Comparison of enteric methane production predicted from the CH_4/CO_2 ratio and measured in respiration chambers

A L F Hellwing[1], P Lund[1], J Madsen[2], M R Weisbjerg[1]
[1]Aarhus University, Tjele, Denmark, [2]University of Copenhagen, Fredriksberg, Denmark
Email:annelouise.hellwing@agrsci.dk

Introduction There is focus on strategies that can reduce the enteric CH_4 production from livestock worldwide. However, the effect of different strategies has to be verified, and measuring methods applicable under different production situations are needed. Indirect calorimetry gives the best measurement of enteric CH_4 production, but the method is time consuming, expensive and not suited to document effects in some production systems as e.g. cattle on pasture. Madsen *et al.* (2010) suggested use of CO_2 as internal marker, where the ratio between CH_4/CO_2 measured in the breath of the animal combined with an estimate of the total CO_2 production from the animal could be used to predict the enteric CH_4 emission. However, the method needs to be verified against other methods as e.g. indirect calorimetry. The aim of this study was to analyse the difference between the predicted enteric CH_4 production and the production measured in dairy cows in respiration chambers.

Material and methods A data set with 157 observations on lactating cows from 8 different experiments covering 30 different diets was used in the analysis. All data were obtained from respiration chambers at Aarhus University (Hellwing *et al.*, 2012). The CH_4/CO_2 ratio was calculated from the mean daily excretion of CH_4 and CO_2. Predicted CH_4 (P_CH4 (L/day)) was calculated as heat producing units (HPU) per day x CO_2/HPU x CH_4/CO_2. HPU (watt) was calculated as 5.6 x kg body mass$^{0.75}$ + 22 x kg energy corrected milk produced per day + 1.6 x 10^{-5} x number of days in pregnancy^3 (CIGR, 1984). Madsen *et al.* (2010) used a CO_2 production of 4.32 m^3/day/HPU. The difference (DIF_CH4) between P_CH4 and measured CH_4 (M_CH4 (L/day)) was calculated as P_CH4 – M_CH4. Data on body weight (W), parity (Lac) (first lactation and others), days in milk (DIM), dry matter intake (DMI), yield of energy corrected milk (ECM, 3.14MJ/kg), daily CO_2 production, the respiratory coefficient (RQ=CO_2/O_2), % of gross energy lost as methane (CH4%) and concentration in dry matter of crude protein (CP), fat (FAT) and carbohydrate (CHO) were compiled. The relationship between P_CH4 and M_CH4 was analysed by PROC REG in SAS. The systematic variation in DIF_CH4 was studied using PROC STEPWISE in SAS. Variables entered the model if P<0.15 and were omitted again if the P>0.15.

Results and discussion A simple regression of P_CH4 on M_CH4 gave the equation: P_CH4 = 147± 24 (s.e.) + 0.58±0.04 x M_CH4 (RMSE=49.8, R^2=0.55). Despite a reasonable high R^2, DIF_CH4 varied between -337 L/day and 48 L/day with a mean of -96 ± 5 L/day and seemed to be systematic. The stepwise analysis of DIF_CH4 included the variables for M_CH4, ECM, CO_2, W, DIM and CH4%. Mean, standard deviation, estimate, P, partial and model R^2 are given in Table 1 for all variables explaining more than one per cent of the total variation in DIF_CH4.

Table 1. Mean, standard error (s.e.), parameter estimate, P, partial and model R^2 for variables used to predict difference (DIF_CH4) between predicted and observed methane production.

Variable	Mean±s.e.	Estimate	P	Partial R-square	Model R-Square
Intercept		35	<0.001		
M_CH4 [L/d]	577±7.6	-0.24	<0.001	0.40	0.40
ECM [kg/d]	29±0.6	7.9	<0.001	0.39	0.79
CO_2 [L/d]	6856±74	-0.06	<0.001	0.13	0.91
Weight [kg]	593±5.5	0.3	<0.001	0.06	0.98

The M_CH4 and ECM production both explained around 40% of the variation. Inclusion of daily CO_2 production explained additional 13%, whereas the weight only explained 6% of the variation. Both ECM production and weight were used to predict the HPU, and the analysis indicates that the estimate for the HPU equation suggested by CIGR (1984) and/or the CO_2/HPU factor were not optimal when applied to the current dataset. Comparison of the heat production (HP) estimated with the Brouwer equation from the measured values of O_2 consumption and CO_2 and M_CH4 production with the predicted HPU showed a regression line where HPU=0.58±0.09 (s.e.) + 0.49±0.48 x HP (watt). This indicates that the HPU equation used by Madsen *et al.* (2010) underestimates the calculated HP in the dataset.

Conclusions Although explaining 55% of the variation in measured methane production, the method suggested by Madsen *et al.* (2010) underestimated the CH_4 production from the current dataset. Analysis of the difference between predicted and measured methane showed that daily CH_4, ECM and CO_2 production and body weight explained nearly all the difference. This indicates that there is a potential for improvement in the prediction of CO_2 production from cows' live weight and milk production and thereby improvement of the CH_4/CO_2 ratio method for estimation of methane production.

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Methane emissions of the South African sheep industry

C J L Du Toit[1], W A van Niekerk[2], H H Meissner[1]
[1]Tshwane University of Technology, Pretoria, South Africa, [2]University of Pretoria, Pretoria, South Africa
Email:dutoitcjl@tut.ac.za

Introduction Methane (CH_4) has been identified as a significant contributor to climate change. Enteric methane emissions in South Africa contribute approximately 90% of the total livestock related CO_2 emissions and approximately 65% of the total agricultural CO_2 emissions (Meissner *et al*., 2012). Sheep contribute approximately 15% of the South African livestock sectors methane emissions. The aim of the study was to generate an updated methane emissions inventory for the South African mutton and wool industry using recent census data and coefficients calculated from country specific data based on international guidelines.

Materials and Methods The methane emissions were estimated by animal category, commercial/ communal production systems, age groups and across seasons on a provincial basis to reduce errors associated with averaging input data across areas with large physical and management differences. The inventory is based on a combination of IPCC (2006) as well as the Australian national inventory report methodology (2010). Sheep population data across the 9 provinces was sourced from relevant governmental departments and industry associations. Primary data used in the algorithms (live weight, live weight gain, pasture digestibilities and lambing rates) are based on reviews of published data in literature and expert assessments.

Results and discussion The South African sheep population is estimated at 24.5 million, mainly concentrated in the drier provinces. The sheep industry represents 14.4% of the total livestock methane emissions with an annual emissions total of 166.66Gg. The Eastern Cape, Northern Cape and Free State are the largest contributors to methane emissions originating from sheep production practices as indicated in Table 1.

Table 1 Methane emissions (Gg) from the South African sheep industry

Province	Population	CH_4 emissions	Contribution (%)
Eastern Cape	7317172	49.08	29.5
Northern Cape	6119979	42.05	25
Free State	4875780	33.3	20
Western Cape	2715934	18.3	11
Mpumalanga	1751284	11.66	7
Kwa-zulu Natal	771123	5.14	3
North West	698111	4.75	2.9
Limpopo	258041	1.77	1
Gauteng	104018	0.7	0.6

Table 2 reports on the four main breed types of the South African sheep industry namely, Merino, other wool, non-wool and Karakul sheep each contributing respectively 48.9%, 21.6%, 29.4% and 0.1% to the national sheep emissions total. The average daily CH_4 emission across all breed types was calculated as 18.55g/d. This corresponds well with other researchers and the IPCC (2006) where values of between 13.7 and 21.92 g/d were reported.

Table 2 Methane emissions (Gg) per sheep breed type in South Africa

Breed type	Enteric CH_4	Manure CH_4	Total	% of total
Merino	81.38	0.05	81.43	48.9
Other wool	36.06	0.01	36.07	21.6
Non wool	48.94	0.01	48.95	29.4
Karakul	0.20	0.0001	0.20	0.1

Conclusion The sheep industry is the second largest methane emitter in the livestock sector contributing 14.4% to the livestock sector's methane emissions total with Merino sheep contributing approximately 50% of the industries methane emissions.

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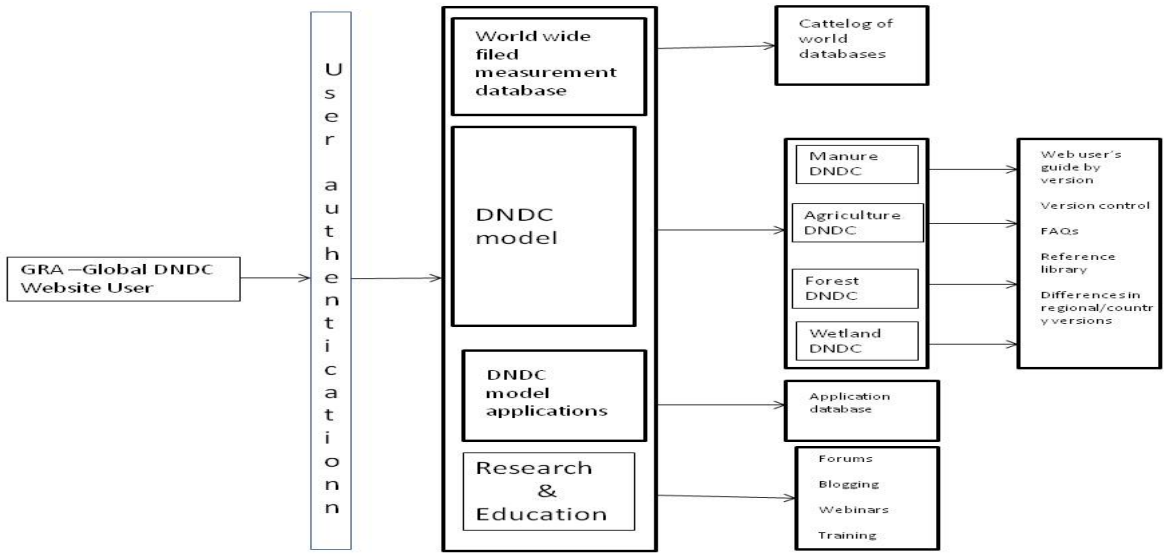
Global Research Alliance Modelling Platform (GRAMP) – developing a unified modelling approach in the context of climate change.

J Yeluripati, B Rees, C Li, D Chadwick, E Tilston, K Topp, L Cardenas, S Gilhespy, S Anthony, W Salas, P Smith
[1]University of Aberdeen, Aberdeen, UK, [2]SRUC, Edinburgh, UK, [3]University of New Hampshire, Durham, USA, [4]Bangor University, Bangor, UK, [5]SRUC, Edinburgh, UK, [6]SRUC, SRUC, UK, [7]Rothamsted Research, Harpenden, UK, [8]Rothamsted Research, Harpenden, UK, [9]ADAS, Wolverhampton, UK, [10]Applied Geosolutions, Durham, UK, [11]University of Aberdeen, Aberdeen, UK *Email:j.yeluripati@abdn.ac.uk*

Introduction C & N process-based models are important tools in prediction and reporting of GHG emissions and soil C stocks. There is a need for continuous evaluation, development and adaptation of these models to improve national inventories and assessment of mitigation across the world. The new UK funded Global Research Alliance Modelling Platform (GRAMP) Project will develop a web-based modelling platform to link researchers with appropriate datasets, models and training material. It will be piloted using the DNDC model. The intention is to form a worldwide collaborative network (virtual lab) via an interactive website with access to: models and best practice guidelines; appropriate datasets for testing, calibrating and evaluating models; on-line tutorials and links to modelling and data provider research groups, and their associated publications. The major objectives of this project are initially to 1) review and catalogue the differences between the number of DNDC versions developing a model 'family tree' , with, in the first instance, particularly emphasis being given to cataloguing the differences between DNDC and UK-DNDC, 2) undertake a stocktake of suitable experimental databases across the world, and identify benchmark sites for DNDC model runs under different land uses (climates, soils, ecosystems), and 3) develop an online infrastructure for DNDC model version control and virtual labs for model development with model-user interaction.

Material and methods These objectives are met by a programme that 1) integrates and establishes a vibrant collaborative network by creating an interactive web-site 2) links up a network of sites for testing, calibrating and evaluating models 3) develop the DNDC platform with a content management system and database system: searchable by region, crop, version, etc, Map system - Web GIS linked with reference library and a database system, Training materials (case studies, Demos, Videos) , RSS type feed will be setup to provide updates to users of new versions of models, papers and workshops etc.

Results: a schematic representation of the GRA-Global DNDC network website is shown below :



This contains a web user authentication system: multi-tiered users with different access and editing authority. A system to manage overall global DNDC user's database will be created. The DNDC platform will contain a Content management system, a Database system: searchable by region, crop, version, etc, Map system - Web GIS linked with reference library and database system, Training materials (case studies, Demos, Videos) , RSS type feed will be setup to provide updates to users on new version, papers and workshops etc. We will explore with the funder the use of social media type tools (e.g. linkedin) for dialog and communication.

Conclusions GRAMP will advance the fundamental understanding of C-N interactions at different scales and improve the interaction between modelers, experimentalists and users to synthesize the problem areas of model application and validation. This project will develop global communication between research teams and model users specifically interested in the measurement and modelling of GHG mitigation.

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Shallow injection of livestock slurry to grassland: effects on the balance of nitrous oxide and ammonia emissions

J R Williams[1], L Sagoo[1], R E Thorman[1], B J Chambers[2], T H Misselbrook[3], D R Chadwick[4]
[1]ADAS, Boxworth, UK, [2]ADAS, Gleadthorpe, UK, [3]Rothamsted Research, North Wyke, UK, [4]Bangor University, Bangor, UK *Email:john.williams@adas.co.uk*

Introduction Reducing nitrogen (N) losses (ammonia and nitrous oxide to air and nitrate to water) following slurry application is important to minimise the environmental impact of livestock farming systems**.** On grassland, shallow injection is effective at reducing ammonia losses compared with surface broadcasting and will increase the soil mineral N pool available for crop uptake, along with reducing the need for manufactured fertiliser applications to meet crop N demand. However, ammonia loss reductions may increase the risk of nitrous oxide emissions; an example of "pollution swapping".

Materials and methods Five experiments were carried out at four sites across England, with contrasting soil textures and climatic conditions (Table 1). Slurry was applied by shallow injection and surface broadcasting at 35m^3/ha, using the ADAS small-plot applicator. There were 3 replicates of each application treatment arranged in a randomised block design. Ammonia emissions were measured for 7 days after application using windtunnels. Nitrous oxide emissions were measured for three months after application from both treatments and an untreated control using the static chamber technique (five chambers per plot).

Table 1 Site details

Site	Topsoil texture	Average annual rainfall (mm)	Slurry type	Application timing
Burleydam (Cheshire)	Clay loam	750	Cattle	November 2003
North Wyke (Devon)	Clay loam	1100	Cattle	June 2004
North Wyke (Devon)	Clay loam	1100	Pig	June 2005
Boxworth (Cambridgeshire)	Clay	570	Cattle	July 2005
Gleadthorpe (Nottinghamshire)	Loamy sand	600	Cattle	March 2006

Results At Boxworth (July 2005) and North Wyke (June 2004), nitrous oxide (N_2O) emissions from the shallow injected cattle slurry were greater (P<0.05) than from surface broadcasting (Table 2). The higher emissions from the shallow injection treatment reflected measured ammonia loss reductions of 27% at Boxworth and 50% at North Wyke, which would have increased the pool of soil mineral N available for loss as N_2O compared with the surface broadcast treatment. However, at Burleydam and Gleadthorpe, there was no effect (P>0.05) of shallow injection on nitrous oxide losses, despite measured ammonia loss reductions at both sites compared with surface broadcasting. At North Wyke in 2005, shallow injection did not reduce ammonia losses, or influence nitrous oxide emissions compared with surface broadcasting, as wet soil conditions caused smearing of the injection slot and the slurry was not able to infiltrate into the soil.

Table 2 Ammonia and nitrous oxide emissions following shallow injection and surface broadcast slurry applications

Site	Application timing	Ammonia emissions (% total N applied)		% change by shallow injection (P>0.05)	Nitrous oxide emission (% total N applied)		Sig diff
		Shallow injection	Surface broadcast		Shallow injection	Surface broadcast	
Burleydam	November 2003	5	12	- 58	2.44	2.41	No
North Wyke	June 2004	3	6	- 50	0.54	0.18	Yes (P<0.05)
North Wyke	June 2005	59	43	+37	1.08	2.30	No
Boxworth	July 2005	11	15	- 27	1.72	0.71	Yes (P<0.05)
Gleadthorpe	March 2006	4	11	- 64	0.37	1.22	No

Conclusions Shallow injection reduced measured ammonia emissions at four of the five sites, although it was not possible to prove the differences statistically. At two of the four sites nitrous oxide emissions were increased (P<0.05) reflecting the increased pool of soil mineral nitrogen available for loss as N_2O. Although, soil and environmental conditions also influenced the balance of ammonia and nitrous oxide emissions measured.

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Effect of progressive inoculation of fauna-free sheep with holotrich protozoa and total-fauna on the hindgut populations of bacteria and methanogenic archaea

A Belanche, G de la Fuente, J M Moorby, C J Newbold
IBERS, Aberystwyth University, Aberystwyth, UK *Email:aib@aber.ac.uk*

Introduction Methane represents about half of the greenhouse gases emitted from livestock agriculture and enteric methane production from ruminants is the most important source, responsible for about 80% of methane emissions (Gill *et al*., 2010). Rumen protozoa are associated with methane emissions as a result of two factors: i) protozoal fibrolytic activity and ii) protozoal interactions with bacteria and methanogenic archaea. These factors determine rumen methanogenesis as a result of the inter-species H_2 transfer in the rumen. However, little is known about the indirect effects of rumen protozoa on bacterial and methanogen populations living in the large intestine, in which about 13% of emitted methane is generated (Murray *et al*., 1976). This study investigated the effect of changing the rumen protozoal population on the composition and numbers of faecal microbial populations.

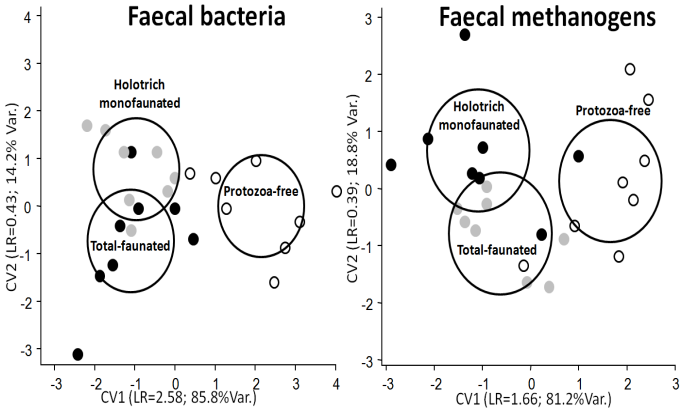
Material and methods Eight mature Texel-crossbreed sheep (approximately 4 years old) were used in a straight-through design experiment with three consecutive periods, with 3 months adaptation between each period. All sheep were naturally fauna-free by separation from their mothers within 24 h of birth and had been kept isolated from adult animals. The sheep were fed twice per day with a diet designed to meet maintenance requirements (66% ryegrass hay and 33% ground barley). For the 1st period animals remained fauna-free (FF); for the 2nd period they were inoculated orally with a mixed holotrich population (HOL), and for the 3rd period animals were faunated with rumen fluid obtained from control animals with a natural protozoal population (FAU). In each period methane emissions were determined from each animal using respiration chambers for 4 days. Faeces were collected daily, pooled and freeze-dried before DNA extraction. Concentrations of DNA from bacterial and methanogen origins in faeces were measured using quantitative PCR while the structure of microbial populations was studied targeting the 16S rDNA and subsequent TRFLP analysis (Belanche *et al*., 2012). Bacterial DNA was amplified using primers *27F* and *1389R* (labelled), while methanogen DNA was amplified using primers Met86F (labelled) and Met1340R. Amplification products were digested with 4 restrictions enzymes (*Hha I, Hae III, Msp I* and *RSA I* for bacteria and *Hha I, Hae III, Msp I* and *Taq I* for methanogens). Terminal restriction fragments (TRF) were analyzed by PERMANOVA after square root normalization, treatments effects were depicted using Canonical Variate Analysis (containing the information of the 8 first principal components) and diversity indices were calculated.

