Milk carbon footprint in French dairy systems

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Introduction In today's environmental context, it becomes crucial to quantify precisely the levels of greenhouse gas emissions (GHG) for different ruminant livestock systems by using Life Cycle Assessment (LCA). The task is to know the levels of GHG and carbon sequestration, and to identify the sources of these emissions to propose mitigation techniques. Focus on 214 dairy farms, this study aims to determine the GHG emissions and the milk carbon footprint at farm scale of the various French production systems.

Materials and methods A methodology based on life cycle assessment has been built to assess the GHG emissions for dairy farms. Following the LCA methodology and IPCC recommendations, the French Livestock Institute developed a Tier 3 methodology adapted to the dairy sector. This methodology proposes methods of evaluation and emission factors specific to the French territory: soil and climate conditions, animal and crop production techniques, French energy mix, origin of inputs adapted to the supply of French agricultural holdings (Doreau *et al* 2011). On the boundary from cradle to the farm gate, an inventory has been made of the direct emissions related to the on-farm production process together with the indirect emissions inherent to the inputs. Beyond the GHG emissions linked to production processes, carbon fluxes related to land use change, have been also considered in the GHG assessment. These carbon sequestration or GHG emissions related respectively to the storage or release of carbon must be assessed. This is particularly the case of imported feeds (soybean) and for feed produced in French farms following land conversion (grassland…). Furthermore, in "stabilized" situations without significant land use changes, several publications (Soussana *et al* 2007) highlight an annual carbon flux on permanent grassland areas over the long term. The GHG balance considering emissions and carbon sequestration allows determining the net emissions on a dairy unit. To allocate the burdens between the two products produced in dairy system (milk and meat), the biophysical allocation rule has been retained. This allocation, based on the technical functioning of the production system, consists to separate energy needed for dairy cows and heifers. It is considered that the total energy of the feed intake by cows is needed to produce milk and the total energy needed by heifers for their growth is to produce meat.

Results For the 214 dairy farms from the French Breeding Network database, the average gross carbon footprint is 0.94 kg CO_2/kg milk (table 1). These emission levels are coherent with the bibliography which reveals gross carbon footprints ranging from 0.8 and 1.5 kg CO2 per liter milk (Cederberg *et al* 2004). Carbon sequestration under grassland and hedges compensates for GHG emissions ranging from 10 to 49% according to the permanent grassland proportion in the system. Consequently the average net carbon footprint is 0.64 kg CO_2 / kg milk. Somme differences are observed on gross carbon footprint between the production systems but the highest difference concern the net carbon footprint in relation with grass vs. maize area in the dairy unit. The high methane contribution in the milk carbon footprint (63%) is linked to the preponderant share of enteric fermentation (69% of CH_4 emissions). The other emissions are divided between nitrous oxide (17%), influenced by emissions during grazing, representing 41%, and carbon dioxide (20%), produced by on farm fossil energy combustion and the impact of inputs. Within this population of 214 farms, this assessment shows variability in the inter-system carbon footprint but even more specifically show significant variability in the optimization of practices. The differential observed can be as much as 30% between optimized and non-optimized farms. The variations in carbon footprint are strongly related to herd management (herd turnover, feed strategy…) and crop production practices (fertilization, manure management…). This explains why the most optimized farms which have the lowest consumption of concentrates per liter of milk but also the most efficient nitrogen balances are the most favorable environment friendly.

Table 1 Milk carbon footprint on 5 French dairy systems (kg CO_2/kg milk)

	Plain system			Mountain system		
	> 30 % maize	10 – 30 % maize	< 10 % maize	maize	grass	Total
Number of farms	38	45	37	27	67	214
Gross carbon footprint	0.88	0.92	0.93	0.94	1,00	0.94
Carbon sequestration	0.09	0.19	0.38	0.17	0.50	0.30
Net carbon footprint	0.80	0.73	0.55	0.76	0.51	0.64

Conclusion This study highlights the differences observed between farms on the milk carbon footprint and the link between environmental issues and practices. Carbon sequestration has considerable potential to abate GHG emissions in grassland livestock systems. These observations emphasize some ways of mitigation adapted to the different production systems. Some of them concern management practices (dietary intake, fertilization, grassland management, etc.) which result in substantial savings in agricultural expenses. Others require installation of new technologies which would need additional funds to improve the production processes.

