams and Coleman, 1992).

Rumen microbial genomes Genome sequence information is only publically available for a small number of rumen bacteria and two methanogens. Several bacteria have been investigated because of their role in the processes of plant polysaccharide degradation and SCFA production which are central to the growth and productivity of ruminant animals. Consequently, some of the first genomes to be sequenced from the rumen were those of cultivated bacteria considered to have key roles in breakdown of the cellulose (*Fibrobacter* and *Ruminococcus*) and hemicellulose (*Butyrivibrio* and *Prevotella*) components of plant cell walls. As expected, these fibrolytic bacteria have a particularly wide range of degradative abilities and produce acetate, butyrate and propionate that can be used by the animal, as well as CO_2, formate, methanol and H_2 which are substrates for methanogenesis.

Progress Sequencing of the first 100 bacterial genomes is underway and genomic DNA is being prepared from additional cultures.The criteria for selection of cultures for inclusion in the Hungate1000 project will include: 1) novel isolates, 2) coverage of the phylogenetic diversity found in the rumen, 3) known species that are not yet sequenced, 4) multiple strains belonging to genera that show high phenotypic diversity, 5) numerically dominant organisms identified from analysis of metagenome data, 6) different host animals and diets, 7) a most wanted list of the cultures as recommended by rumen microbiology researchers. We invite researchers interested in this project to contact the authors at hungate1000@agresearch.co.nz.

Acknowledgements Research is funded by the New Zealand Government to support the objectives of the Livestock Research Group of the Global Research Alliance on Agricultural Greenhouse Gases (http://www.globalresearchalliance.org/). "Disclaimer: the information contained in this abstract should not be taken to represent the views of the GRA as a whole or its partners".

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Effects of Propionibacterium freudenreichii on rumen methane production of beef finishing diets containing flax oils using a semi-continuous fermentation system (RUSITEC)

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Introduction The use of propionibacteria as a direct fed microbial has the potential to produce more propionate as an alternative hydrogen sink to methanogenesis in ruminants (McAllister and Newbold, 2008). Flaxseed oil has been shown to inhibit methanogenesis, likely by direct toxic effects on methanogens and to a lesser extent, by biohydrogenation (Johnson and Johnson, 1995). However, no studies on the ability of propionibacterium to mitigate methane from diets supplemented with flaxseed oils using artificial rumen simulation technique (RUSITEC) have been undertaken. Thus, this study aimed to identify the effects of including *Propionibacterium freudenreichii* (strain T54) or P. *freudenreichii* (strain T54) and flaxseed oil in a beef finishing diet, on *in vitro* methane production and ruminal fermentation parameters using the RUSITEC.

Material and methods The experimental period was 23 d, 8 d of adaptation and 15 d of inoculation with *P. freudenreichii* (strain T54). The experiment was a factorial design with a total of four treatments. All treatments were based on TMR finishing diet (TMR) with either: 1) CON: (10 g TMR+ 23 mL autoclaved mixture of sodium lactate broth (SLB) with 10^9 CFU of P. *freudenreichii*); 2) PB: (10 g TMR + 10^9 CFU of *P. freudenreichii* /23 mL inoculated SLB); 3) FO: (10 g 6% DM flaxseed oil- based TMR + 10^9 CFU of *P. freudenreichii* / 23 mL autoclaved mixture of SLB); 4) FOPB (10 g 6% DM flaxseed oil- based TMR + 10^9 CFU of P. *freudenreichii* / 23 mL mixture of inoculated SLB). *In situ* dry matter disappearance (*in situ* DMD) was determined at 48 h on d 10 to 13. Total gas production was measured daily from each fermenter. On d 9 to 13, concentrations of methane as a percentage of total gas were sampled from the gas bags; VFA and ammonia concentrations were measured at 24 h. Total microbial protein synthesis and microbial protein incorporation of N^{15} were determined on d 14 to 18. Within each run (n=2), treatments were replicated twice so that there were four replicates per treatment. Two RUSITEC apparatuses were used in this study, each equipped with eight 920 mL volume anaerobic fermenters immersed in a water bath maintained at 39ºC. To begin the experiment, each fermenter was filled with 180 mL of McDougalls buffer, 720 mL of strained rumen fluid, two pre-labeled nylon bags (5 × 10 cm, ANKOM), one containing 10 g of solid rumen digesta, and the other containing 10 g DM of diet substrate either with or without oil depending on the treatment. After 24 h, the nylon bag containing solid rumen digesta was removed and replaced with a single nylon bag containing 10 g of the appropriate diet. Thereafter, one bag was replaced daily (10:30 AM) so that each bag remained in the fermenter for 48 h, except on the last day when one bag in each vessel were removed after 24 h. The study was statistically analysed using the PROC Mixed SAS procedures (2012) with treatment as fixed effect, and run and run × treatments interactions as random effects. The two replicates from each treatment were averaged prior to statistical analysis and those averages by run contributed to the statistical unit.

Results *In situ* DMD (%) at 48 h and daily gas production (mL) were not affected ($P>0.10$) by either flaxseed oil or T54. Methane production (mL/g DMD) was reduced ($P<0.01$) by T54 (21%) or flaxseed oil alone (8%), or in combination (12%). Treatments with flaxseed oil had higher ($P<0.01$) total VFA production (mM) in comparison with non - oil groups. No difference in butyrate percentage was observed ($P>0.10$). However, T54 or flaxseed oil alone, or in combination, decreased ($P<0.02$) acetate production and the A:P ratio ($P<0.01$). *P. freudenreichii* supplementation increased ($P<0.01$) the percentage of propionic acid by 14% compared to the control. Similarly, T54 or flaxseed oil alone, or in combination increased ($P<0.01$) total microbial protein (mg) by 24 – 27% compared to the control. *P. freudenreichii* increased ($P<0.01$) total microbial incorporation of N^{15} (μg) 17 – 43% as compared to other treatments.

Conclusions Supplementation with *P. freudenreichii* T54 or flaxseed oil alone or in combination resulted in reductions in methane emissions with the greatest reduction occurring when only T54 was added to the fermenters. It was anticipated that oil would reduce methane emissions, but this reduction was further enhanced by the addition of *P. freudenreichii*, suggesting that these treatments may alter methane emissions by different mechanisms. Some of the response associated with *P. freudenreichii* does appear to arise as a result of increased propionate production during fermentation.

Acknowledgements This project was partially supported by the Norway - Canada BILAT project.

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Effect of crude glycerin on performance and methane emission of Nellore young bulls finished in feedlot

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Introduction Crude glycerine (CG) is a by-product of biodiesel industry and has been considered as a viable energy source for cattle, particularly when it is included in up to 10% of total diet dry matter (Drouillard, 2012).This trial aimed to evaluate the effects of feeding CG - 80% glycerol - included on 10% of DM diet, replacing corn or soybean hulls in different concentrate level (CL) 40 or 60% on enteric methane (CH_4) production of young bulls finished in feedlot.

Material and Methods Thirty six young bulls (Nellore), with 374.11 ± 24.77 initial BW, were randomly assigned to six treatments, with six replicates. The diets were: without CG plus corn (Cn); association of CG and corn (CGc), association of CG plus soybean hulls (CGsh). These three diets were combined with two CL (40 or 60%), resulting in six diets isonitrogenous. Corn silage was used as the only source of roughage and concentrates were composed of grounded corn or soybean hulls, soybean meal, urea/ammonium sulphate and mineral mixture. The urea was used for adjusted the crude protein in diets - maximum 1% of diet DM. Diets were fed as total mixed ration and cattle were fed twice daily (at 0700 and 1500) allowing for up to 10% of orts. Animals were assigned in individuals pens and after 94 days of feeding, the animals were slaughtered with average of 495.50 kg BW. The average daily gain (ADG) was obtained at the beginning and end of the experimental period. Ruminal CH_4 was measured after 87 d of feed using a sulphur hexafluoride (SF_6) gas tracer every 24 h during five consecutive days in one experimental period with 15 prior adaptation days according to the method described by Johnson and Johnson (1995). CH_4 flux produced by animals was calculated in relation to the SF_6 tracer gas flux from a permeation capsule lodged in the rumen minus the basal CH_4 concentration in the air (Westberg *et al*., 1998). Following equation was used: $Q_{CH4} = Q_{SF6}$ x $([CH_4]_y - [CH_4]_b) / [SF_6]$, where Q_{CH4} = CH_4 emission tax by animal; Q_{SF6} = know SF_6 emission tax from capsule in rumen; $[CH_4]_y$ = CH_4 concentrations in collection apparatus; $[CH_4]_b$ = basal CH_4 concentration; and $[SF_6]$ = SF_6 concentration in collection apparatus. CH_4 outputs (g/d) proportional to DM intake DMI (kg/d) and organic matter intake OMI (kg/d) were calculated by dividing the daily CH_4 output of each animal by their daily DMI, OMI (during CH_4 sampling) and ADG (throughout the entire experimental period). The experiment was conducted according to a completely randomized design in a factorial arrangement 2x3 (two CL x three feeding regimes; FR). Data were analyzed by the GLM procedure of SAS, and the Tukey test used considering 5% probability.

Results There was no interaction ($P > 0.05$) between CL and FR for any of the variables evaluated. There was no effect ($P > 0.05$) of CL on ADG, CH_4 emitted per day (g $CH_4.day^{-1}$), CH_4 per kilogram of ADG (g $CH_4.kg\ ADG^{-1}$), CH_4 per kilogram of DM intake (g $CH_4.kg\ DMI^{-1}$) and CH_4 per kilogram of organic matter intake (g $CH_4.kg\ OMI^{-1}$). The inclusion of CG in diets did not affect ($P > 0.05$) the ADG, g $CH_4.day^{-1}$, g $CH_4.kg\ ADG^{-1}$, g $CH_4.kg\ DMI^{-1}$ and g $CH_4.kg\ OMI^{-1}$. However, on FR there was a tendency ($P = 0.0959$) to increase g $CH_4.kg\ DMI^{-1}$ in diets with CG plus corn.

Table 1 Average daily gain and methane emission of Nellore young bulls finished in feedlot

Item[1]	Concentrate Level (CL)		P[2]	Feeding regimes (FR)[3]			P[2]	CL x FR P[2]
	60:40	40:60		Cn	CGc	CGsh		
ADG, kg	1.27±0.05	1.32±0.04	0.4922	1.25±0.08	1.36±0.04	1.29±0.06	0.4511	0.9334
CH_4, g/d	171.30±10.31	171.17±7.73	0.9920	162.11±10.18	183.05±13.83	168.55±7.82	0.4113	0.4897
CH_4, g/kg ADG	138.75±7.72	127.74±4.82	0.2560	131.48±7.47	134.55±10.62	133.71±5.52	0.9625	0.5910
CH_4, g/kg DMI	20.83±0.91	21.18±0.86	0.7597	19.32±0.84	22.32±1.25	21.38±0.94	0.0959	0.0671
CH_4, g/kg OMI	30.08±1.38	27.64±1.19	0.1512	26.51±1.43	30.60±1.87	29.47±1.29	0.1264	0.0823

[1/]ADG = average daily gain; DMI = dry matter intake; OMI = organic matter intake; [2/]Probability ($P < 0.05$); [3/]Cn = without crude glycerin; CGc = crude glycerin plus corn; CGsh = crude glycerin plus soybean hulls.

Conclusion Animals fed with low or high level of concentrate showed similar performance and methane enteric emissions. Animals fed with crude glycerin in 10% of DM showed similar methane enteric emissions than animals fed without crude glycerin on the diet.

Acknowledgements Bellman® Animal Nutrition; FAPESP

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Effect of lipids sources in diets with crude glycerin on methane emission of Nellore young bulls finished in feedlot

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Introduction The inclusion of lipids in the diets reduces methane emissions (Johnson and Johnson, 1994). The association of crude glycerin (increase production of propionate in the rumen) and lipid sources has great potential for further impacts on methane production. The trial aimed to evaluate the effects of feeding crude glycerin (CG) - 80% glycerol - included on 10% of DM diet with lipids sources on enteric methane (CH_4) production of young bulls finished in feedlot.

Material and Methods Twenty four young bulls (Nellore), with 426.00 ± 30.20 initial BW, were randomly assigned to four treatments, with six replicates. The diets (30% of corn silage and 70% concentrate) were: with 10% of CG being control diet (Cn); with 10% of CG plus soybean oil (SO), with 10% of CG plus whole soybean grain (SG) or with 10% of CG plus bypass fat (BF). Animals were assigned in individuals pens and after 97 days of feeding, the animals were slaughtered with average of 521.30 ± 44.27 kg BW. The average daily gain (ADG) was obtained at the beginning and end of the experimental period. Ruminal CH_4 was yielded using a sulphur hexafluoride (SF_6) gas tracer every 24 h during five consecutive days in one experimental period with 15 prior adaptation days according to the method described by Johnson and Johnson (1995). CH_4 flux produced by animals was calculated in relation to the SF_6 tracer gas flux from a permeation capsule lodged in the rumen minus the basal CH_4 concentration in the air (Westberg *et al*. 1998). Following equation was used: $Q_{CH4} = Q_{SF6} \times ([CH_4]_y - [CH_4]_b) / [SF_6]$, where Q_{CH4} = CH_4 emission rate by animal; Q_{SF6} = know SF_6 emission rate from capsule in rumen; $[CH_4]_y$ = CH_4 concentrations in collection apparatus; $[CH_4]_b$ = basal CH_4 concentration; and $[SF_6]$ = SF_6 concentration in collection apparatus. Methane outputs (g/d) proportional to dry matter intake (DMI, kg/d), organic matter intake (OMI, kg/d) and percentage of gross energy intake (%GEI) were calculated by dividing the daily methane output of each animal by their daily DMI, OMI, GEI (during methane sampling) and ADG, MBW (throughout the entire experimental period). The experiment was conducted according to a completely randomized. Data were analyzed by the GLM procedure of SAS, and the Tukey test used considering 5% probability.

Results There was no effect ($P > 0.05$) of crude glycerin plus lipids sources on CH_4 emitted per day (CH_4, g/d), CH_4 per kilogram of ADG (CH_4, g/kg ADG), CH_4 per kilogram of MBW (CH_4, g/kg MBW), CH_4 per kilogram of dry matter intake (CH_4, g/kg DMI), CH_4 per kilogram of organic matter intake (CH_4, g/kg OMI) and % of gross energy intake converted into methane (%GEI).

Table 1 Methane emission of Nellore young bulls finished in feedlot with one of four diets

Item[1]	Diets[2]				s.e.m.	P[3]
	Cn	SO	SG	BF		
CH_4, g/d	133	137	136	109	17.86	0.698
CH_4, g/kg MBW	1.23	1.23	1.27	0.98	0.142	0.536
CH_4, g/kg ADG	157	110	184	151	21.28	0.131
CH_4, g/kg DMI	20.8	16.0	18.3	18.2	1.933	0.398
CH_4, g/kg OMI	22.2	17.1	19.6	19.5	2.251	0.398
CH_4 %GEI	11.5	8.07	9.02	9.39	1.214	0.255

[1/] MBW = metabolic body weight; ADG = average daily gain; DMI = dry matter intake; OMI = organic matter intake and %GEI = % of gross energy intake converted into methane
[2/]Cn = without additional fat; SO = addition of soybean oil; SG = addition of whole soybean grain; BF = addition of bypass fat
[3/]Probability ($P < 0.05$)

Conclusion The inclusion of soybean oil, whole soybean grain or bypass fat in Nellore young bulls diets containing 10% crude glycerin (DM basis) did not alter the enteric methane production.

Acknowledgements FAPESP; EMBRAPA Meio Ambiente; Cargill®

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The genome sequence of Methanobrevibacter sp. AbM4: conservation of methanogenesis associated functions in Methanobrevibacter genomes from the mammalian gut

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Introduction The methanogen strain AbM4 was isolated from the abomasal contents of a sheep and has been placed phylogenetically as a member of the *Methanobrevibacter wolinii* clade. While AbM4 does not make up a large methanogen population in the rumen, its 16S ribosomal RNA gene sequence has been found widely distributed in ruminant species under different rumen and gut conditions including sheep, cattle, and yak, and also in alpacas and the feces of the manatee. AbM4 was genome sequenced as part of a rumen methanogen genomics project which aims to identify methanogen specific genes as targets for ruminant methane mitigation.

Materials and Methods AbM4 cells grown in BY medium (with added trace elements, selenite/tungstate and vitamin solutions) with H_2 as substrate in a 80:20 mixture of H_2:CO_2 were DNA extracted using a liquid N_2 freezing and grinding method (Leahy *et al*. 2010). The DNA sequence of AbM4 was determined using pyrosequencing of 3kb mate paired end sequence libraries using a 454 GS FLX platform with titanium chemistry (Macrogen, USA). Sequence data were assembled and managed using the software programs Newbler (Roche 454 Life Sciences, USA) and the Staden package into a 30 contig assembly. Gaps were closed using additional sequencing by PCR-based techniques. Quality improvement of the genome sequence was performed using standard PCRs to ensure correct assembly and to resolve any remaining base-conflicts. GAMOLA and Artemis were used to manage genome annotation. Pulsed field gel electrophoresis was used to estimate genome size and to confirm genome sequence assembly. For electron microscopy, AbM4 cells were negatively stained with 1% phosphotungstic acid, mounted on Formvar-coated copper grids, and examined using a Philips model 201C electron microscope.

Results AbM4 has a single chromosome of 1.99 Mb with an overall base composition of 29% G+C, AbM4 is predicted to encode 1671 CDSs (coding domain sequences), 3 ribosomal RNA operons and 36 transfer RNAs. Putative functions could be assigned for 1259 CDSs, while the remaining CDSs were annotated as hypothetical proteins. Each of the 7 enzymatic steps expected for the hydrogenotrophic methanogenesis pathway are present in AbM4 enabling the reduction of CO_2 (or formate) through to methane using H_2. Only the Mcr I system for the final methyl-CoM reduction step was found. Genome comparisons showed that the AbM4 genome is syntenous with the draft genome of *Methanobrevibacter* sp. JH1 isolated from the rumen of native Korean cattle (BioProject PRJDB198, Genbank BAGX00000000). Genome comparisons of AbM4 and JH1 with *M. ruminantium* M1 show high levels of similarity, suggesting that the methanogenesis pathway and central metabolism of these organisms are conserved. Although the JH1 sequence is still in draft phase, it appears to contain a prophage element which is absent in AbM4. A complete comparison between the closed AbM4 and M1 genomes identified a number of genes that are unique to AbM4, and of those with annotated functions there is enrichment for genes involved in Type I and Type II restriction-modification systems (8 ORFs), transposases (8 ORFs), and phosphate metabolism and transport (4 ORFs). AbM4 has fewer genes encoding adhesin-like proteins compared to M1, (38 v 105) and fewer transporters (90 v 122). It is notable that AbM4 has a complete coenzyme M biosynthesis pathway (*com*ABCDE), and unlike M1, is likely not to require exogenous coenzyme M for growth. AbM4 also contains a BioY transporter, which is predicted to allow biotin uptake from the rumen environment, and a complete set of cobalamin biosynthesis genes are found scattered throughout the genome. AbM4 does not have non-ribosomal peptide synthase genes as are found in M1, and encodes a large (~16 kb) CRISPR region. The genome of the human gut methanogen, *M. smithii* PS, is distinguished from the AbM4 and M1 genomes by the presence of methanol:cobalamin methyltransferase genes (MtaABC) which have been shown to mediate syntrophic methanol utilisation by *M. smithii* PS in a mouse gut model (Samuel *et al*., 2007).

Conclusions The fewer genes encoding adhesin-like proteins in AbM4 indicates it invests less on external interactions with its environment, while its cofactor and coenzyme biosynthetic genes indicates it depends less on other rumen microbes for the supply of cofactors for growth in the rumen. This suggests that AbM4 occupies a ruminal niche slightly different from that of M1. Overall however, the AbM4 genome is similar to *M. ruminantium* M1 and *M. smithii* PS, and very syntenous with that of *Methanobrevibacter* sp. JH1. The hydrogenotrophic, methane-forming metabolism of these mammalian gut methanogens is highly conserved and suggests that species of the Methanobacteriales order will be amenable to inhibition by small molecule inhibitors and vaccine-based methane mitigation technologies targeting these genes.

Acknowledgements The authors acknowledge the financial support of the New Zealand Agricultural Greenhouse Gas Research Centre and the Pastoral Greenhouse Gas Research Consortium for provision of the AbM4 culture.

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High concordance of CH_4 emissions is possible between the SF_6 tracer and respiration chamber techniques

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Introduction Respiration chambers and the sulphur hexafluoride (SF_6) tracer technique are commonly used to determine methane (CH_4) emissions from individual animals. Chambers provide an accurate CH_4 measurement but are expensive to build and operate. The SF_6 tracer technique provides an indirect method for estimating emissions from individual cows and can be used with grazing animals. However, measurement variability has been reported to be greater with the tracer technique (e.g.: Grainger *et al*. 2007). If the SF_6 tracer technique could consistently give individual cow measurements similar to chambers, it could be used for screening large numbers of animals as required for genetic studies. The objective of this experiment was to assess the concordance between enteric CH_4 yield of individual dairy cows determined using a SF_6 tracer technique and by the respiration chamber technique.

Material and methods Fourteen multiparous, Holstein-Friesian cows, with average 169 days in milk, were offered a wheat-based diet for 4 weeks and a corn based diet for 4 weeks in a randomized crossover experiment at the Department of Primary Industries, Ellinbank, Victoria, Australia (38°14' S, 145°56' E). Cows were offered a common amount of lucerne hay (10 kg DM/day), cold pressed canola (2 kg DM/day) and minerals (0.2 kg DM/d) supplemented with either wheat grain (10 kg DM/day) or corn grain (10 kg DM/day). Equal quantities of feed were offered at 0630 and 1530 hours. Dry matter intake was recorded for each feeding. Methane yield of individual cows was measured using the SF_6 tracer technique (3 days) and 4 to 6 days later by the respiration chamber method (last 2 days of diet period). Methane measurements began following at least 16 d acclimatisation to each diet. During the SF_6 measurement, cows were housed indoors for 6 h per day and spent the remaining 18 h on a rectangular outdoor loafing pad. Samples of background gases indoors were collected as per Williams *et al*. (2011) while outdoor samples were collected using one canister on each fence, about 1 m above ground level. Mean daily concentrations of background gases for individual cows were calculated using a time weighted average then corrections were made using equation 2 of Williams *et al*. (2011). Concordance analysis using the method of Lin (2000) was carried out on the CH_4 yield data from the 19 data pairs that had similar feed intakes while undergoing methane measurement by both the SF_6 and chamber technique. A paired t-test was used to test for a difference between mean CH_4 emission measured by the two techniques on the same cows and diets.

Results Lin's concordance correlation coefficient between the two methods used to measure methane yield of individual cows was 0.835. Mean CH_4 yield determined via the two techniques did not differ (P = 0.265) although results from the SF_6 tracer technique were generally numerically greater than those from the respiration chambers (Figure 1).

Conclusions Based on the high concordance between results for individual cows when measured by the SF_6 tracer and respiration techniques, we conclude that our implementation of the SF_6 tracer technique can be used to accurately measure the CH_4 yield (g/kg DM intake) of individual cows. While previous studies have reported similar results (e.g. Grainger *et al.* 2007), most implemented the SF_6 tracer technique within respiration chambers or reported only correlations between the two techniques. The observed concordance between techniques indicates that our implementation of the SF_6 technique enables accurate determination of the CH_4 emissions of individual animals. Thus the SF_6 technique could be used to screen large numbers of dairy cows to identify individuals with a genetic predisposition for low CH_4 yield.

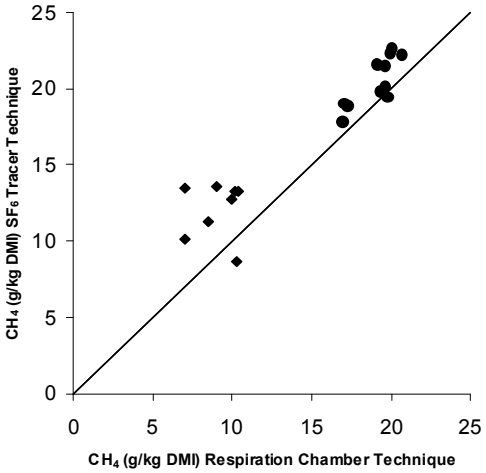

Figure 1. Concordance between the methane yields of individual cows, determined using the SF_6 tracer and respiration chambers (4-6 days apart) when fed a wheat-based (◆) and maize-based (●) diet. Line is y = x.

Acknowledgements This research was funded by the Department of Primary Industries – Victoria, Dairy Australia, Meat and Livestock Australia, and the Australian Government Department of Agriculture, Fisheries and Forestry under its Australia's Farming Future Climate Change Research Program.

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Genome wide association study for enteric methane emissions in New Zealand dual purpose sheep

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Introduction Enteric ruminant methane is the most important greenhouse gas emitted from the pastoral industry. Repeated measurement of methane emissions (using respiration chambers) and feed intake allows the calculation of high accuracy estimated breeding values (eBVs) for methane yield (gCH_4/kgDMI). When these results are combined with high-density SNP genotypes genomic regions associated with the trait can be identified. To identify genomic regions associated with gCH_4/kgDMI a genome wide association study (GWAS) was performed using eBVs for methane yield.

Material and methods
A GWAS was performed consisting of 1,872 New Zealand dual purpose sheep with de-regressed, parent average removed gCH_4/kgDMI eBVs (eBV*s) that had reliabilities at least $0.80*h^2$ (i.e. > 0.1). All animals were genotyped with the Illumina OvineSNP50 BeadChip (50K). Genotypes were checked for quality control before analysis (Dodds *et al*., 2009) and the SNPs not retained as part of the Ovine HapMap study (Kijas *et al*., 2012) were also discarded; 47,028 SNPs remained. All Romney, Perendale and Coopworth animals (n = 32, 11, and 733 respectively) and 735 Composite animals, the latter born prior to 2011, were used as a training set. The validation set consisted of the 361 Composites born in 2011. SNP coefficients (b_i) were calculated using the genomic BLUP (GBLUP) model using the methods of VanRaden (2008). A mixed model was fitted to the eBV*s, with fixed effects of the first 6 principal components of the G_1 matrix (VanRaden, 2008), animal as a random term with effects distributed as $N(0,G_1\ \sigma^2_u)$, and residuals distributed as N(0,R) where R is a diagonal matrix with diagonal elements $(1-r^2)/r^2$ where r^2 is the reliability of the eBV*s. The analysis uses a fixed heritability. The b_i were obtained using equation 2 of VanRaden (2008). Resulting nominal probability values were calculated assuming the b_i follow a normal distribution with mean zero and variance $2p_i(1-p_i)m*var(b_i)/\sum 2p_i(1-p_i)$, where p_i is the minor allele frequency for the i^{th} SNP and m is the number of SNPs. The significance threshold was calculated using the 5% Bonferroni correction of $P<0.05/m \approx 10^{-6}$, and a 'suggestive' threshold set at $P<10^{-4}$.

Results The $-\log_{10}(P)$ values were graphed in a Manhatten plot on Ovine genome v3 (Figure 1). There were no SNPs below the Bonferroni significance threshold. At the 'suggestive' threshold of $P<10^{-4}$ there were 10 SNPs across chromosomes 2, 3, 5, 7, 17, 22, 23 and 25. All SNPs were positively associated with gCH_4/kgDMI eBVs.