Results Inoculation of protozoa-free sheep with holotrich protozoa promoted an increase in methane emissions (Table 1). This was accompanied with an increase in bacterial DNA concentration in faeces, bacterial biodiversity and a shift in the bacterial community living in the hindgut (Figure 1, $P < 0.001$). No further changes in terms of methane emissions, diversity and structure of the faecal bacterial community were observed when holotrich monofaunated sheep were inoculated with total fauna. The structure of the methanogenic population living in the hindgut was modified in a similar manner to that observed for bacteria ($P = 0.015$), however neither methanogen concentration nor their diversity indices were affected by the experimental treatments, suggesting a fairly constant number of methanogens in the hindgut.

Table 1 Microbial populations in faeces.

	FF	HOL	FAU	s.e.d.	P
CH_4 (L/kg DMI)	19.2^b	31.2^a	31.8^a	1.38	<0.001
Bacteria					
DNA (mg/g DM)	1.43^b	1.65^b	2.07^a	0.17	0.006
Richness (TRF)	51.6^b	56.9^a	54.9^a	1.29	0.003
Shannon index	3.25^b	3.43^a	3.41^a	0.055	0.011
Shannon evenness	0.82^b	0.85^a	0.85^a	0.010	0.032
Methanogens					
10^6 copies/mg DM	0.57	0.71	0.86	0.248	0.52
Richness (TRF)	23.8	24.4	25.7	4.03	0.60
Shannon index	2.23	2.25	2.26	0.090	0.95
Shannon evenness	0.70	0.71	0.70	0.017	0.90

Figure 1 CVA depicting the microbial populations in faeces.



Conclusions Our results show that rumen colonization by protozoa led to substantial increases in the concentration and biodiversity of the hindgut bacteria, as well as changes in the bacterial and methanogenic communities. This effect may rely on the compositional change in digesta which is used as a substrate for hindgut microorganisms and could explain, to some extent, the differences observed in terms of methane emissions. More research is needed to study the methanogenic potential of the hindgut microbial communities.

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Changes in the rumen and faecal microbial communities of sheep inoculated with different protozoal populations

A Belanche, G de la Fuente, C J Newbold
IBERS, Aberystwyth University, Aberystwyth, UK *Email:aib@aber.ac.uk*

Introduction Enteric methane production from ruminants represents the most important greenhouse gas derived from the livestock agriculture. These emissions rely on two main factors: i) the type of diet consumed by the ruminant, which determines the fermentation pattern and, ii) the type of gut microorganisms and their interactions, which influences inter-species H_2 transfer. Traditionally most efforts have been focused on the study of rumen microbial populations which are responsible for most of the enteric methane emissions. However little is know about the microorganisms living in the large intestine, from which about 13% of emitted methane is generated (Murray *et al*., 1976). Rumen sampling is not feasible in farm conditions and an attractive alternatively might be to study faecal samples to provide insight about gut microbiology. This study aims to investigate whether ruminal and faecal microbial populations are similarly affected by experimental treatments. Here a progressive inoculation of protozoa-free sheep with different rumen protozoal species was chosen to induce changes in the microbial communities with no diet modifications.

Material and methods Eight mature Texel-crossbreed sheep were used in a straight-through design experiment with 3 consecutive periods. All sheep were naturally fauna-free by separation from their mothers within 24 h of birth and had been kept isolated from other ruminants. The sheep were fed twice per day with a maintenance diet (67% ryegrass hay and 33% ground barley). For period 1 sheep remained fauna-free (FF); for period 2 sheep were inoculated orally with a mixed holotrich population (HOL), and for period 3 animals were faunated with rumen fluid obtained from control animals with a natural protozoal population (FAU). Methane emissions were determined using respiration chambers for 4d. Rumen fluid was extracted by oesophageal tube and faeces were collected daily for DNA studies. After DNA extraction, the absolute concentrations of DNA from bacterial, protozoal, fungal and methanogens originating in the rumen and faeces were measured using quantitative PCR, and the relative abundance of 13 different bacterial species was measured in respect to total bacteria.

Results Successive inoculation of protozoa-free sheep with protozoa increased the methane emissions (19.2^b vs. 31.2^a vs. 31.8^a L/kg DMI in FF, HOL and FAU, P<0.001). This increase in methane emissions was accompanied by an increase in methanogens concentration in the rumen, but not in faeces. Moreover, FAU animals showed the highest concentrations of bacteria, protozoa and fungi in ruminal and faecal samples suggesting a symbiotic relation between fibrolytic microorganisms. Similarly, the presence of a mixed protozoal population increased the relative abundance of certain amylolytic bacteria in the rumen (i.e. *Prevotella spp., S. ruminantium, E. ruminantium* and *A. lipolytica*), and decreased others (i.e. *B. fibrisolvens, S. bovis* and *M. elsdenii*). These changes in the abundance of ruminal bacteria were accompanied by small and inconsistent differences when faecal samples were analyzed.

Table 1 Microbial concentrations in rumen and faecal samples (P1=protozoa free, P2=Holotrich monofaunated, P3=total fauna).

	RUMEN					FAECES				
	FF	HOL	FAU	s.e.d.	P	FF	HOL	FAU	s.e.d.	P
Bacteria (mg DNA/g DM)	1.35^b	1.09^b	2.69^a	0.279	***	1.43^b	1.65^b	2.07^a	0.169	**
Protozoa (mg DNA/g DM)	ND	0.07^b	0.65^a	0.074	***	ND	4×10^{-15b}	2×10^{-13a}	2.122	***
Fungi (µg DNA/g DM)	0.05^b	0.09^b	0.54^a	0.068	***	0.004^b	0.021^a	0.036^a	0.008	**
Methanogens (10^6 copies/g DM)	6.87^b	17.0^a	22.1^a	3.360	**	0.57	0.71	0.86	0.248	n.s.
Relative abundance [1] ($10^3\times2^{-\Delta Ct}$)										
Ruminococcus albus	2.27	3.24	2.03	0.344	n.s.	0.05	0.70	0.28	0.551	n.s.
Ruminococcus flavefaciens	12.5	17.2	5.91	0.292	n.s.	0.74	0.18	0.11	0.246	n.s.
Fibrobacter succinogenes	38.7	35.7	54.3	0.259	n.s.	318^a	167^b	162^b	0.132	n.s.
Butyrivibrio fibrisolvens	35.8^a	16.0^{ab}	7.30^b	0.151	*	104^a	67.9^b	58.1^b	0.078	**
Streptococcus bovis	50.9^a	6.12^a	1.38^b	0.218	***	183^a	10.2^b	15.2^b	0.282	**
Prevotella spp.	3.75^b	4.36^b	79.9^a	0.180	***	2.94	2.50	2.91	0.114	n.s.
Prevotella bryantii	0.004	0.002	0.002	0.206	n.s.	0.0004^b	0.002^a	0.002^a	0.160	***
Prevotella albensis	0.084^b	0.109^b	0.162^a	0.126	*	0.052	0.030	0.039	0.135	†
Selenomonas ruminantium	0.003^b	0.001^b	0.007^a	0.316	n.s.	n.d.	n.d.	n.d.		
Megasphaera elsdenii	0.045^a	0.049^a	0.011^b	0.208	*	0.076^a	0.019^b	0.011^b	0.151	***
Eubacterium ruminantium	0.005^b	0.004^b	0.030^a	0.205	**	0.010	0.024	0.037	0.326	†
Anaerovibrio lipolytica	0.003^b	0.001^b	0.046^a	0.388	**	0.002	0.001	0.001	0.339	n.s.
Lactobacillus spp.	50.3^a	38.5^b	59.1^a	0.047	**	47.4	41.5	48.1	0.053	n.s.

[1]ANOVA conducted in log-transformed data. *** P < 0.001; ** P < 0.01; * P < 0.05; † P < 0.1; n.s., not significant.

Conclusions Our results show that methane emissions can be explained, to some extent, by differences in the rumen microbial populations. Microbial communities living in the large intestine tended to change in a similar manner to that described in the rumen; however the differences were smaller and often inconsistent. This indicates that study of faecal microorganism can be considered complementary, but not substitutive, to rumen studies.

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Changes in the ratio of tetraether to diether lipids in cattle faeces in response to altered dietary ratio of grass silage and concentrates

C A McCartney[1,2], I D Bull[2], L Van Rooyen[1], R J Dewhurst[1]
[1]Teagasc, Animal and Grassland Research and Innovation Centre, Grange, Dunsany, Co. Meath, Ireland, [2]Organic Geochemistry Unit, University of Bristol, School of Chemistry, Bristol, UK *Email:christine.mccartney@abdn.ac.uk*

Introduction The membrane lipids of Archaea are comprised of either diether (archaeol) or tetraether (glycerol dialkyl glycerol tetraether; GDGT) lipids. These lipids are distinctive to ruminant faeces, being derived from the dominant rumen Archaea, which are methanogens (Gill *et al*., 2010). They have become a useful tool for the detection of methanogens in the ruminant digestive tract (McCartney *et al*., 2013). Recent studies have focussed on archaeol concentrations, without quantification of GDGTs. However, GDGTs must not be overlooked since they have an important effect on membrane permeability, which in turn affects maintenance of the chemiosmotic potential and maintenance energy requirements of methanogens. Valentine (2007) suggested that adaptation of archaeal membrane lipids could be useful in times of chronic energy stress. We hypothesised that methanogens increase the proportion of GDGTs when rumen conditions are challenging in order to conserve energy. To test this hypothesis, the GDGT:archaeol ratio was determined in faeces from animals consuming diets that result in rumen conditions that are either favourable (i.e. grass silage) or challenging (i.e. concentrates) for rumen methanogens.

Materials and methods Faecal samples from six beef steers consuming 72% (DM basis) grass silage and six beef steers consuming 89% (DM basis) concentrates were obtained from a previous study (McGeough *et al*., 2010). Dried, ground faeces (300 mg) were weighed in triplicate and 43.4 µg of diether standard (1,2-di-*O*-hexadecyl-*rac*-glycerol) and 1.07 µg of tetraether standard (C_{46} GDGT) were added to each sample. The total lipid extract (TLE) was then obtained using a modified Bligh-Dyer method, and then subjected to acid methanolysis to remove the polar head groups. The TLE was then separated into apolar and alcohol fractions using column chromatography. For archaeol analysis, the alcohol fraction was trimethylsilylated, dissolved in ethyl acetate, and then analysed by GC-MS. For GDGT analysis, the alcohol fraction was dissolved in hexane:isopropanol 99:1 (*v,v*), passed through a 0.45 µm filter, then analysed by HPLC-MS. Both archaeol and GDGT were quantified by comparison of peak areas to their respective internal standards. A one-way ANOVA with diet as treatment factor was applied to the data using Genstat software (14th Edition, VSN International).

Results and Discussion Dietary treatment effects on faecal concentrations and ratios of ether lipids are shown in Table 1. Isoprenoid GDGT-0 was the only GDGT detected. There were significantly lower concentrations of archaeol and GDGT-0 in faeces from animals consuming the high-concentrate diet, which was likely due to rumen conditions that were unfavourable for methanogens and methanogenesis (i.e. higher passage rates and reduced pH). With both diets, there was more GDGT-0 than archaeol in the faeces. Similar high ratios of GDGT to archaeol were reported in membrane lipids of similar methanogens (Methanobacteria) in other ecosystems (Mancuso *et al.*, 1986). This effect may help to explain the non-linearity of the relationship between faecal archaeol and methane production noted by McCartney *et al.* (2013), since concentrations of GDGT-0 and total ether lipids were more proportional to measured methane production (15.0 and 37.4 g/kg DM intake respectively; McGeough *et al*., 2010) than archaeol concentrations. It is unclear whether the higher proportion of GDGTs results from a change in methanogen species or changes in membrane composition of the same species.

Table 1 Effects of dietary treatment on the concentrations (mg/kg DM) and ratio (g/g) of diether (archaeol) and tetraether (GDGT-0) in faeces.

	Dietary treatment			
	Concentrates	Grass silage	s.e.d.	P
Archaeol	9.4	71.1	6.57	<0.001
GDGT-0	87	147	36.9	0.138
Ratio	10.4	2.09	1.95	0.002

Conclusions Changes in the ratio GDGT-0 to archaeol in methanogen membranes are likely to be associated with altered rumen conditions when feeding a high level of concentrates. An increase in the proportion of GDGT-0 may help methanogens under conditions of low pH by reducing the permeability of the cell membrane and thus conserving energy for growth. It will be important to consider both diether and tetraether membrane lipids when looking for relationships with methanogens and methanogenesis.

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RFI phenotype and diet type affect abundance of rumen methanogenic genotypes

C A Carberry[1,2], S M Waters[1], D A Kenny[1], C J Creevey[1]
[1]Teagasc, Animal and Bioscience Research Department, Grange, Dunsany, Co. Meath, Ireland, [2]UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland *Email:sinead.waters@teagasc.ie*

Introduction Methane (CH_4), a potent greenhouse gas, is an undesirable end product of rumen methanogenic fermentative activity. Improved host feed efficiency has been associated with lower methane emissions in cattle fed cereal based diets. Residual feed intake (RFI) has emerged as the measure of choice when determining feed efficiency potential in cattle. Recent published data suggest, that although repeatable within diet type (Kelly *et al*. 2010), the relative ranking of animals for RFI may not be consistent across diets (Durunna *et al*., 2011). Although Carberry *et al*. (2012) showed that the effect of RFI on bacterial profiles in the rumen was influenced by diet, there is little information available on whether constituent ruminal methanogenic species is affected by RFI across contrasting diets. Therefore, the objective of our study was to characterise rumen microbial methanogenic populations in beef cattle divergently selected for RFI while offered (a) a high forage (HF) followed by (b) a low forage (LF) diet.

Materials and Methods Beef heifers (n = 86), initially selected on the basis of sire EBV for RFI, were ranked for phenotypic RFI while offered a 30:70 maize silage to concentrate (LF) diet *ad libitum* for 80 days. The 7 highest (HRFI; least efficient) and 7 lowest (LRFI; most efficient) ranking animals were selected for use in this study. Both groups had similar mean bodyweight and average daily gain (ADG) at ranking but HRFI had, on average, 20% higher DMI. Following ranking on RFI all animals were offered a grass silage (HF) diet *ad libitum* for a 6 week period. Ruminal fluid was sampled at the end of each period using a specialised trans-oesophageal sampling device. Total microbial DNA was isolated and tag encoded amplicon pyrosequencing conducted using barcoded fusion primers, designed to amplify a 550bp methanogen-specific region of the 16S rRNA gene. From all animals, 2,823 raw sequences were obtained which were screened and quality trimmed with read lengths no shorter than 500bp and quality score ≥ 20 considered for further analysis. Trimmed sequences were clustered using CD-HIT-OTU applying the optimum clustering percentage identity of 98%. The automated phylogenetic tree-based Small Subunit Taxonomy and Alignment Pipeline (STAP) was used for taxonomic identification. Sequences from the resulting operational taxonomic units (OTUs) were then clustered at 99.5% similarity to identify different genotypes within each OTU. The number and distribution of each genotype was assessed across all animals. The normalised values were tested for statistically significant difference in abundance between either the HF and LF diets or between HRFI and LRFI animals using a binomial test.

Results Pyrosequencing confirmed *Methanobrevibacter* spp. as the dominant methanogen species in the rumen, with *Methanobrevibacter smithii* the most abundant species. At the genotype level, two genotypes of *Methanobrevibacter smithii* were found to be significantly over-represented ($P = 0.05$ and $P = 0.01$) in HRFI compared to LRFI animals. Two genotypes were found to be significantly over-represented in HF diet, one from *Methanobacterium sp.* ($P = 0.03$) and one from *Methanobrevibater smithii* 1 ($P = 0.02$). Two genotypes were found to be significantly over-represented in the LF diet, one from *Methanobrevibacter smithii* 1 ($P < 0.001$) and one from *Methanosphaera stadtmanae* 1 ($P < 0.001$), with the latter the only genotype identified in *Methanosphaera stadtmanae* 1 and represents the entire OTU. A further 11 genotypes were not different ($P > 0.05$) between animals on the LF and HF diets or between HRFI and LRFI animals.

Conclusion Our results demonstrate that a core group of methanogen OTUs exist across diet and phenotype, however significant differences exist in the distribution of genotypes within these OTUs. These changes in genotype abundance may drive the observed changes in methane emissions between efficient and inefficient animals. In the future a greater understanding of these methanogen genotypes may lead to greater exploitation of the inherent differences in methane emissions between H- and L-RFI phenotypes which could ultimately be utilised for the identification of cattle that are more economically and environmentally sustainable to produce.

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In vitro effects of bacterial direct fed microbials on methane production and volatile fatty acid profiles

J Jeyanathan, C Martin, D Morgavi
Institut National de la Recherche Agronomique, Clermont-Ferrand, Theix, France
Email:jeyamalar.jeyanathan@clermont.inra.fr

Introduction Methane (CH_4) emission from ruminants is the single largest source of agricultural CH_4 emission and contributes significantly to the global emission of greenhouse gases. It also responsible for the loss of 5-9% gross energy consumed by the host animal. Modulating rumen biochemical pathways by direct fed microbials (DFM) is one possible mitigation option but it had received little attention so far. The objective of this study was to examine the effect of selected bacteria on rumen CH_4 production and volatile fatty acid (VFA) profiles *in vitro*.

Material and Methods A detailed literature survey was performed to identify potential bacterial species that could reduce methanogenesis through modulation of rumen biochemical pathways. From identified groups and genera, 45 bacterial isolates were obtained for testing. Two Holstein cows fitted with permanent rumen cannula were used as rumen fluid donors. Cows were fed once per day 80% hay - 20% concentrate diet. Rumen contents (500 g) were obtained through the cannula 2 h after feeding and strained through a polyester monofilament fabric (400 μm) into an Erlenmeyer flask with an O_2-free headspace. Equal volume of rumen fluid from each cow were mixed together and used for the *in vitro* incubations. Bacterial DFM were prepared by growing them in appropriate media. Before use, the number of bacterial cells was estimated by turbidimetry and concentrated by centrifugation. Forage (alfalfa hay) and concentrate (maize and wheat) mixture was used as the substrate and were dried and ground through a 1-mm screen. Each substrate was weighed into 120-mL serum bottles into which 25 ml of Goering and Van Soest buffer (1970), 15 ml of rumen fluid and the prepared bacterial DFM (10^8 colony forming units/ml incubation media) were added under continuous flushing with CO_2. The bottles were sealed with rubber stoppers and aluminium crimps and incubated at 39°C for 24 h. For each experimental batch, control (without DFM) and blank vials (without substrate) were included. After 24 h incubation, gas production was measured with a pressure transducer and 5 ml of gas sample was removed and analysed for gas composition. pH was measured immediately and samples of incubation media were taken for VFA analysis. Each DFM preparation was tested three times on different days. Student's t test was performed to check the difference between controls and treatments ($P \leq 0.05$).