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The effect of lauric acid on methane production and cell viability of Methanobrevibacter ruminantium

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Introduction Lauric acid (C_{12}), a saturated fatty acid, was reported to be a potential inhibitor of ruminal methanogenesis *in vitro* (Dohme *et al*. 2001) and, as the main component of coconut oil, *in vivo* (Hollmann *et al*. 2012). Non-ruminal methanogens (Zeitz 2011) are inhibited by C_{12} as well. However, information about the effect of C_{12} on individual ruminal methanogen species and the way it exerts its influence in the absence of further influencing factors, like fibrolytic bacteria and feed, is limited. This is very important to be able to differentiate direct and indirect C_{12} effects on methanogens and to identify the inhibitory mechanisms.

Material and methods The effects of C_{12} on methanogenesis, cell membrane permeability and cell viability were tested in cell suspensions of the rumen archaea *Methanobrevibacter ruminantium* (DSM 1093). A pure culture of *M. ruminantium* was anaerobically cultivated in the strain-specific cultivation medium (No. 119 of DSMZ, the German Collection of Microorganisms and Cell Cultures) in an incubator shaker at 37°C with a shaking speed of 150 rpm. The culture was harvested anaerobically in the mid exponential growth phase, the cell pellet was washed twice with an autoclaved K-free phosphate buffer containing: 0.025 M $Na_2NH_4HPO_4$, 0.025 M $NH_4H_2PO_4$, 0.01 M NaCl, 1 mM $MgCl_2$, 0.5 mM Ticitrate, and was resuspended in the same buffer to reach a final cell dry matter concentration of 6 mg/mL. C_{12} was dissolved in DMSO and supplemented into the 1-ml cell suspensions in 25-mL serum bottles to reach concentrations of 10, 15 and 30 µg C_{12}/mL. Suspensions supplemented with DMSO only served as control group, and suspensions without any supplementation served as blank. The reaction started when cell suspensions, gassed with a H_2:CO_2 mixture (80:20) to 1.5 bar, were incubated in a water bath at 37°C shaking at 150 rpm. After 3 and 24 h, 150 µL of gas from the head space was withdrawn for CH_4 detection. The CH_4 production was calculated according to the ideal gas law. After 3 and 24 h, 100 µL of cell suspension was treated with the LIVE/DEAD® BacLight™ Bacterial Viability Kit for microscopy and quantitative assays, and green (living) and red (dead) stained cells were specified under a fluorescence microscope. The proportion of living cells of total cell counts was defined as cell viability. Besides, 300 µL of cell suspension was centrifuged and the extracellular K concentration was detected in the supernatant by Inductively Coupled Plasma - Optical Emission Spectrometry. The experiment was performed in triplicate. Analysis of variance was performed using SAS with treatment group as fixed and replicate as random factor.

Results C_{12} addition inhibited the CH_4 production rate (µmol/mg cell DM/min) by *M. ruminantium* in a dose-dependent way and it started exerting its influence immediately (Figure 1A). While concentrations of 10 µg C_{12}/mL markedly decreased the CH_4 production rate, 15 and 30 µg/mL stopped methanogenesis completely after 3 h. Consistent with the pattern of methanogenesis inhibition, a fast increase in extracellular K concentration occurred with 15 and 30 µg/mL of C_{12} after 3 h of incubation, and this effect did not increase further as reaction time progressed (Figure 1B). After 24 h, extracellular K was higher in all treated groups than in the control and blank groups. The effects on cell viability after 3 h and 24 h are shown in Figure 1C. Although methanogenesis was completely inhibited and marked K leakage occurred in groups treated with 15 and 30 µg C_{12}/mL, not all cells were dead after 3 and 24 h. However, cell viability was reduced compared to the control. With 10 µg C_{12}/mL, cell viability was only partly reduced.