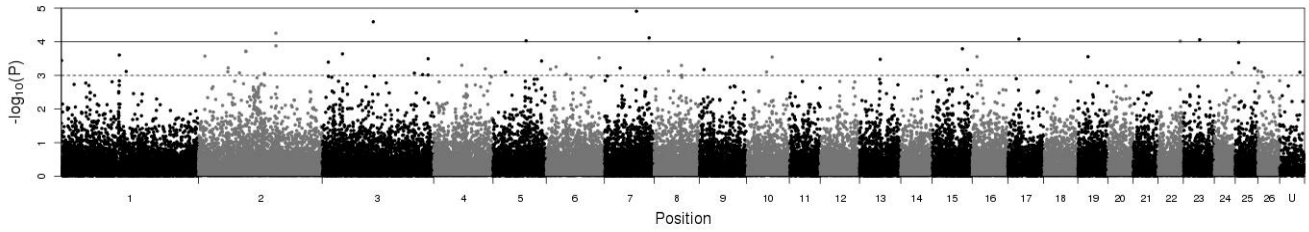

Figure 1 Graph of $-\log_{10}(P)$ values of SNPs for gCH_4/kgDMI. Ordered on Ovine genome v3. P<0.0001 (solid line) P<0.001 (dash line).

Conclusions Nine regions were identified. However, the action of the underlying genes in reducing methane emissions is as yet unknown and additional research is required to demonstrate a causal link. The results are promising and additional regions are likely to be identified by measuring further animals coupled with use of a higher density SNP chip, or resequencing, combined with imputation. The same data can also be used directly for genomic selection in relevant industry flocks.

Acknowledgments This work was funded by New Zealand Agricultural Greenhouse Gas Research Centre and used Pastoral Greenhouse Gas Research Consortium and Beef + Lamb NZ funded resources. The New Zealand Government in support of the Livestock Research Group of the Global Research Alliance (GRA) on Agricultural Greenhouse Gases has funded Natalie Pickering's postdoctoral fellowship.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Vegetation indices variability in grasslands belonging to the Pampa phytophysiognomic units in South Brazil and Uruguay

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Introduction The Pampa in South Brazil and Uruguay form a unique region of the planet and is considered an endemic biome. It is complex with predominance of large areas of grasslands and variable vegetation structure, in response to its diversity and scale. The knowledge of the average conditions and annual dynamics throughout the biome is essential to support studies to assess change trends, due the anthropogenic or climatic causes, which can be done with vegetation indices. The main objective of this study was to characterize the behaviour of the vegetation indices annual and seasonal variability, across the different grassland types. This study is part of the Animal Change project.

Material and methods Time series images from Feb-2000 to Jul-2011 of the NDVI (Normalized Difference Vegetation Index) and EVI (Enhanced Vegetation Index) from MODIS/Terra, product MOD13Q1, with temporal and spatial resolution of 16 days and 250m, respectively were used in the study. The Envi image processing software was used and the methodology included the creation of mosaics, projecting, and resizing. The time series profiles were extracted from 13 phytophysiognomic units for the several grasslands types mapped by Hasenack et. al. (2010). For each unit the indices average, maximum, minimum, and standard deviation were extracted using the Idrisi Taiga software.The spectral separability was based on the vegetation indices characteristic, through the normalized difference between infrared and red of the electromagnetic spectrum for both indices, and included the EVI blue bands (Huete *et al*, 2002). Also, each vegetation index had the maximum image over the temporal series subtracted by the minimum correspondent image. The annual monthly average for each class was calculated for each grassland type.

Results The inter-annual average variability between the maximum and minimum values, in both vegetation indices, was higher in January (summer) decreasing until October (spring), especially in the grasslands with *Stipa* and in shallow soils. NDVI and EVI, over the temporal series, had similar behaviour and low standard-deviation in each grasslands unit. The annual average profiles revealed two vegetative maximums development, one in March (autumn) and the other in October (spring), which could to be correlated with dry summers. The phytophysiognomic separability was higher during summer for the two indices, although during winter-spring (July-November) the EVI performed better, probably due to the dominance of C3 species during winter.

Figure 1 Maximum and minimum difference (2000-2011) of the vegetation indices on January (summer) and October (spring).

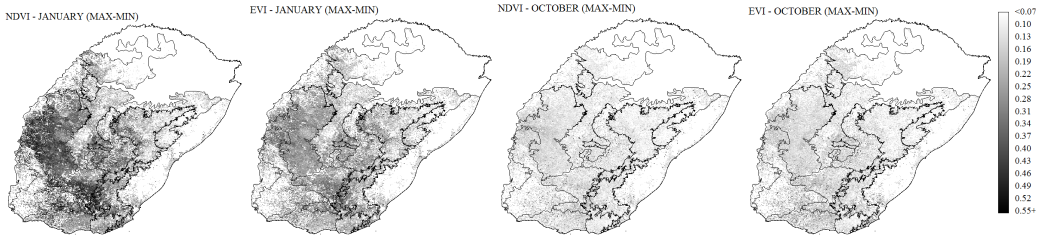
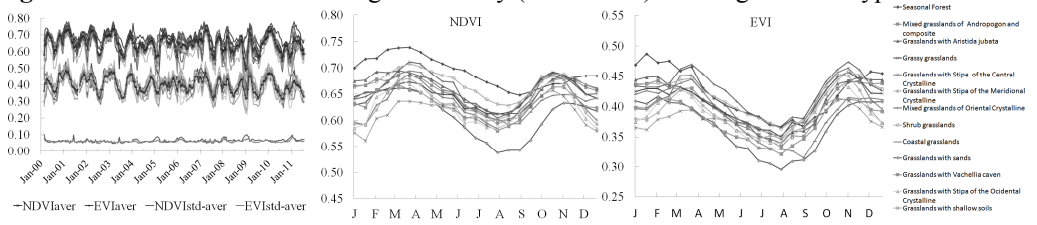


Figure 2 Profiles and annual average variability (NDVI/EVI) for the grasslands types.



Conclusions NDVI and EVI presented variability in their evolution under different soil conditions, time of year, rainfall distribution, and phytophysiognomic units, with annual average patterns of dry summer and rainy spring. The EVI stood out better for its ability to distinguish C3 species types. Although the analysis was conducted only in grasslands, there was separability between phytophysiognomic units, showing the potential for using these indices to spatially and temporally characterize grasslands patterns.

Acknowledgements CAPES (Coordination for the Improvement of Higher Level -or Education- Personnel).

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Effect of nitrate and nitrate reducing bacteria on methane production in growing Murrah buffaloes

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Introduction Presently, India possesses 57% of world's buffalo and 16% of cattle population. Contribution of methane emission by Indian buffaloes is 42% (Chhabra *et al.,* 2009). The addition of alternate electron acceptors to the rumen seems to be a logical mean of reducing methane emission. So the aim of the present study was to assess the effect of nitrate on methane production in growing Murrah buffaloes.

Materials and methods Fifteen growing male Murrah buffaloes with an average body weight of 241.28 ± 4.72 kg were randomly divided into three homogeneous groups in completely randomized design. The animals of group one served as control (T1) and of second group were fed sodium and potassium nitrate @ 2.0% dry matter intake (T2) and those of group three were fed as in group two along with 0.5 ml of nitrate reducing bacterial culture- NRBB 57 (isolated from rumen of buffalo adapted to nitrate rich diet) per kg body weight (T3). Animals were fed their respective iso-nitrogen and iso-caloric concentrate mixture containing 18% crude protein (CP) and 70% total digestible nutrients (TDN) and wheat straw in 1 : 1 proportion targeting 500 g per day growth (ICAR, 1998). The nitrate treatment groups, sodium and potassium nitrate fed incrementally at 0.50% Dry Matter Intake (DMI) per week, starting from 0.50% to 2.0% of DMI. Third group animals were fed nitrate reducing bacterial culture in 200 g of concentrate mixture prior to feeding. After an adaptation period of 45 days feeding, four animals from each treatment on rotational basis were used to study the methane emission from the animals. The selected animal was weighed in morning prior to feeding and watering and kept in respiration chamber for three days for acclimatization to chamber environment and recording of the respiration calorimetric data for three consecutive days. Initial chamber gas composition was recorded. Recording of temperature of dry and wet bulbs, air flow rate, volume and atmospheric pressure were recorded manually at hourly interval. The samples of outgoing air were collected in Douglas bags separately with continuous sampling device. Methane content of pooled sample of the outgoing air from the respiratory chamber was determined. The chamber was opened after 22 hours. The residual feed was collected and measured and representative sub-samples were preserved for further analysis. Total urine voided was collected, measured and representative sub-samples were taken for nitrogen excreted by the animal. Total faeces voided by the animal was collected, quantified and representative sub-samples were taken for determination of dry matter to estimate DM digestibility of feed. The total volume of methane produced was computed as per the following formula
CH_4 (l) = V_{STP} (M_f - M_i)/100. Where, M_f = Average % of CH_4 in outgoing air, M_i = Average % of CH_4 in incoming air. Statistical analysis was performed as per standard method (Snedecor and Cochran, 1989) by using SPSS version 17.0 computer package. For comparison of multiple groups generalized linear model of ANOVA procedures and Tukey's multiple range tests were used.

Results

Table 1 Effect of feeding nitrate and nitrate + live culture of NRBB 57 on methane emission and energy metabolism in growing buffaloes

Attributes	Control (T_1)	Nitrate (T_2)	Nitrate + NRBB 57 (T_3)	P
DMI (kg/day)	5.41±0.27	5.04±0.11	5.56±0.31	0.35
Methane (l/day)	169.62^{a}±11.98	132.10^{b} ± 6.23	141.29^{ab}±7.05	0.04
Methane (l/kg DMI)	31.07^{a}±0. 64	26.32^{b}±0.77	25.34^{b}±0.53	0.00
Methane (l/kg DDMI)	55.12^{a}±2.21	46.00^{b}±1.83	43.67^{b}±0.90	0.00

Methane emission (l/day) was significantly (P<0.05) lower in nitrate fed group than that in control group. Methane emission (litre/kg DMI and litre/kg DDMI) was significantly lower (P<0.05) for nitrate and nitrate plus NRBB 57 fed groups than in control group. Nitrate and nitrate plus NRBB 57 group had 16.55 and 20.77% lower methane emission (l/kg DDMI) than that in control.

Conclusions Feeding of nitrate at 2.0% of DMI and nitrate (2% of DMI) plus NRBB 57 (0.5 ml/kg body weight) reduced methane (l/kg DDMI) production by 16.55 and 20.77%, respectively as compared to that in control group.

Acknowledgements The authors gratefully acknowledge funding from ICAR, New Delhi

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Development of an oligonucleotide microarray chip for analysis of community composition and dynamics of archaea, anaerobic fungi, and protozoa in rumen

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Introduction The fermentation processes carried out by the rumen microbiome, comprising bacteria, archaea, protozoa and fungi, produce vital nutrients from fibrous feeds for the host. However, methane, carbon dioxide and hydrogen are also produced as byproducts that not only result in loss of feed energy but also contribute to greenhouse gas emission. Owing to their vast diversity and mostly uncultivated status, detection, characterization and quantification of rumen microorganisms are very challenging. Attempts to manipulate rumen function have largely remained unsuccessful due to limited understanding of and difficulty in monitoring dynamics of microbial community structure and function. Microarray-based genomic technology has shown a great promise of overcoming such obstacles for comprehensive analysis of microbiomes in various environments. Here, we present a study in which a microarray was developed for comprehensive and simultaneous analysis of archaeal, fungal and protozoal communities in rumen.

Material and methods All available target sequences (16S rRNA gene and methyl coenzyme-M reductase (mcrA) gene sequences of rumen archaea, internal transcribed spacer (ITS) and 28S rRNA gene sequences of anaerobic fungi (AF), and 18S rRNA gene sequences of rumen protozoa) were downloaded from GenBank. The downloaded sequences were subjected to quality check and OTU (operational taxonomic unit) clustering. Consensus sequences were generated for design of probes at genus level or higher taxonomic ranks, while representative sequences of OTUs were used for probe design at species-equivalent level. Oligonucleotide probes were designed using Picky 2.20 (Chou, 2010). The probes generated were subjected to in silico specificity check against GenBank and other databases. The final 895 probes, including 2 internal control probes, were synthesized onto a 6 × 5K custom microarray format, with each probe represented in 5 replicates and each probe spot having about 1.5 x 10^9 probe molecules. The probes were tested for specificity and linear range of detection through hybridization experiments using 26 synthetic oligonucleotides and 13 clones. The chip was also used for analysis of samples from several rumen fermentation studies. An Axon Genepix 4000B scanner (Axon Instruments, Union City, CA, USA) was used to scan the microarray slides. Data were normalized using signal from the control probes and statistical analysis (t-test) was performed using TM4 microarray suite (Saeed *et al.*, 2006).

Results After in silico analysis, the following probes were incorporated in the microarray: a) 67 genus- or higher-level probes and 702 species level probes targeting 16S rRNA; b) 32 genus- or higher-level probes targeting archaeal mcrA; c) 20 genus-level probes and 48 species-level probes targeting protozoal 18S rRNA; d) 13 genus- or higher-level probes targeting ITS of anaerobic fungi; e) 11 genus- or higher-level probes targeting 28S rRNA of anaerobic fungi; and f) control probes targeting cow mitochondrial rRNA. The specificity tests indicated high specificity of probes, and the optimum copies of synthetic oligonucleotides for hybridization was 10^9 oligos per 54 ul of hybridization mix applied to each microarray. With PCR amplicon as targets, it was observed that the microarray had a dynamic linear range of at least four order of magnitude (10^8 to 10^{11} copies of each target), and the dose response relationship in term of median signal intensity had very high correlation coefficient (R^2=0.997). The developed chip was successfully used in examining the dynamics of archaeal, fungal and protozoal communities in samples collected from several rumen-manipulation studies. The preliminary results showed that the new chip can be used to assess how dietary interventions modulate populations of rumen archaea, fungi and protozoa of rumen.

Conclusions The Microarray chip developed in this study supports simultaneous and rapid analysis of predominant ruminal archaea, protozoa and fungi at genus and species levels. This is the first microarray made available for comprehensive and simultaneous analysis of archaeal, fungal and protozoal communities in rumen. When combined with the RumenArray (Kim, 2011), ruminal bacteria archaea, fungi, and protozoa can be simultaneously analysed in a comparative manner. It can be a useful analytical tool in studies that aim to evaluate dietary interventions to mitigate methane emission from ruminants.

Acknowledgements This work was supported in part by an OARDC grant (2010-007). S. S. Paul's tenure at The Ohio State University was supported by a Cutting Edge Research Enhancement and Scientific Training Award Overseas Fellowship from Department of Biotechnology, India.

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Effect of temperature on the rate of sulphur hexafluoride release from permeation-tubes

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Introduction Accurate prediction of sulphur hexafluoride (SF_6) release rate (RR) from permeation-tubes is critical for determination of enteric methane (CH_4) emissions from ruminants by the calibrated tracer technique. The accepted practice for determination of SF_6 RR from permeation tubes is measurement of mass loss at 39°C (Lassey *et al*. 2001). Permeation rate of gases is temperature dependent and can be estimated using the Arrhenius relationship as described by Equation 1 (Namiešnik 1984):

$$\log\frac{R_2}{R_1} = 2950\times\left(\frac{1}{T_1}-\frac{1}{T_2}\right)$$

Equation 1.

where R_1 and R_2 are permeation rates at absolute temperatures T_1 and T_2 (°K). This equation can be used to predict the effect of temperature change upon RR of SF_6 across the polytetrafluoroethylene (PTFE) membrane of a permeation tube. Namiešnik (1984) reported that permeation rate may vary by as much as 10% with a 1°C change in temperature. Bárbaro *et al*. (2008) reported that SF_6 RR from 10 permeation tubes increased by 3% per °K between 35 and 43°C. However, they did not describe the experimental procedure used to draw this conclusion. Hence the temperature sensitivity of SF_6 RR from permeation tubes remains ambiguous. The purpose of this investigation was to determine the effect of temperature upon the rate of SF_6 release from permeation tubes.

Material and methods The effect of temperature upon SF_6 RR from twenty-four permeation tubes was investigated at three temperatures each applied for 14 d. Treatment temperatures were; 37, 39 and 41°C. Permeation tubes as described by Lassey *et al*. (2001) and containing (mean ± S.D.) 1.50 ± 0.204 g SF_6 and with a SF_6 RR of 7.3 ± 0.49 mg/d, were obtained from NIWA, Wellington, New Zealand. Pre-experimental SF_6 RR of individual permeation-tubes was determined gravimetrically during dry storage at 39°C in a laboratory incubator (Heratherm Advanced Protocol; Thermo Fisher Scientific, Waltham, USA). All mass determinations were undertaken using a calibrated balance (CP224S; Satorius AG., Goettingen, Germany). Permeation tubes were blocked (n = 4) in ascending order of SF_6 RR and randomly allocated to treatment within block. Treatments were applied according to an orthogonal Latin square design to allow treatment effects to be adjusted for carry-over effects (Cochran *et al*. 1941). Tube mass was determined to within 0.1 mg before and after each 14 d incubation period. Treatments were applied using three identical water baths each fitted with a 0.1°C resolution digital thermo-regulator and stirrer (ET22 and TH5; Ratek, Melbourne, Australia). Each bath was filled to capacity and calibrated using a partial immersion thermometer with 0.1°C scale (Extreme Precision; ICL Calibration Laboratories Inc., Stuart, FL, USA). During each period, eight permeation tubes were stored in air within 0.5 L glass bottles (Duran GLS 80, Schott AG., Mainz, Germany) fully submerged in each bath. Bottles were vented to the atmosphere to maintain atmospheric pressure and allow release of SF_6 via ⅛" OD plastic tubes. Vent tubes were partially (40 cm) submerged in each bath to equilibrate temperature. Temperature within each bottle was recorded every 20 minutes using logging thermometers (TidbiT v2; Onset Computer Corp., Bourne, MA, USA). The effect of temperature upon SF_6 release rate from permeation tubes was analysed using ReML in GenStat 14 and the model;

$$Y_{ijk} = \mu + B_k + T_i + P_j + PB_{jk} + \tau_{d(i,j)} + \lambda_{d(i,j-1)} + \varepsilon_{ijk}$$

where Y_{ijk} = observation, μ = population mean, B_k = block effect, T_i = tube effect, P_j = period effect, PB_{ik} = the effect of period within block, $\tau_{d(i,j)}$ = treatment effect, and $\lambda_{d(i,j)}$ = carry-over effect of treatment, ε_{ijk} is the residual measurement error.

Results Release of SF_6 increased by 0.18 mg/d (2.5%) per degree Celsius increase in temperature between 37 and 41°C ($P < 0.001$). Mean SF_6 RR (± s.e.) was; 6.63 ± 0.023 mg/d at 37°C, 7.03 ± 0.023 mg/d at 39°C and 7.33 ± 0.023 mg/d at 41°C.

Conclusions The RR of SF_6 from permeation tubes is sensitive to temperature change about 39°C. This finding is in agreement with Bárbaro *et al*. (2008). Variation of reticulorumen temperature from the calibration temperature (39°C) will affect SF_6 RR of permeation tubes by 2.5% per degree Celsius. Further research is required to determine the effect of this variation upon the accuracy of the SF_6 tracer technique and to minimise its impact upon calculated CH_4 emissions from individual animals.

Acknowledgements This research was funded by the Department of Primary Industries – Victoria, Dairy Australia, Meat and Livestock Australia, and the Australian Government Department of Agriculture, Fisheries and Forestry under its Australia's Farming Future Climate Change Research Program.

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Effects of gas composition in headspace and bicarbonate concentrations in media on gas and methane production, digestibility, and rumen fermentation using in vitro gas production techniques

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Introduction *In vitro* batch fermentation, rumen simulation technique (Rusitec) and 'Hohenheim gas test' have been routinely used for the past six decades to evaluate feeds and feed additives, predict their nutritive and energetic values, and investigate their effects on rumen fermentation, microbial protein synthesis, methane production, and rumen microbial ecology. Different researchers have modified *in vitro* batch fermentation techniques, but all use gas production as a measurement of the fermentation. Several factors, including source and collection time of inoculum, inoculum size and preparation, apparatus design, headspace gas pressure, and medium composition have been evaluated for their effect on *in vitro* gas production. However, studies investigating how headspace gas compositions affect *in vitro* gas production, especially methane and rumen fermentation are lacking. Although bicarbonate concentrations in media have been assessed for their effect on gas production, their effects on methane production and rumen fermentation have not been studied. The objective of this study was to investigate the effects of headspace gas composition and bicarbonate concentration in medium on gas and methane production, digestibility and volatile fatty acid (VFA) profiles.

Material and methods Three headspace compositions (N_2+CO_2+H_2, CO_2, and N_2) with two substrate types in a 3 × 2 factorial design (Experiment 1) and three headspace gas composition (N_2, N_2+CO_2, and CO_2) with three bicarbonate concentrations (80, 100 and 120 mM) in a 3 × 3 factorial design (Experiment 2) were evaluated. Fresh rumen fluid was collected from two cannulated lactating Jersey cows at approximately 10 h post morning feeding and used as inoculum. These two cows were fed a total mixed ration composed (% dry matter) of corn silage (45%), alfalfa hay (10%), Cargill dairy protein product (20%) and a concentrate mixture (25%). The *in vitro* batch fermentation was carried out in 120-mL serum bottles as described earlier (Patra and Yu, 2012). In Experiment 1, the buffered medium for *in vitro* rumen fermentation was prepared as described by Menke and Steingass (1988). In Experiment 2, the medium was prepared similarly, but with three bicarbonate concentrations: 80, 100 and 120 mM. The anaerobic medium (30 ml) and the rumen inoculum (10 ml) were dispensed into each serum bottle containing 400 mg of ground feed substrates. Serum bottles were incubated in triplicates for each headspace gas composition x substrate type, headspace gas composition x bicarbonate concentration, and no-substrate blank culture. The headspace gas compositions were achieved by exchange with the gas mixture in an anaerobic chamber or flushing with N_2 or CO_2 (in Experiment 1); or with N_2, CO_2, or a mixture of N_2 and CO_2 at 50:50 ratio (in Experiment 2). After 24 h incubation, gas pressure in the bottle was measured using a manometer to determine total gas production. Then 10 ml of the headspace gas was collected into a tube prefilled with distilled water by displacement for methane analysis. Then the cultures were filtered through filter bags (ANKOM) to determine feed dry matter degradability. The filtrates were sampled into 2 mL microfuge tubes for VFA analysis. The concentrations of methane in the gas samples, VFA concentrations in the fermentation culture, apparent degradabilities of the substrates were determined as described earlier (Patra and Yu, 2012). Net gas or net methane production was calculated by subtracting the mean gas or methane production in the blank from total gas or methane production in each treatment. The data were analyzed using the mixed model procedure of SAS in 3×2 (Experiment 1) and 3×3 (Experiment 2) factorial designs.

Results In Experiment 1, total and net gas productions were lower ($P<0.01$) for CO_2 headspace, followed by N_2 and then the gas mixture in headspace. However, methane concentration was greater for CO_2 headspace (28.3%) than N_2 (18%) and gas-mixture headspace (19.6%), but were similar for N_2 and gas mixture. Total and net methane productions (ml) were greatest for CO_2 (34.4 and 23.6, respectively) followed by gas mixture (26.9 and 21.4, respectively) and then N_2 (24.2 and 17.8, respectively). Headspace gas composition did not influenced ($P>0.10$) digestibility or most of the VFA profiles, except molar percentages of propionate. In experiment 2, with increasing CO_2 concentration in headspace, total and net gas production showed quadratic relationships ($P<0.001$), while methane content, net methane production, and fiber digestibility increased linearly ($P<0.01$), and total methane production increased quadratically ($P<0.01$). Total and net gas productions and fiber digestibility increased linearly ($P<0.001$) with increasing bicarbonate concentration. Methane content and net methane production were unaffected ($P>0.10$) by bicarbonate concentrations, but total methane production tended ($P=0.10$) to increase with increasing bicarbonate concentrations. Although total VFA concentrations and molar percentages of butyrate were unchanged ($P>0.10$), molar percentage of acetate and acetate to propionate ratio decreased, while molar percentage of propionate increased quadratically ($P<0.001$) with increasing bicarbonate concentration in the medium.

Conclusions This study for the first time demonstrated that headspace composition and bicarbonate concentration in media can significantly affect gas and methane production and rumen fermentation, and these effects should be take into account when results from different studies are compared.

Acknowledgements This work was supported in part by an OARDC grant (2010-007). A.K. Patra's tenure at The Ohio State University was supported by a BOYSCAST fellowship from the Department of Science and Technology, India.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Effects of vanillin, quillaja saponin, and essential oils on in vitro fermentation and protein-degrading microorganisms of the rumen

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Introduction Presently, livestock farming faces challenges in reducing its environmental impacts. There is an urgent need to meet these challenges by decreasing methane and nitrous oxide emissions and nitrogen excretion, while improving utilization of nutrients. In recent years, natural plant products containing essential oils (EO), saponins, or tannins have been widely evaluated as non-antibiotic feed additives to mitigate methane emission, suppress protein degradation in rumen, to increase feed utilization efficiency, and to improve characteristics of rumen fermentation. Recently, we noted that EO was effective in inhibiting methane production and modulating rumen fermentation and bacterial and archaeal diversity in an EO type and dose dependent manner (Patra and Yu, 2012). We also investigated that saponins altered rumen fermentation and microbial diversity (Patra *et al*., 2012). However, effects of EO and saponins on various protein-degrading and amino acid-deaminating microbes in mixed cultures were not studied. Castillejos *et al.* (2006) reported that vanillin can modulate rumen volatile fatty acid (VFA) profiles, but its effects on other aspects of rumen fermentation and rumen microbial populations remain uninvestigated. The objective of this study was hence to investigate the effects of vanillin on methane production, fermentation characteristics, abundance of archaea, protozoa and cellulolytic and protein-degrading bacterial population. The effects of EO and saponins on protein-degrading microorganisms were also investigated.

Material and methods In experiment 1**,** vanillin (Sigma-Aldrich, St. Louis, USA) was used at two doses (0, 5 and 10 mM). In experiment 2, clove oil (CLO, *Eugenia* spp.), origanum oil (ORO, *Thymus capitatus* L. Hoffmanns & Link), and peppermint oil (PEO, *Mentha piperita* L.) (Sigma-Aldrich) were used at 0.5 and 1.0 g/L, while quillaja saponins (from the bark of *Quillaja saponaria* Molina plants; Sigma-Aldrich) was used at 0.6 g/L. These doses of EO and saponin were used as they were found to decrease ammonia concentrations in previous studies (Patra *et al.,* 2012; Patra and Yu, 2012). In both experiments, controls were included in parallel that contained none of the above phytochemicals. The *in vitro* incubation was carried out in 120-mL serum bottles in triplicates for each treatment (including controls). The *in vitro* buffered medium was prepared anaerobically as described earlier (Patra *et al.,* 2012; Patra and Yu, 2012), and 30 ml of the anaerobic medium and 10 ml of rumen fluid were dispensed into each serum bottles containing 400 mg of the ground feed substrate (alfalfa hay and a dairy concentrate mixture at 50:50 ratio). The serum bottles were incubated at 39 °C for 24 h in a water bath. Gas production was measured after 24 of incubation, and headspace gas was sampled for methane analysis using a GC (HP 5890 series, Agilent Technologies). Fermented cultures were collected for VFA analysis using GC and ammonia analysis. Metagenomic DNA from the cultures was extracted, and abundances of archaea, protozoa, three cellulolytic and nine protein-degrading bacteria were analysed using real-time PCR. The data on rumen fermentation characteristics and abundances (log *rrs* gene copies/ml samples) of quantified ruminal microorganisms were analyzed using the PROC MIXED procedure of SAS. Significance was declared at $P \leq 0.05$, whereas $0.05 < P \leq 0.10$ values were considered to be a trend.