Result The effect of some selected bacterial DFM on CH_4 production and VFA profiles are shown in Table 1. Most isolates tested did not have a significant reducing effect on CH_4 production. A reduction in CH_4 production was observed only with DFM1. Although CH_4 production increased with other bacterial isolates, the percentage increase in CH_4 production was lower when compared to the percentage increase in total gas production. Total VFA production increased ($P \leq 0.05$) with DFM2, DFM4, DFM6 and DFM7. Increased propionate and decreased acetate ($P \leq 0.05$) proportions were observed with DFM2 and DFM6, whereas DFM3 showed increased propionate proportion ($P \leq 0.05$).

Table 1 Effect of bacterial DFM on rumen CH_4 production and volatile fatty acid profiles *in vitro*

	Bacterial species						
	DFM1	DFM2	DFM3	DFM4	DFM5	DFM6	DFM7
Percentage change compared to control							
Total gas (24 h)	10.9 ± 11.1	20.0 ± 3.7	14.0 ± 6.5	8.6 ± 5.7	20.0 ± 8.6	33.0 ± 12.3	28.5 ± 12.0
CH_4 (24 h)	-4.0 ± 2.0	9.0 ± 4.9	1.7 ± 2.2	5.5 ± 0.0	11.6 ± 9.7	21.6 ± 15.0	20.4 ± 1.3
Total VFA (mM)	65.7 ± 9.9	84.6 ± 6.3*	90.2 ± 14.1	87.8± 1.1*	83.4 ± 21	98.0± 4.0*	87.6 ± 9.2
Acetate (mol/100 mol)	58.2 ± 3.9	59.5 ± 0.7*	57.9 ± 2.4	61.4 ± 0.8	59.6 ± 1.7	60.4 ± 0.7*	52.8 ± 5.6
Propionate (mol/100 mol)	18.6 ± 0.9	21.0 ± 0.7*	20.9 ± 0.7*	19.8 ± 0.9	17.5 ± 1.5	19.5 ± 0.3*	18.2 ± 3.0
Butyrate (mol/100 mol)	14.1 ± 2.3	12.1 ± 1.1	11.8 ± 1.8	11.1 ± 1.2	13.1 ± 1.9	14.0 ± 1.4	16.3 ± 3.0
Others[a] (mol/100 mol)	9.1 ± 2.0	7.4 ± 0.4	9.5 ± 1.2	7.7 ± 0.6	9.8 ± 0.7	6.2 ± 1.2	10.9 ± 2.1
Acetate/Propionate	3.1 ± 0.3	2.8 ± 0.1*	2.8 ± 0.1*	3.1 ± 0.1	3.4 ± 0.3*	3.1 ± 0.1*	3.0 ± 0.7

[a] includes sum of iso-butyrate, iso-valerate, valerate and caproate, * Significant difference compared to the control ($P \leq 0.05$)

Conclusions Only one bacterial species (DFM1) reduced CH_4 production compared to the control. Other bacterial species reduced CH_4 production compared to total gas production potentially decreasing rumen methanogenesis as well. Validation of the observed results by i*n vivo* trials is essential as DFM might both influence the rumen environment and induce shifts in the microbiota that will not occur *in vitro*.

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Direct and indirect nitrous oxide emissions from contrasting cattle slurry application timings

B J Chambers[1], R E Thorman[2], L Sagoo[2], J R Williams[2], T H Misselbrook[3], D R Chadwick[4]
[1]ADAS Gleadthorpe, Meden Vale, Mansfield, UK, [2]ADAS Boxworth, Cambridge, UK, [3]Rothamsted Research North Wyke, Okehampton, Devon, UK, [4]Bangor University, Gwynedd, UK *Email:john.williams@adas.co.uk*

Introduction Slurry application timing has a significant impact on the balance of nitrogen (N) losses to air (i.e. nitrous oxide and ammonia) and water (nitrate leaching), because of differences in soil conditions (i.e. moisture, temperature, soil structure) at the time of, and drainage volumes, after application. The Nitrate Vulnerable Zone Action Programme in Britain (which applies to *c.*60% of England, 14% of Scotland and 3% of Wales) restricts the application of livestock slurries in the autumn/early winter period to reduce the risk of nitrate leaching losses. On medium and heavy soils (which cover an estimated 70% of agricultural land in Britain) autumn application timings are often the most practical for farmers because soils are usually dry enough to carry the weight of heavy application machinery without causing soil compaction. In order to develop slurry management practices that minimise N losses to the environment it is necessary to quantify the impacts of contrasting application timings on different N loss pathways. This paper reports results from an experiment which quantified the effects of contrasting cattle slurry application timings to an arable clay soil on ammonia and nitrous oxide emissions to air and nitrate leaching losses to water.

Materials and methods An experiment was carried out at ADAS Boxworth (Cambridgeshire, average annual rainfall 550mm) on a clay soil of the Hanslope Association (35% clay) in harvest season 2010. Cattle slurry (*c.*40m^3/ha; 3.5% dry matter) was applied to field-scale (12 m x 48 m) hydrologically isolated plots using trailing hose equipment, in August 2009 (to stubble) and March and May 2010 (to a growing winter wheat crop). Each plot was drained with lateral drains at 24 m spacing and 90 cm depth, with gravel backfill to within 30 cm of the surface and secondary mole drains installed at right angles to the lateral drains at 50cm depth and 2 m spacing. There were 3 replicates of each treatment arranged in a randomised block design. Direct nitrous oxide emissions were measured from each treatment and an untreated control for 12 months using the static chamber technique (5 chambers per plot). Ammonia emissions were measured for 7 days after application using the micro-meteorological mass balance technique. Drainage volumes were measured continuously from each plot and drainage water samples (collected on a flow-proportional basis) were analysed for nitrate-N. Indirect nitrous oxide-N emissions were estimated by applying IPCC (2006) default emission factors to the measured ammonia-N (1%) and nitrate-N (0.75%) losses.

Results Direct nitrous oxide -N emissions following each slurry application were well below the IPCC default factor of 1% of total N applied. There were no measureable emissions following the August application, with emissions from the March and May applications equivalent to 0.18% and 0.16% of total N applied, respectively.

Table 1 Slurry N applied and nitrate, ammonia and nitrous oxide emissions following contrasting cattle slurry applications at Boxworth 2009/10

		Slurry N loss (%total N applied)				
Application timing	Slurry total N applied (kg/ha N)	Nitrate-N leached	Ammonia-N emissions	Nitrous oxide-N emissions		
				Direct	Indirect	Total
August 2009	89	4	11	<0.01	0.14	0.14
March 2010	95	0	14	0.18	0.14	0.32
May 2010	126	0	10	0.16	0.10	0.26

Measured ammonia emissions were similar following all three application timings, in the range 10-14% of total N applied (Table 1). Over winter drainage was 111mm, with nitrate-N leaching losses following the autumn timing equivalent to 4% of total N applied; there was no nitrate leaching following the March and May applications because there was insufficient rainfall following application to cause drainflow. Indirect nitrous oxide-N emissions following the August application timing (0.14 % of total N applied) were similar to indirect emissions following the March and May timings of 0.14 and 0.10% of total N applied, respectively.

Conclusions: The largest N loss following each slurry application was by ammonia volatilisation (representing between 73% - 98% of total N losses). Nitrate leaching losses accounted for 27% of total N losses following the autumn application timing. Notably, indirect nitrous oxide emissions accounted for all nitrous oxide emissions from the August application, 44% following the March and 38% following the May timings. This study emphasises the need to account for all N loss pathways when developing policies to minimise the environmental impacts of livestock farming systems and maximise the N use efficiency of slurry applications.

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Reducing Emissions from Livestock Research Program: Australia's response to mitigation of ruminant methane emissions

T B Davison[1], J Hill[2]
[1]Meat & Livestock Australia, North Sydney, NSW, Australia, [2]Ternes Agricultural Consulting Pty Ltd, Upwey, Victoria, Australia *Email:tdavison@mla.com.au*

Introduction In 2008, the Australian Government commenced a four-year program to assist Australia's primary industries to adapt and adjust to the impacts of climate change and manage their greenhouse gas emissions. Within this strategy, the Climate Change Research Program (CCRP), administered through the Department of Agriculture, Fisheries and Forestry (DAFF), provided funding for research projects and on-farm demonstration activities. A national approach to emissions research was taken, reflecting the diversity of agricultural systems in Australia and the relatively large contribution of agriculture to Australia's total emissions. In a parallel process, the national research, development and extension agency for the red meat industry, Meat & Livestock Australia, started to plan a major industry-lead research program in abatement of greenhouse gases from livestock systems. The Reducing Emissions from Livestock Research Program (RELRP) was established with the support of both the red meat industry's initiative and the government's CCRP. The CCRP activities were originally designed to inform decisions about the treatment of agricultural emissions under the then proposed Australian emissions trading scheme. Since the commissioning of the CCRP in 2008, agriculture has been excluded from coverage under Australia's carbon price mechanism, which commenced in 2012. Instead, farmers and land managers can voluntarily provide offsets through the Carbon Farming Initiative (CFI). The CFI is an Australian Government legislative scheme that enables farmers, forest growers and landholders to earn abatement credits in return for reduced or avoided greenhouse gas emissions (mainly agricultural CH_4 or N_2O), or carbon sequestration through changes to soil and land management practices or systems biology. This approach provides farmers, forest growers and landholders with opportunities to generate and trade greenhouse gas abatement credits know as Australian Carbon Credit Units (ACCU). ACCU's will be linked to Australia's carbon price mechanism that has been established through the Clean Energy Future Bill (2011) and to international markets where credits are compliant with Kyoto Protocol rules. Work conducted in RELRP was therefore relevant to the red meat industries as it provided the underpinning knowledge to develop a range of CFI methodologies producers could deploy to claim ACCUs and participate actively in emissions abatement. Furthermore, the program quantified emissions from a range of ruminant production systems thereby supplying a source of information for the Australian National Inventory as well as for setting baseline emissions for claims for ACCU.

Reducing Emissions from Livestock Research Program This program of work commenced in 2009 and was completed in June 2012. The program was designed to align with other national research programs focussed on nitrous oxide, soil carbon, biochar and the adaptation of farming system to future climate change. The major objective of RELRP was to deliver knowledge and technologies to enable producers to breed and/or manage ruminants to significantly reduce methane emissions while maintaining livestock productivity for a viable agriculture industry. Thirty-nine research projects were commissioned in RELRP with MLA co-investing $3.42 million, the Australian Government committing $11.25 million and other RDC and commercial partners providing $0.83million. The total value of the program was $28.71 million. Six themes were developed: (1) national coordination,(2) quantifying methane emissions,(3) genetic approaches in sheep and cattle to reduce emissions, (4) manipulation of rumen function to achieve lower emissions, (5) improved management of waste, and (6) farming systems for lower methane emissions, demonstration and information delivery and is delivering new knowledge and tools to the livestock industries. The key industry questions developed were (1) What level of reduction in methane output is possible without affecting performance and profitability? (2) Is there enough information concerning the underlying biology of methane emissions to allow rapid uptake and adoption of abatement strategies? (3) Are there abatement strategies that allow reductions in greenhouse gas emissions under 'business as usual' conditions? (4) Can methane emissions from ruminant livestock systems be measured and verified reliably? (5) Are there circumstances where abatement of methane leads to increases in emissions of other greenhouse gases? and (6) What strategies are required for the communication of a voluntary policy to ensure uptake and adoption of abatement technologies and what are the co-benefits to methane abatement in the ruminant livestock sector and how are these communicated to industry?

Outcomes from the national research program The main outcomes of the research conducted were the development of an intra-ruminal methane measurement device to measure methane, CO2 and hydrogen production *in situ;* baseline methane emissions measurements from at least 10 different farming systems using open path laser & FTIR technologies yielding important new data suggesting northern beef production systems produce 30% less methane, dependent on DM intake, than was first thought ; the demonstration of the genetic basis of the low methane trait in sheep and beef cattle (beef cattle h^2 = 0.29; sheep h^2=0.04 to 0.18); basic research conducted on rumen microbiology identifying key processes that result in hydrogen production, hydrogen flow through the dissolved rumen pool and methane production; the role of a range of feeds and supplements that reduce emissions (5 to 18% *in vivo*) from both intensive and extensive ruminant systems (including new information on plant-derived fats, tannins and plant essential oils on controlling microbial methane production); work conducted to develop strategies to reduce the total greenhouse gas emissions from beef feedlots by focusing on nitrogen based emissions using nitrification inhibitors; and the development of four national demonstration sites and a range of modeling studies to understand total methane production from farming systems as well as a new decision support tool - the farmGAS calculator. The communication of results to the industry from the whole program was through 154 journal papers and conference proceedings nationally and internationally, 517 media alerts and a range of fact sheets and producer focussed DVD.

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Animal agriculture for a changing climate – using new ways of educating extension agents

E Whitefield[2], R Stowell[1], C Powers[1], M Risse[3], P Knox[3], G Hawkins[3], J Harrison[2], S Mukhtar[4], D Smith[4], L Jacobson[5], D Schmidt[5], C Gooch[6], J Pronto[6]
[1]University of Nebraska, Lincoln, Nebraska, USA, [2]Washington State University, Puyallup, Washington, USA, [3]University of Georgia, Athens, Georgia, USA, [4]Texas A&M University, College Station, Texas, USA, [5]University of Minnesota, St. Paul, Minnesota, USA, [6]Cornell University, Ithica, New York, USA *Email:e.whitefield@wsu.edu*

Purpose Given the cross-cutting social-environmental-economic implications of climate change issues, there is some urgency to generate new knowledge on this topic and also to disseminate knowledge in ways that can induce beneficial changes in practice. This is especially true for animal agriculture, which, although it is identified as a relatively minor source (1-3%) of total U.S. greenhouse gas (GHG) emissions (US EPA, 2010), has been the target of much negative press, calls for consumer avoidance, and regulatory attention to lessen America's contribution to global warming. Climate change adaptation and mitigation is an emerging issue for animal agriculture research and extension. Recent weather trends indicate that precipitation and temperature patterns are changing, and are already impacting agricultural production in a variety of ways. Farmers and ranchers must continue to adapt in a manner that minimizes risks to production and efficiency. In addition, animal agriculture producers must also become more informed about on-farm contributions of greenhouse gas emissions and possible methods for mitigation and sequestration.

Background For many Extension educators and other livestock advisors, this is a new topic. A nation-wide team and U.S. Extension professionals have set out to build capacity amongst Extension with the goal of informing and influencing livestock and poultry producers and consumers of animal products in all regions of the U.S. to foster animal production practices that are environmentally sound, climatically compatible, and economically viable. The United States Department of Agriculture (USDA) awarded six partnering Land-Grant Universities $4.1 million for this 5-year project to address issues associated with climate change and animal agriculture. The funding comes from the National Institute of Food and Agriculture (NIFA) via its Agriculture and Food Research Initiative (AFRI) competitive grants program. Five regions of the U.S. were identified to allow each to focus on regionally appropriate topics & methods.

Outcomes This project is facilitated through the online, web-based Livestock & Poultry Environmental Learning Center (LPELC). This national, web-based environment facilitates networking and extends the availability and utility of extension resources. The LPELC focuses on the environmental concerns of animal agriculture and maintains up-to-date information and Extension resources in a variety of media (webcasts, videos, newsletters, fact sheets, ask the expert). Resources have been developed and are currently available on the following topics: Air Quality, Pathogens, Environmental Planning, Regulation, Feed Management, Small Farms, Manure Nutrient Management, Manure Storage, Handling & Mortality, Manure Treatment Technologies, Value and Economics of Manure, and now with this project, Climate Change. The LPELC has engaged more than 100 experts to develop a comprehensive web presence for animal manure issues and exceeds 200,000 page views annually. The Learning Center is competitive among web search engines, accounting for 70+% of site visits. The LPELC has attracted a large and diverse audience interested in animal environmental issues.One of the first products of this effort is a web-based educational course for extension agents and educators using the latest research and tools. The objectives of the course and companion website are: 1) to build a foundation of knowledge; 2) facilitate learning across U.S. regions; and 3) provide a shorter time from research to extension to application. The educational modules are web-based using the Moodle platform and include introductory materials followed by specie & region specific information on: 1) climate impacts to animal agriculture; 2) risk management and adaptation; 3) climate science; 4) contributions and solutions to GHG emissions; 5) science communication during controversy. Each course module includes presentations, voice over commentary, video, interactive participation, certification test, downloadable extension materials, links for further reading, and complete bibliography. The presenter(s) will discuss methods used for developing a national curriculum that is regionally accessible and relevant & demonstrate selected course materials.The project team will have also facilitated a National Animal Agriculture and Climate Change Symposium. The goals of the symposium are to build capacity and networking amongst Extension educators, agents, and other livestock advisers. The symposium has invited speakers covering each of the topics listed above, submitted talks featuring ready-to-implement research & education methods, and a farmer and industry panel. The symposium will conclude with a stakeholder focus group discussion of barriers to education and how this project can help address their needs.In order to better assess the needs and perceptions in each region and amongst different groups, clicker polling is used to collect survey information. Clickers are interactive handheld keypad devices that allow for real time feedback by students electronically submitting answers to given questions at national and regional Extension, producer or industry meetings. Information is collected on regional issues and the current status and/or future plans of making on-farm management decisions based on the risk and impact of climate change. This information is used to deliver appropriate educational and information materials. Also, this information will provide yearly evaluation throughout the project to determine if knowledge is gained, if adaptation and/or mitigation practices are adopted, and if overall Extension capacity has been built. The presenter will discuss that latest evaluation results, and how they are being used to guide the project.Climate change adaptation and mitigation is an emerging issue for animal agriculture research and Extension. This Extension project aims for serving Extension agents with the tools they need to provide science-based educational materials to producers that will use the information to make decisions based on the risk of climate change.

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The ratio of archaeal and bacterial 16S rRNA genes in ruminal digesta as determined by qPCR correlates with methane emissions from beef cattle, but the relation is highly dependent on diet

R J Wallace[1], J A Rooke[2], C A Duthie[2], J J Hyslop[2], D W Ross[2], A Waterhouse[2], R Roehe[2]
[1]Rowett Institute of Nutrition and Health, Aberdeen, UK, [2]SRUC, Edinburgh, UK *Email:john.wallace@abdn.ac.uk*

Introduction Although it might be expected intuitively that the number of methanogenic archaea present in the rumen should be related to the quantity of methane emitted by individual ruminant animals, evidence for such a relation has been difficult to find (e.g. Danielsson *et al*., 2012). Indeed, an 'activity' determination based on transcribed genes of the methanogenesis pathway could, it has been suggested, provide a better indicator of ruminal methanogenic activity (Popova *et al.,* 2012). Theoretically, because the growth yield of methanogens is directly proportional to the flux of metabolites through the pathway, and because the ATP yield per mole of methane formed will be similar for the most abundant ruminal methanogens (Thauer *et al.*, 2008), genomic estimates should in principle provide a better index. Genomic analyses would avoid issues such as metabolic flux control that are well known to have a major influence how gene expression can have only a minor influence on the flux through a metabolic pathway. The aim of this study was therefore to examine dietary and genetic factors that influence the relation between the abundance of ruminal archaea from genomic DNA measurements and methane emissions in beef cattle as measured in respiration chambers.

Material and methods Thirty-six Aberdeen Angus and 36 Limousin steers were housed at the Beef Research Centre of SRUC, Edinburgh. The steers received two diets, one mainly concentrate-based and the other a forage-concentrate-based diet, with forage:concentrate ratios (DM basis) of 8:92 and 48:52, respectively. Eighteen animals of each breed received each diet. Methane emissions were measured individually for 48 h in 6 respiration chambers. The animals were allocated to the chambers in a randomised block design with 3 replicates. Measurements from 4 animals were discarded due to health issues and an air leak from the respiration chamber. Samples of ruminal digesta were recovered within two weeks at slaughter. Digesta were strained immediately, mixed with glycerol/buffer solution as cryoprotectant and stored at -20 °C. Samples were subsequently thawed and DNA was extracted by the RBB+C method. Bacterial 16S rRNA genes were analyzed by qPCR using primers UniF and UniR and a BioRad iQ5 analyser. Archaea were amplified using the universal archaeal primers Met630F and Met803R. Analyses of the ratio of archaea:bacteria (A:B) and methane emissions were performed using the GLM procedure of SAS, fitting different models, including A:B ratio, diet, breed, sire within breed, chamber and block effect, with the main emphasis to identify the underlying regression of methane emissions on ruminal digesta from individual animals.