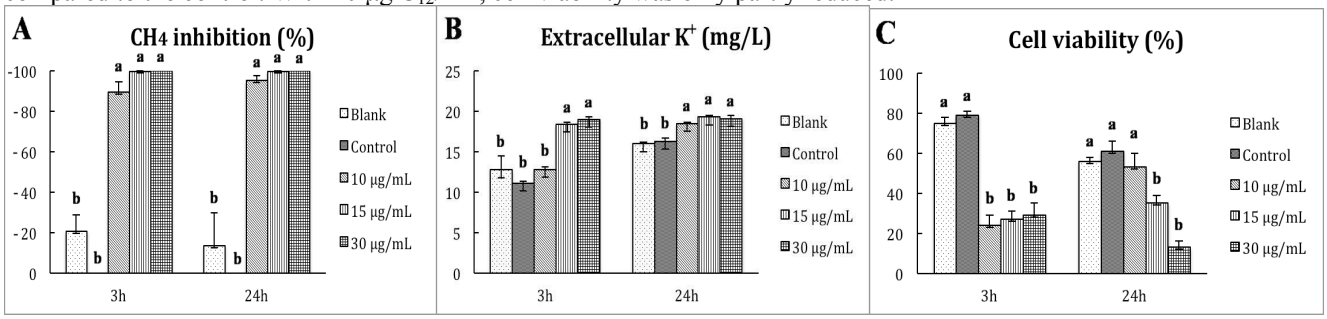

Figure 1 A. CH_4 inhibition (% decline in CH_4 relative to control), **B.** Extracellular K and **C.** Cell viability in cell suspensions treated with C_{12}. Means within time point with unequal letters (a, b) are different at $P < 0.05$. Bars represent standard errors.

Conclusions C_{12} inhibited methanogenesis, increased cell membrane permeability and decreased survival of *M. ruminantium* in a dose- and time-dependent way. These results provide new insights into why C_{12} suppresses methane formation in methanogens.

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In vitro gas production as a means to measure methane produced by a variety of upland plants when incubated with rumen fluid

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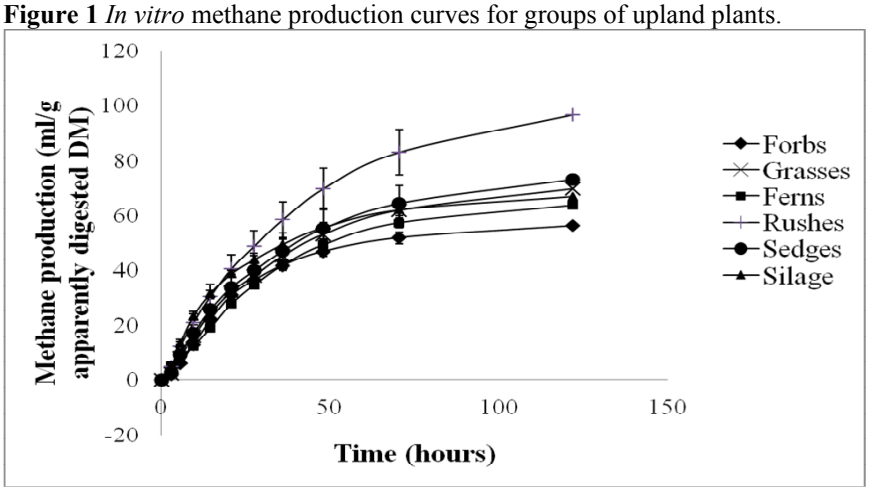
Introduction Very little is known about the methane production of ruminants grazing upland plant species, and yet the uplands of the UK are used extensively for sheep production. The aim of this experiment was to use *in vitro* gas production to estimate and compare the volumes and rates of methane production by a variety of species of upland plants, to investigate whether the potential for methane emissions by grazing sheep could be affected by the different upland plant species they consume.