Results Methane production, degradabilities of feed substrate, and ammonia concentration decreased linearly ($P<0.05$) with increasing doses of vanillin. Concentration of total VFA also decreased ($P<0.05$), whereas proportion of butyrate tended to increase linearly ($P<0.10$) with increasing doses of vanillin. Protozoa population decreased ($P<0.05$), but abundances of *Ruminococcus flavefaciens*, *Prevotella bryantii, Butyrivibrio fibrisolvens*, *Prevotella ruminicola, Clostridium aminophilum*, and *Ruminobacter amylophilus* increased ($P<0.05$) with increasing doses of vanillin. ORO and CLO resulted in lower ammonia concentrations compared to control and PEO. All the tested EO decreased ($P<0.05$) abundances of protozoa, *Selenomonus ruminantium, R. amylophilus, P. ruminicola* and *P. bryantii*, with the largest decrease resulted from ORO followed by CLO and PEO. The abundances of *Megasphaera elsdenii*, *C. aminophilum* and *Clostridium sticklandii* were deceased ($P<0.05$) by ORO, while that of *Butyrivibrio fibrisolvens* was lowered by both ORO and CLO. Saponin decreased ($P<0.05$) ammonia concentration (by 24%) and protozoal population (9.23 versus 8.51 log unit), but increased the abundances of *S. ruminantium, R. amylophilus, P. ruminicola* and *P. bryantii*, though the magnitude was small (less than one log unit).

Conclusions The results suggest that reduction of ammonia production by vanillin and saponin may not be caused by direct inhibition of major known proteolytic bacteria, and essential oils can have different inhibitory effects on different proteolytic bacteria, resulting in varying reduction in ammonia production. Collectively, the phytochemicals tested in this study have potential to mitigate methane emission and ammonia excretion from ruminant animals, but future studies including (meta)transcriptomic and (meta)proteomic analysis of the ruminal microbiome are needed to better understand their modes of action.

Acknowledgements This work was supported in part by an OARDC grant (2010-007). A.K. Patra's tenure at The Ohio State University was supported by a BOYSCAST fellowship from the Department of Science and Technology, India.

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Assessment of forage preparation on in vitro methane production

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Introduction Formulating diets to meet the nutritional needs of cattle is important to ensure that animal production and health are optimised. *In vitro* incubations are a widely accepted and commonly used method to determine the nutritional value of ruminant feed. This procedure allows simulation of the rumen environment in a laboratory setting and involves material being inoculated with buffered rumen fluid. Analysis is usually conducted on feed that has been dried and then ground to pass a 1-mm sieve. This technique may be appropriate for the analysis of concentrate feed, however may not be the most suitable method for analysing fresh forages. The Australian dairy industry is predominantly pasture based, with pasture accounting for 70-75% of the diet. It is therefore essential that an appropriate method of fresh forage preparation be used in *in vitro* incubations. To our knowledge no studies have been published evaluating the effects of preparation method on methane or gas production. The objective of this study was to evaluate the effects of four different forages prepared by frozen minced procedure or oven dried at 55^{o}C and ground to 1-mm on the fermentation characteristics from *in vitro* batch culture incubation.

Material and methods Samples of kikuyu (*Pennisetum clandestinum*), oats (*Avena fatua*), forage rape (*Brassica napas*) and maize (*Zea mays*) silage were randomly collected from 3 paddocks and 2 silo pits, respectively, at the Costorphine dairy farm, University of Sydney, near Cobbitty, NSW, Australia. Approximately 2 kg of each sample dry matter (DM) was harvested from each paddock/silo and stored at -20°C prior to preparation. This experiment involved 8 treatment groups. The treatment groups consisted of the 4 plant species, prepared 2 different ways. The 2 different preparations were frozen minced or dried ground. On the day prior to incubation, 0.5 g DM of forage samples (dried ground) were weighed into pre-weighed filter bags (F57 Ankom) and placed in each incubation bottle or weighed into each incubation bottle for the frozen minced samples. On the day of incubation, the bottles were warmed to 39°C in the incubator for 60 minutes, and gassed with oxygen-free nitrogen before adding 25 ml of incubation media (rumen liquor:buffer 1:2 ratio). The bottles were then capped and returned to the incubator. This incubation procedure was repeated 3 times with 2 replicates for each treatment for each sampling time (6, 12 and 24 hours). Only 24h data will be presented. Two bottles containing only incubation media were prepared for each sampling time (i.e. a total of six bottles) to act as blanks. Measurements taken from the *in vitro* bottles included cumulative gas production, methane production, pH, *in vitro* dry matter disappearance (IVDMD), and yield of volatile fatty acids (VFA). Gas samples were measured at 6, 12 and 24 hours of incubation. The two replicates were averaged prior to statistical analysis and those averages, within run, was the statistical unit. Data were analysed as a completely randomised design using mixed procedure with forage type, preparation and forage type × preparation interaction in the model as fixed effects. Differences among means were tested using the least squares mean linear hypothesis test with significance declared if $P<0.05$.

Results Cumulative gas production expressed as ml/g DM or ml/g DDM was higher ($P<0.01$) for the frozen minced preparation than the dried ground preparation for all forages after 24 hours of incubation (Table 1). A similar trend was also observed for methane expressed as ml CH_4/g DM or ml CH_4/g DDM with higher ($P<0.01$) gas production for the frozen minced preparation compared to the dried ground preparation for all forages after 24 hours incubation. There was no forage × preparation interaction ($P=0.31$) or preparation effect ($P=0.22$) after 24 hours of incubation but there was a forage effect ($P<0.01$) with IVDMD (g/kg) being higher and similar ($P>0.05$) for forage rape and oats, followed by kikuyu and then maize silage.

Table 1 - Effects of forage type and preparation method on *in vitro* batch culture rumen microbial fermentation

	Forage rape		Kikuyu		Maize silage		Oats			P		
	DG	FM	DG	FM	DG	FM	DG	FM	s.e.m.	F	Prep	F×Prep
Cumulative gas, ml/g DM	101.6	171.9	98.3	135.9	93.0	117.4	103.4	152.4	3.93	<0.01	<0.01	<0.01
Cumulative gas, ml/g DDM	145.1	251.6	163.4	221.9	182.6	219.4	167.8	219.9	9.09	ns	<0.01	<0.01
Methane, ml/g DM	11.0	26.6	13.8	22.9	9.3	16.5	12.8	19.7	1.09	<0.01	<0.01	<0.01
Methane, ml/g DDM	15.7	39.0	22.9	37.4	18.5	30.8	18.9	28.7	1.98	<0.05	<0.01	<0.01
pH	6.1	5.4	5.8	5.7	5.7	5.4	5.9	5.5	0.08	<0.05	<0.01	<0.01
IVDMD, g/kg	700.8	682.7	600.8	616.7	510.7	535.3	676.0	698.3	12.69	<0.01	ns	ns
Total VFA, mM	146.3	153.8	137.4	135.5	123.6	139.0	144.5	138.6	3.36	<0.01	ns	<0.05

DG, Dried ground 1-mm; FM, frozen minced; F, forages; Prep, preparation; ns, non-significant.

Conclusions This study is the first to our knowledge to demonstrate that preparation method does have an effect on methane and gas production, with forages prepared by the frozen minced preparation producing higher amounts of methane and cumulative gas than the dried ground preparation.

https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Effect of methane inhibitors on molecular hydrogen emissions and total tract digestibility in sheep

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Introduction Inhibition of methanogens leads to increased hydrogen emissions *in vitro* and decreases fibre digestibility (Janssen 2010). To our knowledge however, this has not been demonstrated *in vivo* where hydrogen concentrations are expected to be much lower compared to fully enclosed *in vitro* systems. In this experiment, methanogens in sheep were inhibited using a combination of chloroform (CF) and 9,10-anthraquinone (AQ) over a period of 4 weeks to study the effect on hydrogen emissions and dry matter digestibility (DMD).

Materials and methods A total of 15 sheep were housed indoors and adapted to a general purpose diet (GP) for 4 weeks. The diet was offered at 1 kg DM/d in two equal meals and consisted of 500g chaffed grass hay, 290 g, barley 100 g soybean meal, 100 g molasses and 10 g salt and minerals. The animals were kept for 4 weeks in individual pens on the diet before being transferred to metabolic crates for measurement of methane and hydrogen emissions in respiration chambers (Pinares 2008) for two days followed by a 6 day faeces collection to determine baseline DMD. Following this pre-treatment period, the animals were randomly allocated to one of 3 treatment groups: Control (GP diet only); Combination (GP diet + a combination of CF and AQ dosed at 62mg/kg DM and 500 mg/kg DM respectively) and Sequence (CF and AQ added as sole inhibitor and changed every three days at the same dosed as above). After 4-weeks of treatment application, methane emissions and DMD measurements were made as for the pre-treatment period. Dry matter intake (DMI) was determined on a daily basis during the whole experiment and rumen samples for short chain fatty acid analysis were collected via stomach tube after each methane measurement.

Results Methane emissions and DMD during the pre-treatment period was similar to those from the Control group in the treatment period (data not shown). Treatments did not affect DMI. Methane emissions were decreased ($p = 0.017$) by 24 and 44% for the Sequence and Combination groups, respectively; while hydrogen emissions increased ($p = 0.020$). DMD was not affected by treatment ($P = 0.079$) but tended to be lower in the combination group. In the rumen, the proportion of propionate increased ($P < 0.001$) mainly at the expense of acetate ($P < 0.001$) indicating the redirection of hydrogen into fermentation pathways that produce less hydrogen. The proportion of butyrate was not affected by any of the treatments ($P = 0.711$), but group C showed a reduction of ammonia concentration by 40% in the rumen fluid ($P = 0.039$).

Table 1 Effect of a combination or sequence of methane inhibitors on methane emissions, total tract digestibility and rumen fermentation.

Parameter	Control	Sequence	Combination	s.e.d.	P
DMI (kg/d)	0.99	0.88	0.93	0.090	0.459
Methane (g/kg DMI)	27.6	21.0	15.6	3.53	0.017
Hydrogen (g/kg DMI)	0.05	0.45	0.41	0.125	0.020
DMD(g/kg)	719	728	694	14.3	0.079
Ammonia (mM)	13.1	12.1	7.8	1.91	0.039
Acetate (% of VFA)	64.1	52.5	55.3	1.75	>.001
Propionate (% of VFA)	14.9	27.8	23.8	2.49	>.001
Butyrate (% of VFA)	16.4	15.0	16.1	1.86	0.711

Conclusion Molecular hydrogen does not appear to be one of the major end products of fermentation when methanogenesis is inhibited in sheep. Hydrogen is diverted into fermentation pathways that produce less hydrogen (i.e. propionate) as described in Jansen (2010). Furthermore, that molecular hydrogen accumulation does not lead to a decrease in rumen fermentation. Further analysis of faecal fibre components and the microbial community composition will provide greater insights into the direct effect of methane inhibition on fibre degradation. This evaluation is currently underway.

Acknowledgements This work was funded by the Ministry of Primary Industries, New Zealand under the Sustainable Land Management and Climate Change programme.

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Rate of release of SF_6 from permeation tubes is described by Michaelis-Menten kinetics

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Introduction The SF_6 tracer technique which is used to estimate enteric methane emissions from ruminants requires knowledge of, or at least accurate prediction of, the rate of release of SF_6 from permeation tubes (Williams *et al.* 2011). The rate of release of SF_6 from permeation tubes is generally determined by placing them in an incubator set at 39ºC and the weight loss from individual tubes is monitored over a number of weeks (Lassey *et al.* 1997). Simple linear regression (zero order kinetics) of tube weight versus time has often been used for determining rate of release of SF_6. However, Lassey *et al.* (1997) showed that the rate of release of SF_6 from permeation tubes declines with time. To circumvent this problem, Lassey *et al.* (1997) recommended fitting a quadratic equation to the tube weight vs time data. However, we have noticed that the shape of the curve describing weight of SF_6 remaining in the tube vs. time, is not parabolic (as described by a quadratic equation), but can more accurately be described as resembling a "hockey stick", a shape typical of Michaelis-Menten (MM) kinetics. In this research we investigated the potential for MM kinetics to accurately describe the release rate of SF_6 from permeation tubes.

Material and methods Eight SF_6 permeation tubes for cattle were purchased from NIWA New Zealand (www.niwa.co.nz). Permeation tubes were incubated at 39ºC in a Contherm series 5 incubator (www.contherm.co.nz) and tubes weighed at approximately weekly intervals over 800 days. The weight of SF_6 remaining in the permeation tubes over the first 70 days was analysed by linear regression, and SF_6 weight loss occurring over 800 days was analysed by MM kinetics as described by Equation 1:

$$\frac{dW(t)}{d(t)} = \{(W(t) - Z)V_{\max}\} / \{K_m + (W(t) - Z)\} \qquad \text{Equation 1}$$

Where $W(t)$ is the weight (mg) of SF_6 in a tube at time t (days), $W(0)$ is the initial weight of SF_6 in each tube; V_{max} (mg/day) is related to the maximum rate of disappearance of SF_6 from the device, K_m (mg) is the MM constant, and it is the weight of SF_6 in the tube when the rate of weight loss is $V_{max}/2$; Z (mg) is a constant perhaps related to residue or contaminant in the tube.

Results Figure 1 shows a typical example of SF_6 weight loss from a tube, the linear regression (dashed line) and the MM fit to the data (solid line). Figure 2 shows the corresponding predicted rates of release of SF_6 by the two methods. The mean ± s.d. of the MM parameters for the eight tubes were: V_{max} 6.34 ± 1.05; K_m 388 ± 85.8; $W(0)$ 2593 ± 227.2; Z 168 ± 29.2.

Figure 1. Michaelis-Menten description of weight of SF_6 loss from permeation tubes

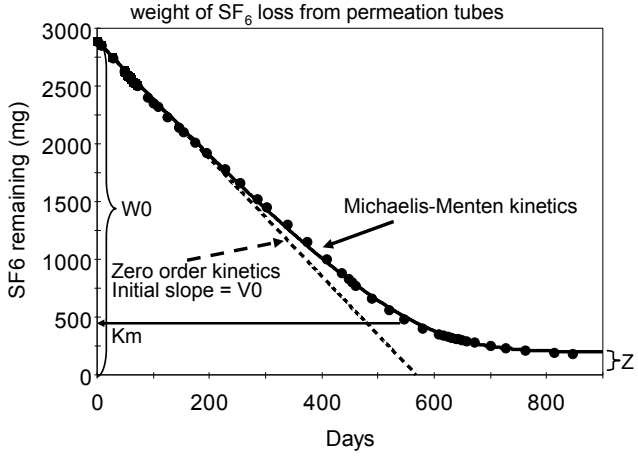
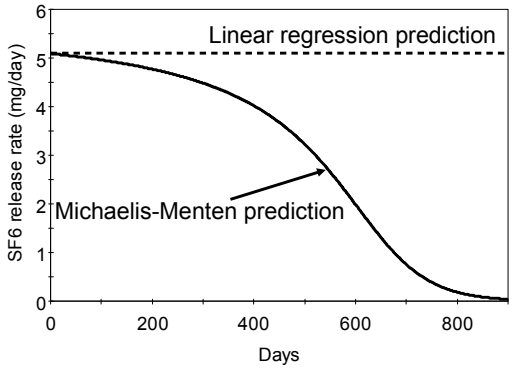


Figure 2. Predictions of SF_6 release rate by linear regression and by Michaelis-Menten kinetics



Conclusion Michaelis-Menten kinetics can be used to accurately describe the time course of the release rate of SF_6 from permeation tubes out to 600 days. The implications of this finding are that MM kinetics will enable more accurate estimation of CH_4 emissions by the SF_6 technique and extend the useful lifetime of permeation tubes by at least 2 or 3 fold.

Acknowledgements This research was funded by the Department of Primary Industries - Victoria, Dairy Australia, Meat and Livestock Australia, and the Australian Government Department of Agriculture, Fisheries and Forestry under its Australia's Farming Future Climate Change Research Program.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Comparison of enantiomers of organic acids for their effects on methane production in vitro

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Introduction Organic acids, such as malic and fumaric have the potential to decrease CH_4 emissions by acting as an alternative H_2 sink in the rumen. Both malate and fumarate are key intermediates in the succinate–propionate pathway, which directs H_2 away from methanogenesis, thus potentially reducing CH_4 production in the rumen (Carro and Ranilla, 2003). Both malic and fumaric acid have been extensively studied *in vitro* with numerous studies reporting dose dependent reductions in CH_4 production (Carro and Ranilla, 2003: Bayaru *et al.*, 2001), but some *in vitro* studies have reported that some organic acids stimulate CH_4 production (Czerkawski and Breckenridge, 1972). Tartaric acid is similar to both fumaric and malic acid in that all three are dicarboxylic acids. Futhermore, malic acid and tartaric acid exist in chiral forms in that both of these acids have D- and L- enantiomers. There has been little research comparing the relative effectiveness of these organic acids in terms of their methane mitigation potential, and no studies have specifically examined the comparitive effectiveness of the D- and L- enantiomers of malic and tartaric acids. This study compared the *in vitro* effects of 15 mM concentrations of L-tartaric, D-tartaric, DL-tartaric, L-malic, DL-malic, fumaric and citric acids for their effects on total gas production and CH_4 production.

Materials and methods Two 24 h *in vitro* batch culture incubations were conducted to examine the effects of supplementing Lucerne hay with either L-tartaric, D-tartaric, DL-tartaric, L-malic, DL-malic, fumaric and citric acids. Lucerne hay was dried at 55^{o}C and ground through a 1 mm screen prior to incubation. Rumen fluid was pooled from three lactating Holstein-Friesian dairy cows and strained through four layers of cheesecloth into a pre-warmed Thermos and immediately transported to the laboratory. Incubation bottles (50 mL) contained 500 mg of forage, modified McDougall's buffer (16.7 mL) and rumen fluid (8.3 mL), and sufficient added specific organic acid such that the specific organic acid concentrations were either 0, or 15 mM. Incubations took place under anaerobic conditions at 39^{o}C. After 24 h of incubation total gas production was determined using the water displacement technique. A sample of the gas headspace was removed for determination of CH_4 production (mL) via gas chromatography. Culture pH and IVDMD were determined after 24 h of incubation. Data were analysed using the mixed model procedure of SAS.

Results All organic acids decreased ($P<0.05$) culture pH and increased total gas production and production of CH_4 (Table 1). Only fumaric acid was associated with an increase ($P<0.05$) in IVDMD. There were no differences between L- and D-tartaric acid and between L- and DL- malic acid in terms of their effects on all measured parameters. These findings are somewhat surprising in that contrary to a number of published studies, none of the acids decreased CH_4 production.

Table 1 In vitro effects of various organic acids on cumulative gas production, CH_4 production, pH and IVDMD.

	Control	L-Tartaric	D-Tartaric	DL-Tartaric	L-Malic	DL-Malic	Fumaric	Citric	s.e.m.
Cumulative gas (mL/g DDM)	215.1	256.3*	257.8*	253.8*	244.8*	242.9*	235.9*	256.9*	2.84
Methane (mL/g DDM)	33.2	38.3*	38.6*	38.1*	35.1	36.1	32.8	37.4*	0.79
Culture pH	6.35	6.20*	6.20*	6.20*	6.15*	6.16*	6.15*	5.98*	0.029
IVDMD (g/kg DM)	547	561	560	570	568	581	599*	585	10.7

* Means in the same row followed by an asterisk, differ ($P<0.05$) from the control treatment

Conclusions These findings add to a growing body of scientific literature that together suggest that we still do not understand the mechanisms and situations under which organic acids may inhibit or stimulate CH_4 production by rumen methanogens.

Acknowledgements This research was funded by the Department of Primary Industries - Victoria, Dairy Australia, Meat and Livestock Australia, and the Australian Government Department of Agriculture, Fisheries and Forestry under its Australia's Farming Future Climate Change Research Program.

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Influence of rumen cannulation on feed intake, milk production, enteric methane production and composition of rumen headspace gas

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Introduction Rumen cannulated cattle and sheep are routinely used in research to investigate rumen fermentation and to quantify rumen methanogenesis. Rumen methanogenesis is an anaerobic process, but it is possible that rumen cannulae may allow the ingress of air into the rumen, thereby inhibiting methanogenesis. This research investigated the leakage of air into the rumen headspace of rumen cannulated dairy cows and quantified the effect of rumen cannulation on enteric methane production.

Material and methods Fourteen Holstein dairy cows in late lactation were used in this research. Six of the cows were non-fistulated and eight were fitted with rumen cannulae (www. rumencannula.com). The cows were fed individually in pens on a diet containing a grain mix (82% crushed corn, 16% cold-pressed canola, and 2% minerals) and alfalfa hay. Individual cow feed intakes and milk yields were measured daily. On days 27 and 28, cows were individually held in respiration chambers and methane emissions measured by methods previously described (Moate *et al.* 2011). On day 29, at 7.00AM (before the morning feed), rumen headspace gas in non-cannulated cows was sampled by rumenocentesis using a gas sampling syringe. For rumen cannulated cows, the gas sampling needle was inserted into the rumen through the plug of the rumen cannula. Rumen headspace gas (% by volume) analysis was by gas chromatography.

Results

Table 1 Influence of rumen cannulation on feed intake, milk production, enteric methane production and rumen headspace gas composition.

Parameter	Non-cannulated	Cannulated	s.e.d.	P
Grain intake (kg DM/cow/day)	11.5	12.0	0.86	0.54
Alfalfa intake (kg DM/cow/day)	10.1	10.1	0.08	0.67
Total intake (kg DM/cow/day)	21.6	22.1	0.86	0.51
Milk (kg/cow/day)	26.5	28.0	1.30	0.27
Methane (g/cow/day)	439	407	16.0	0.07
Methane yield (g/kg DMI)	20.5	18.5	0.75	0.02
Methane intensity (g/kg milk)	16.8	14.6	0.85	0.03
Rumen headspace gasses				
CO_2 (%)	49.8	13.4	7.73	0.001
CH_4 (%)	26.1	3.8	4.64	0.001
N_2 (%)	21.0	70.8	10.06	0.001
O_2 (%)	3.0	12.0	1.88	0.001
H_2 (%)	0.14	0.03	0.036	0.01

Rumen cannulation was associated with significant ($P < 0.01$) effects on all rumen headspace gases with the concentration of oxygen in cannulated cows being 4 times higher than in non-cannulated cows. Daily methane production in rumen cannulated cows was not significantly different to that in non-cannulated cows, but methane yield and methane intensity were significantly ($P < 0.05$) less in rumen-cannulated cows compared to non-cannulated cows.

Conclusion These results show that rumen cannulae allowed considerable amounts air to ingress into the rumen headspace, but total methane emissions were non-significantly decreased by about 7% and methane yield was significantly decreased by about 10%. In view of the magnitude of these effects, we interpret these findings as indicating that rumen-fistulated animals are probably valid experimental tools for researching rumen fermentation. However, their use in experiments intended to accurately quantify the effects of treatments on methane emissions is somewhat problematic. Further research is required to confirm these important findings.

Acknowledgements This research was funded by the Department of Primary Industries - Victoria, Dairy Australia, Meat and Livestock Australia, and the Australian Government Department of Agriculture, Fisheries and Forestry under its Australia's Farming Future Climate Change Research Program.

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Potential effects of time of cutting and plant genotypes on gas production from fermentation of perennial ryegrass (Lolium perenne) using dairy cow rumen fluid

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Introduction Volatile fatty acid (VFA) profiles in the rumen are important factors affecting methane production (Ellis *et al.* 2011). This study assessed the VFA, methane and total gas production from 'Standard' (commercially available 'PG150' cultivar) and 'high-energy' (genetically modified) perennial ryegrass *in vitro* using dairy cow rumen fluid.

Material and methods In October 2012 samples of 'Standard' and 'high-energy' (HE) perennial ryegrass (*Lolium* perenne) were collected at 6am and 4pm from a trial grown in a randomised complete block design in Hamilton, Victoria, Australia. Plant material was cut and freeze dried before being analysed for water soluble carbohydrate concentration (WSC) using a method described by Liu *et al.* 2011. Rumen fluid was collected from two Holstein-Friesian dairy cows located in Ellinbank, Victoria, Australia. One gram samples of each treatment of plant material were added to separate 250mL serum bottles and distributed between five 39°C water baths in a randomised incomplete block design. Added to each serum bottle was a 0.75:0.25 mixture of Cones buffer (with a pH of 6.8) and fresh rumen fluid. ANKOM^{RF} Technology modules were screwed onto the bottles to record cumulative gas pressure from the microbial activity over a 48 hour period. Twelve replicates of each treatment were run twice over two consecutive weeks. Samples of rumen fluid taken at the end of the fermentation period were frozen to -20°C and analysed for VFA composition using the gas chromatograph method described by Taylor 2002. Gas production was analysed using regression analysis fitted to an exponential curve, while WSC data were analysed using ANOVA. Volatile fatty acid analysis was performed with REML using a linear mixed model with pasture treatment and time of cutting as fixed effects.

Results Total WSC in the HE perennial ryegrass plants were higher than from the Standard plants ($P<0.05$) and higher in plants cut at 4pm compared to at 6am ($P<0.05$) (Table 1). Lower rumen fluid pH was recorded for the HE perennial ryegrass ($P <0.001$) compared to the Standard perennial ryegrass after 48 hours fermentation. Total gas production over 48 hours (A) and production of propionate was higher for the HE perennial ryegrass material compared to the Standard perennial ryegrass ($P<0.01$). Acetate production was also significantly higher (<0.001) in the HE compared to the Standard perennial ryegrass treatments, but there was no significant difference in butyrate production (data not shown).

Table 1 Rumen pH; total gas production (A); the concentration of propionate, and methane for plant material with varied water soluble carbohydrate concentration (WSC) after 48 hours *in vitro* fermentation for 'Standard' and 'high-energy' (HE) perennial ryegrass cut at 6AM and 4PM.