Results The ratio of archaea:bacteria (A:B) varied more than ten-fold in digesta taken from individual animals by stomach tube immediately after animals left the chambers or subsequently at slaughter. We have demonstrated previously that there is strong correspondence between the A:B ratio in both types of sample. Analysis of the *post-mortem* samples showed that the regression of methane emissions on the A:B ratio was most strongly dependent on the diet. Making no allowance for diet, a R^2 of 0.35 was found across all samples/animals. A model fitting the regression of methane emissions on A:B within diet increased the R^2 to 0.58. Over all samples, a significant nonlinear regression was found, whereas within diet the regression was linear. The linear regression coefficients in units of methane (g/kg DMI) per A:B(%) were very different between diets, 1.325 ±0.505 and 0.234 ±0.244 for concentrate and forage-based diet, respectively. A major reason for the different regression within diet was different means and standard deviations of the A:B, which were 2.83% and 1.263%, respectively, for the concentrate-based diet and 6.52% and 2.538%, respectively, for forage-based diet. Standardising the A:B ratios for their means and variations within diet resulted in a similar fit of the model (R^2 = 0.57), indicating that heterogeneous variances for A:B ratio between diets were one cause for different regressions within diet. The resulting regression of methane emissions on 1% increase in standardised A:B ratio was 1.119 ± 0.446 g/kg DMI. However, different regressions of methane emissions on A:B ratio depending on diet can not be excluded by this analysis. Using the full model with diet, A:B ratio within diet, breed, sire within breed, chamber and block effect explained most of the variation in methane emissions with a R^2 = 0.81.

Conclusions The archaea:bacteria ratio of ruminal digesta at slaughter varied according to the methane emissions of individual animals, though the relation was variable between diets. The amounts of methane produced from cattle on the concentrate-based diet increased more rapidly with increasing A:B than with the higher-forage diet, and the correlation was weaker with the latter diet. Thus, A:B ratio can be a useful tool for comparing methane emissions in animals within diets, but for across diets comparison the different means and heterogeneous variances for A:B ratios depending on diet have to be considered. Further refinement of the analysis of bacterial and archaeal communities may be required.

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Measuring high accuracy fluxes of N_2O from soils using the closed loop high accuracy N_2O (CLHAN) chamber

N Cowan[1,2], D Famulari[1], P Levy[1], B Rees[3], M Bell[3], U Skiba[1]
[1]Centre of Ecology and Hydrology, Edinburgh, UK, [2]University of Edinburgh, Edinburgh, UK, [3]Scotland's Rural College, Edinburgh, UK *Email:nicwan11@ceh.ac.uk*

Introduction Nitrous oxide (N_2O) is a potent greenhouse gas (GHG) and the single largest contributor to global stratospheric ozone depletion. Of the 3 dominant GHGs (carbon dioxide, methane and nitrous oxide) the estimated anthropogenic fluxes of N_2O have the largest uncertainty associated with them. The aim of this study was to develop a chamber methodology using modern infrared laser equipment, which is capable of measuring N_2O fluxes from soils with a greater precision than by previous chamber methods.

Material and methods An Aerodyne Research Inc. compact continuous wave quantum cascade laser (CW-QCL) was used to measure gas concentrations from within a sealed PVC plastic chamber inserted into the soil. The QCL instrument is a rapid gas analyser capable of measuring N_2O with a resolution of 30 parts per trillion (pptv) at high frequencies based on tuneable infrared absorption spectroscopy (TILDAS). The closed loop high accuracy N_2O (CLHAN) chamber was designed specifically for use with the QCL instrument. Air is pumped in a closed loop system from the CLHAN chamber to the QCL at a flow rate of 7 L min^{-1}. Constant airflow allows measurements of N_2O within the sealed system to be measured at a rate of 1 Hz. Air temperature and pressure were measured during the enclosure period. The method is similar to that used by Hensen *et al*. (2006). A comparison of results collected by both the CLHAN chamber method and the more commonly used static chamber method was made on an intensively managed grassland, grazed by dairy cows in SW Scotland (September, 2012). Fluxes were measured using both methods immediately after nitrogen fertilisers had been applied, then again 3 weeks later which provided a total of 150 measurements over a large concentration gradient for comparison of the methods.

Results The CLHAN chamber method provides high resolution N_2O flux measurements over a period of just a few minutes. Measuring change in concentration of N_2O within the system at 1 Hz significantly reduces the uncertainty associated with the regression analysis of the data. It is possible to use both linear and non-linear regression models with each measurement, which allows a best fit approach for calculating fluxes (See Figure 1). The results of the comparison experiment showed that the precision of the measurements made using the CLHAN chamber method was far greater than the static chamber method. This was highlighted in the comparison of very low fluxes which the static chamber has difficulties measuring due to the detection limit of the GC instrument (minimum of approximately 15.32 µg N_2O-N m^{-2} h^{-1}). Uncertainty values in measurements carried out using the CLHAN method are calculated by combining the relative volume, instrumental and regression uncertainty. For low flux measurements this can be as low as 0.15 µg N_2O-N m^{-2} h^{-1}.

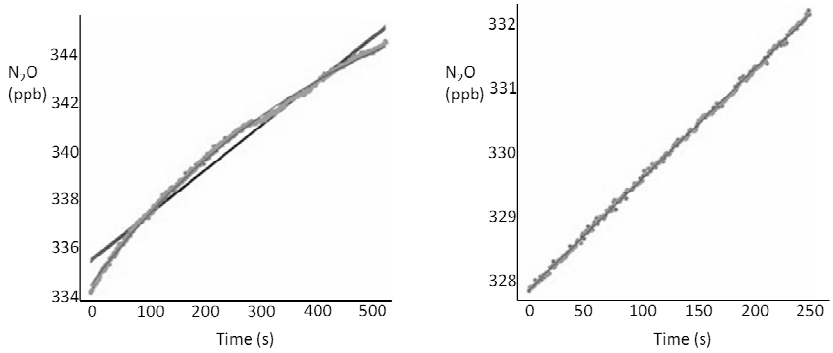

Figure 1 A comparison of non-linear and linear regression used to calculate two different fluxes measured using the CLHAN chamber

Conclusions These results show that it is possible to improve how N_2O is measured using modern instrumentation and methodology. The CLHAN chamber method has the ability to investigate low flux phenomena such as soil uptake of N_2O which previous methods have been unable measure with confidence. Further high precision measurements are required to achieve a better understanding of how soils react to anthropogenic influences such as agricultural practices and changes in land use.

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Characterization of the rumen methanogenic communities and their contribution for the methane emission

N Friedman[1], I Mizrahi[2]
[1]Volcani Research Center, Beit Dagan, Israel, [2]The porter school of environmental studies, Tel-Aviv University, Tel-Aviv, Israel *Email:frinir.1@gmail.com*

Introduction Methane emission from ruminant livestock constitutes a considerable portion from the global greenhouse gas emission. This emission is the outcome of methanogenesis activity of the methanogenic archaea that reside in the rumen. Ruminant animals fed on plant material based diets that are composed from varying fiber contents. The effect of diet on the number and composition of rumen methanogens is ambiguous. Hence, in the present study using pyrosequencing approach together with quantitative real time PCR (qRT-PCR) and *In Vitro* methane measurements we characterized the overall archaeal communities composition as well as their methane emission potential as a function of two diets each represent the upper or lower limit of the dietary fiber in the feed.

Material and methods Both experimental diets consisted of the same dietary fiber and varied only at its ratio (Table 1). These diets were fed to 5 Holstein Friesian cannulated cows in a 69 days experiment which was divided into two feeding periods. On the first period, day 1-39, the cows were fed 95% fiber diet (high fiber diet). On the second period, starting from day 43 until day 69, 3 experimental cows were fed 30% fiber diet (low fiber diet) and 2 cows remained on 95% fiber diet as a control. In each feeding period, rumen content from each of the 5 cows was sampled every 4-7 days. The DNA from those samples was extracted and administrated to 454 pyrosequencing of the V3 - V5 region of the archaeal 16S rRNA gene. In order to determine the kinetics of the change within the different methanogenic orders, Quantitative RT-PCR was employed on all rumen samples taken in this experiment. Furthermore, 8 rumen samples from both diets were analysed for their methane emission potential using *In-Vitro* methane emission assay.

Results Our findings reveal that the change in diet affects the methanogenesis potential. While the overall archaea frequency did not change with the diet, substantial shifts in taxonomic composition of the methanogenic communities were documented as revealed by pyrosequencing and qRT-PCR results. Some archaeal operational taxonomic units (OTUs) were highly diet specific and could be found in all samples from a specific diet rather than the other.

Table 1 Ration content of both diets.

	High fiber -95% fiber	Low fiber – 30% fiber
Corn	0 %	65%
Soy bean	3.1%	4%
Salts	1.1 %	1%
Clover	41.8 %	12.7 %
Urea	0.4 %	0.4 %
Oat	53.6 %	16.3 %

Conclusions Our results suggest an advantage that each of the tested taxonomic units possess at different ecological niches created by these diets. Furthermore, these results also demonstrate the methanogenesis potential of each diet. These novel findings may have important future implications both at the rumen microbial ecology and at the environmental green house gas perspectives.

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Emission inventory of ammonia and methane from a commercial Holstein dairy farm

D J Mali, W J Van Heyst, C Wagner-Riddle
University of Guelph, Guelph, Ontario, Canada *Email:dmali@uoguelph.ca*

Introduction There has been a trend in agricultural livestock production in Canada that has seen small family owned farm operations move to larger more industrialized production facilities. This expansion comes with an increase in volume in waste streams simply due to the increased mass of animals. Coinciding with this increase in waste stream are the associated environmental problems. Farms and agricultural operations have long been associated with greenhouse gas (GHG) production. The quantities of these GHGs have been estimated but there are still gaps in the data, especially with regards to the variability introduced by geographic location and seasonal climatology. This study will examine how emissions are effected seasonally, and identify influential factors are driving ammonia and methane production.

Materials and Methods The dairy operation has the capacity to milk 168 cows, and house an additional 158 heifers, 40 dry cows, 65 young heifers, and 70 bulls. The operation is somewhat unique in Canada as it uses a combined barn, housing all types of Holstein's under the same roof. The barn is a combination of tie-stall, for the milking cows, and free stall for the rest of the heifers, calves, cows, and bulls. The barn is equipped with a scraping arm system to collect the manure on the free stall side and a gutter collection system for the tie stall section. The manure is directly drained to an outdoor lagoon. The barn is tunnel ventilated via a bank of fourteen 1.2m diameter fans. In order to capture the barn air, a heated (124^{o}C) sample line was installed that transported barn air into an enclosed trailer that housed a tower of Thermo Electron analyzers used to determine the concentrations of the gases. Once in the trailer, the air was split and distributed to an ammonia and methane/non-methane hydrocarbon analyser. To capture the exhaust rate from the barn, the exhaust rate from each of the 14 fans was measured using a Flow Assessment Numeration System (FANS) unit. The FANS unit consists of an aluminium frame supporting six propeller anemometers that traverse across a known area to produce a velocity profile which is multiplied by the area to get an exhaust rate. Information about the animals was collected throughout the study including animal population, estimated animal mass, feed composition and mass as well as faecal and urine profiles. Other influential factors that were collected included manure cleanout schedule, lighting schedule, feeding schedule relative humidity, barn temperature and outdoor temperature.

Results In all instances the emission factors were highest in the summer and lowest in the winter. The barn concentrations were highest in the summer, followed by fall, than winter. The reduced ventilation in the winter and fall accounted for the lower emission factors. The emissions presented are on the high end, but the barn had very high summer exhaust rates which influence the emission factor. For other barns with mechanical ventilation in the similar geographic region the data agrees well (Aguerre, *et al*. 2011). The average weight of a cow was 800kg, which is much higher than the typical 600kg more commonly seen. The program SAS was used to preform statistical analysis. Statistical evidence for a t-test shows that the three sampling campaigns were significantly different with a P of <0.0001 for both ammonia and methane. The time of day also impacted the emission factor significantly due to barn activities. For methane, the hourly averages spiked twice a day and were shown to be dependent on each other. The least squares difference showed that the means of these spikes are very similar with a P of 0.009. No other time of day exhibited this behaviour. The ammonia peaked only once and no time of day dependency was found using the least squares difference.

Table 1 Ammonia and Methane Emission Factors for Three Sampling Periods

	June-July 2012				September-November 2012				January-March 2013			
(g hr^{-1} AU^{-1})	Avg	Min	Max	s.d.	Avg	Min	Max	s.d.	Avg	Min	Max	s.d.
NH_3	5.82	1.98	14.57	2.30	3.08	0.39	10.36	1.65	2.11	0.45	12.16	1.62
CH_4	101.85	38.50	273.61	38.32	78.22	25.60	302.97	35.96	63.15	32.30	437.21	39.49

Conclusions There are many influential factors that contribute to the emissions of methane and ammonia. For methane the only influential factor that occurs twice a day are the feedings. The dependency of the spikes does correlate to feeding times although there is a lag time after the feeding and when the spikes occur. For ammonia the spike occurs after the feeding times. Since ammonia is produced from the mixing of urine and faecal matter, the spike is presumably due to the animals now defecating and urinating after the feeding period has taken place. More research is being conducted on other seasons and to narrow down other influential factors in order to model and mitigate the emissions of methane and ammonia.

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Methanogen community structure in the rumens of dairy cattle grazing tannin-free and condensed tannin-containing forages under Colombian tropical conditions by using 454 pyrosequencing techniques

E A Angarita[1,2], O L Mayorga[1,2]
[1]CORPOICA, Bogota, Cundinamarca, Colombia, [2]National University Of Colombia, Bogota, Cundinamarca, Colombia
Email:olumayorga@gmail.com

Introduction Several studies have demonstrated that condensed tannin (CT) rich forages contribute to a decrease the methanogenesis, nevertheless relatively little is known about their effects on the community of rumen methanogens. This study describes the composition of rumen methanogenic archaea in cows grazing tannin-free and condensed tannin-containing forages under Colombia tropical conditions.

Material and methods The experimental trial was performed using twelve lactating cows grazing on Pennisetum clandestinum (PC), Lotus uliginosus (LU) contained 7.3% of CT and their association 70:30% (PL) with 2.3 % in CT, in a crossover design trial with three 21-d periods. The diversity of methanogens was evaluated from total DNA extracted from rumen contents. Archaeal 16S rRNA genes were amplified by PCR using two pairs of methanogenic archea-specific primers[2] and PCR products were sequenced using tag-encoded FLX amplicon pyrosequencing (TEFAP).

Results A total number of 15390 sequences (322±81 pb) with an average 1710±753 per rumen sample were identified and analyzed based on their best match using MG-RAST and RDB II database at ≥95% nucleotide sequence identity. The true diversity estimated by Chao1 was 86, 92 and 116 OTUs (operational taxonomic units) and the species diversity assessment by Shannon-Wiener index (H') were 3.1, 2.5 and 3.0 from animals grazing on PC, LU and PL, respectively. Based on the taxonomic analysis of data set, 99.4% of methanogens detected were placed in three genus: Methanobrevibacter (92,1%), Metanobacterium (3,95%), Methanosphaera (1.54%) and a group of uncultured rumen archaea (1.81%), observing changes in the abundance of Metanobacterium 4.42%, 2.19 and 5.24%, and Methanosphaera 0.8%, 1.47% and 2.35% for PC, LU and PL, respectively.

Conclusions These results revealed that dominant methanogens were presented in all rumen samples taken from all of cows fed with any of grazing forages used in this experiment, while methanogens with low abundance were not shared by all samples. These results also showed a characteristic fingerprint in terms of relative presence of rumen methanogens, which was affected by grazing forage type, indicated that these microorganisms differ depending on diet of the host, and consequently could affect the methanogenesis by modifying dietary composition or by plant compounds within the diet as tannins. These observations increase the understanding on effect of intake forage on rumen methanogens, and raise new questions for further study on methane emissions.

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Diversity of rumen methanogens from cattle grazing on a tannin-free gramineae Pennisetum clandestinum and a tanniniferous legume Lotus uliginosus using PCR-DGGE and qPCR analyses

E A Angarita[1,2], O L Mayorga[1,2]
[1]CORPOICA, Bogota, Cundinamarca, Colombia, [2]National University of Colombia, Bogota, Cundinamarca, Colombia
Email:olumayorga@gmail.com

Introduction The molecular based ecology techniques have provided insight on rumen methanogens, which should lead to strategies for improving production by reducing methanogenesis. The aim of this study was to determine the effects on diversity of rumen methanogens from cattle grazing on tannin-free gramineae and tanniniferous legume using PCR-DGGE and qPCR analyses.

Material and methods The diversity of rumen methanogens was assessed in nine non-lactating cows grazing on the pastures: Pennisetum clandestinum (PC), Lotus uliginosus (LU), and their association70:30% (PL) in a completely randomized design. The total DNA was extracted from rumen contents, followed by amplification of the V3 hypervariable region of 16S rADN by conventional PCR and finally PCR products were separated by electrophoresis in denaturing gradient gel (PCR-DGGE). The similarity of the DGGE profiles was calculated using the average Dice similarity coefficient (Dsc) index using the UPGMA algorithm and the copy number of targeted methanogens 16S rRNA gene per mg of rumen fluid was calculated by qPCR.

Results UPGMA analysis showed the formation of three clusters of methanogens which were associated with type of forage supplied. A first cluster with 69% similarity consisted by a animal feeding PC, a second cluster (87%) was composed by two animals grazing PC and PL respectively, and a final cluster 91% all the animals grazing LU. The average number of bands was 22, 18 and 17 for PC, PU and LU respectively. The total number of methanogens was significantly different ($P<0.001$): 6.75, 6.30 and 8.17 [Log10 ADN methanogens (ng/g dry matter)] in animals fed with PC, LU and PL, respectively.

Conclusions These results showed a characteristic fingerprint of rumen methanogens, which was affected by grazing forage type, indicated that these microorganisms differ depending on diet of the host, and consequently could affect the methanogenesis by modifying dietary composition or by plant compounds within the diet as tannins.

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Methane emissions of two divergent breeds of beef suckler cows offered a straw based diet with either grass silage or brewers grains

C A Duthie, J A Rooke, J J Hyslop, D W Ross, A Waterhouse
SRUC, Edinburgh, UK *Email:carol-anne.duthie@sruc.ac.uk*

Introduction Nutritional manipulation has been shown to be an effective tool for reducing methane emissions from beef production systems. There is some evidence to show that replacement of forage in the diet with brewers grains, a by-product of the beer and ethanol grain industry and high in oil content, reduces methane production in cattle. Utilising the difference between breeds has also been suggested as an alternative tool. There is currently limited knowledge about differences in methane output between hill type breeds and the more common beef breeds in the UK. This study investigated methane output from two divergent breeds (Limousin x Aberdeen Angus (LIMx) and purebred Luings (LUI)) of non-lactating cows fed a straw based diet with either grass silage or brewers grains. LIMx represent the most common genotype of suckler beef within the UK, whilst the LUI is a hardy, hill breed.