Materials and methods Fourteen upland plant species, representing 5 plant groups (forbs, grasses, ferns, rushes and sedges) that are potentially consumed by grazing sheep, were collected on a monthly basis for one year from a site near Aberystwyth, UK, and were freeze-dried and ground. For this experiment, all fourteen species collected on one day (in July 2011) were analysed in triplicate using the *in vitro* gas production technique (Davies *et al.*, 2000), together with a standard grass silage sample and blank containing no substrate. Briefly, plant material was incubated with rumen fluid and a digestion medium in sealed serum bottles. Gases were periodically removed from bottles using a syringe to equalise the pressure in the bottle with atmospheric pressure. The removed gas was then analysed to determine the proportion of methane in the gas. Cumulative methane production data was collected over a 122 hour period. Apparent digestibility of each sample was also measured, and used to calculate methane production per gram of dry matter apparently digested. The data was fitted to the France *et al.* (1993) model for gas production using Genstat. Analysis of variance was used to compare the total methane production potential in the system (ml/g apparently digested DM), the cumulative methane production at 36 hours to represent typical rumen emissions, and the fractional rate of degradation for each sample (h^{-1}). Samples were also analysed for neutral detergent fibre (NDF) and water soluble carbohydrate (WSC). Type II regression was used to correlate NDF and WSC with total methane production potential in the system.

Results and Discussion Figure 1 shows mean plant group cumulative methane production over the period of the experiment. There were significant differences ($P<0.01$) between plant species in terms of the total methane potential in the system, 36 hour cumulative methane production and the fractional rate of degradation. The lowest yields of methane were from the forbs, including *Cirsium palustre*, *Calluna vulgaris*, and *Vaccinium myrtillis*, while the greatest yields were from the rushes, *Juncus effusus* and *Juncus squarosus*. It is possible that secondary compounds (e.g. condensed tannins) in forbs such as heather contribute to the relatively lower yields of methane produced from the fermentation of these plants. There was a significant correlation between NDF concentrations and total methane production in the system ($P<0.01$, $R^2=0.67$), but there was no significant correlation between total methane production and WSC concentrations. Plant NDF concentrations may be useful for predictions of total methane production potential. Further work will be carried out using samples of feed offered to animals during methane chamber experiments.

Figure 1 *In vitro* methane production curves for groups of upland plants.



Conclusions Significant differences in methane production were found *in vitro* among a range of upland plants, which is likely to affect methane produced by ruminants grazing them.

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In vitro gas production to test methane production from mixtures of two contrasting upland plant samples in varying proportions

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Introduction The *in vitro* gas production technique (Davies *et al.*, 2000) can be used to measure methane production potentials of ruminant feeds, and could thereby be used as a method to predict methane output by ruminants fed on different diets. The aim of this experiment was to use the technique to test the methane produced by graded mixtures of two contrasting upland plant species in varying proportions that were shown to yield different amounts of methane (Doran *et al.,* this meeting).

Materials and methods Dried and ground samples of heather (*Calluna vulgaris*) and fescue (*Festuca spp.*), collected from an upland site in July 2011, were analysed in triplicate using the gas production technique (Davies *et al.*, 2000) in proportions varying from 100% fescue to 100% heather in 20% increments. A grass silage standard and a blank containing no sample were also analysed. Cumulative methane production data were collected over a 100 hour period. Apparent digestibility of each sample was also recorded. The digestibility data was used to calculate methane production per gram of dry matter apparently digested. The data were fitted to the France *et al.* (1993) model for gas production using Genstat. Analysis of variance with polynomial contrasts was used to compare the total methane potential in the system (ml/g apparently digested DM), the cumulative methane production at 36 hours (because this would be a typical rumen retention time), and the fractional rate of degradation for each sample (h^{-1}).