Treatment	Time of cutting	WSC (mg/g DM)	pH	A (mL/g DM)	Propionate (mmol/L)	Methane (ppm)
HE	AM	234.3	6.06	169.2	21.56	71327
HE	PM	253.0	6.01	169.4	21.47	62925
Standard	AM	202.2	6.15	151.3	20.61	73008
Standard	PM	224.8	6.11	165.3	20.14	61798
P	Pasture	0.008	<0.001	0.001	<0.001	0.983
P	Time	0.008	<0.001	0.017	0.099	0.141
P	Pasture.Time	0.972	0.798	0.079	0.453	0.940
s.e.d		11.87	0.016	4.32	0.24	10033

Conclusions This *in vitro* study indicated there was no difference in total methane production from HE perennial ryegrass compared to a Standard perennial ryegrass. However, the increase in propionate and no change to butyrate production suggests there could be an effect on methane if this high WSC plant material were to be fed to dairy cows in an animal trial. Further plant sampling is planned for different growth stages and points in time. These data are required before more conclusive assessments of the VFA and methane implications of changing WSC in perennial ryegrass can be made and used to prioritize plant breeding targets and optimise rumen nutrition models.

Acknowledgements The authors are grateful for financial support from Dairy Futures CRC, the Australian Association of Ruminant Nutrition and technical support from staff at Hamilton Department of Primary Industries and the University of Melbourne's Animal House.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Alteration of the rumen ecosystem of buffaloes by feed additives of plant origin

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Introduction The use of plants containing secondary metabolites (PSM) as rumen modifiers have been worked on extensively (Soliva *et al*., 2008; Sallam *et al*., 2009) but in the majority of studies conducted so far, extracts of the plant parts have been used in in vitro systems (Agarwal *et al*., 2009; Patra *et al*., 2010). In field conditions, it is not feasible to use extracts of the plants for the feeding of animals because it is less cost effective and the organic solvents used for the preparation of extracts might affect the animals adversely. There are varying reports of PSM on rumen fermentation and rumen microbial ecosystem depending upon the dose, chemical structure of PSM, nutrient composition of diets and physiological status of animals. Hence, the present study was conducted to establish the possibility of using feed additives of plant origin as natural manipulators of rumen fermentation for eco-friendly livestock production.

Material and methods Four fistulated adult buffaloes were fed in 4x4 Latin square design. All the animals were fed on a basal diet consisting of wheat straw and concentrate mixture in 70:30 ratio. The four groups were, control with no additive (CON), second with Mix 1 (essential oil mixture), third with Mix 2 (mixture of seeds rich in saponin and essential oil) and fourth with Mix 3 (mixture of seeds rich in saponin and fruit pulp rich in tannin and essential oil). In each phase of 30 d duration, after 19 d of feeding, rumen liquor was sampled for 2 consecutive days at 0, 2, 4, 6 and 8 h post feeding, whereas rumen content was sampled at 0 h feeding. The pH of the rumen liquor was recorded at every collection and then the rumen liquor of every collection was pooled by day and animal. The 0 h and pooled samples were used for estimation of rumen metabolites. Rumen contents collected at 0 h from different locations of rumen were processed for enzyme estimation immediately. The changes in population density of various rumen microbes at 0 h feeding were estimated by real time PCR. Data were statistically analyzed using generalized linear model (GLM) procedures and difference between treatments and periods was analyzed by using analysis of variance (ANOVA).

Results Daily intake of concentrate mixture, wheat straw and total dry matter were similar (P>0.05) in all the four groups. Feeding of any of the dietary treatments did not affect (P>0.05) rumen fluid pH. The concentration of ammonia nitrogen (mg/dL) was significantly (P=0.000) lower in the treatment groups than the control group. There was no effect of addition of feed additives on lactic acid. The ruminal enzymes *viz.* carboxymethyl-cellulase, avicelase, xylanase, protease and acetyl esterase activities were not affected (P>0.05) by supplementation of any of the feed additives to the diet of fistulated buffaloes. The changes in population density of various rumen microbes at 0 h feeding as estimated by real time PCR showed that supplementation of Mix 1, Mix 2 and Mix 3 did not affect the population of total bacteria, methanogens and *Ruminococcus flavefaciens*, whereas, *Fibrobacter succinogenes* and fungi populations increased and protozoa decreased in all the treated groups (Table 1).

Table 1 Effect of feeding plants containing secondary metabolites on microbial population in rumen liquor of fistulated buffaloes assessed by real time PCR

Rumen microbes	CON	Mix 1	Mix 2	Mix 3
Methanogens	1.00	1.347	0.785	1.357
Fibrobacter succinogenes	1.00	15.739	22.445	22.943
Ruminococcus flavefaciens	1.00	1.495	1.187	0.722
Fungi	1.00	5.579	4.807	5.178
Ciliate protozoa	1.00	0.112	0.010	0.015

Conclusions Supplementation of feed additives (Mix 1, Mix 2 and Mix 3) to fistulated buffaloes did not affect feed intake, rumen fermentation pattern and enzyme profile. However, there was a shift in the microbial profile of buffalo rumen. The population density of *Fibrobacter succinogenes* was highest in Mix 3 group followed by Mix 2 and Mix 1 group than control. The fungi population was increased by 5-fold in all the supplemented groups than the control group. Maximum reduction in ciliate protozoa population was observed in Mix 2 group followed by Mix 3 and Mix 1 group than control.

Acknowledgements The authors gratefully acknowledge funding from Indian Council of Agricultural Research (ICAR), New Delhi.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Effects of herbicide application, tillage and winter grazing of a forage crop on nitrous oxide emissions during pasture renewal

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Introduction Pasture renewal practices in New Zealand often include a single winter grazed forage crop established using tillage following herbicide spraying of the old pasture. There is limited data on the N_2O emissions that result from this form of pasture renewal, including the effects herbicide application, tillage and winter grazing and management to mitigate the emissions.

Methods and experimental design A replicated, factorial field trial was established at Lincoln, Canterbury, New Zealand on a Pahau silt loam (Mottled Argillic Pallic, NZ classification). The main crop treatments were rape, established following inversion ploughing to 15 cm, harrowing and rolling (IT) or no-tillage (NT), and new grass (G) established with no-tillage in autumn 2012 (Table 1). Six weeks before the tillage treatments were applied, the pasture (>15 years old) was sprayed with glyphosate. Two split applications of 50kg fertiliser N ha^{-1} were applied in March and April. In July 2012 we simulated winter grazing on the rape and grass plot. Treading and synthetic urine treatments were applied to split plots (6 m x 2 m). Cow treading was simulated at a pressure of approx. 250 kPa. Synthetic urine (Kool *et al.* 2006) was applied at a rate of 600 kg N ha^{-1}. Fluxes of N_2O were estimated from three chamber headspace samples taken from each plot; two or three times weekly. Cumulative N_2O emissions were analysed using ANOVA.

Table 1 Experimental stages and treatments

Stage/Activity	Date	Treatments	Treatment (reps) nos.
Spraying off	24 Jan 2012	(i) Old pasture, (ii) sprayed off pasture	2 (4 and 12)
Crop period	5 Mar 2012	Rape established by (i) IT and (ii) NT, (iii) G	3 (4)
Simulated winter grazing	11 Jul 2012	+/- treading, +/-urine split plots applied to the main rape plots established with (i) IT and (ii) NT, (iii) G.	12 (4)
New pasture sown (autumn)	25 Sep 2012	New pasture sown into +/- treading, +/-urine split plots with (i) IT and (ii) NT.	12 (4)

Results Spraying off the pasture significantly increased N_2O emissions ($P = 0.003$). Soil organic matter mineralisation, and a lack of plant N and water uptake contributed to these losses. Nitrous oxide emissions from IT plots during 2 weeks following tillage (0.7 kg N ha^{-1}) were almost double that of NT ($P = 0.009$). Cumulative N_2O emissions during the growing season were similar for all treatments ($P = 0.122$, Table 2) and similar to those during the 6-week period after spraying off the pasture. Tillage, ($P < 0.001$), urine ($P < 0.001$) and treading ($P = 0.020$) had strong effects on N_2O emissions, while there were strong interactions between tillage and treading ($P=0.004$) and tillage and urine ($P < 0.001$). Highest emissions followed rain when soils were saturated and contained high levels of mineral N (>200 kg ha^{-1}) contributing to larger emissions than previously measured from this soil (Thomas *et al.* 2008).

Table 2 Cumulative N_2O emissions (kg N ha^{-1}) between herbicide application and winter grazing.

	Herbicide application - Pre-cultivation (41 d)	Post-cultivation to Pre-grazing (128 d)	l.s.d. (5%)
Old grass	0.03		
Sprayed off grass	2.2		0.95 (d.f. = 11)
Intensive Tillage Rape (IT)		2.4	
Direct Drilled Rape (NT)		2.5	
New Pasture (G)		1.8	0.9 (d.f. = 6)

Table 3 Cumulative N_2O emissions (kg N ha^{-1}) over 70-d following simulated grazing. Data are back transformed from log values.

	- Treading/-urine	- Treading/ + Urine	+ Treading/ - Urine	+ Treading/ + Urine	Approx. l.s.r. (5%)
IT	15.2	58.0	49.0	109.8	
NT	8.2	37.9	7.4	46.0	
G	0.4	33.7	0.4	28.7	1.3(d.f. = 27)

Conclusions Soil damage from grazing increases the risk of large N_2O emissions. Direct drilling to establish crops reduces the risk of large emissions from intensive winter grazing practices that damage and compact soils, have large excretal N inputs, and long fallow periods when water contents are high. Periods of fallow following herbicide spraying also increases the risk of large N_2O losses.

Acknowledgement The work was funded by the Plant &cFood Research Core “Land Use Change & Intensification” programme.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Nitrous oxide production by micromycetes isolated from soils under cattle overwintering husbandry

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Introduction In addition to bacterial denitrification, production of nitrous oxide (N_2O) was demonstrated in filamentous fungi and yeasts in the 1970s (Bollag and Tung, 1972). The role of key enzyme (P450nor) and the metabolic pathway of fungal N_2O production were revealed in *Fusarium oxysporum* (Shoun *et al*., 1992), leading to further detection of producers randomly across different fugal taxa isolated from various environments (Morozkina and Kurakov, 1998). The aim of this work was to investigate the potential for N_2O production among fungi isolated from a pasture soil under beef cattle overwintering husbandry.

Material and methods Soil samples were collected from an upland pasture used for overwintering cattle since 1995, located on the Borová farm in South Bohemia, Czech Republic. Soil samples were collected several days after cattle left the winter pasture. Soil suspension from 2 g of fresh sample (n = 3) was diluted 1/1000 with sterile tap water, and agar plates with Sabouraud dextrose agar amended with rose bengal were inoculated with 0.25 mL of this suspension. After 7 days at 25 °C, the mature colonies were counted. All morphologically different colonies were isolated and identified on the basis of morphological features, as well as ITS DNA sequencing. Pure cultures were used according to the method of Kurakov *et al*. (2000). Cultures were aerobically grown under submerged conditions to obtain sufficient amount of biomass, and checked for bacterial contamination. Mycelium (100 mg ww) was sterile-filtered and transferred into 100 mL flasks with screw cap, butyl rubber stopper, and 20 mL of medium. Nitrite (10 mM $NaNO_2$) was selected as the sole nitrogen source resulting in 2800 µg NO_2^--N in the medium at the start of the anaerobic phase. Anaerobic conditions were established after inoculation by replacing headspace atmosphere with argon. After 3 and 8 days in a horizontal shaker at 28 °C, N_2O concentrations in the headspaces were measured using a gas chromatograph equipped with a 3 m, 0.318 cm i.d. stainless steel Porapak Q column and electron capture detector (GC-ECD, Agilent HP 5890). Control flasks were filled with a sterile medium to deduct N_2O evolved chemically. The rate of N_2O production in the flasks was expressed as µg N_2O-N d^{-1}.

Results From total 36 isolates, 63.8% were able to produce measurable amounts of N_2O. Two groups of fungal N_2O producers were described: (1) species utilising < 1% of the initial NO_2^--N stock and (2) species converting 1 - 18.5% of the initial NO_2^--N stock. Second group showed substantially higher average N_2O production rates (up to 58.6 ± 8.2 µg N_2O-N d^{-1}) (Table 1). *Fusarium*, *Monographella*, *Acremonium*, *Gibberella*, and *Eurotium* were the most important genera for N_2O production in soil under cattle impact, explaining variability in previous findings concerning N_2O emissions at the experimental area.

Table 1 Characteristics of most potent genera of micromycetes isolated from soils under cattle impact (AV ± s.d.; n=3)

Fungal genus	Productive isolates	Average N_2O production rate [µg N_2O-N d^{-1}]	NO_2^--N transformed into N_2O-N after 8 days [%]
Acremonium	1	52.6 ± 3.1	15.1 ± 1.7
Aspergillus	5	3.2 ± 1.7	1.1 ± 0.6
Clonostachys	1	11.8 ± 2.3	3.3 ± 0.9
Eurotium	1	43.6 ± 23.2	12.6 ± 6.7
Fusarium	3	46.4 ± 10.8	13.4 ± 3.4
Giberella	1	44.9 ± 11.8	16.2 ± 3.7
Monographella	1	58.6 ± 8.2	18.5 ± 1.3
Penicillium	4	23.6 ± 23.7	7.0 ± 6.9
Pseudallescheria	1	29.5 ± 19.7	8.4 ± 5.6
Verticillium	1	8.9 ± 5.6	2.6 ± 1.6

Conclusions Results presented confirmed the ability of a wide range of common soil fungi to produce significant amounts of N_2O. Although such capability was proven under laboratory conditions, the role of fungi in N_2O emissions from soils should not be neglected. Since N_2O is the dominant gaseous end product of fungal denitrification, fungi are responsible for most of the N_2O production in certain ecosystems, especially grasslands or pastures (Laughlin and Stevens, 2002).

Acknowledgements The authors gratefully acknowledge funding from GACR (P504/12/P752).

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Effects of diallyl sulfide and illite on methane production using different feed formula diets for Korean native cattle

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Introduction Manipulation of rumen microbes and microbial fermentation through the use of feed additives is seen to be a promising approach in mitigating methane emission from livestock. Feed additives such as diallyl disulfide was found to improve fiber digestion and decrease methane production (Busquet *et al*., 2005; Klevenhusena *et al*., 2011) while illite was found to be an antibiotic replacement which also increase growth performance in cattle (Sarker and Yang, 2010). In this study, we investigated the effects of chemically produced diallyl disulfide and illite on methane production using different feed formulation for Korean native cattle through *in vitro* rumen fermentation technique.

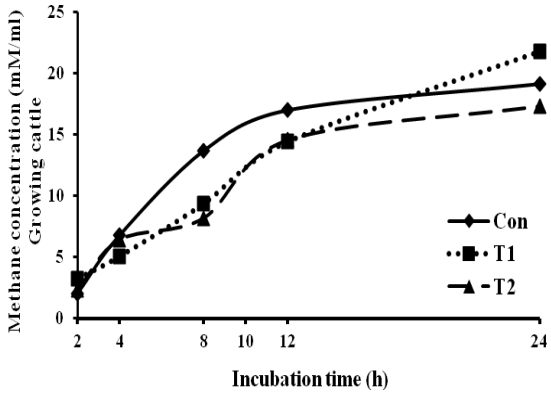

Fig. 1 Methane concentration of growing cattle at different incubation time

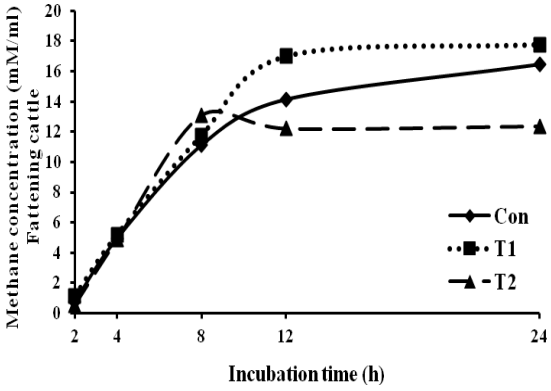

Fig. 2 Methane concentration of fattening cattle at different incubation time

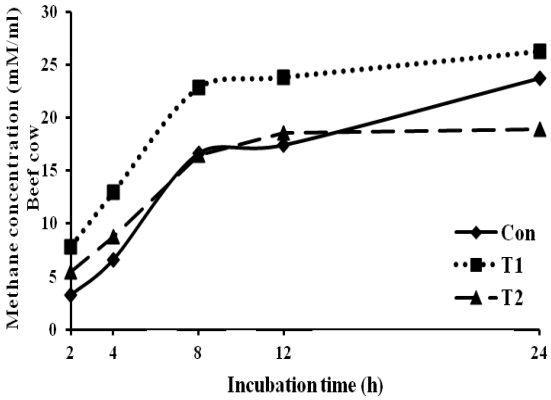

Fig. 3 Methane concentration of beef cow at different incubation time

Materials and methods Rumen content was collected from cannulated cattle with body weight of 600±47 kg at the experimental farm. One percent dry matter of different feed formula diets for growing cattle, fattening cattle and beef cow were used as substrates. The formulations were treated with of either 1% illite (T1) or 0.5% diallyl disulfide (T2). One hundred ml of 1:3 mixed filtered rumen fluid and Asanuma *et al.* (1999) buffer was anaerobically dispensed under a stream of O_2-free N_2 gas. The filled serum bottles were sealed with rubber stopper and aluminum cap and then incubated at 39˚C, 80 rpm for 0, 2, 4, 8, 12 and 24 h. Total gas production, ammonia-nitrogen, pH, volatile fatty acid (VFA) and other metabolites, methane, DGGE and methanogen quantitative real time PCR were analyzed from each of the serum bottles. All analyses were carried out using Statistical Analysis Systems (SAS) version 9.1 (2002).

Results Different feed formula diets for Korean native cattle produced different concentrations of ammonia nitrogen, volatile fatty acids, methane and carbon dioxide. Treatment containing diallyl disulfide (T2) had higher ($P<0.05$) pH than illite (T1) and control (Con) in all feed formulations. Moreover, total gas production and methane concentration in T2 were apparently lower than T1 and Con. Additionally, VFA production in all treatments was found to be not significantly different. Beef cow formula diet produced the highest methane production followed by growing and then fattening cattle feed formula diets. In growing cattle feed formula diet, methane production in T1 and T2 was significantly lower ($P<0.05$) than control at 8 and 12h of incubation. On the other hand in fattening cattle feed formula diet, T1 and Con had lower methane production than T2 at 8 h of incubation but became the lowest methane production at 12 and 24 h of incubation. Furthermore, T2 had the lowest methane production in beef cow feed formula diet.

Conclusions Different feed formula diets for Korean native cattle produced different concentrations of methane. The addition of diallyl disulfide reduced methane production on all feed formula diets while illite did not have a significant effect. Use of diallyl disulfide as feed additive in different formula diets for cattle may provide a means of decreasing ruminal methane production in cattle.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Trifluoromethyl sulphur pentafluoride (SF_5CF_3) as a new tracer gas for diffuse emissions

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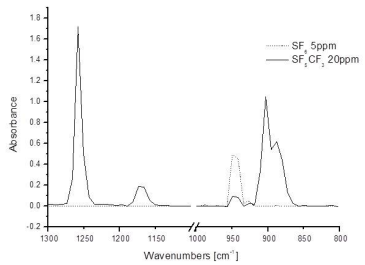
Introduction Diffuse emissions are increasingly important compared to conducted systems. Examples include gas leakage, industrial production and emissions from agricultural livestock.
The quantification of a diffuse source, such as NH_3 or greenhouse gases (GHG) in a naturally ventilated loose housing system with an outdoor exercise area, is difficult, mainly because the volume flow is badly defined and cannot be measured directly. Additional difficulties are: (i) a considerable spatial extension, (ii) an inhomogeneous source structure, and (iii) an emission mass flow that varies with time and may strongly depend on meteorology.

Tracer Ratio Method The scientific literature describes various methods to determine emissions from naturally ventilated housing and from diffuse sources. The constant tracer gas method is especially attractive because it does not depend on a direct determination of the ventilation rate. The overall source strength is determined through the release of a tracer gas which mimics the position and relative strength of the unknown source. If both gases disperse in the same way, then the ratio of known and unknown emissions is equal to the concentration ratio of tracer and target substances and the following equation applies:

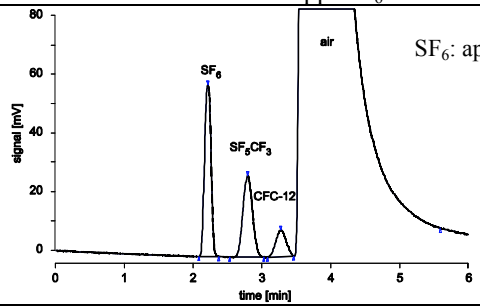
$$\dot{m}_{target} = \dot{m}_{tracer} \cdot \frac{c_{target}}{c_{tracer}}$$

where $\dot{m}$ is mass flow of target and tracer substances, and c is the concentration at the receptor. The above challenges can largely be met by using two traces gases, representing the housing and outdoor exercise area. In addition to the well-established SF_6 (sulphur hexafluoride), SF_5CF_3 (trifluoromethyl sulphur pentafluoride) was used as a second tracer gas. SF_5CF_3 has a similar chemical structure and physical behaviour as SF_6. In the atmosphere, the concentration is about 0.15 ppt.

Production and quantification of SF_5CF_3 and SF_6 tracer gases Provided the availability of an adequate analytical method, it is advantageous to measure both SF_6 and SF_5CF_3 near their background concentration, i.e. in the lower ppt range. This allows the use of diluted gases for dosing of a few hundred ppm, to mimic the emission source. Gases at such low concentrations have a density which is similar to air and can thus be expected to disperse like the emission source for most applications. While certified SF_6 standards can be obtained in both in ppm and the ppt range commercial standards are not available for SF_5CF_3. To overcome this limitation, we produced gravimetrically a primary reference gas mixture of ~4 ppm by diluting pure SF_6 and SF_5CF_3, and determined their concentrations by FTIR. This gas was then diluted to about 100 ppt and used as a working standard for GC-ECD measurements. The dilution factor was assumed to be the same for both components, and its value was determined by quantifying SF_6 based on a commercial 100 ppt SF_6 standard.



FTIR spectra measured using a GASMET CX-4000 with Peltier cooled MCT, optical resolution of 16 cm^{-1}, 10 scans min^{-1}, optical path length of 980 cm and cell temperature of 40°C.



SF_6: approx. 108 ppt

GC Chromatogram: Varian 3400CX with ECD-Detector, T=300°C,
custom-made columns: molecular sieve 5A(60/80 mesh, ID=3.7 mm, L=4 m, Applied Aldrich), Hayesep-Q (80/100 mesh, ID=3.7 mm, L=50 cm, Sigma Aldrich), T=120°C

Results and conclusions FTIR and GC-ECD based analytical techniques were successfully developed for the determination of SF_5CF_3 and SF_6 at ppm and ppt range. During the further procedure the dual tracer method was successfully implemented for the determination of ammonia emission in naturally ventilated cattle housings with an outdoor exercise.

Acknowledgements The authors gratefully acknowledge funding from the Federal Office for Environment, FOEN, Switzerland

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Methanogen diversity in the indigenous ruminants on the Qinghai Tibetan plateau

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Introduction The Qinghai Tibetan Plateau, frequently referred to as the Earth's "third pole", is one of the major drivers of global climate. Large numbers of yak, Tibetan sheep and goats graze the native pastures of the Tibetan plateau. These indigenous Tibetan ruminants have evolved special adaptations to withstand the severe cold, high altitude, strong UV radiation and poor forage resources of the alpine rangelands. Yaks also appear to have lower methane emission (per unit of live weight) compared to other ruminants such as cattle (Ding *et al*., 2010). Enteric fermentation of ruminant livestock is the largest source of global anthropogenic methane emissions contributing between 20% and 25%. Based on the analysis of global data sets available in public databases, the majority (>90%) of rumen archaea are affiliated with the genera; Methanobrevibacter (> 60%), Methanomicrobium (~ 15%), and a group of uncultured rumen archaea commonly referred to as rumen cluster C (RCC, ~16%) also known as Thermoplasmatales-Affiliated Lineage C (TALC) (Janssen and Kirs, 2008; St-Pierre and Wright, 2013). Huang *et al*. (2012) have reported that members of the TALC represent a large proportion of archaea in the yak rumen.
In this study the diversity of methanogens in Tibetan sheep and yak from the Tibetran Plateau were investigated by 454 pyrosequencing, to provide fundamental knowledge about archaea that may lead to reduced methane emissions from these ruminants.

Material and methods A total of 6 castrated male animals comprising three animals from each of the following species, yaks (*Bos grunniens*) and Tibetan sheep (*Ovis aries*), were fed with oaten hay and barley (7:3) for 14 days ad libitum in covered pens. Rumen fluid were obtained after 14 days on the oaten hay/barley diet by stomach tube, filtered through four layers of sterilized gauze, immediately transferred into sterile bottles and stored in liquid nitrogen until needed for DNA extraction and the following pyrosequencing analysis of the rumen archaeal community.

Results The archaeal communities in both Tibetan sheep and yak were dominated by the taxa associated with the Thermoplasma affiliated linage C group (Tibetan sheep, 78.2%; yak, 53.61%). Members of the Methanobacteriaceae family were also highly prevalent (Tibetan sheep 21.3%; yak, 44.9). Less than 2% of the archaeal population was represented by the Methanosarcinaceae family in all animals. A small amount of sequences from Crenarchaeota phylum was found in Tibetan sheep (0.14%).

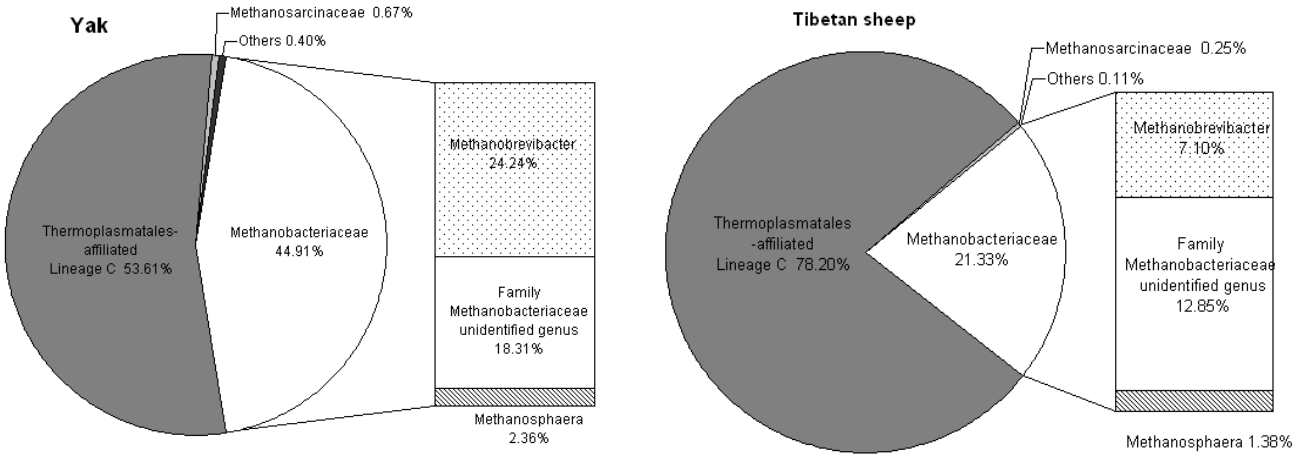

Figure 1 Diversity of methanogen in Tibetan sheep and yak on the Qinghai Tibetan Plateau.