Material and methods The experiment was of a 2x2 factorial design, comprising of non-lactating cows of 2 breed types (LIMx and LUI), and 2 diet types (a straw-silage based diet (677, 311 and 12 g/kg DM of barley straw, grass silage, and minerals, respectively) and a straw-brewers grains based diet (771, 218 and 11 g/kg DM of barley straw, brewers grains, and minerals, respectively)). Cows (n=48) were group-housed in even numbers of each breed type across 2 pens, and each diet type was allocated to 1 pen. Diets were offered *ad libitum* to cows twice daily through electronic feed intake recorders to measure individual feed intake. Prior to chamber-based gas production measurements, feed intake and weekly live-weight (LW) had been measured for a minimum of 3 weeks, following a 4 week adaptation period to acclimatise to the diets. Cows were allocated to respiration chambers using a replicated (2 times) randomised block design so that allocation was balanced for breed, diet and LW. Six indirect open-circuit respiration chambers were used with CH_4 production being recorded for the last 48h of a 72h measurement period. Within the chambers (76 m^3) cows were loose-housed in 4x3 m pens. Measurements of ambient CH_4 concentrations were obtained from samples of inlet air. CH_4 concentrations (9 per h for each chamber and ambient air) were measured by infrared absorption (MGA3000, ADC Ltd., Hoddesdon, UK). Dry air flow, corrected to standard temperature and pressure was calculated for each individual record of CH_4 concentration. Daily gas production was calculated as the average of individual values and converted to a mass basis. Due to occasional noted failures of equipment the experiment was run over 9 weeks giving n=10 for each breed x treatment combination. Data were analysed using Genstat using linear mixed models where the factors were the 2 x 2 arrangement of breed and diet, chamber and week of experiment. Data are reported as means and standard error of difference.

Results Although both breeds of cattle showed higher DMI on the straw-silage based diet (9.85 *vs.* 9.3 kg/day) than the straw-brewers grains diet (Table 1), this was not significant. Cattle offered the straw-brewers grains diet produced significantly less ($P<0.01$) CH_4 per day than the straw-silage fed cows. When expressed as g/kg DMI, the straw-silage fed cows produced higher CH_4 than cows which were offered the straw-brewers grains diet, however this was not significant ($P=0.076$). When expressed as kJ/MJ GEI, cows offered the straw-silage based diet produced significantly more ($P<0.05$) CH_4 than the straw-brewers grain fed cows. Although the LUI cows showed consistently higher CH_4 per day than the LIMx cows (145.0 *vs.* 132.5 g/day) on both diets, this was not significant. No significant breed effects were identified for DMI or CH_4 expressed as g/kg DMI or kJ/MJ GEI.

Table 1 Dry matter intake (DMI), and methane (CH_4) production of two breeds of non-lactating cows fed one of two diet treatments

Diet	Straw-silage		Straw-brewers grains			P		
Breed	LIMx	LUI	LIMx	LUI	s.e.d.	Breed	Diet	Breed x Diet
DMI (kg/day)	9.9	9.8	9.2	9.4	0.89	n.s.	n.s.	n.s.
CH_4 (g/d)	140.1	161.2	124.9	128.8	18.27	n.s.	0.006	n.s.
CH_4 (g/kg DMI)	14.6	15.8	14.2	13.5	2.21	n.s.	n.s.	n.s.
CH_4 (kJ/MJ GEI)	43.35	46.98	40.97	38.94	6.51	n.s.	0.037	n.s.

GEI, gross energy intake.

Conclusions The results of this study indicate the potential of increasing the level of oil in the diet as a method of mitigating CH_4 emissions from cattle production. This strategy is suggested to reduce CH_4 production through decreased methanogenesis (inhibition of protozoa), increased production of propionate *vs.* acetate and higher use of hydrogen in the biohydrogenation of unsaturated fatty acids. The results indicate that cattle offered a diet containing brewers grains, instead of grass silage produced less CH_4 per day as well as per MJ GEI. This may be due to the higher levels of oil in the straw-brewers grains diet compared with the straw-silage diet (36 *vs.* 21 g/kg DM). Utilising breed differences for lower CH_4 production has been suggested, however no significant breed differences were identified in the current study.

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Estimated methane emissions, animal performance and Net Feed Efficiency (NFE) in young breeding bulls and finishing steers offered the same diet

J J Hyslop[1], R Fuller[2], U Taylor[2], D Thirlwell[3], S Wareing[4]
[1]SRUC (Beef & Sheep Select), Bush Estate, Penicuik, UK, [2]BIG, Southburn Offices, Driffield, UK, [3]JSR Farming Ltd, Southburn Offices, Driffiled, UK, [4]Richard Keenan (UK) Ltd, Stoneleigh Park, Warks, UK
Email:jimmy.hyslop@sruc.ac.uk

Introduction It is widely recognised that improving the efficiency of beef production is the most viable way to reduce the Greenhouse Gas (GHG) emissions from these animals. Net Feed Efficiency (NFE) is a measure of feed use efficiency that allows identification of individual future breeding stock that are more efficient for any given level of animal productivity over the longer term. The objective of this study was to determine voluntary feed intakes, animal performance, NFE and estimated methane (CH_4) outputs in young bulls prior to their selection as future breeding stock and finishing steers prior to slaughter.

Materials and methods A total of 38 Stabiliser bulls, 5 Beef Shorthorn bulls between 10-13 months of age and 39 Stabiliser steers (14-16 months of age) were offered a mixed forage/concentrate complete diet (CD) *ad libitum* for an adaptation period of 4 weeks and a subsequent measurement period of 56 days *via* electronic feed intake bins (Growsafe). The CD contained (g/kg DM) wholecrop wheat (344), maize silage (102), barley straw (39), barley (182), sugar beet pulp (129), wheat distillers grains (156), molasses (40) and minerals (8) - (DM: 549 g/kg; ME: 11.5 MJ/kg DM; CP: 134 g/kg DM). Dry matter intakes (DMI) were recorded continuously by the feed intake bins, individual electronic ear tags and their associated computer software. Individual bull and steer liveweights (LW) were determined weekly and individual carcass fat depths were determined once at the end of the recording period by ultrasound scanning. Daily liveweight gain (LWG) was determined by linear regression of weekly LW measurements over the 56 day period whilst data for bull and steer groups were analysed separately such that NFE was derived for individual bulls and steers (within groups) as the difference between actual DMI against estimated DMI using a multiple regression model including metabolic LW ($LW^{0.75}$), LWG and fat depth as predictor variables. CH_4 emissions (l/day) for each individual bull and steer was then predicted as CH_4 output = 14.7+35.1xDMI (Yan, *et al*, 2009). Within bull and steers groups, data was grouped into three groups where low NFE = < -0.5 s.d. of mean NFE, MID NFE = > -0.5 but < +0.5 s.d. of mean NFE and high NFE = > +0.5 s.d. of the mean NFE value respectively. Differences between these three groups were then tested using the residual maximum likelihood (REML) facility in Genstat 15.

Results Feed intake, animal performance, NFE and predicted CH_4 production are shown in table 1. For both bulls and steers, no significant differences between the three groups were seen in mean $LW^{0.75}$, LWG or fat depth parameters. However, low NFE bulls and steers ate less, were significantly more efficient ($P<0.05$) in terms of NFE values and produced lower predicted CH_4 outputs compared to the high NFE groups of either bulls or steers. Bulls required an average of 82 days (1.84 kg/d LWG) whilst steers required an average of 102 days (1.47 kg/d LWG) to achieve 150 kg of liveweight gain. Assuming the average DMI figures observed here to achieve this 150 kg of gain, this equates to predicted CH_4 emissions of 38048 and 47328 (l) for bulls and steers respectively.

Table 1 Feed intake, animal performance, NFE and predicted CH_4 output in young Stabiliser bulls and steers

	Low NFE	MID NFE	High NFE	s.e.d.	Significance	
Bulls						
DMI (kg/d)	12.2[a]	12.8[b]	13.5[c]	0.226	*	[a,b,c]
Mean $LW^{0.75}$ (kg)	119	120	120	2.27		Values not
LWG (kg/day)	1.81	1.87	1.83	0.059		sharing
Mean fat depth (mm)	5.7	4.8	5.0	0.579		common
NFE (kg DMI/day)	-0.68[a]	0.05[b]	+0.77[c]	0.148	***	superscripts
Predicted CH_4 (l/day)	442[a]	466[b]	490[c]	7.95	**	differ
Steers						significantly
DMI (kg/d)	12.4[a]	12.6[b]	13.5[c]	0.233	***	
Mean $LW^{0.75}$ (kg)	120	117	118	2.48		
LWG (kg/day)	1.47	1.47	1.48	0.046		
Mean fat depth (mm)	7.2	7.5	7.4	0.475		
NFE (kg DMI/day)	-0.69[a]	-0.07[b]	+0.63[c]	0.130	***	
Predicted CH_4 (l/day)	449[a]	455[b]	487[c]	8.19	**	

Conclusions Low NFE bulls and steers consumed 8-10% less feed, produced 8-10% lower CH_4 emissions and cost £14-£17 less to feed over the 12 week period (at £155/t DM) on the NFE unit compared with high NFE bulls. Measurement of NFE offers significant opportunities to select future breeding stock with improved feed efficiency and lower CH_4 emission characteristics. The overall increase of 24.4% in predicted CH_4 emissions from steers confirms the critical importance of production system choice (e.g bulls *vs* steers) and of reducing days to finish as mitigation strategies in beef production systems.

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Effects of in vitro and in vivo dietary supplementation with saponins on rumen fermentation with particular reference to volatile fatty acids, ammonia and methane

A Budan[1,2], N Tessier[1], C Pierre[1], D Guilet[2], D R Yáñez-Ruiz[4], V Fievez[3]
[1]Nor-Feed Sud, Beaucouzé, France, [2]laboratoire substances d'origine naturelle et analogues structuraux, Université d'Angers, France, [3]Laboratory for animal nutrition and animal products, Ghent University, Belgium, [4]Estación Experimental del Zaidín, CSIC, Granada, Spain *Email:alexandre.budan@nor-feedsud.fr*

Introduction Saponins occur in plants as secondary metabolites. These compounds are able to shift the rumen fermentations resulting in decreased ammonia (NH_3) concentration in the rumen fluid and mitigation in methane (CH_4) production (Wina *et al.*, 2005). *In vivo* results are not always consistent with data obtained *in vitro*. The aim of this study was (i) to evaluate *in vitro* the effects of a product based on saponins (Yuquina® M extract, Nor-Feed Sud, France) on VFA, CH_4 and NH_3, and (ii) to compare the *in vivo* results to data obtained from *in vitro* experiments on goats using similar diets and amount of Yuquina® M extract/mL of rumen fluid.

Material and methods The total saponin content in Yuquina® M extract (YME) was estimated by gravimetric method according to Yao *et al.* (2010). The saponin content was calculated as the mass of dried butanolic extract. A substrate based on grass silage/maize silage/concentrate (30/30/40) was incubated without or added with any of 12 doses of YME (from 0.04 to 40 g/L DM, with results of 0.1, 0.2 and 1.0 g/L DM shown in Table 1) for *in vitro* rumen fermentations, conducted as described by Castro-Montoya *et al.* (2012). After 24 h at 39 °C, flasks were sampled for gas, NH_3 and VFA determination. A 17 days *in vivo* trial was conducted with 24 dry Murciano-Granadina goats split into 1 control group and 3 treated groups. Animals were fed twice a day a diet similar to the substrate used *in vitro*. Concentration in YME for treated animals was 0.525, 1.050 and 5.250 g/goat/day DM. Methane measurement was done at the end of the experiment in respiratory chambers during two consecutive days. Afterwards rumen content was sampled for VFA and NH_3 analysis. Feed intake was recorded and urine was collected for purine derivative analysis. Means were compared by two-way unbalanced variance analysis (ANOVA) with subsequent post-hoc multiple comparison test of Duncan using XLSTAT (version 2011.2.04, Addinsoft, USA).

Results Total saponin content was estimated at 341 mg/g in YME by gravimetric method. Similar effects were observed *in vitro* and *in vivo*. Compared to the control, methane production decreased significantly from 0.2 g YME/L DM *in vitro* and from 0.525 g YME/goat/day DM *in vivo* ($p<0.05$). Total VFA production (*in vitro*) and concentration (*in vivo*) increased significantly at the highest dosages ($p<0.05$). Ammonia, isobutyrate and isovalerate decreased from the lowest dosages *in vitro* and *in vivo*. On the contrary, the acetate/propionate ratio decreased *in vitro* only. YME significantly decreased feed intake (-8%, $p<0.05$) at the highest dose in goats. The concentration of total urinary purine derivatives was numerically higher (+25%) from 0.525 g/goat/day DM.

Table 1 Rumen fluid parameters

	In vitro (g YME/L DM)					*In vivo* (g YME/goat/day DM)				
Concentration	0.00	0.1	0.2	1.0	s.e.d	0.000	0.525	1.050	5.250	s.e.d
Total VFA (mmol/L)	40.6^{a}	39.2^{a}	41.4^{a}	46.7^{b}	1.0	36.5^{a}	41.8^{ab}	40.7^{a}	58.9^{b}	3.7
Molar proportion (%)										
Acetate	63.2^{a}	63.7^{a}	63.7^{a}	61.0^{b}	0.3	60.1	63.4	63.2	62.8	0.8
Propionate	21.3^{a}	21.6^{a}	22.3^{ab}	26.2^{c}	0.6	15.9	15.7	17.2	16.8	0.5
Butyrate	11.9^{a}	11.6^{ab}	11.3^{b}	10.2^{c}	0.2	12.5	12.0	10.4	13.1	0.5
Isobutyrate	0.8^{a}	0.6^{b}	0.5^{b}	0.4^{b}	0.1	4.7^{a}	3.5^{ab}	3.8^{ab}	3.0^{b}	0.2
Valerate	1.2	1.2	1.2	1.1	0.3	1.9	1.5	1.6	1.5	0.1
Isovalerate	1.2^{a}	1.1^{b}	0.9^{c}	0.8^{d}	0.1	5.0^{a}	3.9^{b}	3.8^{b}	3.0^{b}	0.3
Acetate/propionate	2.97^{a}	2.95^{a}	2.86^{b}	2.33^{c}	0.1	3.85	4.06	3.78	3.79	0.1
NH_3 (mg/100mL)	14.5^{a}	11.3^{ab}	10.1^{b}	10.8^{b}	0.7	21.7^{a}	18.6^{ab}	17.8^{ab}	15.1^{b}	1.2
CH_4 (mmol/day)	0.337^{a}	0.332^{a}	0.322^{b}	0.298^{c}	0.005	798.5^{a}	582.8^{b}	666.8^{ab}	495.8^{b}	42.8
Relative CH_4 *	332.9^{a}	340.5^{a}	310.0^{a}	255.9^{b}	10.7	27.7	22.1	25.3	31.8	1.9

* CH_4/VFA (mmol/mol) *in vitro* and CH_4/feed intake (L/kg) *in vivo*
[a, b, c]: Means in the same row with different superscripts differ ($P < 0.05$ for *in vitro* and $P < 0.10$ for *in vivo* trial)

Conclusions These results show that the concentration in YME to reduce rumen NH_3 is lower than the concentration to decrease CH_4 production *in vitro*. The lowest dose was enough to observe both NH_3 and CH_4 inhibition *in vivo*. Consequently, dose effect was observed only *in vitro* and it could be interesting to evaluate the lowest efficient dose of YME *in vivo*.

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Methane emission by sheep fed tropical grasses

D G Jayme[1], N M Norberto[1,4], G O Ribeiro Junior[1], F S Machado[2], T R Tomich[2], A M Teixeira[1], F O Velasco[1], J A G Azevedo[4], F P Possas[1], F A Magalhaes[1], M N Ribas[1], W Carvalho[1], L G R P Pereira[2,4], L C G Gonçalves[1,4]
[1]Universidade Fedreal de Minas Gerais, Belo Horizonte, MG, Brazil, [2]Embrapa Dairy Cattle, Juiz de Fora, MG, Brazil, [3]Universidade Estadual de Santa Cruz, Ilheus, BA, Brazil, [4]Bolsista de Produtividade do CNPq, Brasilia, DF, Brazil
Email:luiz.gustavo@embrapa.br

Introduction There is a scarcity of published literature regarding the methane emissions by ruminants fed tropical forages. Respiration calorimetry has been a usual method to determine methane. The introduction of calorimetric studies in tropical conditions is important for conceptual advances in roughage evaluation, enabling the best way of utilization, optimizing livestock performance and methane mitigation. The purpose of the present study was to estimate the average impact of species and stages of maturity at harvest of tropical grasses on methane emission by sheep.

Material and methods Data from 213 experimental units in nine calorimetric studies with sheep fed tropical grasses conducted at the Department of Animal Science, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil, were analyzed for average impact of methane emissions. These calorimetric studies evaluated seven tropical grasses (*Andropogon gaianus*, *Brachiaria decumbens*, *Cynodon spp*, *Pennisetum purpureum*, *Sorghum bicolor*, *Sorghum bicolor* x *Sorghum sudanence, Zea mays*) harvested at different stages of maturity (27 to 56, 57 to 84 and 85 to 114 days at harvest). Individual animal emissions (g of CH_4/day and g of CH_4/kg of dry matter intake) were registered using respiration calorimetry method. The equipments and experimental procedures utilized for the respiration calorimetry studies were described by Rodriguez *et al*. (2007). The data were grouped as grass type and stage of maturity and subjected to analysis of variance, followed by Tukey post-test.

Results Intake of different tropical grasses significantly affects methane emission by sheep (see Table 1). The daily emissions ranged from 14.1 g CH_4/day to 19.8 g CH_4/day and emissions per unit of forage consumed was 9.9 to 22.7 g of CH_4/kg of dry matter intake. The daily emissions of methane by sheep fed tropical grasses observed in this study were near the lower emissions that can be calculated from literature data (Czerkawski, 1969; McAllister *et al*., 1996). Furthermore, the wide variation observed in daily emissions or per unit of food consumed depending upon the forage provided to sheep indicates the possibility of developing feeding systems to mitigate methane emissions by animals of this species fed tropical forages.

Table 1 Methane emission by sheep fed different tropical grasses

	Grass								
	Andropogon gaianus	*Brachiaria decumbens*	*Cynodon spp*	*Pennisetum purpureum*	*Sorghum bicolor*	*Sorghum bicolor x sudanense*	*Zea mays*	s.e.m.	P
Methane – g/day	14.1^{bc}	19.5^{ab}	19.8^{a}	16.8^{abc}	14.1^{c}	15.0^{abc}	18.3^{ab}	0.4	< 0.0001
Methane – g/kg of dry matter intake	12.3^{cd}	22.7^{a}	17.5^{bc}	16.9^{bc}	14.5^{cd}	9.9^{d}	19.6^{ab}	0.5	< 0.0001

Values with different superscript letters within the same line significantly differ (Tukey's test, P < 0.1)

The stages of maturity of tropical grasses influenced (P < 0.1) daily methane emission by sheep, highlighting the smaller emission for the animals that consumed forage in intermediate stages of maturity (15.7 g of CH_4/day) compared to those that consumed forage in more advanced stage of maturity (18.3 g of CH_4/day). However, the stages of maturity did not significantly affect methane production in relation to dry matter intake, being observed an overall mean of 18.3 g of CH_4/kg dry matter intake (see Table 2).

Table 2 Methane emission by sheep fed tropical grasses harvest at different stages of maturity

	Stage of maturity (days at harvest)			s.e.m.	P
	27 - 56	57 - 84	85 - 114		
Methane – g/day	18.0^{ab}	15.7^{b}	18.3^{a}	0.4	0.060
Methane – g/kg of dry matter intake	18.2	17.3	19.2	0.5	n.s.

Values with different superscript letters within the same line significantly differ (Tukey's test, P < 0.1)

Conclusions

These results indicate that both type of tropical grass and stage of maturity of tropical grasses influence the methane emissions by sheep. The lowest emission observed for some grasses and stage of maturity indicate to possibility of development of strategies for the best way of utilization of tropical grasses, optimizing livestock performance and promoting strategies to mitigate methane in tropics.