Conclusions The current study has described the general rumen archaeal communities of the indigenous ruminants in Qinghai Tibetan Plateau (QTP). The community structure was different among these ruminants but the predominant populations were similar at a higher taxonomic level. Members of the Thermoplasma affiliated linage C dominated the methanogen communities in the ruminants in Qinghai Tibetan Plateau which has rarely been seen in other ruminants globally. However the metabolic activities of the TALC clade are relatively unknown. Therefore understanding the physiology and nutritional requirements of the organisms and their contribution to methane emissions may aid in the development of technology to manipulate this microbial population in the future.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Long-term impacts of grazing season on soil carbon sequestration in arid areas of South Africa

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Introduction Understanding factors controlling C sequestration by landscapes under different management scenarios has become a central concern for science, policy, and management. Soils of grasslands have a great potential for C sequestration. Like most other African countries, little is known about the level of C sequestration under various grazing strategies in the vast dry grassland areas of South Africa. It is well known that long-term studies with various grazing strategies would be able to shed light on the level of C sequestration in varying soil types (Peneiro *et al.,* 2010). The objective of this study was to assess the long-term impacts of grazing at various seasons on soil organic carbon (SOC) storage in the dry sandy clay soils of Grootfontein, South Africa.

Materials and methods Seasonal rotational grazing camps namely spring (SG), summer (SUG), winter grazing (WG) and Exclosure (Excl), which has not been grazed for more than 75 years, were used in this study. The camps were laid out in the form of parallel rectangular strips (width: length ratio of approximately 1:10) of 8.5 ha (seasonal grazing treatments) or 3.4 ha (exclosure) along the slope on gently sloping mixed Karoo apron veld. Merino wethers were introduced to the grazing treatment sites at the "two-tooth" stage and replaced after three to four years. The soil of the area is sandy clay, shigalo series with calciferous layers (Donaldson, 2012). Vegetation status of the area has been addressed in previous studies (Du Toit, 2000). Each camp was grouped into five homogenous grids located every 100 m along the slope. Each grid was considered as a replicate for each treatment. Soil samples were collected every 20 m along the grid with 15 m left as border on either side of each transect. . The samples were collected using auger from the 0-0.10, 0.10-0.20, 0.20-0.30, 0.30-0.40, and 0.40-0.60 m layers. The samples from each layer of a sampling point in each transact were combined and mixed to make a single homogeneous soil sample per layer. The samples were air dried and pulverized to pass through a 150-µm screen and analyzed for total C and N using a Carlo Erba NA1500 C/N analyzer (Carlo Erba Strumentazione, Milan, Italy). The data were analyzed using the GLM procedures in the statistical package of SAS, with grazing treatment (fixed effects) and error (random effect). Duncan's multiple range tests were used to determine significance differences in the mean SOC and C: N among different grazing managements at $P<0.05$.

Results This study showed that grazing significantly reduced carbon sequestration ($P<0.05$). It seems that spring grazing reduced carbon sequestration compared to summer and winter grazing, though not statistically significant. The C: N ratio of the exclosure was higher than SG at 30-60 ($P<0.05$) while it was consistently ($P<0.05$) higher at all depths except 0-10 and 20-30 compared to SUG and WG, respectively. However, no difference was observed in C: N ratio among seasonal grazing treatments.

Table 1 Soil organic carbon content (%) in different grazing management systems (mean ± s.e) of Grootfontein

Soil depth(cm)	SG	SUG	WG	Excl
0-10	0.460 ± 0.20^{b}	0.528 ± 0.13^{b}	0.537 ± 0.18^{b}	0.663 ± 0.19^{a}
10-20	0.470 ± 0.14^{b}	0.527 ± 0.10^{b}	0.550 ± 0.14^{b}	0.659 ± 0.16^{a}
20-30	0.494 ± 0.11^{a}	0.531 ± 0.09^{a}	0.545 ± 0.13^{a}	0.583 ± 0.13^{a}
30-60	0.372 ± 0.11^{b}	0.385 ± 0.09^{b}	0.406 ± 0.11^{b}	0.587 ± 0.14^{a}

Table 2 C:N ratio in different grazing management systems (mean ± s.e) of Grootfontein

Soil depth(cm)	SG	SUG	WG	Excl
0-10	9.520 ± 2.13^{a}	10.856 ± 2.52^{a}	9.548 ± 1.03^{a}	10.413 ± 2.34^{a}
10-20	8.984 ± 1.65^{b}	8.920 ± 1.63^{b}	9.534 ± 0.74^{b}	10.138 ± 1.97^{a}
20-30	7.791 ± 1.44^{ab}	6.855 ± 1.73^{b}	7.877 ± 1.39^{ab}	8.440 ± 2.41^{a}
30-60	7.656 ± 1.49^{b}	8.192 ± 0.99^{b}	7.895 ± 0.66^{b}	10.681 ± 3.27^{a}
0-60	8.318 ± 1.09^{b}	8.188 ± 0.80^{b}	8.865 ± 1.21^{b}	10.050 ± 2.08^{a}

Conclusion Higher SOC in the exclosure treatment is an indicator for long-term organic matter accumulation and this could be a viable management option in sandy soils. There is a necessity to develop a sustainable veld management for reducing degradation & C losses through stock exclusion practises. However, the minimum period required to detect differences in C stocks need to be investigated by re-sampling.

Acknowledgements The authors gratefully acknowledge funding from the University of Pretoria, Dept. of Science and Technology and the European communities, 7th framework programme under the grant agreement No. 266018, ANIMALCHANGE project.

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Quantifying methane mitigation potential for beef cattle at the herd scale in NW Western Australia

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Introduction Australia's Carbon Farming Initiative (CFI) is a carbon offset scheme legislated by Parliament in August 2011. The scheme allows farmers and land managers to earn carbon credits by storing carbon or reducing greenhouse gas (GHG) emissions. These credits can be sold on to third parties wishing to offset their emissions. Offset projects established under the CFI will require approved methodologies which detail implementation and monitoring activities to generate carbon credits. Currently six offset methodologies have been identified, with one specifically targeting management of methane (CH_4) from livestock. Unique to Australia is the existence of large corporate pastoralists, both publicly owned companies and private operators, who manage vast tracts of land across N Australia for grazing purposes. The property portfolio of some of these companies may include several extensive cattle stations encompassing grazing lands measured in millions of hectares and running hundreds of thousands of cattle. These significant operations manage integrated cattle breeding, growing and finishing operations which are essentially based on native pastures. However, across these northern rangelands beef productivity is associated with high CH_4 emissions intensity/unit animal product. Potentially, large pastoralists have much to gain from the CFI, but monitoring emissions and implementing new and persistent management practices will be crucial to qualify for carbon credits. A number of methodologies are currently available to measure individual animal emissions, but these are not suitable at the herd scale which is regarded as the smallest unit of measure when characterising livestock GHG emission for mitigation management purposes for N Australia. The objective of this study was to measure baseline CH_4 emissions for beef cattle in NW Australia under current practice prior to the implementation of significant change in management using irrigated and improved pastures. The study is part of the CSIRO Sustainable Agriculture Flagship Cluster "Measuring and Managing Emissions from Livestock: from Laboratory to Landscape".

Material and methods The study was carried out on a property in NW Western Australia, where methane flux from 60, 2-3 year old Droughtmaster heifers was measured when confined to a water point over a 14 d period (6 h/d) in August 2012. Available pastures consisted of Curly Mitchell (*Astrebla lappacea*), Kangaroo grass (*Themeda triandra*) and Roebourne Plains grass (*Eragrostis xerophila*). Methane flux was measured using open-path lasers (OPL) (GasFinder, Boreal Lasers, Alberta Canada) and FTIR (OP-FTIR) spectrometers. Instruments were on motorised mounts which automatically aligned to retro-reflectors terminating at paths on two sides of the animal enclosure and at multiple distances from the animals, ensuring the CH_4 plume was well sampled. Other stationary lasers and FTIR spectrometer measured ambient CH_4 concentration along the predominant upwind path. A micrometeorological mast with a three-dimensional sonic anemometer (CSAT3, Campbell Scientific Inc, USA), barometric pressure sensor and temperature humidity sensors, a cup anemometer and wind sentry collected data including wind speed, turbulence and direction. Herd scale methane emission values were generated using two analysis techniques. Surface-source assumptions were used in a backward Lagrangian Stochastic dispersion model (bLS) to derive methane flux, with laser and FTIR spectrometer data merged with sonic anemometer and other micrometeorological data before modelling in WindTrax (WindTrax dispersion model V.2.0.8.3, Thunder Beach Scientific, Halifax, NS, Canada) (Tomkins *et al*., 2011). Also a tracer-gas was released from gas canisters located on the panels of the enclosure, with both the tracer-gas and CH_4 measured by the downwind OP-FTIR spectrometer. This allowed for the CH_4 emission rate to be calculated from the known release rate of the tracer-gas and the ratio of the measured CH_4 and tracer- gas concentrations (Jones *et al.,* 2011).

Results Mean daily methane emission estimates were calculated from 10 min average data over 12 d. Emissions tended to be greatest in the morning immediately after animals arrived at the water point with hourly averaged emissions ranging from 190 to 60 g CH_4 /hd per day over the ~ 6 h of measurement. Daily averaged (± s.d.) emissions ranged from 113.4 ± 35.1 to 146.6 ± 57.9 g CH_4 /hd per day measured over the 12 days.

Conclusion & Discussion The data collected in this study represent baselines values for a current grazing scenario typical of NW Western Australia. Under the current CFI a management change from grazing native unimproved pastures to an intensive irrigated finishing system could qualify as a suitable methodology if a reduction in GHG emissions can be quantified. Methane emissions at the herd scale can be benchmarked using both the open-path and FTIR spectroscopic / bLS dispersion method making it possible to validate C credits in a scenario where cattle are finished on irrigated pastures. Ongoing work in 2013 will measure methane emissions from cattle grazing Pangola grass (*Digitaria eriantha subsp. pentzii*) cultivated under a series of centre pivot irrigators covering approximately 16 ha. Results from this work will be the first to quantify methane mitigation potential under a CFI methodology with direct relevance to the northern beef industry where intensive animal and pasture management are viable options.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Prediction of the individual enteric methane emission of dairy cows from milk mid-infrared spectra

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Introduction The livestock sector is considered the largest producer of methane (CH_4) from anthropogenic sources, world wide contributing 37% of emissions (FAO, 2006). An important step to study and develop mitigation methods for livestock emissions is to be able to measure them on a large scale. However, it is difficult to obtain a large number of individual CH_4 measurements with the currently available techniques (chambers or SF_6). The aim of this study was to develop a high throughput tool for determination of CH_4 emissions from dairy cows. Anaerobic fermentation of food in the reticulorumen is the basis of enteric CH_4 production. End-products of that enteric fermentation can be found in the milk (e.g., volatile fatty acids). Therefore individual enteric CH_4 emissions could be quantified from whole milk mid-infrared (MIR) spectra which reflect milk composition and can be obtained at low cost (e.g., national milk recording). Prediction equations of individual CH_4 emissions (determined using the SF_6 method) from milk MIR spectra have been established (Dehareng *et al.,* 2012; Soyeurt *et al.*, 2013). The results presented here are the improvement of this methodology by using a multiple breed and country approach.

Material and methods A total of 452 daily CH_4 measurements were obtained using the SF_6 technique (Johnson, 1994). During the measurement period a 40ml sample of milk was collected from each cow at each milking (morning and evening) and was analyzed by MIR spectrometry. These two spectra were averaged proportionally to milk production to generate one spectrum per CH_4 measurement. The reference data used have two origins: Holstein cows at the CRA-W in Belgium (performed in duplicate); and Jersey, Holstein and Holstein-Jersey crossbred cows at Teagasc Moorepark in Ireland. To include as much variability as possible, measurements were performed on 146 different cows of mixed parity (63, 36, 18, 29 cows in parity one to four+, respectively). Cows had also received many different diets: a basic diet enriched in maize or fresh grass or with supplemented linseed, total mixed ration, starch based (corn silage) morning ration with a fibrous (straw, grass silage) evening ration or grazed perennial ryegrass. The calibration model used to relate milk spectral data to CH_4 emissions was developed using Foss WINISI 4 software. The spectral regions used were: 972-1,589cm^{-1}, 1,720-1,782cm^{-1} and 2,746-2,970cm^{-1}. A first derivative was applied to spectral data followed by PLS regression. The number of factors was determined by a 50-group cross-validation which was also used to estimate the robustness of the equation. Calibration coefficient of determination (R^2c), cross-validation coefficient of determination (R^2cv), standard error of calibration (SEC), and the standard error of cross-validation (SECV) were calculated.

Results Calculated R^2c and R^2cv are greater than 0.70 (Table 1). R^2c (0.76) was lower than in previous equations 0.85 (Dehareng, *et al.,* 2012), 0.81 (Soyeurt *et al*., 2013), yet the difference between the R^2c and the R^2cv was slighter (0.06 vs. 0.13 and 0.09, respectively) as was the difference between the SEC and the SECV (6.1 g/d vs. 27 g/d and 8.5 g/d, respectively). This reflects an increase of the robustness of the equation. The present equation is based on 452 measurements (in place of 77 and 196, respectively) and additional variability has been introduced through inclusion of data from Jersey and crossbred cows with no decrease in statistical parameters.

Table 1 Equation statistical parameters

	N	s.d.	R^2c	R^2cv	SEC	SECV
g CH_4/day	452	126.4	0.76	0.70	62.0	68.7

Conclusions These results confirm the possibility to predict enteric CH_4 emissions from whole milk MIR spectra. This equation used a calibration dataset of wider variability than those used in previous analyses, yet the robustness of prediction was much improved. This improved equation will be useful in large scale studies to link enteric CH_4 emission to diet, genetics (Kandel *et al*., 2013), management and geographical location, with the objective to develop tools to mitigate enteric CH_4 emissions.

Acknowledgements The authors gratefully acknowledge the Agricultural Head Office of the Walloon Region DGO-3 (Belgium) for support of the MethaMilk project, the EU GreenHouseMilk project and Teagasc Moorepark (Ireland) for their collaboration.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Microbial inoculation of silage – an overlooked greenhouse gas mitigation option?

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Introduction Inoculation of silage with lactic acid bacteria is well recognised for securing a fast fermentation and improving aerobic stability at feed out. A literature-review by Weinberg & Muck (1996) showed that not only pH reduction is secured, but also that 40% of the trials significantly improved milk yield. Improving animal efficiency is the most recognised enteric CH_4 mitigation option. However, recent reviews from e.g. Eckhard *et al.* (2010), Martin *et al.* (2010) and Cottle *et al*. (2011) discuss various different dietary methods and farm management mitigation options, but none of them include microbial silage inoculation into their analysis. The literature review therefore aims at describing differences in CH_4 emission for dairy cows fed silage treated with silage inoculants or untreated silage.

Material and methods In 37 experiments with inoculated silage versus untreated control silage. The cows were fed either TMR or fixed amount of concentrate. Information on milk yield and feed intake provided. The trials were divided into four silage categories: Grass (n=12), Grass/Legume (n=5), Lucerne (n=7) and Maize (n=13). The percentage difference between control and microbial inoculated silage on milk yield, dry matter intake (DMI), estimated daily CH_4 emission and CH_4 emission per kg of milk was averaged and the mean standard error calculated. The CH_4 prediction equation of Kirchgessner *et al.* (1991) was used since DMI is the only variable factor and could therefore be used to estimate CH_4 emission in all studies.

Results and discussion The preliminary results from the current study shows that silage inoculation increased DM intake (kg) and energy corrected milk (ECM, kg), and reduced CH_4/ECM (g/kg) 68%, 68%, and 57% of the studies, respectively (figure 1 & 2). The most constant effect was found when inoculating lucerne silage, where all seven studies improved milk yield. The function between DMI and milk yield across silage forages could be predicted as follow: milk yield (kg) =1.3 x DMI (kg) + 4.0; R^2=0.580 and milk yield (kg) =1.2 x DMI (kg) + 5.5; R^2=0.498 for control and inoculated silage, respectively. Methane emission per kg of ECM was first of all reduced in grass and Lucerne, but not in grass/legume or maize (figure 2). The increased milk yield in both these two forages gives an opportunity to reduce herd size with 3.8% and 2.5%. A reduced herd size and including reduced CH_4/kg ECM would produce 5.4% and 2.9% less CH_4/kg ECM compared to a herd where grass and lucerne silage is not inoculated. An *in vitro* study by Jalc *et al.* (2009 a, b) showed also a significant CH_4/g digested DM reduction of 20 – 31 % in inoculated grass silage, while inoculated maize silage had no significant effect. The different species and strains of lactic acid bacteria used to inoculate silage vary greatly on pathogens and fungi inhibition and their effect is fairly well understood. However, the mode of action of silage inoculants on milk yield is not well investigated and understood. Most of the silages included in the study were well preserved and the nutritional differences between treated and untreated control silage were not likely to explain milk yield increase. Possible mode of action can be improved palatability, rumen modification or even a probiotic effect as discussed by Muck (2012).

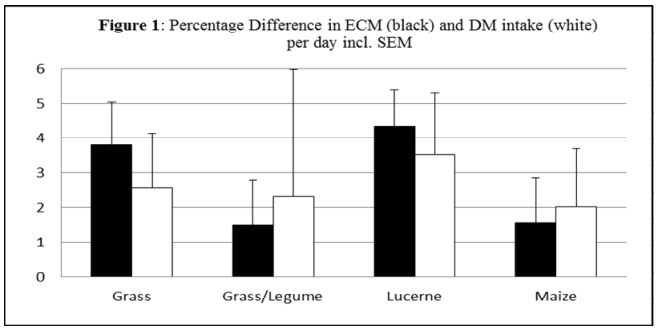

Figure 1: Percentage Difference in ECM (black) and DM intake (white) per day incl. SEM

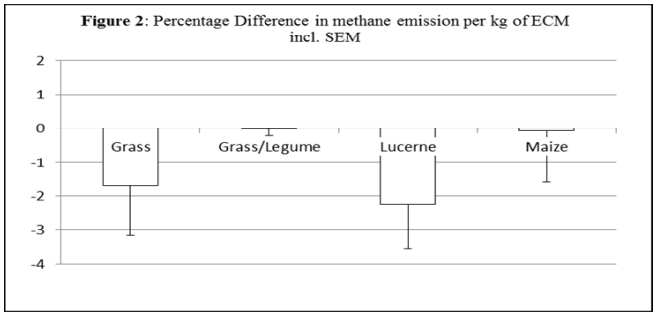

Figure 2: Percentage Difference in methane emission per kg of ECM incl. SEM

Conclusions

The current literature shows that silage inoculation is a promising CH_4 mitigation option in dairy cattle, since milk yield is improved and CH_4 emission per ECM reduced, especially in grass and lucerne silage. The mode of action of the silage inoculants on the performance of dairy cows requires further investigations, however.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Beef production systems and greenhouse gasses in South Africa

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Introduction Livestock agriculture is the world's largest user of land resources and South Africa is not different. In South Africa approximately 84% of the surface area is available for farming. However, a large part is not suitable for crop cultivation, with approximately only 13% that can be cultivated. The greater part of South Africa (71%) is only suitable for extensive livestock farming (RMRD SA, 2012). Thus, this is the only system by means of which the major part of the country can contribute to food security, which is also the case in the rest of Africa. In spite of primary beef cattle farming (cow-calf production cycle) being largely extensive in South Africa, more than 75% of cattle slaughtered in the formal sector is finished in feedlots on maize and maize by-products (RMRD SA, 2012).

Discussion The cow-calf portion of the production cycle (the extensive part in South Africa) accounts for 72% of the nutrient requirements from conception to harvest. Under natural rangeland conditions decomposition of manure is aerobic, leading to production of CO_2 and H_2O as end products. Part of this CO_2 from the manure is absorbed by the regrowth of the surrounding vegetation rather than released into the atmosphere, but carbon sequestration measurements have been neglected and therefore the quantitative effect is not known (Scholtz *et al.*2013). This is in sharp contrast to the intensive systems in large parts of Europe and North America, where large quantities of manure are stockpiled, often for long periods, and undergoes anaerobic decomposition. Anaerobic decomposition of manure, as found in intensive cow-calf systems, feedlots and intensive dairy systems, produces CH_4 as one of the major end products. It is estimated that the cow-calf portion of the production cycle accounts for approximately 80% of GHG from beef in the North American temperate zone production systems (Beauchemin *et al.,* 2010). It is also relevant to consider the calf finishing systems, or the post weaning phase. Cattle in feedlots fatten over approximately 110 days in South Africa, which means that they produce GHG for only 110 days before they are slaughtered. For cattle on rangeland/pasture it requires more than 200 days to finish to the same carcase classification because of the lower quality feed compared to a feedlot diet. Furthermore, the lower quality feed (mainly pastures that they are consuming) also produce more GHG per kilogram feed intake than the concentrates used in feedlots (Capper, 2011; Meissner *et al*., 2012). The bottom line is that feedlots finish cattle faster and maximize efficiency of meat production resulting in a lower carbon footprint. Furthermore, there is substantial evidence indicating that organic production systems consume more energy and have a bigger carbon footprint than conventional production systems. For example, organic grass-fed cattle requires approximately three times more energy per kilogramme of weight gain and release more than double the quantity of GHG's per kilogram of weight gain than conventional feedlot cattle (Capper, 2010). Most consumers purchasing organic products do not know that such systems have a higher carbon footprint. It is also important to keep in mind that livestock, such as cattle, are important to mankind since most of the world's vegetation biomass is rich in fibre. Only livestock can convert this high fibre containing vegetation into high quality protein sources for human consumption and this will need to be balanced against the concomitant production of methane. In addition to the formulation of strategies aimed at greener food environments and lower carbon footprints, health considerations such as the nutrient-density of livestock products, should also be considered. In Africa, subsistence farmers keep livestock for multiple purposes and rural households depend on livestock for the milk, meat, hides, horns, fertilizer and income (Dovie *et al*., 2006). Livestock is therefore central to the livelihoods and wellbeing of rural communities.

Conclusion Estimates of GHG emissions from livestock are subject to uncertainty because generic coefficients applicable to all animals are commonly used which takes no account of differences in production efficiency and production systems. It is therefore important that the GHG emissions, of for example beef cattle in South Africa and other developing countries, be modelled properly since the production systems and in many cases the genotypes used, are significantly different from the developed countries in the northern hemisphere.

Acknowledgements This study is financially supported by RMRD SA (Red Meat Research and Development South Africa) and THRIP (Technology and Human Resources for Industry Programme).

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Implementation of GreenFeed in a dairy herd grazing pasture

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Introduction. All techniques for measuring methane from dairy cows fed fresh pasture involve compromise; restrictions in activity and dietary choice with chambers, variable accuracy and absence of intake measurements with SF_6 at pasture, etc. The GreenFeed system does not impose restrictions on behaviour or diet, and cows access the unit of their own free will. There are no requirements for laboratory equipment and expertise for gas sample analysis and although cows are given small amounts of feed to encourage visits for methane measurement, the number of visits by individuals can be restricted. The operation of two GreenFeed units was evaluated by placing them with cows grazed on pasture (given new pasture twice a day) to provide information about cow willingness to access the units, chute designs for ensuring one animal accessed the unit at a time, requirements and success of training individual cows, and to estimate the time required to obtain robust data for individual animals.

Methods. The evaluation was undertaken in Hamilton, New Zealand, with two GreenFeed units accessible to 24 cows (including 4 with a permanent rumen fistula) over a 5 week period, and concluded with the addition of a further 20 cows in the herd. At the commencement of the trial, the cows were about 50 days in milk and grazed on pasture as the sole diet in a self-contained herd. They were milked twice daily and new pasture was given after each milking. Two GreenFeed units were placed in the paddock, close to the new area of pasture to be grazed next, to minimise frequency of moving. A chute was designed that could be towed behind the units when moved between paddocks, to control access to the units. Measurements included number of cows visiting, number of visits/day (restricted to 6/24h), preference for a grain or forage based attractant, time taken for individuals to enter the units, benefits of either people training cows (encouraging entry to the units) or cows training cows (when 20 additional animals were added to the initial group). The cows were ages 2, 3 and 4 years, and milk production was measured daily, with weekly measures of composition and cow live-weight. These data, with pasture composition enabled intakes to be calculated from energy requirements for maintenance and production, so the values for methane emissions (provided by the manufacturers) could be compared to estimated production determined from calculated intake and methane yield (21 g/kg dry matter intake). Calibration was undertaken weekly using CH_4 and nitrogen/CO_2 gases. Comparisons between pellet preferences used mixed models fitted by REML using Genstat V 14.1.

Results and Discussion. The Holstein/Friesian cows used in this evaluation produced about 21 kg milk/day and liveweight (average 496 kg) changes over the measurement period were minor. They were in good health and accustomed to handling. Pasture was good quality and dominated by perennial ryegrass, with about 0.25 ha allocated to the 24 cows after each milking to meet their feed requirements. Of the 24 cows given access to the GreenFeed units, 17 became frequent users. Cows showed no apprehension toward the units, but many were inquisitive and chewed on all accessible wiring and other parts of the system. Some animals that had no interest in the units at all, despite training and frequent visits to the units by most cows. Training, by placing a trail of pellets toward the chute resulted in about half the “infrequent users” becoming routine users, but “cows did not train cows”. When an additional 20 cows were introduced to the herd, GreenFeed access was very low (4 of the 20 provided useful data after 7 days, compared with 11 of the first 24 after 7 days). Cows did not exhibit a preference for either grain-based or alfalfa-based pellets used as an attractant and the attractant did not affect estimates of methane (P=0.098), but the contribution of pellets to their intake (about 220g/visit) was important, as it effectively altered their diet; hence the use of forage-based pellets in the evaluation. Methane yield averaged from 14 cows estimated by GreenFeed over 38 days of measurement averaged 339 ± s.d. 47.8 g/d, and the calculated values based on metabolisable energy (ME) requirements, the ME content of the feed (12.1 MJ/kg DM) and a methane yield of 21.6 g/kg DM intake was 345 g/d. There was a positive correlation between GreenFeed measurement and calculated methane emission (R^2=0.72; P= 0.004). GreenFeed values varied between days, but the coefficient of variation for daily methane production did not decline appreciably after 20 days of measurements. It was not possible to show improvements in methane estimates from prolonged measurements with the experimental design used here. Methane emissions from cows with a rumen fistula averaged 111 g/day, probably as a consequence of leakage from the fistula.

Conclusion. GreenFeed technology provided estimates of methane emissions from lactating dairy cows grazing pasture that corresponded well with estimates based on calculated values. More than half the cows in the herd adapted rapidly to the GreenFeed units and all cows that used GreenFeed had their access restricted to prevent over-use. Cows could be trained to use the units, however the type of attractant had no effect on cow behaviour. Damage to pastures and creation of deep (20 cm) ruts in the chutes leading to the units must be prevented in future evaluations in wet conditions, and the system is not appropriate for cows with a rumen fistula. Principle areas of concern in pastoral grazing systems are possibilities of excessive contribution of the attractant to the cow diet, the time taken for cows to use the system and our limited understanding of algorithms used to determine values for methane emissions, that are provided by C-lock Inc.

This project was funded by the New Zealand Government in support of the livestock research group of the global research alliance on greenhouse gases. Disclaimer: the information contained in this abstract should not be taken to represent the views of the alliance as a whole or its partners.

Meta-analysis of mitigation options for enteric methane emissions from ruminants

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Introduction The animal science sector has seen a proliferation of potential mitigation strategies, aimed at tackling emissions from enteric fermentation. Broadly speaking, mitigation can be achieved by manipulating the whole animal through breeding, manipulating the diet or manipulating the rumen environment (Eckard *et al*. 2010). By bringing together data from studies on the many mitigation options available, we aimed to evaluate the overall mitigation potential for these broad strategies. The data collected also allowed an investigation of the potential moderating variables explaining the great variability in results between studies.

Methods A structured search for published data was performed using a range of databases and relevant search terms for type of animal, methane and mitigation type. Papers with data on *in vivo* methane measurements for treatment and control groups were added to a database which currently contains 294 papers. Analysis was limited to those papers which reported emissions relative to intake and reported a measure of variance of the mean. A total of 147 papers were included in the current analysis, representing a wide range of countries and animal breeds. The effect size was calculated as the log transformed ratio of means. For each mitigation category, a random effects model meta-analysis was performed with a DerSimonian-Laird estimator using the "metafor" package in *R*. Where the overall treatment effect was found to be significant, further explanatory variables (chosen *a priori* to include animal species, treatment type, duration and dose) were explored as moderators in the model.

Results Lipid supplementation and addition of tannins or saponins was found to be effective in all animals, while the addition of H-sinks or ionophores were found to be effective in cattle and sheep only (Table 1). The basic model left no significant remaining heterogeneity for data breeding where the data was very limited. Treatment was a significant explanatory variable for feed quality, lipid supplementation, tannins, H-sinks and inhibitors. Effectiveness varied significantly with dose for lipid supplementation and tannins. Duration was only a significant moderator for lipid supplementation.

Table 1 Effectiveness of mitigation strategies on a per feed intake basis. Effect size represents mean ratio of treatment to control. Strategies producing a significant (P<0.05) reduction in methane are highlighted in bold.

Mitigation strategy	Effect size	Number of comparisons	95% CI	Model moderators			
				Animal	Treatment	Duration	Dose
Animal manipulation							
Breeding	**0.79**	6	0.69-0.91				
Diet manipulation							
Feed quality	**0.94**	94	0.91-0.97	**X**	**X**		
Enzymes	1.05	3	0.93-1.19				
Lipid supplementation	**0.81**	81	0.78-0.84	**X**	**X**	**X**	**X**
Probiotics	0.97	14	0.93-1.01				
Tannins, saponins	**0.85**	59	0.81-0.89	**X**	**X**		**X**
Essential oils	0.95	17	0.91-1.00				
H-sinks	**0.89**	20	0.81-0.95	**X**	**X**		
Rumen manipulation							
Chemical inhibitors	**0.67**	25	0.64-0.70	**X**	**X**		
Ionophores	**0.91**	33	0.88-0.94	**X**			
Vaccination	1.18	6	1.14-1.22				
Defaunation	0.94	7	0.84-1.05				

Conclusions The most effective mitigation strategy appears to be the addition of chemical inhibitors which alter the rumen community. This strategy does, however, raise concerns for animal health and food safety and is therefore not likely to be a viable option. Vaccination or defaunation do not appear to be viable strategies, though data are scarce. Diet manipulation provides many viable options, including manipulation of forage quality or the supplementation with lipids or tannins. There is significant heterogeneity in the effectiveness of these strategies which warrants further exploration. An important aspect highlighted here is the treatment type which varies greatly in such broad categories, and for some strategies there is a dose dependent response which needs to be clarified. There are also significant differences between animal types for some strategies which warrant further exploration.

Acknowledgements Research conducted as part of Animal Change funded by EC-FP7/2007-2013 (grant agreement no. 266018)

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Variation in enteric methane emissions among cows on commercial dairy farms

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Introduction Measuring the rate of eructed methane (CH_4) emissions during milking (MERm) has been shown to provide an adequate and repeatable measure of individual cow enteric CH_4 emissions under experimental conditions (Garnsworthy *et al.*, 2012). The technique uses an infrared analyser to continuously measure CH_4 concentration in the feeding bin within a robotic milking station. The method can be applied to commercial farms at a relatively low cost. The aim of the present study was to assess variation in MERm among cows on commercial dairy farms.

Material and methods A measurement of MERm (g/min) was obtained from 16,870 milkings from 490 individual cows across 7 commercial dairy farms with robotic milking systems. Cows with at least ten milkings and MERm measurements were retained in the analysis. Farms were visited between September 2011 and July 2012, and CH_4 analysers on each milking station recorded continuously for at least 7 days. Cows were predominantly of Holstein Friesian (HF) breed and remained on the same feeding system during the sampling period. All cows received concentrate feed during milking with the amount dependent on daily milk yield. The remainder of the diet consisted of a total mixed ration at farms A to C where cows were housed, or grazed grass at farms D to G where cows were allowed access to pasture (with additional conserved forage being fed at farms D to F). Effects of explanatory variables on average MERm per individual cow were assessed by fitting a linear mixed model. The variables assessed were: farm, breed (1 = HF, 2 = HF cross, 3 = other), season (1 = October to March, 2 = April to September), average number of milkings per day, lactation number (1, 2 and 3+), week of lactation at start of sampling (1, 2, 3,..., 57), age at calving (months), average daily concentrate intake, live weight and milk yield. The most significant variables identified from univariate analysis were added first to a multivariate model with only significant ($P < 0.05$) variables being retained. In the multivariate model, residual variance estimates were allowed to differ among farms. The proportion of variation associated with each explanatory variable was determined. The deviance statistic was used to test whether a model for MERm per cow that used a daily average of 2 or more CH_4 measurements per day was better than a model that averaged all measurements over the sampling period.

Results Variation in MERm (Y_{ijkl}, g/min) of individual cows measured across farms was best described by: $Y_{ijkl} = \mu + F_i + W_j + b_1M + E_{ijkl}$; where, μ = overall mean; F_i = fixed effect of farm; W_j = fixed effect of week of lactation; b_1M = linear regression of Y on daily milk yield ($b_1 = 0.03$, $P < 0.001$); E_{ijkl} = random error term. The model showed that there were significant differences in predicted mean MERm between farms (similar to observed means in Table 1, s.e.d = 0.25, $P < 0.001$) and week of lactation (a curvilinear response ranging from 2.1 g/min at week 2 to 3.9 g/min at week 55, s.e.d. = 0.51, $P < 0.001$). There was also considerable variation among cows within farms (Table 1). The largest proportion (0.66) of variation in MERm can be explained by differences between farms and by the average daily milk yield of the cow (0.30). Removing sampling days with a single CH_4 measurement and using a daily average MERm value increased the deviance from 555 to 577, and hence did not improve the model.

Table 1 The average (s.d.) observed CH_4 emission rate (MERm) at each farm and the minimum and maximum values

		MERm (g/min)		
Farm	No. of cows	Mean (s.d.)	Minimum	Maximum
A	39	2.2 (0.6)	1.1	3.7
B	56	2.8 (0.7)	1.4	5.0
C	61	2.0 (0.8)	0.7	4.3
D	53	3.6 (1.9)	1.0	11.0
E	70	3.9 (2.7)	1.1	15.7
F	44	4.3 (1.3)	1.9	7.4
G	149	1.8 (0.8)	0.5	5.0
All	472	2.7 (1.7)	0.5	15.7

Conclusions This study suggests there is considerable variation in the MERm among commercial dairy cows. Further CH_4 measurements from farms used in this study, when animals are on a different feeding system (i.e. either housed or with access to grazing), may help explain some of the variation seen among farms and test interactions between variables.

Acknowledgements This work was funded by Defra, the Scottish Government, DARD, and the Welsh Government as part of the UK's Agricultural GHG Research Platform project (www.ghgplatform.org.uk).

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Development of an exergetic life cycle assessment (ELCA) tool to evaluate environmental impact of dairy farms in Flanders (Belgium)

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Introduction The evaluation of the potential environmental impact of the entire production chain of agricultural products is to date most commonly done through Life Cycle Assessment (LCA). Yet, LCA tools have limitations. They mainly focus on environmental impacts related to emissions, while environmental impact can also be related to the use of (non-) renewable resources. Very often, they are even restricted to only one environmental impact, e.g. global warming caused by greenhouse gas emissions and expressed as Carbon Footprint. The drawback of such a narrow approach is the risk for environmental problem shifting between emission- and resource-related impacts and within emission-related impacts. Moreover, LCA tools often lack a holistic system logic with transparent links between the inputs and outputs of processes and thus lack the required flexibility to search for improvement opportunities in a dynamic way. If we want to address sustainability in a world of increasing scarcity, comprehensive and dynamic tools that integrate the analysis of environmental impacts related to both emissions and resource use along agricultural supply chains, are required.

Material and methods Exergy analysis is a method to account for the consumption of resources, both material and energy inputs, on one common scale (Joule of exergy). The exergy of a resource is defined as the maximum amount of useful work that can be retrieved from this resource when bringing it into equilibrium through reversible processes with the natural environment (Szargut *et al.*, 1988). Through an Exergy Analysis (EA), exergetic efficiencies can be calculated at process level. At life cycle level, an Exergy Analysis can be extended to an Exergetic Life Cycle Assessment (ELCA). In order to calculate efficiencies at process and life cycle level, this method considers inputs (materials and energy) as well as outputs (products, co-products, wastes and emissions). In this way hotspots of high and inefficient resource use, possibly related with undesired emissions, can be identified. In combination with the LCA methodology, in which emission-based impacts, such as global warming, are evaluated, an ELCA tool can be promising for the evaluation of the environmental impact of dairy farms in Flanders. The impact assessment method "Cumulative Exergy Extraction from the Natural Environment" (CEENE) (Dewulf *et al.*, 2007) is used to quantify total resource use throughout the production chains of dairy farms in Flanders. To build the ELCA tool, we have chosen the dairy life cycle from cradle to farm gate because of the existing systems' links between animal and plant production at the dairy farm. We performed a case study on two Flemish dairy farms that made their accounting data available for primary data collection.

Results First, dairy farms in Flanders were analysed in detail and generic process diagrams at system and process level were drawn. The dairy farm, i.e. the foreground system (β), was divided in five subsystems "dairy production", "crop production", "water supply and pretreatment", "renewable energy/heat/hot water production" and "wastewater treatment" (Figure 1). The α-core system "dairy production" was divided in five subsystems "animal breeding", "milking", "manure storing", "feeding" and "housing". The side systems "renewable energy/heat/hot water production" (solar panels, -boilers and digesters) (β_3) and "wastewater treatment" (β_4) were not present at the two dairy farms under study, but these systems were included because a generic tool is developed. Second, flows corresponding with a certain mass or energy requirement were quantified within and across the β-system and interrelations between these flows were identified. Physical data were retrieved directly from the accounting data of the farms for 48% of the identified flows. Another 23% of the identified flows were not applicable in the case study (11% were related to the β_3 and β_4 systems). For the remaining 29% of the flows, no physical data was available in the farm accounting data. These flows include waste flows (e.g. wastewater), emissions (e.g. methane), small flows (e.g. disinfectant) and non-traded internal flows of the β-system (e.g. manure and forage crop products). Internal flow data are necessary for analyses at process level.

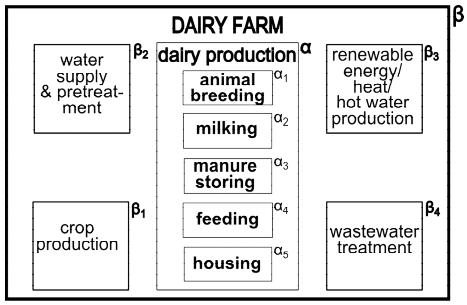

Figure 1 Simplified schematic overview of the identified system boundaries at the dairy farm level.

Conclusion A generic framework was established to build the ELCA tool. Special attention was paid to (i) transparency of the data sources used, (ii) flexibility and (iii) interrelations. Data gaps at the foreground system level were filled with special attention to the representativeness for the Flemish situation and exergy analysis was performed.

Acknowledgements The authors gratefully acknowledge funding from the Agency for Innovation by Science and Technology in Flanders (IWT).

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Growth, efficiency and carcass attributes of feedlot cattle supplemented with calcium nitrate or urea

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Introduction Dietary nitrate (NO_3) can serve as a source of non-protein nitrogen for the rumen microbiota, as well as reducing emission of enteric methane from ruminants (Zijderveld *et al*., 2010). NO_3-supplemented animals have a higher acetate proportion in the rumen volatile fatty acids and an increased microbial protein outflow compared with similar animals offered a diet with iso-nitrogenous urea (Li *et al*., 2012). There is also recent evidence that NO_3-supplemented cattle exhibit higher feed conversion efficiency (FCE, kg gain/kg feed) than do urea-supplemented cattle. The following study was undertaken to quantify the growth, intake, FCE and carcass attributes of feedlot cattle supplemented with NO_3 or urea.

Material and methods A 2 x 2 factorial study of the effects of level (N-Level) and source (N-Source) of nitrogen supplement in feedlot finished cattle was conducted. Composite breed steers (Brahman-based, n=384; initial LW = 414 kg) were introduced to the feedlot and adapted to one of 4 diets; i.e. low urea (LU: 0.50% urea in DM); low nitrate (LN: 0.95% NO3 as calcium nitrate in DM); high urea (1.00% urea in DM); or high nitrate (1.90% NO3 in DM). LU and LN were iso-nitrogenous (11.9%CP in DM) as were HU and HN (13.6% CP in DM) measured in the mixed rations. The diet was based on cracked barley (70.3%) and maize silage (8.5%) with an NIR derived ME content of 13.1MJ/kg DM. Urea and nitrate supplements were included as liquid supplements mixed into the dry ration prior to feeding. All cattle were introduced to their diets over 20d, with 96 head allocated using stratified randomisation to each of the 4 dietary treatments, using 18 bunk pens and 14 auto-feeder pens (Bindon 2001) with12 head/pen. Animals which did not adapt to the auto-feeder were transferred to a bunk-fed pen of that treatment. Cattle were offered a finisher diet for 102d, and then transported to a commercial abattoir for slaughter. A statistical model allowing for pen, feeder-type, age (assessed by dentition at slaughter) and genotype (visual assessment of composites) was used to test for effects of N-level, N-Source and their interaction.

Results Compared with the low N inclusion, high supplementary N was associated with significantly reduced feed intake, reduced ADG, reduced LW from day 38 onwards, and reduced carcass weight (Table 1). FCE was not affected by N-level or N-Source. NO3-supplemented cattle ate less and grew slower for a lighter final LW and carcass weight than did urea supplemented cattle. Main effects of N-level and N-source were consistent across the experiment, with no N-Source x N-Level interactions.

Table 1 Least square means for average daily gain (ADG), final liveweight (LW), average dry matter intake (DMI), feed conversion efficiency (FCE) and carcass weight of feedlot finisher cattle given supplementary nitrogen as urea or calcium nitrate (N-Source) at a low or high level (N-Level).

	N Source			N Level			N Source × N Level
Item	Urea	Nitrate	P	Low	High	P	P
ADG (kg/d)	1.71	1.59	0.003	1.71	1.59	0.002	0.64
Final LW (kg)	596.8	585.4	0.005	596.4	585.8	0.013	0.68
DMI (kg/d)	11.02	10.32	<0.001	10.95	10.38	<0.001	0.09
FCE (kg LWG/kg DMI	0 .154	0.154	0.95	0.154	0.153	0.74	0.17
Carcass wt (kg)	334.8	326.1	<0.001	334.9	326.0	<0.001	0.28

Conclusions Both the higher level of supplementary N and provision of N as nitrate in place of urea led to significant reductions in feed intake, growth and carcass weight of feedlot steers. Since neither source nor level of supplementary N affected FCE, growth responses to both variables can largely be ascribed to the lower ME or nutrient intake. From the absence of a N-Level x N-Source interaction for intake or growth traits, it can be concluded that the negative effects of high N and inclusion of NO_3 in place of urea in the diet are additive.

Acknowledgements This project is funded by Cargill Animal Nutrition and the Australian Government Department of Agriculture, Fisheries and Forestry Carbon Farming Futures - Action on the Ground programme.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Modelling the effect of diet composition on enteric methane emissions

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Introduction Individual feeds can vary considerably in their methanogenic potential and in their composition. Although several empirical and mechanistic prediction equations in the literature estimate enteric methane (CH_4) emissions based on elements of dry matter (DM) intake and diet nutrients, few studies have identified the effect on CH_4 losses of diets differing in nutrient composition. The aim of the present study was to quantify the effect of diet composition on enteric CH_4 emissions.

Material and methods The response in enteric CH_4 emissions in grams per kg DM intake was estimated from a detailed dataset published by the Rowett Institute (Wainman *et al.*, 1981). Data consisted of 288 calorimetric measurements for individual sheep (adult wethers). Sheep were fed at the maintenance level (875 g DM per day) and full energy balance measurements were taken for a period of 12 days. Diets consisted of silage or hay fed with concentrate feeds at ratios of 75:25, 50:50 and 25:75. Diets were designed to contain wide ranges of crude protein (130 to 215 g per kg DM), ether extract (20 to 75 g per kg DM; EE) and crude fibre (35 to 165 g per kg DM). Effects on CH_4 emissions were assessed for these components and also energy (gross, digestible and metabolisable; all MJ/kg DM), digestible DM and organic matter (OM), sugar, starch, lignin, cellulose, hemicellulose, neutral detergent fibre, acid detergent fibre, ash, non-fibre carbohydrate (NFC) and the reciprocal of DM content minus digestible OM content (UOM), expressed as g per kg DM. The most significant feed components for predicting CH_4 from univariate analysis were added first to a multivariate model with only significant variables being retained. Predictions of the multivariate model (Equation 1) were tested against two independent data sets of individual CH_4 measurements from lactating dairy cows at Ellinbank Research Farm in Australia (62 cows) and the Nottingham University Dairy Centre in the UK (84 cows). Cows were fed forage and concentrate diets to meet requirements for milk production. Associations between CH_4 emissions and diet components in the Rowett data were assessed using a linear mixed model, with diet added as a random effect. Pearson's correlation coefficient and square root of the mean square prediction error (RMSPE) were used to test associations between CH_4 emissions observed in cows and predicted from diet composition using Equation 1.

Results At the maintenance level of intake in sheep, the significant diet components that affected CH_4 emissions were OM, EE and either NFC or sugar content (Table 1). In the univariate analysis, UOM was also a significant variable, but was not retained in the multivariate model for sheep fed at maintenance. In cows fed at production level, however, CH_4 was reduced at approximately 1.978 (s.e. 0.5) times the UOM content of the diet. Therefore UOM was included in the multivariate model at production intake levels. The multivariate model (Equation 1), which included OM, EE, NFC and UOM, was found to adequately predict CH_4 production in cows, with a positive correlation and relatively low prediction error across diets fed to cows at both Ellinbank ($r = 0.232$, $P = 0.07$; RMSPE = 9 to 20%) and Nottingham ($r = 0.801$, $P < 0.001$; RMSPE = 14 to 16%).

Table 1 Significant effects on CH_4 emissions when sheep were fed at maintenance intake level

	Effect	s.e.	% of variation	Effect	s.e.	% of variation
Component	Including non-structural carbohydrate			Including sugar		
Organic matter	0.05642	0.007	27.2	0.05092	0.006	26.5
Ether extract	-0.1986	0.029	60.6	-0.1718	0.029	58.6
Non-structural carbohydrate or sugar	-0.009214	0.003	10.8	-0.04164	0.012	13.5

Equation (1) used to predict CH_4 emitted from cows at production intake level:

$$CH_4\,(g/kg\ DM) = 0.05642 \times OM - 0.1986 \times EE - 0.009214 \times NFC - 1.978 \times UOM$$

Conclusions This study identified the important feed components for estimating responses in CH_4 emissions per unit DM intake. The models developed for sheep at maintenance or cows at production level of intake can be used to predict effects of OM, EE and NFC content of diets on enteric CH_4 losses per unit DM intake.

Acknowledgements This work was funded by Defra, the Scottish Government, DARD, and the Welsh Government as part of the UK's Agricultural GHG Research Platform project (www.ghgplatform.org.uk).

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Implication of soy meal content on feed rations carbon footprint – a Swedish case study

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Introduction There is an on-going discussion in Sweden whether to use imported soymeal or domestic protein feed to reduce greenhouse gas (GHG) emissions from animal production. Due to a voluntary agreement in dairy industry, all feed must be GMO-free and as a consequence Sweden imports soy meal mainly from Brazil. Similar policies occur in other Nordic countries (e.g. Finland) and discussed elsewhere in Europe (e.g. Switzerland). Soybean production in Brazil is associated with transition of natural ecosystem into cropland, implicating GHG emissions from land use change (LUC). Present study aimed to analyse feed rations carbon footprint (CF, i.e. emitted GHGs in a life cycle perspective) in relation to soy meal content when LUC is included or not.

Materials and methods Feed rations were optimized with the feed evaluation system NorFor (Volden 2011) and same animal parameters (milk yield etc.) for five different dairy regions. All rations included ~60% roughages (normally grass/clover silage), ~15% grain and ~25% protein concentrates which varied in composition and shares of protein sources (mainly soy meal, rapeseed meal and distiller's dried grain) and were produced at regional feed industries. Soy meal content in the rations therefore varied from 6 to15% crude protein (CP) of total CP in the ration, which varied between regions from 15.4 to17.0% of total dry matter intake (DMI). Rations representing the national dairy regions were characterised as: *North*; grain and protein concentrate have longer transports (+200 km) than others regions, *West*; locally produced horse bean (*Vicia faba var. equina*) replaced some protein in purchased concentrate, *S West*; high yields of grass dominating silage, *S East*; maize silage (~25% of total roughages), *South*; pressed beet pulp (~16% of total roughages) and grass dominating silage. GHG emissions were estimated according the standardised method of life cycle assessment (LCA) and included all emissions in the chain of producing feed until fed in feed trough: from extraction of raw materials through fertilizer production, crop cultivation, feed processing and feeding. The GHG carbon dioxide, methane and nitrous oxide were converted to carbon dioxide equivalents (CO_2e) using the factors of 1, 25 and 298 respectively. The reference unit was 'feed required to produce 1 kg energy corrected milk (ECM) at farm from a cow producing 9900 kg ECM/year'. The LUC-factor 7.38 kg CO_2e/kg soy meal (Gerber *et al.*, 2010) was chosen to show potential impact of LUC, however lower factors have been published.

Results Emitted GHGs varied between feed diets with ~30% and increased significantly when LUC-emissions were included, also the ranking between rations changed from lowest to highest CF (table 1). Soy meal contributed with only 2.7 to 6.9% of emitted GHG when not including LUC, but increased up to almost 50% (23-49%) taking LUC-emissions into account (table1). Regression analysis of rations' CF with no LUC versus g DMI of soy meal/kg ECM showed no significant correlation (R^2=0.17, P=0.489), however correlation increased (R^2=0.65, P=0.096) when including emissions from LUC. Among protein feed ingredients, soy meal had the highest GHG emissions per kg product (578 g CO_2e/kg without LUC-emissions) compared to rapeseed meal (534 g/kg) and horse bean (311 g/kg) but per kg CP soy meal (1.35 g CO_2e/kg) fell between horse bean (1.23 g/kg) and rape seed meal (1.54 g/kg) and when relating the emissions to g AAT_{20} (amino acids absorbed in the small intestine), soy meal had the lowest (3.03 g CO_2e/kg) compared to horse bean (3.28 g/kg) and rapeseed meal (4.14 g/kg).

Table 1 Contribution of GHG emission from soy meal to feed rations carbon footprint for a cow producing 9900 kg ECM/year.

	Feed rations carbon footprint				Change after incl. LUC	
Feed ration	excl. LUC g CO_2e/kg ECM	soy part %	incl. LUC g CO_2e/kg ECM	soy part %	CF increase %	soy part % units
North	369	2.7	491	26.4	33	23.7
West	338	4.2	505	35.8	49	31.6
S West	428	2.3	545	23.3	27	21.0
S East	353	6.9	639	48.6	81	41.7
South	326	4.1	483	30.4	26	26.3

Conclusions LUC-emissions related to soy meal can have a significant impact on feed rations CF (thereby also affect milk CF) depending on the size of the estimated LUC emissions which mostly depend on the method to calculate LUC-factors and to some extent also on where the soy bean is produced. Production of other ingredients in cow rations, especially if used in large rations (e.g. roughages), have larger impact on rations' CF than soy meal with no or only minor LUC-emissions, indicating the importance of recognizing all feed ingredients when analysing the rations impact on GHG emissions. LUC emissions are important, but there is a need for methodology agreement and development since large variations are found in published LUC–factors (Flysjö *et al.*, 2012).

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Estimation of methane emissions from enteric fermentation and manure management of domestic livestock in Indonesia.

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Introduction Methane is the second highest contributor of green house gases after carbon dioxide. However in term of an *index of global warming potential*, methane is more than 20 times of carbon dioxide (Iqbal *et al*., 2008). Increasing methane concentration in the last 150 years indicate that ruminant animals in one of many contributors with the production of 80 million ton methane/year (Beauchemin *et al*., 2008). It is represent about 28% of total methane antropogenic emision (EPA, 2010). Ruminant population in Indonesia increased year by year following the increasing of meat and milk demand for consumption. However, there are no formal data released by the Indonesian Government on the methane contributed from the livestock. A simple calculation technique adopted from IPCC 1996 Book, was used to estimated the methane emission from enteric fermentation and manure management from livestock in Indonesia. This summary is presenting the estimation of methane emission from livestock in Indonesia based on animal population in year 2011.

Material and Method. Estimation of methane emision from ruminant animals was calculated by using the population number of ruminant animals during year 2011 that collected from statistic book of Indonesia livestock year 2012 (Direktorat Jenderal Peternakan dan Kesehatan Hewan, 2012). Calculation of the emission from livestock and manure was followed the procedure in Module 4 for Agriculture in *Revised IPCC 1996 Guidelines Greenhouse Gas Inventories : Workbook*. The calculation was only used the population of dairy cattle, non-dairy cattle, buffalo, sheep and goat, swine and poultry. Non-lactating dairy cattle and heifers were included in non-dairy cattle population. Emission factor for enteric fermentation used was the emission factors for developing country. Specific for dairy cattle, the calculation used emission factor for Asia region. In the calculation of emission from manure management, emission factors used was for developing country, which has annual average temperature range from 15^{o}C to 25^{o}C or temperate region.

Results. Non-dairy cattle contribute the higher enteric methane (73.34%) followed by sheep and goat (14.94%) and buffalo (7.46%). Emission from manure management was dominated by swine (36.29%) then poultry (23.54%) and non-dairy cattle (22.09%). Total methane emission from domestic livestock in year 2012 is 1.107.068 Gg per year, increased by 16.25 % during the last 5 year. An increasing in enteric methane emission was 0.12% and was 25.18% from manure management compared to year 2007.

Table 1 Estimation of enteric methane and methane from manure management of domestik livestock.