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Short-term emission measurements in beef feedlot cattle to demonstrate enteric methane mitigation from dietary nitrate

J I Velazco[1,4], G Bremner[1], L Li[1], K Luijben[3], R S Hegarty[1], H Perdok[2]
[1]The University of New England, Armidale, New South Wales, Australia, [2]Cargill Animal Nutrition, Velddriel, The Netherlands, [3]Wageningen University, Wageningen, The Netherlands, [4]National Agricultural Research Institute, Treinta y tres, Uruguay *Email:jvelazco@myune.edu.au*

Introduction Methane emissions from ruminants make a significant contribution to anthropogenic greenhouse gas emissions in pastoral countries such as Uruguay and Australia. In those countries cattle are routinely supplemented with non-protein nitrogen (NPN) to promote rumen fermentation and microbial protein synthesis. Nitrate has been identified as an alternative NPN source to urea, offering the additional advantage of reducing enteric methane emissions (Nolan *et al*., 2010). Measurement of enteric emissions in the production environment is difficult, but short-term emission measures show promise in quantifying daily methane emissions. In association with a larger study (Hegarty *et al*., 2013), this investigation aimed to test the ability of short-term emission measurements to detect nitrate-induced methane mitigation in beef cattle using a GreenFeed emission monitoring unit (GF, C-Lock Inc, USA).

Material and methods Composite-breed steers (n=22; 521.3kg initial LW and 32.7kg s.d.) were allocated to two isonitrogenous and isoenergetic feedlot diets based on 70.25% cracked-barley and 8.5% maize silage as feed (13.6% crude protein, 13.1 MJ ME/kg DM) using stratified randomization based on LW. The treatments differed in their NPN source, being either urea (1.00% in DM) or calcium nitrate (1.90% NO_3 in DM) (Hegarty *et al*., 2013). Each treatment was fed to a pen of 11 steers, with the ration provided in auto-feeders (GrowSafe Systems Ltd., Canada) which also recorded individual feed intakes. Animals were acclimated to the rations with inclusion rates of NPN and grain progressively increased. Steers were fed the finisher rations *ad libitum* for a period of six weeks and the GF device was swapped between treatments on a weekly basis (3 x 1 week periods/treatment) to provide estimations of methane and carbon dioxide production. The GF device records gas concentrations when animals voluntarily visit the unit (eructation + breath events are recorded when animals are close and standing in the front of the unit). Animals receive a small food reward when visits the unit (200 grams in average in this case). Data was only accepted when cattle kept their head in the GF for at least 2 minutes to ensure inclusion of adequate eructation events. For statistical analysis, only animals with at least 3 measures in the period were considered. Parameters were estimated methane production (CH_4, g/d), estimated carbon dioxide production (CO_2, g/d), feed intake (kg DM/d), length of the visits at the GF (min) and number of visits (visits/week). Analysis of variance was performed for all repeated measurements. Period and week effect were not significant so were dropped for final analysis.

Results No effect of dietary NPN source on the number (7.54 visits/week) or length (3.37 min) of the visits to the GreenFeed unit was apparent (P=0.626 and P=0.271 respectively). Average daily feed intake (kg DM/d) over the 6 weeks also did not differ (P=0.145; Table 1) between cattle on the urea or nitrate-containing rations. Calcium nitrate tended to be effective in reducing methane emissions relative to when urea alone supplied dietary NPN (P=0.056), but no differences were detected for carbon dioxide emissions (P=0.300). Dietary nitrate led to a significant reduction in the methane yield (g CH_4/kg DMI) of cattle (P<0.05; Table 1)

Table 1 Feed intake, breath emissions and methane yield of cattle offered isoenergetic and isonitrogenous diets containing non-protein nitrogen as only urea or with calcium nitrate

	Urea	Calcium Nitrate	s.e.	P
Intake (kg DM/d)	9.19	10.04	0.332	0.145
CH_4 (g/d)	106.8	86.1	5.53	0.056
CO_2 (g/d)	4455	5005	326.8	0.300
CH4 yield (g CH4/kg DM)	12.62	8.61	0.681	0.014

Conclusions The results show that calcium nitrate was effective in reducing enteric methane production without reducing feed intake, although intake reduction was observed in the larger study (Hegarty *et al*., 2013). Additionally, the short-term measurement technique was able to detect significant nitrate-derived methane mitigation (over a 30% of reduction). Methane yield was low consistent with results reported by Hegarty *et al*., 2007 (through the SF6 technique with high-grain ration). Future research should attempt to evaluate short-term measurements to validate mitigation achieved by other means.

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Bayesian calibration of the Pasture Simulation model (PaSim) to simulate emissions from long-term European grassland sites: a case study at Laqueuille (France)

H Ben Touhami[1,2], R Lardy[1,2], K Klumpp[1], V Barra[2], G Bellocchi[1]
[1]French National Institute for Agricultural Researches, Grassland Ecosystems Research Unit, Clermont-Ferrand, France, [2]Clermont University, Blaise Pascal University, LIMOS - UMR 6158CNRS, Aubière, France *Email:haythem.ben-touhami@clermont.inra.fr*

Introduction The Pasture Simulation model (PaSim, **https://www1.clermont.inra.fr/urep/modeles/pasim.htm**, APP ID: 44 IDDN.FR.001.220024.000.R.P.2012.000.10000), a biogeochemical model to simulate water, energy, C and N cycles, is used to investigate GHG emissions from European grasslands. The model is difficult to calibrate and most of its parameters (~150) are assumed to be fixed and identical for all model realizations. Based on a sensitivity analysis of the model, we determined parameters to be calibrated. The study documents the improvements obtained with Bayesian calibration using long term observations to estimate GHG fluxes (NEE: Net Ecosystem Exchange; CH_4, methane) from two semi-natural grazing systems in Massif Central (France).

Material and methods Based on Ben Touhami *et al.* (2012), the Bayesian approach was used to update prior parameter distribution (expression of current imprecise knowledge about parameter values) to achieve a posterior distribution (expression of reduced uncertainty and more precise parameter values) by incorporating the information contained in the measured data of seven multi-year observational grassland sites in Europe (Amplero, Italy; Bugac, Hungary; Easter-Bush, United Kingdom; Früebüel and Oensingen, Switzerland; Laqueuille intensive and extensive plots, France) mainly derived from the FLUXNET network (**http://fluxnet.ornl.gov**). The nine most relevant PaSim vegetation parameters (chosen from Europe-wide sensitivity analysis) were calibrated using a set of soil (temperature, water content), vegetation (leaf area index, harvested biomass) and atmospheric (NEE) measured variables. The calibrated model was used to assess CO_2 (NEE, g C m^{-2} d^{-1}) and CH_4 (g CH_4-C m^{-2} d^{-1}) fluxes based on the eddy covariance measurements (in place since 2002) of Laqueuille in France (45° 38' N, 02° 44' E, 1040 m a.s.l.). Two paddocks were continuously grazed by heifers from May to October with two management options (Klumpp *et al.*, 2011): the intensive management paddock included significant amounts of N fertilization (three times per year for a total of 210 kg N ha^{-1}) and annual average stocking rate of 1.1 LSU ha^{-1}; the extensive management paddock had no fertilization and 0.6 LSU ha^{-1}.

Results The improvement of simulation after parameters calibration is reflected in the posterior estimates (thanks to maximum likelihood) of NEE and CH_4 daily values, which are closer to observations than using the prior distribution. For NEE from multiple years (2004-2008), regression lines (Figure 1) show the improvement obtained with posterior parameter values (higher R^2; slope and intercept closer to 1 and 0, respectively), with no difference between managements. Daily CH_4 observed values were limited to May-October 2010 in the intensive system. Figure 2 shows the improvement obtained with the posterior parameterization but also that the model is not properly simulating the fluctuation in CH_4 values. It is noteworthy that, with posterior simulation, the system emits enteric CH_4 fluxes in summer because grass biomass is available and grazing may occur. This approximates what happens in reality, which is not the case with prior parameterization.

Figure 1 Scatterplots of simulated (prior [-] and posterior [+]) and observed NEE values (g C m^{-2} d^{-1}) at Laqueuille (2004-2008), with regression lines for extensive and intensive management

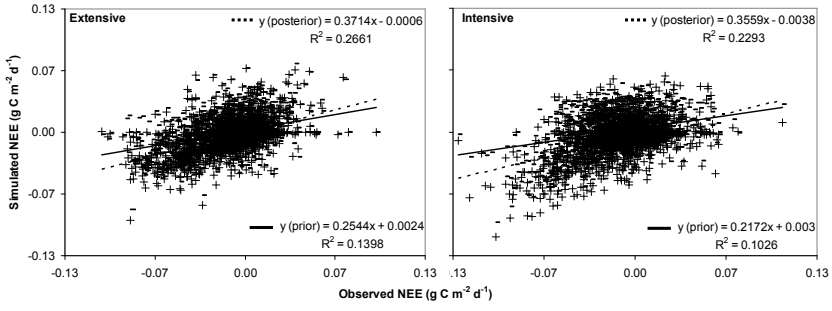
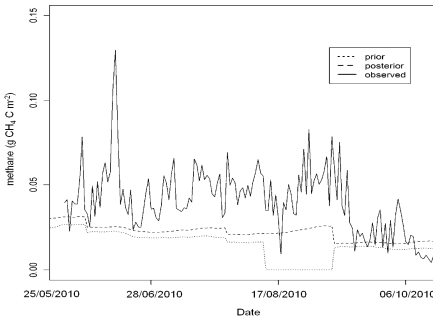


Figure 2 Simulated (prior and posterior) and observed CH_4 values (g C m^{-2} d^{-1}) at Laqueuille (intensive management) over May-October 2010



Conclusions These results show that the parameterization of PaSim obtained via Bayesian calibration at multiple European sites has improved simulation of both CO_2 (NEE) and enteric CH_4 fluxes at Laqueuille, though without compensating for limitations in the model structure. This means that the modelling of GHG fluxes from grasslands merits further investigation. This is a non-trivial task, not only because of unsolved theoretical questions but also because fluxes are affected by large observational uncertainties.

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Effect of tree density on greenhouse gases emissions related to external inputs on agrosilvopastoral systems combining beef cattle, soybeans and Eucalyptus in Central-Brazil

D J Bungenstab, R G Almeida, C Carromeu
Brazilian Agricultural Research Corporation - EMBRAPA, Campo Grande-MS, Brazil
Email:davi.bungenstab@embrapa.br

Introduction Agrosilvopastoral (ASPS) systems are a promising alternative for improving sown pastures and increasing beef production in Central Brazil without clearing new areas and, at the same time, generating other agricultural products like grains and timber as well as several environmental benefits, like carbon sequestration. Regarding greenhouse gases (GHG) balance assessment on these systems, the larger amounts of external inputs used on more intensive systems should not be neglected (Oliveira *et al*., 2009). For this reason this work explores, through a case study, the relation between higher tree densities and GHG emissions as well as embodied energy and shadow areas (carbon footprint) related to external inputs in emerging ASPS, with different tree arrangements, currently recommended for pasture renovation in Central-Brazil,.

Material and methods An ASPS was implemented in December 2008 at Embrapa Beef Cattle Research Centre in Central-Brazil, based on regionally used Brachiaria grass, Nelore breed, soybeans and Eucalyptus trees in three different systems: a) traditional system with plain pasture (S0); b) pasture with 227 trees/ha (S1) and c) pasture with 357 trees/ha (S2). The main goal of the whole trial was to evaluate productive, economic and environmental performance of the systems. For this analysis, amounts of each external industrial input applied in each system were recorded. Carbon content of whole trees after three years was measured. Methodology follows the adapted ecological footprint approach for agriculture, proposed by Bungenstab, 2005, for estimating embodied energy and CO_2-equivalent emissions, having shadow areas as an ecological indicator to compare land use under different intensification levels. For instance, shadow areas correspond to a forest area that would be necessary for carbon sequestration of the equivalent GHG emissions generated by the manufacturing of all production inputs, as well as the emissions from fuels and other energy sources used in the system. This area should be added to the physical area used by the system to calculate its total area appropriation. GHG emissions for each input were obtained from assessments of the manufacturing process or derivate from the embodied energy of each input. Shadow area is therefore the area of forest that would be necessary for permanently sequestering this total amount of carbon equivalent. For this work it was considered that one hectare forest would sequester 6600 kg CO_2 per year.

Results Table 1 shows that total embodied energy increases with intensification especially as a function of higher fertilization rates. The system with 357 trees/ha used in average 41% more energy embodied on external inputs on its implementation than the traditional plain pasture system, reflecting a similar difference on CO_2 emissions and shadow areas.

Table 1 Embodied energy, CO_2-equivalent emissions and shadow areas per hectare on agrosilvopastoral systems with different tree arrangements in Central-Brazil.

System/Indicator	MJ/ha	kgCO_2Equivalent/ha	Shadow area/ha
S0 (traditional – only pasture)	26,686.02	1,636.01	0.25
S1 (227 trees/ha)	34,093.49	2,084.87	0.32
S2 (357 trees/ha)	37,668.74	2,301.45	0.35

Results also show that shadow areas represent a significant part of the total area appropriated by local cattle systems if their ecological footprint is to be considered. They also show that more intensive systems have substantially larger shadow areas. Shadow areas rates per hectare per year will vary according to need of pasture renovation, which is recommended to occur every five years. Experimental results showed that the two systems having trees were able to sequester 13.29 and 24.10 metric tons CO_2 per hectare per year in the 36 months period. Therefore, systems with trees were able to sequester 19.1 and 31.4 times more CO_2 in the period, than they emitted through external inputs used on its implementation. Differently from the traditional plain pasture system, ASPS are able to generate a carbon sink, even if enteric emissions from cattle kept on them is accounted.

Conclusions From this study it can be concluded that emissions related to external inputs on agricultural systems should not be neglected. If converted to area following a carbon footprint approach, they could add as much as 7% in terms of area per year for intensive systems. It can be also concluded that, like on other agricultural systems, fertilizers play a major role as a source of emissions on ASPS, however, these systems are able to compensate, with a large surplus, the emissions from external inputs used on them. This net carbon sequestration represents a potential carbon sink that should be considered for voluntary markets in the future.

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Effect of essential oils and an antioxidant (resveratrol) on in vitro methane production and degradability

C E Souza[1], S L S Cabral Filho[1], S H Kim[2], R Guimarães Junior[3], L G R Pereira[4]
[1]University of Brasília (UnB), Brasília, Distrito Federal, Brazil, [2]Rural Development Administration (RDA), Republic of Korea, Republic of Korea, [3]Embrapa Cerrados (CPAC), Brasília, Distrito Federal, Brazil, [4]Embrapa Gado de Leite (CNPGL), Juiz de Fora, Minas Gerais, Brazil *Email:camila.eds@gmail.com*

Introduction *In vitro* studies have shown that essential oils and antioxidants have antimicrobial properties against ruminal microorganisms producing methane gas (CH_4). This effect might have a positive impact on animal performance by reducing CH_4 emission per unit of animal product. The aim of this study was to assess *in vitro* the effects of essential oils and resveratrol (antioxidant) in two different concentrations on ruminal fermentation.

Material and methods Different feed additives (99.9 % purity) were used to assess the impact on *in vitro* CH_4 yield and degradability of a forage based diet. A sample of *Brachiaria brizantha* and a 22% crude protein concentrate ground to 1 mm weighed in the ratio 80:20 into filter bags (F57 Ankom®) and placed into 100 mL ambar fermentation bottles was used as the control (BC). Other treatments were three essential oils at the concentrations 0.3 and 0.6 mL/L of solution: cinnamon (BCC3, BCC6), oregano (BCO3, BCO6) and garlic (BCG3, BCG6); a plant secondary metabolite and an antioxidant at levels 300 and 600 mg/L of solution: thymol (BCT3, BCT6) and resveratrol (BCR3, BCR6), respectively. The source of the additives was Biomin® e RDA. Incubation medium was prepared according to methodology described by Theodorou *et al.* (1999). Rumen inoculum consisted of a mix from three fistulated adult bovines kept on *B. brizantha* pasture and supplemented with 2 kg/animal/day of a concentrate, the same feeds used as *in vitro* control. *In vitro* gas production was performed by the use of a simple apparatus for measuring gas production through communicating vessels and methane concentration was determined by gas chromatography. Gas samples were collected at 6, 12 and 24 hours after incubation, and the *in vitro* dry matter degradability (IVDMD) determined at 24h. Data were analyzed (ANOVA) as a completely randomized design and means compared by Duncan test at 5% (SAS, 2001).

Results All treatments reduced both the production of methane (VCH_4) as the volume of methane per gram of degraded dry matter (VCH_4/IVDMD) when compared to the control P<(0.05) (Table 1). BCR3 and BCR6 were statistically similar and showed to be the less effective ones regarding to reducing VCH_4 production and VCH_4/IVDMD. BCC6 produced 60% less VCH_4/IVDMD and increased the IVDMD in 34% compared to treatment BCC3 (P<0.05). BCG3 and BCG6 treatments did not differ (P<0.05) for any of the evaluated traits being the same observed for BCO3 and BCO6. BCT3 and BCT6 did not differ (P<0.05) for VCH_4/IVDMD.

Table 1 Volume of methane (VCH_4 - mL), *in vitro* dry matter degradability (IVDMD - %), volume of methane per gram of degraded dry matter (VCH_4/IVDMD – mL CH_4/g IVDMD

Treatment	VCH_4 (mL)	IVDMD (%)	VCH_4/IVDMD (mL CH_4/gIVDMD)
BC	2.69^{A}	50.26^{BCD}	9.95^{A}
BCR3	1.94^{B}	52.81^{ABCD}	7.04^{B}
BCR6	1.86^{B}	48.29^{D}	7.23^{B}
BCC3	1.39^{C}	47.53^{CD}	6.19^{B}
BCG3	1.08^{CD}	58.44^{ABCD}	3.44^{C}
BCC6	0.83^{DE}	63.83^{A}	2.47^{CD}
BCT3	0.79^{DE}	62.78^{AB}	2.34^{CD}
BCG6	0.75^{DE}	61.22^{ABC}	2.30^{CD}
BCO3	0.65^{EF}	64.89^{A}	1.88^{CD}
BCO6	0.34^{F}	66.25^{A}	0.97^{D}
BCT6	0.32^{F}	65.71^{A}	0.89^{D}
CV (%)	17.54	12.09	20.80

Means followed by different letters in the same column differ (P<0.05)

Conclusions Evaluated feed additives decrease *in vitro* CH_4 output and improve the efficiency of use of the diet. Although the treatments with resveratrol have reduced CH_4 production, in general they were less effective in reducing CH_4 compared to the others. The best level of inclusion of cinnamon is 600 mg/L. For the other treatments different levels of inclusion did not cause any benefit regarding diet efficiency of use; nevertheless, the highest level of inclusion of thymol lowered the methane production.

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Farming for a better climate by improving nitrogen use efficiency and reducing greenhouse gas emissions (FarmClim)

B Amon[1], S Zechmeister-Boltenstern[1], M Kaspar[1], J Kantelhardt[1], L Schaller[1], T Moser[1], H Hasenauer[1], E Pötzelsberger[1], M Anderl[2], G Zethner[2], A Baumgarten[3], G Dersch[3], M Prosenbauer[4], B Kitzler[5], E Sigmund[5], W Winiwarter[6]
[1]University of Natural Resources and Life Sciences, Vienna, University of Natural Resources and Life Sciences, Vienna, Austria, [2]Umweltbundesamt GmbH, Vienna, Austria, [3]Austrian Agency for Health and Food Safety (AGES), Vienna, Austria, [4]Chamber of Agriculture of Lower Austria, Sankt Pölten, Austria, [5]Federal Forest Office, Vienna, Austria, [6]University of Graz, Institute for Systems Science, Innovation and Sustainability Research, Graz, Austria
Email:bamon@atb-potsdam.de

Introduction Responding to new challenges, agriculture not only needs to focus on productivity increases but also address environmental concerns. The project FarmClim assesses impacts of agriculture on greenhouse gas fluxes in Austria and proposes measures for mitigating emissions, including their economic assessment. Including stakeholders` views at a very early project state will contribute significantly to closing the science-policy gap in the field of climate friendly farming. The FarmClim consortium comprises the University of Natural Resources and Life Sciences, Vienna, the Austrian Agency for Health and Food Safety, the Austrian Umweltbundesamt GmbH, the Chamber of Agriculture of Lower Austria, the University of Graz and the Research Center Raumberg-Gumpenstein.