	Number of Animals	Emissions from		Total Annual Emissions
		enteric fermentation	Manure Management	From Domestic Livestock
	(x 1000 head)	(t/yr)	(t/yr)	(Gg)
Dairy Cattle	597	33.432	16.119	49.551
Non-Dairy Cattle	17.748	705.496	32.068	737.564
Buffalo	1.305	71.775	3.915	75.69
Sheep & goat	28.737	143.685	6.204	149.889
Swine	7.525	7.525	52.675	60.2
Poultry	1.485.819	0	34.174	34.174

Conclusions Total methane emision from domestic livestock in Indonesia was increased by 16.25 % from 927.2 Gg per year in 2007 to become 1.107.068 Gg per year in 2012. Cosideration should be undertake to maintain or reduce the increasing of methane emision from domestic livestock in Indonesia.

Acknowledgements
The authors gratefully acknowledge funding from AgriSearch New Zealand.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Effect of carbohydrate source of diet on enteric methane emissions from small ruminants

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Introduction Diet manipulation is one of the most cost-effective techniques aimed to reduce enteric methane (CH_4) emissions from ruminants (Beauchemin, 2008). The source of carbohydrates (CHO) in the diet is one of the main factors affecting CH_4 emission. In this regard, diets based on cereal grains, forages or fibrous by-products may lead to find large variations in methane emissions. Small ruminant production is widespread in Spain and other Mediterranean countries, being responsible for a significant portion of total CH_4 emissions. The main objective of this work was to assess the effect of modifying the carbohydrate source of the diet on CH_4 emissions from small ruminants using two species: goats and sheep.

Materials and Methods 18 Murciano-Granadina dairy goats in mid-lactation (average live weight 46.1±8.6 kg) and 20 dry Guirra ewes (average live weight 57.5±3.5) from the experimental flock of the Universitat Politècnica de València (Valencia, Spain) were fed with two different diets. Both diets were mixed including alfalfa hay and a concentrate. This concentrate presented different sources of CHO, fibre (NDF diet) and starch (STA diet). The composition and chemical characteristics of each diet is presented in Table 1.

Table 1 Composition and chemical characteristics (% of DM content) of the two diets used for both animal categories

	Goat		Ewe	
	NDF	STA	NDF	STA
Diet composition				
Alfalfa hay (kg/animal/day)	0.8	0.8	0.3	0.3
Concentrate (kg/animal/day)	1.2	1.2	0.7	0.7
Chemical composition of diets				
Dry matter (DM)	88.31	87.53	88.71	87.77
Crude protein (CP)	17.96	18.17	18.55	19.66
Crude fat (CF)	5.31	4.32	6.25	5.05
Neutral Detergent Fibre (NDF)	46.45	40.60	43.90	36.80
Starch (STA)	7.01	21.87	8.32	26.35
Gross Energy (MJ/kg DM)	19.17	18.97	19.16	18.92

CH_4 emissions were determined following two methods: indirect open-circuit calorimetry (head-box) and a flux chamber. For the first method, emissions from 10 animals were assessed individually. In the flux chamber, animals were introduced in groups (two animals per group for ewes and 3 animals per group in the case of goats) resulting in 6 groups of goats and 10 groups of ewes. All animals were fed with both diets following a cross design. Emissions were measured during 24-h periods with both methods. Daily average emissions related to animals' metabolic body weight (L/h/kg $BW^{0.75}$) were calculated for comparisons.

Results No significant differences were found for different diets on each type of animal in terms of CH_4 emissions when using the head-box. Average CH_4 emissions obtained with this method were 0.10±0.01 L/h/kg $BW^{0.75}$ for lactating goats and 0.05±0.01 L/h/kg $BW^{0.75}$ for dry ewes. When using the flux chamber, significant differences ($P<0.05$) were found for goats' CH_4 emissions, but not for ewes. Goats fed with the STA diet presented higher emissions (0.10±0.01 L/h/kg $BW^{0.75}$) than those fed with the NDF one (0.05±0.01 L/h/kg $BW^{0.75}$). In the case of ewes, an average emission factor of 0.04±0.02 L/h/kg $BW^{0.75}$ was found. According to these results, on overall terms, the effect of both diets on emissions seems to be slight. Emissions differences observed for goats when using the flux chamber were opposite to expected, since the diet with higher starch content led to lower emissions. This effect might be also attributable to the slightly difference in fat content of diets (Grainger and Beauchemin, 2011). On the other hand, the level of agreement between both methods was not as high as expected, mainly for ewes. The high inter-animal variability could explain these results.

Conclusions The effect of different carbohydrate sources (fibre vs. starch) of the diet on CH_4 emissions from small ruminants, at the levels tested in this work, remains unclear. Fat content of the diet might be also affecting emissions. Further work is needed in this field by forcing higher differences in diets and increasing the number of animals evaluated.

Acknowledgements This work was funded by the INIA project RTA-2011-00107-C02.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Quantification of enteric methane in milk and its relationship to milk composition, cow live-weight and dry matter intake in dairy cows

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Introduction Although measurement of methane (CH_4) using respiration chambers is accepted as the most accurate means of estimating CH_4 emissions from ruminant livestock, limitations are the relatively low throughput possible and the need to confine animals individually. With dairy cows, there are opportunities at each milking to measure breath CH_4 directly or to predict CH_4 from spectroscopic analysis of milk samples. Here we report measured CH_4 concentrations in milk and their relationships to related parameters.

Material and Methods Milk samples were obtained from cows housed all year round and offered a complete diet ad libitum containing between 450 and 500 g forage / kg dry matter (DM) at SRUC Dairy Research Centre. Cows were milked three times daily and samples were collected at the 12.00 h milking into sample containers containing preservative and then further processed within 1 h of milking. Approximately 20 ml milk were transferred into 50 ml crimp-top serum bottles through gas-tight septa. Exact quantities of milk were determined gravimetrically. After 24 h equilibration, head-space gas samples were withdrawn from serum bottles and transferred to evacuated 20 ml head space vials and CH_4 concentrations determined. Concentrations of milk CH_4 were calculated from milk and gas volumes assuming a density of 1.03 g/ml for milk and reported as µg/g milk. Samples were obtained on 4 occasions: in August from 40 cows and subsequently in November, December and January from a subset (n=18) of the 40 cows sampled in August which remained in milk throughout the sampling period. For each cow and sampling occasion, milk fat and protein concentrations from the same milk sample and mean weekly milk yields, live-weights and DM intakes were available. Within each sampling time, simple correlation analysis was carried out with available data and multiple stepwise regression to establish best-fit models. For all data relating to the 18 cows over 4 sampling periods, data were scaled by setting a minimum CH_4 value of 5 µg/g and then performing multiple regression analysis using Genstat. Fitted values were generated, and for each cow a mean deviation of actual from fitted values calculated.

Results Mean milk CH_4 from the August sampling was 28.4 µg/g (n=40; s.d. 7.19, minimum 13.8, maximum 48.9) and values were normally distributed. For the 4 sampling periods for 18 cows mean values (µg/g) were 30.40 (s.d. 6.76; August), 28.29 (s.d. 8.32; November), 49.22 (s.d. 9.27; December) and 49.00 (s.d. 9.20; January). Milk CH_4 concentrations were greater ($P<0.001$) in December and January compared to August and November. Examination of simple correlations showed positive correlations with cow DM intake and milk fat concentration and negative correlations with cow live-weight. On the scaled data, there was a significant regression ($P=0.004$) for milk CH_4 concentration (y) = 3.5 + 1.08 DM intake (kg/day) + 2.75 milk fat (g/100g) – 0.026 live-weight (kg). The mean differences for each cow between measured milk CH_4 concentrations and concentration predicted from the above equation for each of the 4 samples are shown in Figure 1; there were significant differences between cows ($P<0.05$).

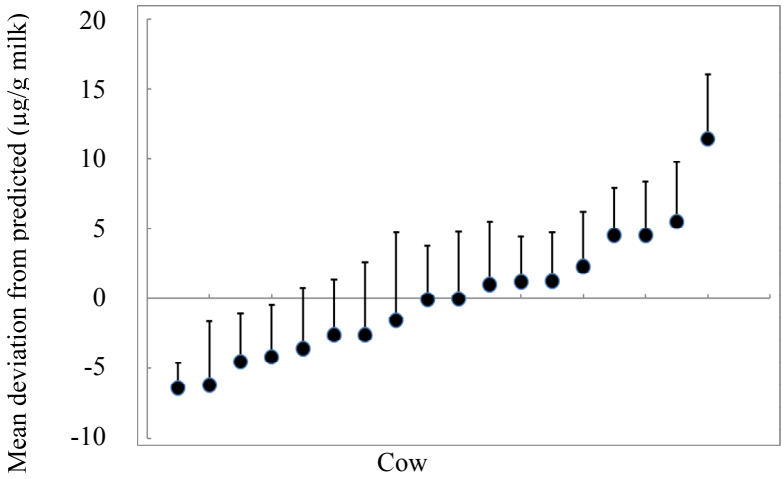

Figure 1 Plot of difference between actual and predicted milk methane concentrations (µg/g milk) for each of 18 cows sampled on 4 occasions

Conclusions Milk contains substantially higher CH_4 concentrations than would be expected on grounds of water solubility, likely due to the presence of fat globules in the milk. Thus milk CH_4 concentrations are positively related to milk fat concentration and to DM intake as an index of CH_4 production. Although preliminary, there are suggestions that there is cow to cow variation in milk CH_4 that could be used to rank cows if milk CH_4 can be correlated with total CH_4 production by the cow

Acknowledgments The authors are grateful for the funding from the Scottish Government and for excellent technical support at SRUC Dairy Research Centre and Analytical Lab.

Measured methane emissions from low and high intensity grazing lamb production systems

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Introduction A greenhouse gas emissions reduction scheme in the form of a carbon tax has been introduced in Australia. While the Agricultural sector is not included in the scheme, the Carbon Farming Initiative (CFI) allows for producers to earn carbon credits on-farm by reducing emissions or sequestering carbon. A sheep production experimental site was established at Armidale on the tablelands of NSW to investigate the relative CH_4 emissions per unit product when sheep are grazed on high fertile pasture at an increased stocking rate compared with lower fertile pasture with a corresponding lower stocking rate. The experiment provided information to producers on current estimates of on-farm emissions and opportunities for emissions mitigation. The site and experiment have previously been described in (McPhee *et al.,* 2010). Briefly it consisted of 6 flocks of sheep, with three flocks grazed at high density (16 ewes/ha plus lambs) on high fertility pastures, located on a creek flat with improved pasture base. The remaining three flocks were grazed at lower density (8 ewes/ha plus lambs) on lower fertility pasture located on hillsides with reduced fertiliser history, predominately native pasture and lower land-use class. Towards the completion of the experiment CH_4 emissions were measured from sheep grazing both the low and high fertility pastures. This paper reports these measured CH_4 emissions.

Methodology Sheep (Merino) were randomly assigned to each flock at the start of experiment. Each flock was rotated monthly between three paddocks, each 2 ha, with paddocks grazed exclusively by the one flock. Animal body weights and condition scores were monitored monthly throughout the experiment. Although the animal live-weight did not differ at the commencement of the experiment, at the conclusion of the experiment when the CH_4 emissions measurements were made, both ewes and weaned lambs of the lower intensity system were significantly lighter compared to those in the high intensity grazing system. Lambs (of Border Leicester sires) had been weaned approximately 3 months previous to the CH_4 emissions measurements by removal from their dams and subsequently returned to their source flocks.
Emissions were measured from only two of the three flocks for each stocking density, due to the time required to measure all 6 flocks. To provide an area suitable for the OP-FTIR measurements sheep were constrained to a subsection of one of the three paddocks. Further, to ensure preferential grazing did not influence the measured CH_4 emissions over the grazing period, the plots were divided into two, with each sub plot providing two days grazing, 4 days measurement for each flock, or 8 days for each stocking density. The paddocks were similar for the high and low fertility plots; however some minor differences were necessary due to different geometries of the paddocks. Paddocks were selected that had not been grazed in the previous month. Emissions were measured using open-path FTIR (OP-FTIR) spectrometers. The OP-FTIR simultaneously measures the mixing-ratio of a range of infrared active gases, including CH_4, CO_2, NH_3 and N_2O in the open atmosphere between the spectrometer and a reflector place up to 130 m from the spectrometer. Instruments were located both up- and down-wind of the animals with the measurement path perpendicular to the predominant wind direction. A tracer-gas (N_2O) was released from a canister mounted in a canvas pouch on the back of the sheep. Both tracer-gas and emitted CH_4 were measured simultaneously down-wind from the animals by the OP-FTIR. The up-wind instrument provided a measure of any CH_4 or N_2O entering the experimental plot from nearby sources. The emission rate was calculated from the know release rate of the tracer-gas and the ratio of mixing-ratios of the emitted CH_4 and tracer-gas (Jones *et al.*, 2011). The average tracer-gas release was calculated from the weight loss of N_2O and the release time. As the release rate is related to temperature, the instantaneous release rate was calculated based on temperature dependence measured in the laboratory. Trace-gas canisters were mounted only on the ewes and were exchanged every 24 hours, when the sheep were bought into a portable yard adjacent to the paddock. The animals were absent from the paddock for ~ 30 min each day to exchange the canisters. Pasture biomass was measured using two methods pre- and post-grazing. Total biomass (kg Dry matter/ha) was estimated using 10 x quadrats taken evenly along a transect passing through the centre of the plot, with pasture in each quadrat cut to ground level and dried for analysis. Green dry biomass was also measured spatially using a Crop Circle™ ACS 210 (Holland Scientific, Lincoln NE USA) sensor coupled to a Trimble ProXRS differential receiver and Ranger data-logger. The plots were surveyed on foot at 4m transects running ~ parallel with the external fence lines. The daily enteric emission likely to arise from sheep in each subplot was estimated with GrasGro (Moore *et al.*, 1997) and Grazfeed (Freer *et al.,*1997).

Results and Discussion The distribution in emissions showed higher emissions in morning, lower emissions in the early afternoon, increasing again slightly in the evening and lowest overnight. The average (±s.d.) daily CH_4 emissions when grazed on low fertility pastures were 19.5±0.45 g CH_4 $hd^{-1}d^{-1}$ (flock 1 and 2: 19.6±0.58 and 21.1±0.65 g CH_4 $hd^{-1}d^{-1}$ respectively). The daily average emissions when grazed on high fertility pastures was17.9±0.41 g CH_4 $hd^{-1}d^{-1}$(15.5±0.99 g CH_4 $hd^{-1}d^{-1}$ and 19.5±0.98 g CH_4 $hd^{-1}d^{-1}$ flock 3 and 4 respectively).

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

On-farm methane measurements in exhaled air of individual Dutch cows obtained during milking using Fourier transformed infrared methods

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Introduction. Methane (CH_4) is a greenhouse gas (GHG) that contributes to climate change. The livestock sector, particularly ruminants (cattle and sheep), is estimated to contribute up to 18% of total global anthropogenic GHG emissions. Preliminary international data suggest that genetic selection to reduce emissions is possible. However, successful breeding programs require large datasets of individual measurements which cannot be generated through respiration chambers. Several researchers have recently investigated use of infrared gas analysers to measure enteric methane, sometimes in combination with CO_2, released during milking or feeding (Garnsworthy *et al.*, 2012; Lassen *et al.*, 2012). In the Netherlands, we have recently started measuring enteric methane, in combination with CO_2, during milking. The aim of this preliminary study is to show whether realistic values for and individual differences in enteric methane emission could be measured during milking, so that a large scale data collection project can be set up for genetic evaluation of CH_4 production in dairy cattle.

Material and methods. The data collection started in September 2012 in an experimental dairy cow house at the Dairy Campus of Wageningen UR Livestock Research. The dairy cow house was equipped with a natural ventilation system, which included large openings in side walls and an open ridge, and slatted floors in the cow area with manure storage below. The equipment was installed in an automatic milking system (AMS) that was visited daily by 60 cows.
Cow breath air was sampled and analysed directly for CH_4 and CO_2 using a portable Fourier Transformed Infrared (FTIR) gas analyser with 2 air inlets (GASMET 4030; Gasmet Technologies Oy, Helsinki, Finland). One air inlet was placed in front of the cow's head in the AMS, and the other air inlet was placed at 2.5 meters height near the AMS to measure background concentrations. Measurements were alternatively taken from one of the 2 air inlets; each measurement involved continuous sampling for 1 minute. Cows visited the AMS between 1 and 4 times per day, and between 3 to 8 measurements of 1 minute were recorded per visit. The total number of unique cows that visited the AMS in October and November 2012 was 73, and 45 of them had at least 1,000 measurements of 1 minute each.

Results. Mean CH_4 concentration was 386 ppm, with a range of individual cow means between 196 and 724 ppm, and a standard deviation of 99 ppm. Mean CO_2 concentration was 6,380 ppm, with a range between 4,280 and 14,500 ppm, and a standard deviation of 1,900 ppm. Daily means of the background concentrations were 35 ppm for CH_4 and 591 ppm for CO_2. The high concentrations measured in the AMS indicate that the sampled air included a high portion of the breath of the milked cow. Individual variation is shown in the mean enteric CH_4 concentration (Figure 1; Coefficient of variation: 3.9), and also in the mean CH_4 concentration per ratio between CH_4 and CO_2 (Figure 2; Correlation coefficient: 0.14). Analysing the ratio might be attractive, because with the ratio method only the gas concentrations in the animal's breath have to be measured, whereas for emitted CH_4 volume both air volumes and CH_4 concentration have to be recorded.

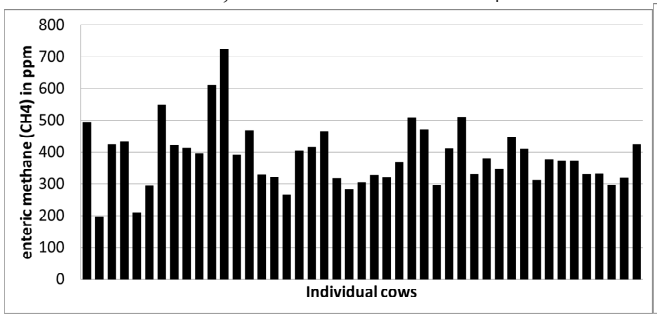

Figure 1 Mean methane concentrations of 45 individual cows.

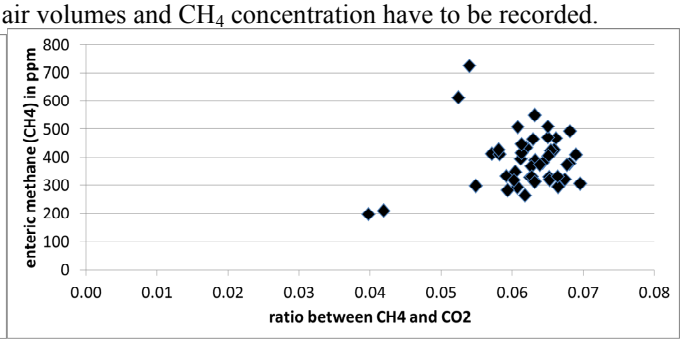

Figure 2 Mean methane concentration per mean ratio CH_4:CO_2.

Further research will focus on determining the repeatability of measurements within a day and between days, both within and between cows, and on defining other traits than the average CH_4 concentration to indicate differences between individuals.

Conclusion. This preliminary study has shown that using a portable FTIR measuring unit in an AMS to measure individual cow CH_4 emissions gave realistic values and ranges. The FTIR instrument combined with AMS may therefore be useful in the future to generate large scale data for genetic evaluation of CH_4 production in dairy cattle.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Greenhouse gas emissions from fermentation of sugarcane silages treated with natamycin or Lactobacillus buchneri

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Introduction Sugarcane is one of the most used crops for silage production in Brazil. The fermentation leads to high dry matter losses (DML) caused by yeasts development, which convert sucrose to ethanol and produce gases. Additives are being tested to avoid yeasts growth and reduce DML. This trial evaluated greenhouse gas emissions and DML from sugarcane silages added natamycin or *Lactobacillus buchneri* as additive.

Material and methods Whole sugarcane plant was harvested at 340 g kg^{-1} of dry matter (DM) and the following treatments were applied at ensilage: C - no additives (control); N10 - natamycin (10 g t^{-1}); LB - *Lactobacillus buchneri* ($5x10^4$ CFU g^{-1}) or NLB – natamycin (5 g t^{-1}) + LB ($2.5x10^4$ CFU g^{-1}) (wet basis), with four replicates for each treatment. Sugarcane silages were produced in 20-L buckets equipped with a mobile apparatus to recover and measure the volume of gas produced during fermentation. The bulk density of the silos was 600 kg m^{-3}, and they were stored for 52 days in a temperature controlled room (22±3 ^{o}C). Using a pipeline and a graduated collection chamber made of low density polyethylene, the total amount of gas of each silo was registered daily. In the first 25 days it was taken every 3 hours. Samples of gas were collected for determination of the concentration of CO_2, N_2O and CH_4 at days 5, 15 and 50. Polypropylene syringes (20 mL) equipped with a valve were used for sampling. Analysis were performed using Gas Chromatography (GC Shimadzu 14-A). Data were analyzed as a completely randomized design and the means of treatments were compared by Tukey's test.

Results No treatment effect was detected on total gas or GHG production, probably due to the high variation among replicates (Table 1). The additives natamycin and LB were effective in reducing DML, but the combination of both additives did not show improvements compared to control silages. The same trend were verified for the gas variables, with a great decrease in GHG production due to natamycin, however not significant (P=0.14).
The average gas production was 2736.8 L per ton of ensiled forage, and carbon dioxide was the main gas produced (24862 ppmv – 99.9%), with low levels of methane (2.1 ppmv) and nitrous oxide (1.1 ppbv). Probably, the high concentration of CO_2 is related to the metabolism of yeasts capable to convert glucose to CO_2 (McDonald *et al*., 1991). The average GHG production found in this trial is higher than those observed in our previous trials with sugarcane or maize silages (36.4 and 14.5 g CO_2-eq t^{-1} forage, respectively) (Schmidt *et al*., 2011; Schmidt *et al*., 2012). It shows that the pattern of fermentation can vary due to microbial population (Ávila *et al*., 2010), which leads to variation in gas production. However, the figures verified for silages are quite low when compared to the emissions from feedlot cattle (Phetteplace *et al.*, 2008).
A poor correlation (0.28) between volume of gas measured and DML was detected. This sounds alarming once the traditional measuring of gases could be inaccurate considering as gases losses the volatile compounds lost during oven drying.

Table 1 Greenhouse gas emissions during ensiling of corn silage

	Treatment					
Variable	C	N10	LB	NLB	Mean	s.e.m
Gas production, L per ton of forage	4748.4	1036.1	2131.3	3031.3	2736.8	644.4
GHG, g CO_2-eq t^{-1} forage	203.0	43.1	84.7	121.9	113.2	27.0
Total DM losses, g kg^{-1}	20.5^{a}	10.4^{b}	7.8^{b}	14.5^{ab}	13.3	1.44

Conclusions Silages are used in most of farms around the world, and contribute to carbon balance of the systems of animal production. Carbon dioxide is the main gas produced during the fermentation of silages. The use of additives reduces DML and can be an important strategy for GHG mitigation from the production of this feed.

Acknowledgements The authors gratefully acknowledge professor Cimélio Bayer from Federal University of Rio Grande do Sul.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Enteric methane emission by male beef cattle finished in feedlots with sources of roughage and crude glycerin

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Introduction Finishing cattle in feedlot has been shown to be an efficient strategy for methane mitigation in ruminants (Beauchemin *et al*., 2011). Due to the advent of biodiesel production, by-products are being generated and should be used in an environmentally appropriate and economically viable way. Crude glycerin, a biodiesel by-product, has been successfully used in ruminant diets replacing corn on concentrate (Mach *et al*., 2009).
Therefore, the objective of this study was to evaluate the enteric methane (CH_4) emission of young bulls fed crude glycerin at feedlot.

Material and methods Thirty Nellore young bulls with 416Kg ± 24.68 initial BW, were randomly assigned to 3 treatments, with 10 replicates during 90 days. The sources of roughage used were: corn silage (T1), sugar cane (T2) and sugar cane bagass (T3). Ten percent of crude glycerin was used to replace corn in the concentrate. Animals were slaughtered at 550.5kg BW. The CH_4 emissions were measured using the sulphur hexafluoride (SF_6) gas tracer technique (Johnson and Johnson, 1995). Measurements were taken every 24 h during five consecutive days from each animal by the end of experimental period with 15 prior adaptation days. Methane flux was calculated in relation to the SF_6 tracer gas flux from a permeation capsule lodged in the rumen minus the basal CH_4 concentrations in the air (Westberg *et al*., 1998). Following equation was used: $Q_{CH4} = Q_{SF6} \times ([CH_4]_y - [CH_4]_b) / [SF_6]$, where Q_{CH4}=CH_4 emission rate by animal; Q_{SF6}=known SF_6 emission rate from capsule in rumen; $[CH_4]y$=CH_4 concentration in collection apparatus; $[CH_4]b$=basal CH_4 concentration; and $[SF_6]$=SF_6 concentration in collection apparatus. Data were analyzed using the GLM procedure of SAS. Dry matter intake was evaluated and expressed per day as a percentage of BW.

Results

Table 1 Mean values of methane emission (ECH_4) and dry matter intake (DMI) of bulls fed sources of roughage

Variable	Treatment			s.e.m	P
	T1	T2	T3		
ECH_4 (g $CH_4.h^{-1}$)	6.79	6.39	6.28	0.22	0.52
ECH_4 (g $CH_4.d^{-1}$)	139	153	151	5.34	0.52
ECH_4 (g/kg DM)	15.0	14.7	14.9	0.58	0.97
ECH_4 (g/kg CG)	176	164	205	13.4	0.45
DMI (kg/day)	9.23	10.6	10.3	0.26	0.07
DMI (%BW)	1.93	2.16	2.12	0.05	0.10

Means followed by different letters in the same line differ by Tukey test at 5% probability.

Dry matter intake expressed in kg/day or %BW was not different (P > 0.05) among diets. Methane emissions were expressed in grams of CH_4 emitted per hour (g $CH_4.h\text{-}^{1}$), grams of CH_4 emitted per day (g $CH_4.d^{-1}$), grams of CH_4 emitted per kg of dry matter intake (g/ kg/day of DMI and) and grams of CH_4 emitted per kg of carcass gain (g/ kg of CG). The methane emission was also not different (P >0.05) among the treatments in any unit expressed.

Conclusions Animals fed with crude glycerin in diets with different sources of roughage showed similar enteric methane production.

Acknowledgements The authors gratefully acknowledge funding from FAPESP.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Carbon footprint of an intensive dairy farm in the Basque Country and the development of a calculator for farm analysis

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Introduction Carbon footprint (CF) indicator has become increasingly popular in the recent years, due to quantify GHGs emitted during the production of a food product, in order to show how that product affects the environment and contributed to the global changed. Animal farms has been one of the main sector to work on this line, in order to associated GHGs to the production of milk for eco-labelling of products. This work shows the results of a project which developed a carbon footprint calculator with the purpose of used it for dairy farms. This communication presents the carbon footprint of a single intensive dairy farm in the Basque Country, as a study case.

Material and methods The analysis was performed in a farm (524 dairy cows, 253 ha of grassland and 98.4 ha of cropland) for the year 2011. A computer tool was development as a carbon footprint calculator. The tool utilises data collected from specific farm and aims to be PAS 2050:2008 compliant and IPCC guidelines. Most of the technical data were provided by personal interviews in the farm and surveys sources from the Minister of Agriculture of Spain.
The system boundary of the study covered the production of a litre of raw milk from cradle-to-farm-gate. The functional unit is one litre of energy corrected milk. Capital, medicines and cleaning products were not included. The emission factors used have been gathered from recognised standard database (Table 1).

Table 1 Source of emissions and models used in the study.

Sources of emissions	Gas	Emission Factor used
Enteric fermentation	CH_4	Merino *et al*.,2011(Tier 3)
Manure storage	CH_4,N_2O	Merino *et al*.,2011(Tier 3)
Nitrogen inputs - direct (fertilisers, crop residue) and indirect (volatilisation, leaching)	N_2O	IPCC 2006
Energy use of farm (fuel and electricity)	CO_2	GES´TIM & IBERDROLA
INPUTS (feed, fertilisers, pesticides..)	CO_2eq	GES´TIM

Results CF was 1.32 kg CO_2 eq/litre of Energy Corrected Milk at farm gate without allocation. Many dairy farms produce more than one economically significant output (in this case live dairy stock). Emissions from the farm are allocated between different outputs using economic allocation (Cederberg and Stadig, 2004) bases on income from the various stated outputs. The percentage of the CF allocated to milk is 93.3% if we use economic allocation and 87.65% if we use mass allocation. Table 2 shows the CF of a litre of milk, using economic allocation and the contribution from different sources of emissions.

Table 2 Carbon footprint of an intense dairy farm at farm gate and the relative contribution from different sources of emission.

	Results
CF (kg CO2eq/litre ECM)	1.23
Enteric fermentation (%)	31.72
Manure management (%)	12.56
N_20 management soils (%)	14.94
Feed (%)	38.46
Other inputs (%)	2.32

Conclusions Our study provides data on the CF of an intensive dairy farm using a tool development with this purpose. These results show that methodological issues were very important because of their influence on the results. The development of a computer application allows calculating the carbon footprint with specific data for a farm with more precision than using models. The tool allows for farm-level, management-relevant GHG assessments to be made easily and a decision-support tool.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

An evaluation of mechanistic and empirical models of methane emissions in grazing dairy cattle

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Introduction Measuring and estimating greenhouse gas emissions of livestock systems is a major issue due to global concerns on climate change and the potential consequences on agriculture regulations and international trade. Methane (CH_4) is the largest contributor of livestock emissions and several models have been developed to predict these emissions. Models for predicting CH_4 emissions can be classified into empirical (relate intake to emissions based on equations derived from experiments at the animal level) or mechanistic (simulate emissions using equations based on the underlying biochemical processes at the rumen level, allowing for predictions outside the range where they were developed). However, these models were evaluated using data from North American and European systems, with diets based on high concentrates. Only recently experimental measurements of CH_4 emissions from grazing cattle have been conducted, using the SF6 tracer technique. The objective of this paper is to evaluate performance of mechanistic and empirical models of CH_4 emissions in dairy grazing systems.

Material and methods A database of 7 published experiments comprising 32 treatments of enteric CH_4 emissions measured with SF6 tracer technique from grazing dairy cattle in Brazil, Uruguay, and New Zealand was assembled. Experiments included heifers, dry and lactating cows, from Holstein and crossbred breeds, fed a broad range of forages including seeded or native pastures, with single species or mixtures of C4 and/or C3 grasses and legumes. Dry matter intake (DMI) ranged from 4.8 to 19.3 kg, neutral detergent fibre (NDF) from 35 to 80%, protein from 4 to 26%, $CH_4$from 5 to 24 MJ/d, and CH_4 yield (Y_m) from 3.7 to 7.8% of gross energy intake. We predicted CH_4 emissions for each treatment based on other three previously published models: the empirical IPCC tier 2 model with fixed Y_m (6.5%), an empirical equation with Y_m as a quadratic function of diet digestibility (Cambra-López *et al.* 2008), and mechanistic model based on rumen fermentation parameters (COWPOLL, Kebreab *et al.* 2008, based on Dijkstra *et al.* 1992). The COWPOLL model is a series of dynamic, deterministic, and nonlinear differential equations of nutrient digestion in rumen, describing utilization of feed particles by microbes, their growth and formation of VFA and H_2 as rumen fermentation end products. The inputs required are: DMI, composition of diet, digestion rate of fractions, protein, non-protein nitrogen, NDF, ADF, lignin, ether extract, starch, and soluble carbohydrates. Therefore, we assembled a nutritional database of feedstuffs compiling information from previous literature reviews and a new literature search from grazing systems in South America and Oceania. We evaluated each model for accuracy (how closely predicted values are to the true values) and precision (how closely individual predicted values are within each other). The square root of the mean square prediction error (the average of the squared deviations of observed minus predicted individual values, MSPE), expressed as a percentage of observed mean, was used as a measure of accuracy (RMSPE). The MSPE was further decomposed into error due to overall bias of prediction (ECT), error due to deviation of the prediction line from unity (ER), and error due to random variation (ED), to assess precision. The concordance correlation coefficient (CCC) was also calculated (Lin, 1989) as the product of the correlation coefficient (r, deviation of observations from the best fit line, a measure of precision) and the bias correlation factor (Cb) that measures how far the regression line deviates from the line of unity (accuracy).

Results The COWPOLL model has the lowest MSPE compared to the other two models (Table 1). The composition of the MSPE suggested a better precision for the COWPOLL model, with smallest error due to overall bias (ECT), and largest error due to random variation (ED). The COWPOLL and IPCC models performed better than the empirical model with variable Y_m, suggesting that the equation for Y_m from Cambra-López *et al.* (2008) does not account for the variation on emissions on grazing systems properly.

Table 1 Parameters of accuracy and precision for three models to estimate CH_4 emissions in dairy cattle evaluated in grazing systems.

Model	MSPE	RMSPE (%)	ECT (%)	ER (%)	ED (%)	r	Cb	CCC
Empirical IPCC tier 2 fixed Y_m	10.8	21	20	10	71	0.83	0.996	0.82
Empirical variable Y_m (Cambra-López *et al*., 2008)	19.1	29	6	28	66	0.62	0.971	0.60
Mechanistic COWPOLL (Kebreab *et al.*, 2008)	9.5	22	2	17	82	0.82	0.988	0.81

Conclusions The COWPOLL model has good potential for predictions of CH_4 in grazing dairy cattle, but measuring diet inputs is required and should be focus of further research. Better empirical models are needed to relate digestibility to Y_m in grazing systems.

Acknowledgements Funding from USDA-Global Research Alliance-Borlaug Fellowship and CSIC-UDELAR, Uruguay.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Batfarm software: A support tool to mitigate gaseous losses at dairy housing in the Atlantic region

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Introduction Documents, in which environmentally friendly best available techniques (BAT) are reported, have been published on intensive rearing of poultry and pigs (BREF, 2003). However, there is not official BREF document for dairy cattle husbandry yet despite the increase in scientific and political interest. BATFARM EU-Atlantic Interreg Project (http://www.batfarm.eu/) aims to set-up a novel model for dairy farms in which the application of different abatement strategies on ammonia (NH_3) and greenhouse gas losses (N_2O, CH_4) will be simulated. This scientific contribution shows preliminary case study results.

Material and methods The model has been set-up by intermediary approaches between traditional empirical and mechanistic models. Data from national inventories (Spain, Portugal, France, UK, Ireland) have preferably been considered throughout the model. In the case of lack of national data, literature values from scientifically tested commercial/experimental farms have been included. Mitigation strategies have been classified into 3 groups: Nutritional (phase feeding and electronic collars for milking cows), Manure Management (manure removal system and removal frequency) and Technological (deep pit aeration, curved slat mats and slatted floors with valves). Enteric CH_4 emissions from confined and grazing cattle have been collected from national inventories or literature review. Ammonia emissions have been assessed including the type of facility (freestall, tie-stall), type of floor (solid, slatted floor) and slurry temperature. IPCC default values have been used to estimate N_2O and CH_4 emissions from manure management (IPPC, 2006). Each country has been divided into different climatic areas for monthly mean temperature and rainfall.
The case study farm is located in the North of Spain under the Oceanic Transition climatic regime (mean temperature, 11ºC; annual rainfall, 912 mm). Herd size has been divided into 50 milking cows, 10 dry cows, 15 calves < 12 months and 15 heifers 12-26 months. Mean milk yield is 7000 l/cow/year. Cattle are grazing outdoors from May to September. When cattle are confined at stall diet is mainly based on grass silage, maize silage and grass hay forages. Nutrient intake (DM, N, P, K, Cu, Zn) is calculated through default values joined to diets based on grass silage/hay, maize silage or grazing. Default values are used for body weights, nutrient retention either in milk or meat and manure production (slurry and solid manure). Two abatement strategies have been considered through this case study: the use of electronic collars for milking cows and the frequent removal of slurry and solid manure.

Results Figure 1 shows the monthly trend on NH_3-N, N_2O-N and CH_4 (enteric and manure) emissions at dairy housing. Ammonia emission is reduced by 10% on annual basis (63 kg NH_3-N/stall/year). Such reduction is remarkable when cattle are confined as the use of electronic collars reduces the concentrate intake. Similarly, N_2O-N emission was reduced by 10.8% (3.8 kg N_2O-N/stall/year) and CH_4 losses by 12.2% (1386 kg CH_4/stall/year).

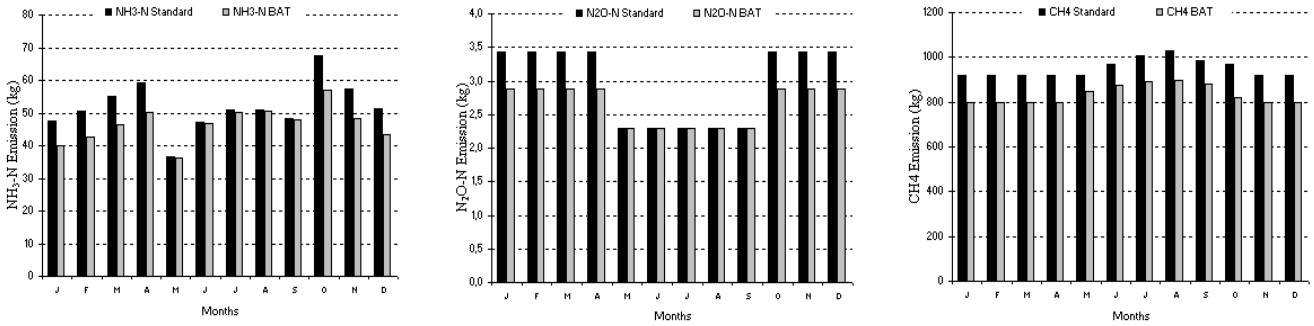

Figure 1 Gaseous emission pattern (NH_3, N_2O and CH_4) after the application of two abatement techniques at dairy housing

Conclusions This preliminary work demonstrates the potential of different abatement strategies implemented at dairy housing to reduce gaseous losses. Further tests will be necessary to validate the results obtained.

Acknowledgements This work has been funded by BATFARM Interreg-Atlantic Area Project (2009-1/071) entitled "Evaluation of best available techniques to decrease air and water pollution in animal farms". This project is supported by the European Union ERDF – Atlantic Area Programme – Investing in our common future.

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An Assessment of the Methane Production Potential of Irish Bovine Manures

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Introduction Biogas is produced by the anaerobic decomposition of animal manures and is predominantly composed of methane (CH_4) and carbon dioxide (CO_2). Emissions of CH_4 are environmentally undesirable due to its effect on global warming. When compared to CO_2, CH_4 levels are relatively low within the atmosphere; however, CH_4 is 21 times higher than CO_2 with regards to global warming potential on the molecular level. Methane production potential (B_o) of manure is a measurement of the degradation of the organic compounds in the manure i.e. carbohydrates, lipids and proteins. "Methane productivity" is a term used to explain the yield of CH_4 per unit variable where the variable is volatile solids (VS) loaded, VS destroyed, volume or animal production. Methane productivity in terms of VS loaded as residence time approaches infinity is referred to as ultimate methane yield (B_o) (Møller *et al*., 2004). Non-dairy cattle are assigned a B_o default value of 0.17 m^3 CH_4/kg volatile solids (VS) for Western Europe, with dairy cattle assigned a default value of 0.24 m^3 CH_4/kg VS. Ireland uses the B_o value of 0.24 m^3 CH_4/kg VS (IPCC, 2009) for all cattle populations, due to some experts advising that there is no difference expected in manure potential due to dairy and non-dairy cattle being fed on grass-based diets (McGettigan *et al*., 2010). The objective of this experiment was to assess the accuracy of the IPCC default B_o values for Irish bovines manures.

Material and methods Manure for this experiment was collected from a previous experiment, where four bovine categories were fed three different diets in succession. The animals were two dry dairy cows, two dry suckler cows, two 13 month steers and two 8 month heifers. Diet one was 100% grass silage, diet two was 50% grass silage and 50% concentrates and diet three was 100% concentrates. Two hundred litres of slurry was collected and frozen until ready for incubation trials. Total ammoniacal nitrogen (TAN), dry matter (DM), volatile solids (VS), fixed solids (FS), total nitrogen, (TN), and carbon (C) were measured within Johnstown Castle Laboratories.This method was adapted from ISO (1999). Each bottle had a volume of 100ml manure substrate (1:9 dilutions, manure: deionised water), from each manure and animal type. The bottles were then sealed, crimped and the headspace flushed with nitrogen gas. Excess pressure was vented until the pressure in the bottles returned to atmospheric pressure (Vedrenne *et al*., 2008). The test bottles were then placed into an incubator at 35°C, for 1 hour. After the initial hour, any excess pressure was removed, and the bottles returned to the incubator. Pressure within the bottles was measured daily and when the pressure exceeded 200mBar on a Digitron^{TM} manometer, gas samples were taken via syringe. Excess pressure was removed and the bottles returned to incubator. The gas samples were analysed for CO_2 and CH_4 and were measured by gas chromatography on a Varian 3800 with a Flame Ionisation Detector (FID).

Results Methane production potential (B_o) was observed to correlate with the % volatile solids in slurry. Methane production potential (B_o) ranged from 80 to 110 L CH_4 kg^{-1} VS with most of the methane emitted during 30 days. This represents a lower methane production potential compared to the IPCC default of 240 L CH_4 kg^{-1} VS.

Conclusions The results of this experiment demonstrated that the IPCC default values for B_o were high compared to measured values for Irish cattle.

Acknowledgements The authors gratefully acknowledge funding from the Atlantic Area Programme, the ERDF and the Teagasc Walsh Fellowship Fund.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Modelling greenhouse gas emissions from the Hanwoo beef production system

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Introduction The beef production is recognized as one of the major greenhouse gas (GHG) emission sector in the livestock industry (Beauchemin *et al*., 2010). There is, thus, strong rationale of estimating GHG emissions for producing beef and establishing a better mitigation strategy. Life cycle inventory (LCI) can be used to quantify GHG emissions of a product from cradle to grave. The objectives of this study were 1) to modelling and quantifying GHG emissions from Hanwoo beef production which is the dominant cattle breed in Korea and 2) to compare GHG emissions from producing Hanwoo beef among different size of farms.

Materials and methods We conducted LCI which consists of two major steps; (1) goal and scope definition including purpose of study, system boundary and functional unit, (2) data collection and inventory establish. The system boundary of this study was animal production section (i.e., methane emissions from enteric fermentation and GHG emissions from energy usage in the farm). The GHG emissions from feed production and manure management were not included. Functional units were 1kg of live, carcass and dressed weights, and 1 kg of protein. Data collection was performed two different ways. Information of a typical feeding condition (e.g., size of farms, number of animals in each physiological stage, feed intake, and types of feed) for Hanwoo operation was obtained from National Statistics of Korea. Because of variations in housing and facilities among farms, the standard animal housing plan for cattle raisers recommended by Korean Ministry for Food, Agriculture, Forestry and Fisheries was used to estimate farm energy usage. The GHG inventory for Hanwoo beef production was developed for each pre-defined size of farms based on National Statistics. It was assumed that calves are produced in the cow-calf operation, and then they are transferred and raised in the growing-finishing operation, which is a typical system in Korea. GHG emissions from producing a calf and from raising a calf to the market weight were separately estimated in each operation, and total GHG emissions to produce beef were calculated. Methane emissions from enteric fermentation of animal were estimated using the IPCC tier 2 methodology, which multiplies GE intake of an animal with CH_4 conversion factor (Y_m; 6.5% for growing, 3.0% for finishing cattle). GE intake was estimated on the basis of feed consumption reported in the National Statistics and estimated GE content of feeds. To estimate energy consumption in the farm, we first calculated a required area for animal based on the standard animal housing plan, and determined electricity usage by equipment (i.e., ventilation system and heated water bowl) per area needed for managing an animal (i.e., growing = 3.5 m^2, finishing=7.0 m^2). CO_2 equivalent was used (1kg of CH_4 = 21kg of CO_2), and usage of 1 kWh of electricity was assumed to produce 0.47 kg of CO_2 equivalent.

Results The GHG emissions from producing a calf in the cow-calf operation were estimated to be 2,838 kg CO_2 eq/head on average. In the Hanwoo growing-finishing operation, the GHG emissions from enteric fermentation and farm energy usage of Hanwoo growing cattle were averagely estimated to be 942.1 (96.1%) and 38.2 (3.9%) kg CO_2 eq/head·year, respectively, and those from enteric fermentation and farm energy usage of Hanwoo finishing cattle were averagely estimated to be 579.8 (88.4%) and 76.4 (11.6%) kg CO_2 eq/head·year, respectively. Total GHG emissions from Hanwoo beef production were estimated to be 4,184 kg CO_2 eq/year. The GHG emissions per functional unit of Hanwoo beef was the highest in the smallest farm size (< 20 heads) while the lowest GHG emissions were observed in the largest farm size (≥ 100 heads).

Table 1 Greenhouse gas emissions from Hanwoo beef production

Farm size	< 20	20 - 49	50 - 99	≥ 100	Average
Total GHG emissions (kg CO_2 eq)	4,231.4	4,145.1	4,166.1	4,174.7	4,184.2
GHG emissions per unit					
kg CO_2 eq/kg live weight	6.17	5.94	6.05	5.73	5.98
kg CO_2 eq/kg carcass weight	10.28	9.90	10.08	9.56	9.96
kg CO_2 eq/kg dressed weight	16.72	16.09	16.39	15.54	16.20
kg CO_2 eq/kg protein	49.71	47.86	48.73	46.22	48.17

Conclusions A large proportion of GHG was produced from enteric fermentation of cattle in the Hanwoo beef production. The contribution of farm energy usage to GHG emissions from Hanwoo beef production was largest during the finishing period. Among different size of farms, the GHG emissions for producing beef was lowest in the largest sized farm (≥ 100 heads), which suggest that a large size of beef operation is more sustainable in terms of GHG emissions.

Acknowledgements This work was financially supported by Agricultural Science and Technology Development Program (No. PJ007338), Rural Development Administration (RDA), Republic of Korea

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

An analysis of extended lactation as a potential mitigation and carbon offset option for pasture-based dairy enterprises in south eastern Australia

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Introduction In 2011 the Australian government introduced a voluntary carbon offset scheme called the Carbon Farming Initiative (CFI), which enables farmers to earn carbon credits by lowering greenhouse gas emissions (GHGE) or sequestering carbon. For farmers to participate in the CFI, the options must be economically attractive to farmers. This research examines the GHGE mitigation potential and economic viability of changing from an annual calving/lactation system to an extended lactation system on pasture-based farm enterprises in south eastern Australia.

Material and methods Eight modelled dairy enterprises were examined, with half using 12 month calving cycle (10 month lactation) and the rest extending to an 18 month calving cycle (16 month lactation). The enterprises were based on Browne *et al.* (2011) and were located in southwest Victoria, Australia. Half the enterprises consumed 20% of feed intake from concentrated supplements and the other half consumed 35% of feed intake as concentrates. An average enterprise and a 'top' enterprise were described for each system, based on operating profit/ha as recorded in Farm Monitor benchmarking studies (Tocker *et al.* 2009), with the main differences between the two groups being stocking rates and pasture species. The 12 month calving enterprises were modelled using the biophysical farm model DairyMod (Johnson *et al.* 2008) with two thirds of the herd calving in September and the rest in April. The extended lactation enterprises were calculated using a spreadsheet model. Dairy cows in both types of calving systems lived for eight years, were in calving cycles for six years and had the same replacement rates when compared over the six years. The results were averaged across these six years. The milk fat plus protein (MFP) for extended lactation was reduced by 2 kg/cow/year, consistent with previous research (Auldist *et al.* 2007). GHGE were calculated using the Australian National Inventory methodology. Operating profit was calculated as income minus variable and fixed costs, including an owner-operator allowance.

Results Extended lactation enterprises produced higher operating profits than 12 month calving cycles (Table 1). GHGE also rose with extended lactation along with MFP production. Extended lactation could not be used as a carbon offset method for the enterprises studied due to an increase in GHGE. The emissions intensity (t CO_2e/t MFP) improved with extended lactation.

Table 1 The difference in GHGE and operating profit as a result of changing to extended lactation from annual calving.

Enterprise type	Stocking rate (cows /ha)	Calving interval (months)	GHGE (t CO_2e/ year)	Operating profit ($/ha)	GHGE (t CO_2e /ha)	Change in GHGE (t CO_2e/ha)	Change in operating profit ($/ha)	Emissions Intensity * (t CO_2e /t MFP)
Dairy 20 Avg	1.7	12	2,389	881	8.0			10.2
Dairy 20 Avg	1.7	18	2,428	1,067	8.1	+0.13	186	9.3
Dairy 20 Top	2.0	12	2,534	1,188	9.4			10.2
Dairy 20 Top	2.0	18	2,575	1,440	9.5	+0.15	253	9.3
Dairy 35 Avg	1.7	12	2,471	894	8.2			9.4
Dairy 35 Avg	1.7	18	2,508	1,055	8.4	+0.12	161	8.6
Dairy 35 Top	2.0	12	2,599	1,215	9.6			9.6
Dairy 35 Top	2.0	18	2,637	1,438	9.8	+0.14	223	8.7

* Emissions intensity used mass allocation to allocate GHGE to MFP. CO_2e, carbon dioxide equivalents; Dairy 20, feeds 20% of total intake from concentrates; Dairy 35, feeds 35% of total intake from concentrates; GHGE, GHG emissions.

Conclusions Extended lactation was more profitable than 12 month calving due to the greater number of days that cows were lactating. Extending lactation to 18 months caused some calving events to occur when pasture supply was limited, but the extra MFP that was produced compensated for these additional feed costs. However, cows required a higher feed intake to produce this additional MFP, which resulted in increased GHGE. As a result extended lactation could not be used as a carbon offset method under the CFI.

Acknowledgements This research was funded by Dairy Australia, Meat and Livestock Australia and the Australian Government Department of Agriculture, Fisheries and Forestry under its Australia's Farming Future Climate Change Research Program.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press

Methane emissions of finishing Nellore young bulls on pasture in the rainy season supplemented with crude glycerin

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Introduction Ruminal methane emission measurements are of great importance because it represents a loss of productive energy for the animal and also contribute for the greenhouse gas emissions. Thus, the development of feeding strategies to mitigate methane emissions may bring not only environmental benefits for the planet but also nutritional benefits for the animal. Therefore, the aim of this research was to evaluate the methane emission of Nellore young bulls maintained in a tropical pasture during the rainy season and supplemented with crude glycerin as a substitute to corn grain.

Material and methods In the rainy season (December 2011 to May 2012), thirty Nellore young bulls with initial shrunk body weight of 428.8 ± 20.08 kg were randomly assigned to five treatments, with six replicates. Animals were distributed in 10 paddocks, of 1.8 hectares each (2 paddocks per treatment), of *Brachiaria brizantha* cv. Xaraés. Treatments were constituted by 5 levels of crude glycerin (CG) inclusion in the supplement: (0, 70, 140, 210 and 280 g/kg dry matter (DM) of CG based on dry matter) as a substitute to corn grain. The CG used was derived from soybean biodiesel production (80% glycerol). Animals were supplemented daily in a proportion of 300 g/100kg of body weight. The supplement was constituted of corn grain, soybean meal, urea, gluten meal and mineralized salt, containing 300 g/kg DM of crude protein based on dry matter. The experiment was conducted in 141 days and by the end of the experimental period all animals were slaughtered. Intake and nutrient digestibility were estimated using three markers: isolated, purified and enriched lignin (LIPE®), titanium dioxide (TiO_2) and indigestible neutral detergent fiber (iNDF), used for estimation of fecal excretion, supplement intake and forage intake, respectively. Ruminal CH_4 was evaluated using a sulphur hexafluoride (SF_6) gas tracer every 24 h during five consecutive days in one experimental period after 15 prior adaptation days according to the method described by Johnson and Johnson (1995). CH_4 flux produced by animals was calculated in relation to the SF_6 tracer gas flux from a permeation capsule lodged in the rumen minus the basal CH_4 concentration in the air (Westberg *et al*. 1998). Following equation was used: $Q_{CH4} = Q_{SF6} \times ([CH_4]_y - [CH_4]_b) / [SF_6]$, where Q_{CH4} = CH_4 emission tax by animal; Q_{SF6} = know SF_6 emission tax from capsule in rumen; $[CH_4]_y$ = CH_4 concentrations in collection apparatus; $[CH_4]_b$ = basal CH_4 concentration; and $[SF_6]$ = SF_6 concentration in collection apparatus. Methane outputs (g/d) proportional to gross energy intake (GEI, Mcal/d), digestible dry matter intake DDMI (kg/d), digestible organic matter intake DOMI (kg/d), digestible neutral detergent fiber intake DNDFI (kg/d) and carcass gain (kg/d) were calculated by dividing the daily methane output of each animal by their daily GE intake DDMI, DOMI and DNDFI (during methane sampling) and carcass gain (throughout the entire experimental period), respectively. Data was analysed using the GLM procedure of SAS program and the effects of treatments (linear and quadratic) were considered significant at $p<0.05$.

Results There was no statistical significance ($P>0.05$) among the treatments, for enteric methane emissions, as well as for the different forms to express the emission (Table 1).

Table 1 Methane emissions of finishing Nellore young bulls on pasture in the rainy season fed crude glycerin

	Glycerin level in supplement (g/kg DM)						
	0	70	140	210	280	S.E.M.	P
CH_4, g/d	125.5	143.6	137.5	158.3	116.7	16.42	0.413
CH4, g/kg DDMI	18.7	21.6	22.9	26.9	19.0	2.98	0.283
CH4, g/kg DOMI	18.6	21.8	23.6	27.5	19.4	2.97	0.223
CH4, g/kg DNDFI	43.4	48.8	53.1	63.1	45.4	6.12	0.171
CH4, g/kg GEI	2.5	2.9	2.9	3.5	2.5	0.38	0.372
CH4, % of GE intake	3.4	3.9	3.9	4.6	3.3	0.50	0.374
CH4, g/kg of carcass gain	216.4	299.4	283.0	286.8	181.8	41.82	0.220

Conclusions The inclusion of crude glycerin until the level of 280 g/kg DM in the supplement did not affect the production of methane enteric of finishing Nellore young bulls on tropical pasture in the rainy season

Acknowledgements The authors gratefully acknowledge from FAPESP and Cargill®.

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https://doi.org/10.1017/S2040470013000101 Published online by Cambridge University Press