Objectives The general objectives of FarmClim are: Optimise N use in Austrian Agriculture; Minimise N and GHG losses to the environment; Identify intervention points in agriculture which are relevant for a general N and GHG strategy; Develop a basis on which guidelines on recommendations for agricultural advisory services on potential optimisation measures and their economic impact can be developed; Close the science-policy gap on the possibilities to optimise N use and minimise GHG losses. FarmClim started in April 2012 and will last until April 2014.

Project layout The tasks of FarmClim are addressed in individual work packages. In attributing parts of the overall work to these work packages, both the respective expertise of partners and the overall project objectives were considered.
First, we address nitrogen and greenhouse gas fluxes in Austrian agriculture, both for animal husbandry and in crop production. As part of these tasks, we not only assess the respective fluxes and possibilities to improve their quantification, but we also provide information on mitigation measures, their efficiency and the related costs of implementation. As a next stage, we use soil modelling to assess the formation of nitrous oxide under specific Austrian conditions, with a scope to provide country-specific emission factors. Mapping these emissions will also allow hot-spots and hot moments to be identified and focus measures towards such high-level situations. The input from previous work packages is used in an economic assessment to determine the economic effects of the mitigation measures proposed, and to compare their diverse effects. In order to allow mitigation measures become accountable under international agreements, the potential of integrating newly derived assessment methodology to reporting obligations, specifically with regard to the Austrian National Inventory Report (NIR) prepared for submission to UNFCCC will be investigated. Emissions reported on the basis of the project results are expected to become more robust and less prone to uncertainties. Finally, in collaboration with an agricultural advisory organization, possibilities for practical application of the recommendations discussed and provided in the project will be examined. The focus here is to liaise the legitimate interest of the farming industry to the requirements of limiting N-related environmental pollution and greenhouse gas release.

Results The first project year focusses on identifying mitigation measures and data input for the economic assessment. With regard to animal husbandry strategies, resulting production levels as well as data on resulting GHG mitigation have been delivered. Three promising mitigation measures in animal husbandry have been identified to undergo a detailed economic assessment: dairy cattle diet, phase feeding for pigs, and anaerobic digestion of animal manures. Data include detailed economic information for further processing. For crop production, upgraded regional yield and N content data of arable crops have been delivered from field experiments. This data will be used to adjust official statistical data which are in use for the Austrian OECD agricultural nutrient balance. This allows to assess the effect of increasing legume crops in crop rotations and reducing fertilizer input on GHG emissions and to derive the economic effects connected with such change.Two model regions have been identified as test regions for soil emission modelling, using the “Landscape DNDC” model. Input data from the first model region - Marchfeld – is being collected from a range of sources. The analysis of present agricultural practices is currently producing its first results, the basis for assessing further mitigation practices specifically focussing on the hot spots and hot moments of nitrogen emissions on a regional scale.
The analysis of adaptation costs with N and GHG mitigation potentials has started. First of all, a format can be demonstrated that provides the most relevant cost factors in a way appropriate for decision makers.
FarmClim recognizes that the effects of all mitigation measures will only come to life if optimisation measures are implemented at farm level. Intensive communication with stakeholders is thus on-going, there is first experience on the process to be reported. Stakeholders’ views and needs are to be integrated into future concepts. The intensive communication with stakeholders from the very beginning of the project is a central feature of FarmClim. It culminates in the final WP7, where recommendations are created that will – after the project end – undergo tests on commercial farms and pass the relevant authorising steps which are necessary for an implementation on commercial farms.

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Methane emissions from alpaca and sheep fed lucerne hay as either chaff or pellets

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

Introduction Methane emission from ruminants is a heritable trait (Pinares-Patiño *et al*., 2011a); hence offering an attractive option for CH_4 mitigation by breeding for low CH_4 emitting animals provided that it has no unwanted effects on production traits. Further, the physiological reasons for between-animal variation within species in CH_4 emissions are poorly understood due to limited variation in digestion parameters. Therefore, this study was conducted using alpaca and sheep, which have known differences in forestomach function (San Martin and Bryant, 1989; Pinares-Patiño *et al*., 2003), as models to elucidate the relationships between forestomach digestion and CH_4 emission.

Material and methods Six male alpacas (2–4 yr old; 64.0 kg mean liveweight; LW) and six cryptorchid sheep (2 yr old; 53.8 kg mean LW) were used in two experiments (E1 and E2). In E1 and E2, the animals were acclimatised for 10 and 7 d, respectively, followed by 8 measurement days. All animals were fed (near *ad libitum*) on chaffed lucerne hay (E1) or lucerne pellets (E2), delivered in two equal size meals (0830 and 1600 h). Feed dry matter (DM) digestibility (DMD) was calculated using 7-d total faecal output. Retention time of feed particles in the forestomach (PFRT) was estimated from a pulse dose of Cr-NDF (Pinares- Patiño *et al*., 2011b). Respiration chambers were used for CH_4 emission measurement (3–4 days). Rumen contents, sampled by stomach tubing (3 h post morning feeding), were analysed for volatile fatty (VFA) acid concentrations. Data were analysed separately for each experimental period. Data for feed DM intake (DMI), CH_4 emission and VFA concentrations were analysed using one-way ANOVA, whereas data for DMD and PFRT were analysed by a t-test.

Results Animal species effects within each experiment are shown in Table 1. The animal species did not differ in their daily DMI when fed on chaffed or pelleted lucerne, but alpaca's feed intakes were more variable and lower on a per LW basis than of sheep. Further, alpaca ate their meals more slowly than sheep (indirect observation). Alpaca had lower CH_4 yields (g/kg DMI) than sheep both on the lucerne chaff and the pellet diets. Feed DMD tended to be higher ($P<0.10$) for alpaca than for sheep on lucerne chaff, whereas on the pellet di*et al*paca had lower DMD than sheep ($P<0.05$). The PFRT tended to be longer ($P<0.10$) in alpaca than in sheep on chaffed diet, but on the pelleted diet the PFRT did not differ between the species. Contrary to what was expected, the pelleted diet yielded longer PFRT than chaffed diet. In both diets, the total concentration of VFA in the rumen of sheep was much higher than in the forestomach of alpaca, but the species did not differ in the ratio of molar concentrations of acetate/propionate (Ac/Pr).

Table 1 Effects of animal species on feed intake, methane emission and forestomach function when fed lucerne chaff or pellets. Values are mean ± s.d.

	Experiment 1 (lucerne chaff)			Experiment 2 (lucerne pellets)		
	Alpaca	Sheep	P	Alpaca	Sheep	P
DMI (g/d)	961±95	965±1	0.920	1377±263	1530±2	0.180
CH_4 (g/kg DMI)	16.7±0.7	20.8±0.6	0.001	13.6±0.5	19.6±0.5	<0.001
DMD (%)	63.2±0.8	61.4±1.8	0.080	57.2±1.3	59.9±2.3	0.020
PFRT (h)	28.4±7.3	23.1±2.7	0.090	30.6±4.7	33.5±4.8	0.370
VFA (mmol/L)	59.1±16.1	99.3±8.3	0.001	77.2±5.7	100.9±3.7	0.030
Ac/Pr (mol/mol)	4.4±0.2	4.0±0.3	0.550	3.2±0.3	3.8±0.4	0.300

Conclusions Results on lucerne chaff, suggest a role of PFRT on DMD, but the lower CH_4 yield in alpaca compared to sheep is contrary to accepted paradigms (e.g., Benchaar *et al*., 2001), suggesting the existence of differential microbial communities in these species. Results on pellets suggest that particle size compromised the forestomach function in both species, but to a major degree in alpaca. Within diets, the differences in total VFA concentration may reflect the effect of feed intake rate (faster in sheep than alpaca).

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Integrating biogeochemical process models with life cycle costing model for quantification of greenhouse gas emissions from US swine production.

J Popp[1], R Ulrich[1], C Li[2], W Salas[3], G Thoma[1], H G Rodriguez[1]
[1]University of Arkansas, Fayetteville, Arkansas, USA, [2]University of New Hampshire, Durham, NH, USA, [3]Applied Geosolutions, Durham, NH, USA *Email:wsalas@agsemail.com*

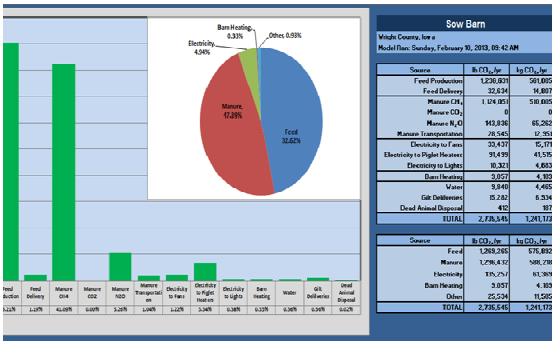
Introduction This poster describes the integration of biogeochemical process modelling with life cycle costing capabilities into a life cycle assessment model of greenhouse gas emissions for US swine production. Combining these tools allows for a sustainable analysis of a process to identify potential production practices which are environmentally friendly and economically feasible.

Material and methods This effort is integrating models (ManureDNDC, Swine Carbon Footprint Calculator, and a Life Cycle Costing model) to develop a decision support tool to better understand the economic and environmental impacts of swine production.

ManureDNDC The DNDC model was developed for quantifying N_2O emissions from agricultural soils in the late 1980s. The first component consists of three sub-models and converts primary drivers (i.e., climate, soil, vegetation and anthropogenic activity) to soil environmental factors (i.e., temperature, moisture, pH, Eh and substrate concentration gradient); and the second component consisting of nitrification, denitrification and fermentation sub-models simulates production/consumption of N_2O, NO, N_2, NH_3 and CH_4 driven by the modeled soil environmental conditions. With the bio-geochemical reactions embedded in the model framework, DNDC can predict the turnover of soil and manure organic matter and the consequent trace gas emissions and nitrate leaching losses. Starting from animal excretion, and during storage and treatment to field application, the manure undergoes decomposition, hydrolysis, ammonification, nitrification, denitrification etc., which have been precisely parameterized in DNDC. Manure-DNDC was created by linking the bio-geochemical processes to the livestock farm components such as feedlot, compost, lagoon, anaerobic digester and field application. Manure DNDC has been tested against datasets of NH_3, CH_4 and N_2O fluxes measured at dairy, swine and poultry farms with encouraging results.

Life Cycle Costs Model Development of the LCC model will take place in five steps. First, the data collection focuses on the cost of the phases and activities above. The relative importance of these costs can fluctuate dramatically given the type of swine operation, geographical location, and costs of major feed ingredients. We will improve the model's cost-estimation routines by collecting location-specific (county level) and time-specific (weekly quarterly or annually, as available) cost data. Data will be collected from several sources including: USDA, the Energy Information Administration, the Farm and Ranch Irrigation Survey, the ARMS Survey private milling companies, private swine barn construction firms, and other sources. Second, these data will be used to develop economic algorithms to calculate the costs of production activities for the farm over a full year. Third, we will integrate all economic components into the GHG model to estimate both sources and amounts of GHG emissions generated and their associated costs. Fourth, we will conduct differential cost analyses by estimating the cost of reducing GHG emissions associated with production activity modifications. In this way, the tool will help users in identifying the relationships between GHG emissions and costs. Finally, we will conduct cost-benefit, risk assessment and optimization analyses by the inclusion of mathematical processes that simulate scenarios hundreds of times and provide ranges of outputs that fall within 95% confidence intervals. While some modification of production activities may reduce GHG emissions, they are expected to increase costs; efforts will be made to determine incentives needed to offset the cost of mitigation strategies.

Swine Carbon Footprint Calculator The model predicts GHG emissions from all parts of a barn-based pig production operation: feed production, feed delivery, barn heating/cooling, fans for cooling and ventilation, emissions of CH_4 and N_2O from manure systems, water, disposal of dead animals, delivery of manure to the fields.



The code is highly detailed, tracking every pig every day. Not an "average animal" approach. For example, the user does not enter the amount of electricity used, the program calculates that for them based on the size and type of operation specified. Predicts usage of feed, electricity, fuels and water; also predicts amount of manure produced. Calculates emissions from all.

Conclusions The final model will be a powerful tool that will allow swine producers to better determine the economic and environmental impacts of their production activities. It will also serve as the foundation for a more detailed LCA and costing being conducted through a grant from the USDA National Institute of Food and Agriculture. This model presents great potential for assisting researchers, policy makers, and especially farm managers in making informed decisions regarding GHG emissions while maintaining production and profitability.

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Win-win mitigations and behavioural change in the livestock sector

D Moran
SRUC, Edinburgh, UK *Email:Dominic.Moran@sruc.ac.uk*

Introduction

Policy discourse on greenhouse gas (GHG) mitigation has been influenced by abatement potentials demonstrated by marginal abatement cost curves (MACCs), which indicate the relative mitigation potential offered by available measures in a specific sector, applied within a time horizon, over and above business as usual activity. Measures are distinguished by the volume of gas (CO2 equivalent) and its cost per tonne. An economically efficient total abatement potential can be established by adding the net contribution of all measures that cost less than a benchmark carbon price (Figure 1).

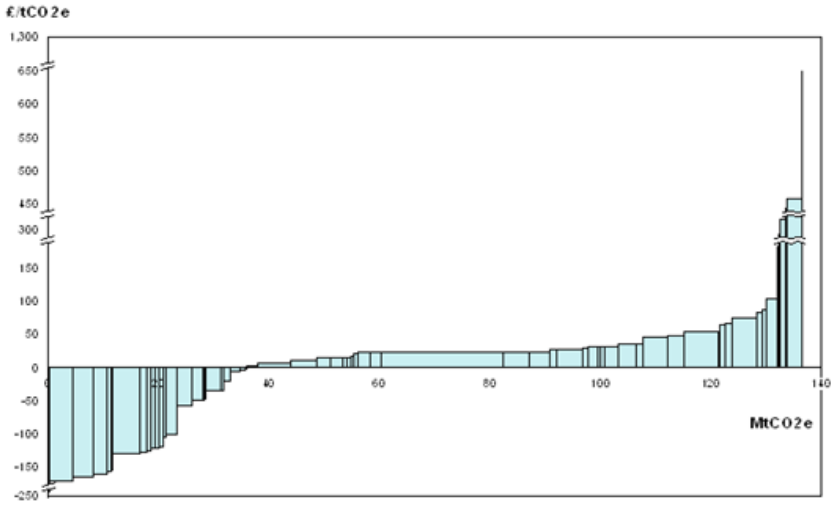

Figure 1 Hypothetical MACC demonstrating mitigation measures differentiated by cost per tonne (height of bar) and quantity (width). Cost-effective measures are those below a given carbon price and include the win-win measures below the x axis.

There are now three fairly comprehensive MACC studies covering measures in the UK, Ireland and France. A compelling observation emerging from them is the opportunity for exploiting so-called 'win-win' measures, which are emission-reducing as well as cost-saving (see left hand side of figure 1). For example, genetic improvement, improved fertility and the use of ionophores. Win-wins present a policy challenge since paradoxically they are often not adopted. and this inertia highlights the deficiency of MACC analysis in converting technical or theoretical potentials (i.e. what could happen) into actual mitigation action through the adoption of largely voluntary measures.

Materials and methods

Drawing on these studies, this paper outlines methodological and behavioural and institutional reasons to explain the non-adoption of livestock mitigation win-wins, thereby preventing the fullest extent of mitigation potential being realised in the livestock sector globally.

Results

Policy options to remove barriers will be considered in the presentation. These options include support to specific research activities to reduce policy uncertainty around key mitigation measures.

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Livestock greenhouse gas emissions from four countries in South East Asia; a collaborative approach to a regional problem

V Hatton[1], K Boonyanuwat[2], L V Kinh[3], S Sithambaram[4], Y Widiawati[5]
[1]New Zealand Agricultural Greenhouse Gas Research Centre, Palmerston North, New Zealand, [2]Department of Livestock Development, Bangkok, Thailand, [3]Institute of Agricultural Sciences of South Viet Nam, Hochiminh, Viet Nam, [4]Malaysia Agriculture Research and Development Institute, Kualalampur, Malaysia, [5]Central Research Institute for Animal Science, Jakarta, Indonesia *Email:victoria.hatton@nzagrc.org.nz*

Introduction A pilot project to develop, for the South East Asia region, a full understanding of the diversity of livestock systems and from that identify priority areas for improving the quantification and mitigation of non CO_2 GHG emissions has been sponsored by the New Zealand government in support of the Livestock Research Group (LRG) of the Global Research Alliance on agricultural greenhouse gases. Livestock production constitutes an integral component of the agriculture sector, major source of food production with consequential GHGs emissions.

Material and Methods

1. Describe the key livestock production systems and associated emissions in South East Asia (Indonesia, Malaysia, Thailand, and Vietnam).
2. Analyse the data set to identify common and, where relevant, country-specific priority areas for improvement of emissions estimates.
3. Identify specific and realistic steps by which livestock emissions inventories can be improved or modified to better reflect regional systems and practices for the identified priority areas and to reduce biases and uncertainties in regional emissions estimates.

Results The study presents a detailed inventory of GHG emissions from different species of livestock categories estimated using the emission coefficients based on IPCC 1996 guidelines. The total CH_4 emission including enteric fermentation and manure management of livestock was estimated at 2.29 Mt/year for the year 2011. Enteric fermentation contributes 80.5 % of the total CH_4 emissions. Non-dairy cattle including native cattle, beef cattle, and non-milking cattle contribute 68.9 % of the enteric CH_4 emissions, and 57.3 % of total CH_4 emission. The total nitrous oxide emission for the year 2011 is estimated at 6.81 Mt/year, with 97.0% contribution from solid storage and dry lot manure management. The total GHGs emission from livestock is estimated at 2,166.00 Mt in terms of CO_2 equivalent emissions. Although the livestock contributes substantially to the CH_4 budget, the per capita emission is only 5.49 kg CH_4/year. The patterns observed in livestock production indicated the countries with high GHG emissions. This project identifies key focus areas for further research to mitigate GHG emissions from the livestock sector in these countries.

Table 1 Methane production from domestic animal

Livestock Type	Number of Animals	Emissions from Enteric Fermentation (Mt/yr)	Emissions from Manure Management (Mt/yr)	Total Annual Emissions from Domestic Livestock (Mt/yr)
Dairy Cattle	1,160,906	0.065	0.025	0.090
Non-dairy Cattle	28,906,969	1.272	0.042	1.314
Buffalo	5,384,796	0.296	0.015	0.311
Sheep	12,048,945	0.060	0.002	0.062
Goats	19,147,379	0.096	0.003	0.099
Camels	177	0.000	0.000	0.000
Horses	504,663	0.009	0.001	0.010
Mules & Asses	1,945	0.000	0.000	0.000
Swine	47,432,013	0.047	0.309	0.357
Poultry	2,463,603,866	0.000	0.049	0.049
Totals		**1.846**	**0.446**	**2.292**

Table 2 N_2O production from domestic animal

Animal Waste Management System (AWMS)	Nitrogen Excretion $Nex_{(AWMS)}$ (Mt N/yr)	Total Annual Emissions of N_2O (Mt)
Anaerobic lagoons	0.238	0.030
Liquid systems	3.976	0.023
Daily spread	0.080	-
Solid storage & dry lot	3.923	6.609
Pasture range and paddock	1.251	-
Other	0.096	0.146
Total	**9.393**	**6.808**

Conclusions The total GHG emissions from livestock across the s.e. Asia region is estimated at 2,166.00 Mt in terms of CO_2 equivalent emissions. Although livestock contributes substantially to the methane budget, the per capita emission is only 5.49 kg CH_4/year. The next steps are to identify how livestock emissions inventories can be improved or modified to better reflect regional systems and practices for the identified priority areas and to reduce biases and uncertainties in regional emissions estimates mitigation measures.

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Repeatability and between cow variability of enteric CH_4 and total CO_2 emissions

P Huhtanen, S J Krizsan, M Hetta, H Gidlund, E H Cabezas Garcia
Swedish University of Agricultural Sciences, Umeå, Sweden *Email:pekka.huhtanen@slu.se*

Introduction Enteric methane (CH_4) emissions from ruminant livestock make significant contribution to global greenhouse gas flux. Several dietary and animal factors influence CH_4 emissions and different mitigations strategies have extensively been investigated during the last decade. Recently, there has been increasing interest to reduce CH_4 emission by animal breeding relating animal genome to rumen microbiome, feed efficiency, and methane emissions. Methane emissions could also be reduced indirectly by breeding the animals for improved feed efficiency. Understanding the relationship between rumen microbiome and CH_4 emissions accurate, repeatable and cost-effective measurements of CH_4 emissions from large number of animals are needed. The objectives of this paper were to investigate between animal variability and repeatability of CH_4 and carbon dioxide (CO_2) emissions in dairy cows in practical production environment.

Material and Methods Measurements CH_4 and CO_2 emissions were conducted in two feeding experiments (Exp.) in dairy cows fed total mixed rations based on grass silage. In Exp. 1 rapeseed and soybean meals were compared at four isonitrogenous levels in a cyclic change-over design with 7 diets, 4 periods and 4 blocks of 7 cows. Measurements of gas emissions were made during the two last experimental periods. Exp. 2 two concentrates were compared in a 3 period switch-back design with 16 cows. Gas emissions were measured using a new standardized technique called GreenFeed (C-Lock Inc, Rapid City, South Dakota, USA) during 7 (Exp. 1) and 14 last days of the period (Exp. 2). Feed intake was automatically recorded. The data was analysed by GLM model: Y = Animal + Period + Diet + Residual. Residual coefficient of variation (CV) was calculated as root mean square error divided by mean. Repeatability (R) was calculated as $R = \delta^2_{Animal} / (\delta^2_{Animal} + \delta^2_{Residual})$. In addition to R measurements, the cows were ranked to the lowest and highest according the first period CH_4 emissions (g/kg dry matter intake; DMI) to evaluate persistency of the differences.

Results and discussion The data of intake, milk yield and gas emissions are presented in Table. Methane emissions were about 6.5% of gross energy intake, which is consistent in respiration studies in dairy cows fed diets based on high quality grass silages. Variability between animals was smaller for CH_4 emissions than for DMI and milk yield. Part of the variation in total CH_4 emission is due to differences in intake and experimental diet. When the values of CH_4/DMI were adjusted for the effects of diet, period and DMI per kg body weight (BW) CV reduced to 8.0 and 9.8% in Exp. 1 and Exp. 2, respectively. Repeatability of CH_4 and CO_2 measurements were high, close to those of intake and milk yield. The difference between the lowest and highest quartile in CH_4/DMI adjusted to constant DMI/BW was approximately 20%, and the differences between low and high emitters remained rather constant between the periods. Variation in CH_4/CO_2 was small and the ratio was poorly correlated to CH_4/DMI. Relatively high repeatability of CO_2 emissions per DMI and ECM suggests that the system has a potential for ranking the cows for feed efficiency and energy utilization. Residual standard deviation in gas production variables was similar to intake and milk production suggesting significant diet effects can be detected with reasonable number of animals.

Table 1 Mean values, variability and repeatability of feed intake, milk production and gas emissions

	Item	DMI[1] (g/d)	Milk (kg/d)	CH_4 (g/d)	CH_4/DMI (g/kg)	CH_4/ECM[2] (g/kg)	CO_2 (g/d)	CO_2/DMI (g/kg)	CO_2/ECM (g/kg)	CH_4/CO_2 (g/kg)
Exp. 1	Mean	20.0	26.3	455	23.0	16.7	11381	575	418	40.0
	CV[3] (%)	12.1	19.0	10.8	9.8	15.0	8.1	9.4	16.1	5.7
	Residual CV (%)	5.7	5.8	6.6	4.4	5.9	3.7	4.2	6.5	4.6
	Repeatability	0.78	0.91	0.64	0.78	0.84	0.80	0.77	0.84	0.34
Exp. 2	Mean	21.3	27.6	453	21.4	15.4	12337	585	418	36.7
	CV (%)	14.9	18.6	12.4	14.2	26.1	11.1	14.3	24.2	6.6
	Residual CV (%)	3.2	3.5	3.7	4.8	5.0	3.7	4.6	5.5	1.9
	Repeatability	0.87	0.88	0.81	0.77	0.80	0.83	0.77	0.77	0.90

[1]DMI = dry matter intake; [2]ECM = energy corrected milk; [3]CV = coefficient of variation

Conclusions Methane emissions measured by the GreenFeed system were similar to values derived from respiration chambers in cows fed similar diets and between animal variability was also within the range observed in respiration chambers. Repeatability of both CH_4 and CO_2 measurements was high when the measurements were made during 1-2 week periods. The system has potential to rank the animals according to CH_4 emissions and energy efficiency cost-efficiently in normal production environment.

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Methylotrophic methanogenic Thermoplasmata implicated in reduced methane emissions from bovine rumen

M Poulsen[1], C Schwab[2], B B Jensen[1], R M Engberg[1], A Spang[2], N Canibe[1], O Højberg[1], G Milinovich[2], L Franger[3], C Schleper[2], W Weckwerth[3], P Lund[2], A Schramm[4], T Urich[2]
[1]Department of Animal Science, Aarhus University, Tjele, Denmark, [2]Department of Genetics in Ecology, University of Vienna, Vienna, Austria, [3]Department of Molecular Systems Biology, University of Vienna, Vienna, Austria, [4]Department of Bioscience, Aarhus University, Aarhus, Denmark *Email:morten.poulsen@agrsci.dk*

Introduction Enteric methane (CH_4) production by ruminants is a major source of anthropogenic CH_4 emissions. The CH_4 is derived from complex anaerobic degradation of plant biomass by the rumen microbiota, the terminal group being methanogenic archaea. The methanogens have been targets of a plethora of methane mitigation strategies, often aiming at reducing concentrations of H_2; the major energy source of most rumen methanogens known to date. However, not all rumen archaea are yet physiologically characterized. Using a metatranscriptomic approach the present study investigated the effect of dietary manipulation, aiming at reducing enteric CH_2 production, on the active rumen microbiota.

Materials and methods Four rumen-fistulated Holstein dairy cows were fed a control diet (total fat: 3.5% DM) and a rapeseed oil (RSO)-supplemented diet (total fat: 6.5% DM), in a conventional 4×2 cross-over design (three weeks per feeding period). One cow was excluded from the full experiment due to health considerations not related to the diets. Methane emission from the cows was quantified at the end of each feeding period in transparent polycarbonate chambers (Hellwing *et al*., 2012). Concomitantly, rumen fluid was sampled from the top and bottom part of the frontal, mid and distal section of the rumen (25 ml per section), pooled, physically disrupted to release microorganisms from organic matter and filtered (pore size: 0.5 mm). Total nucleic acids were extracted using a standard phenol-chloroform bead-beating procedure. DNA was removed and double-stranded complementary DNA (ds-cDNA) was synthetized from purified RNA. The ds-cDNA was sequenced using the Illumina HiSeq2000 system (≈160bp; paired-end sequenced from 2×100bp read lengths). Concatenated small subunit (SSU) rRNA reads were analyzed according to Urich *et al.* (2008) and putative messenger RNA (mRNA)-related reads were analyzed by Meta-Genome Rapid Annotation using Subsystems Technology, v. 3.1.2. (MG-RAST) with subsystem-based annotation based on the SEED database and with MEGAN4 analysis of BlastX searches against the Genbank Refseq protein database (e-value cut-off $1e^{-5}$).

Results RSO significantly reduced CH_4 emission from the cows, resulting in a 6.2% lower CH_4-to-CO_2 emission ratio ($P<0.05$). Illumina deep sequencing resulted in ~10-12 Million high-quality reads per sample, of which putative mRNAs comprised between 380,000 and 485,000 reads. Analysis of SSU rRNA reads revealed that archaea were significantly decreased upon RSO supplementation, whereas bacterial and eukaryotic taxa were basically unaffected. The decrease of archaea could be assigned to a novel group of methanogens distantly related to Thermoplasmata ("Rumen Cluster C", RCC) (Fig. 1A). In contrast to well-known hydrogenotrophic methanogens (Fig. 1B and D), the novel RCC methanogens decreased in abundance and activity upon RSO amendment, as illustrated by the 16S rRNA and methyl-coenzyme M reductase subunit α (*mcrA*) transcript levels (Fig. 1A and C). mRNAs of enzymes involved in methanogenesis from methylamines (MA), only distantly related to known variants from methanogens, were among the most abundant archaeal transcripts. These transcripts, decreased in abundance upon RSO amendment (Fig. 1E), concurrently with 16S rRNA and *mcrA* of RCC, making the RCC methanogens the likely origin of these mRNAs.

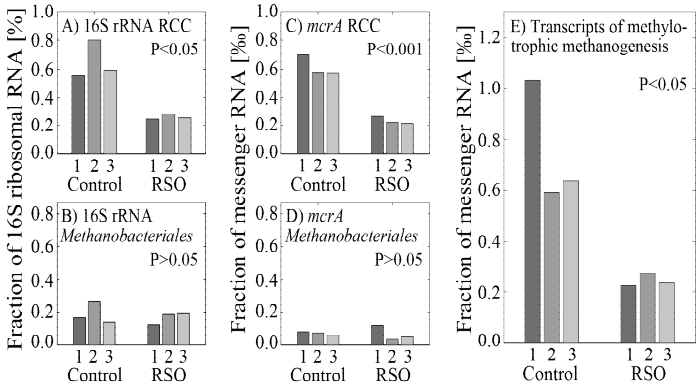

Figure 1 Effect of Control and RSO diets on fraction of archaeal 16S SSU (A, B), on *mcrA* gene transcription (C, D), and on transcription of genes related to methane production from methylated compounds (E) in three cows (1, 2 og 3). P-values below 0.05 indicate significant differences between diets. Modified from Poulsen *et al*. (2013).

Conclusions Metatranscriptomic analysis enabled *in situ* characterization of a novel, yet uncultured group of rumen methanogens. These RCC methanogens are putative methylotrophic archaea, and the second order of methanogens able to utilize methylamines. The RCC methanogens may potentially be a key target for strategies to mitigate CH_4 emissions from ruminant livestock.

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Methane emissions from enteric fermentation and manure management of high yielding dairy herds in Saudi Arabia

A A Aljaloud[1], T Yan[2], A M Abdukader[1]
[1]National Centre for Agriculture Technologies, King Abdulaziz City for Science and Technology, Riyadh 11442, Saudi Arabia, [2]Agri-Food and Biosciences Institute, Hillsborough, Co. Down, UK *Email:tianhai.yan@afbini.gov.uk*

Introduction Specialized dairy enterprises started in Saudi Arabia over 30 years ago by importing high yielding Holstein dairy cows from abroad. These high genetic merit dairy herds are managed under intensive feeding systems with dry lucerne as the main source of forage produced locally and concentrate meals imported from abroad. The objective of the present study was to develop CH_4 emission inventories from enteric fermentation and manure management for this group of animals in Saudi Arabia from 2001 to 2010.

Material and methods Cattle population and milk production data for high yielding dairy herds in Saudi Arabia were collated from the national statistical books from 2001 and 2010 published by Ministry of Agriculture of Saudi Arabia. Information of feeds, feeding regime and manure management was obtained through farm survey and advice of animal production experts. The principle of Tier 2 methodology of IPCC (2006) was used to develop CH_4 emission inventories according to animal physiological states, namely cows, heifer of less than 1 year old and over 1 year old. Enteric CH_4 emissions were calculated from collated data (live weight, milk production/growth rate, dairy energy concentration and calving rate), and default data (e.g., CH_4 energy output as a proportion of GE intake) of IPCC (2006). The majority of manure in dairy farms in Saudi Arabia is managed under the solid storage system. Methane emissions from manure management were estimated from OM output in faeces using collated data and default factors of IPCC (2006).

Results The results of the present study for the high yielding herd in Saudi Arabia are presented in Table 1. There was a dramatic increase, from 2001 to 2010, in populations of cows (85%) and heifers with age of less than 1 year old and over 1 year old (60%). The same period also saw an increase in annual milk yield per cow (18%), which was due to the genetic selection of dairy cows. High yielding cows normally have a large body size and a high growth rate in comparison with low/medium yielding cows. They thus need more nutrient consumption to meet requirements for maintenance and production (milk and live weight gain), consequently producing more CH_4 from enteric fermentation and manure management. The increases in both sources of emissions from 2001 to 2010 were quantified to be 13.0, 8.2 and 7.5% for cows, heifer of less than 1 year old and over 1 year old, respectively. Therefore, total CH_4 emissions from enteric fermentation and manure management for the whole high yielding dairy herd in Saudi Arabia increased from 14757 to 29693 t/y during the 10 year period with an average annual growth equivalent to about 8.19%.

Table 1. Methane emissions from enteric fermentation and manure management of high yielding dairy herds in Saudi Arabia

		2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Dairy cows											
	No of cows	86895	87830	94369	101293	105759	114477	129333	147648	157081	160493
	Milk yield (kg/head/y)	8743	9685	9362	8811	9817	9279	8721	9560	9891	10294
	Enteric CH_4 (kg/head/y)	125.0	133.0	130.8	126.8	135.3	131.4	127.3	134.5	137.6	141.3
	Manure CH_4 (kg/head/y)	9.3	9.9	9.7	9.4	10.0	9.7	9.4	10.0	10.2	10.5
Heifers < 1 year old											
	No of heifers	43573	47099	43320	50188	44155	57971	69570	76986	82729	69879
	Enteric CH_4 (kg/head/y)	27.4	27.6	27.9	28.1	28.4	28.6	28.9	29.1	29.4	29.6
	Manure CH_4 (kg/head/y)	2.6	2.6	2.6	2.7	2.7	2.7	2.7	2.8	2.8	2.8
Heifers > 1 year old											
	No of heifers	35651	38536	35444	41063	36127	47430	56921	62988	67687	57173
	Enteric CH_4 (kg/head/y)	46.6	47.0	47.4	47.7	48.1	48.5	48.9	49.3	49.7	50.1
	Manure CH_4 (kg/head/y)	3.4	3.5	3.5	3.5	3.6	3.6	3.6	3.6	3.7	3.7
Total CH_4 emissions from national dairy herds											
	Enteric CH_4 (t/y)	13716	14790	15228	16213	17298	19000	21259	25203	27404	27604
	Manure CH_4 (t/y)	1041	1123	1154	1231	1308	1443	1617	1915	2082	2089
	Enteric+manure CH_4(t/y)	14757	15914	16382	17444	18606	20442	22876	27118	29486	29693

Conclusions. Methane emissions from enteric fermentation and manure management for the high yielding dairy herd in Saudi Arabia have been developed. These data provide useful information for the relevant authority in Saudi Arabia to develop appropriate mitigation strategies to reduce carbon footprint from dairy production systems.

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Greenhouse gas impacts of interventions to improve cattle health using environmental life cycle assessment

J C Chatterton[1], A G Williams[1], G Hateley[2], A Curwen[3]
[1]Cranfield University, Bedfordshire, UK, [2]Animal Health and Veterinary Laboratories Agency (AHVLA), Yorkshire, UK, [3]XLVet UK Ltd, Suffolk, UK *Email:j.chatterton@cranfield.ac.uk*

Introduction It is well established that deterioration in cattle health leads to losses of productivity, both in terms of milk and meat production (Bennett, 2003). Mitigation measures themselves may cause additional GHGE, e.g. medicine production and delivery. The research question is can productivity gains brought about by improving cattle health lead to an overall reduction in GHGE? This research considered the impact of 10 conditions affecting dairy cattle in the UK, including lameness, mastitis, liver fluke and Johne's disease. The study combined environmental life cycle assessment (LCA), veterinary data and experience and economics to assess the economic cost and GHGE of cattle diseases and interventions to mitigate the ill-effects. This paper focuses on the estimation of GHGE associated with each condition and the impact of individual and consolidated mitigation methods.

Methods GHGE were assessed using the Cranfield systems-based LCA model (Williams *et al.*, 2006). This produces an estimate of GHGE (normalised as global warming potential, GWP) associated with the production of a commodity, accounting for all inputs and outputs crossing a defined system boundary. All is related to the functional unit of output, either 1000 L milk or 1000 kg expected edible carcase weight of beef and the system boundary was cradle to farm-gate. Taking a systems approach enables the production of a commodity to be based on inputs from a range of different production systems and the sensitivity of input parameters such as mortality rates and calving interval to be individually adjusted. Each condition was considered in turn, using veterinary statistical data and literature to estimate impact and prevalence. Productivity losses, such as reduction in milk yield, increased mortality and extension to calving interval were modelled as scenario variables to find the impact of each condition. A comprehensive review of both interventions and effectiveness was provided by *XLVets*, ranging from veterinary interventions (e.g. vaccination) to civil engineering methods, such as limestone tracks to reduce lameness incidence. Any additional GHGE from the interventions were included, e.g. fuel for additional vet visits, extended dry periods, manufacturing medicines or extra forage purchases if wet pastures are fenced to prevent liver fluke.

Results GHGE per unit milk produced are lower with "naturally" healthy cows than the current national herd (Table 1). The full disease impacts increase GHGE by up to 210 kg CO_2e per 1000 L. Interventions increase GHGE by only about 5 kg CO_2e per 1000 L, but deliver net reductions for fully impacted cows of 20 to 200 kg CO_2e per 1000 L. The absolute differences between the baseline and treated animals are relatively small in all cases, but the health and welfare of the cows is better. These values only address the milk output from dairy cows. In all cases, obligatory culling and mortalities are reduced so that dairy cows can produce more calves for beef production. These will incur lower breeding overheads than suckler cows and further help reduce emissions per unit output from cattle production. A major point is that the interventions themselves cause very small net effects on GHGE and so should not be a deterrent on environmental grounds. Various assumptions about farmer responses to loss of milk yield and further work is needed to validate decision making by farmers when informed about the effects of interventions on the carbon footprint of their milk.

Table 1 Effects of impacts and interventions on GHGE for milk production (kg CO_2e/ 1000 L) for four conditions

Condition	Current performance	With healthy cows	With full disease impacts	With full disease impacts and all mitigation interventions, if no recovery	With all mitigation interventions and expected recovery
Lameness	950	890	960	970	920
Liver fluke	950	890	990	990	920
Mastitis	950	890	950	960	930
Salmonella	950	890	1100	1100	940
BVD	950	890	1100	1100	900
Johne's disease	950	890	1100	1100	900

Conclusions For all conditions, veterinary or managerial interventions produced lower GHGE per unit output than from the current herd, but were higher than from healthy cattle. Differences were sometimes marginal, but the GHGE costs of interventions that promote cattle health and welfare are low.

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