Results and Discussion Figure 1 shows the volume of methane produced over the period of the experiment; F represents fescue and H represents heather. There were significant positive linear ($P<0.001$) and quadratic ($P<0.001$) effects of increasing the proportion of fescue in the plant mixture on both the total methane produced, and cumulative methane production at 36 hours. Similarly, there were significant linear and quadratic effects ($P<0.001$ for both) on the fractional rate of degradation. The lower production of methane from heather was probably a result of the lower degradability. However, the inclusion of even a small proportion of fescue with heather increased methane production, perhaps as a result of improved fermentability, as shown in Figure 2.

Figure 1 *In vitro* methane production curves for mixtures of fescue (F) and heather (H)

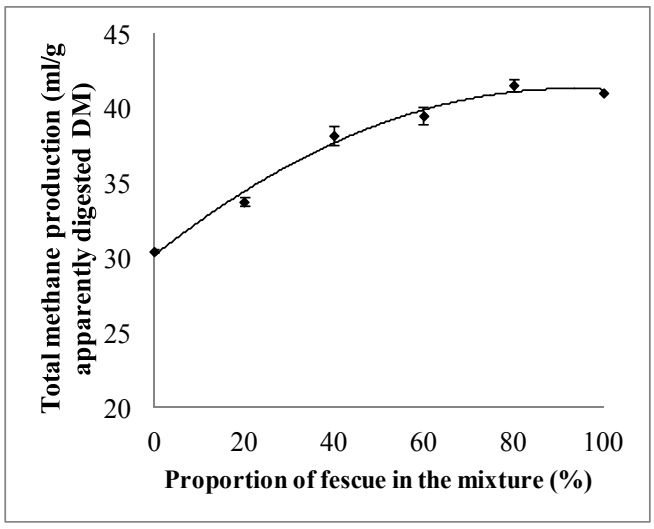
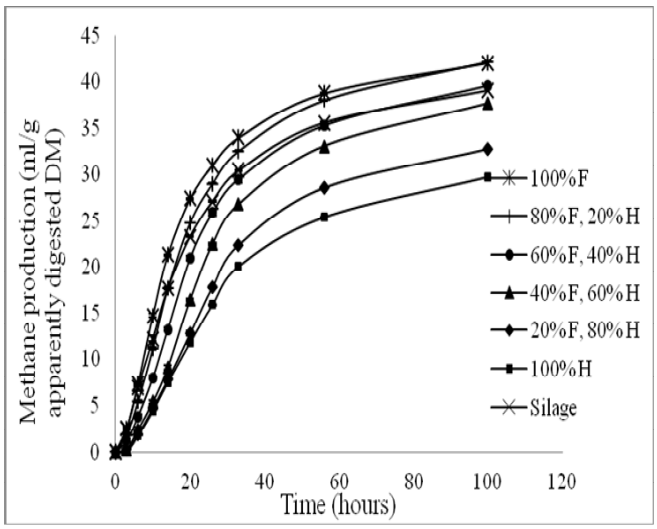


Figure 2 Quadratic relationship between proportion of fescue and total methane production ($R^2 = 0.99$).



Conclusion There is little evidence of the effect of plant secondary compounds in heather, e.g. condensed tannins, affecting methane production from the plant material when incubated with rumen fluid *in vitro*. Changes in methane production are likely to be caused by differences in digestibility of the plants, and the relatively poor fermentability of heather is improved by the addition of even small amounts of grass in the mixture.

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Farm-scale assessment of greenhouse gas mitigation strategies in dairy livestock-cropping-systems

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Introduction As a part of the Government of Canada's Agricultural Greenhouse Gases Program (AGGP, a contribution to the Global Research Alliance), we initiated a study on GHG mitigation strategies in dairy-cropping systems in fall 2011. Several projects are underway to examine feeding strategies, manure management, and cropping systems management. The project's objective are to 1) test new technologies and beneficial management practices that could result in net GHG reduction in integrated system of animal production, manure management, and crops; 2) contribute scientific data to address gaps in the National GHG Inventory; 3) enhance understanding of the fundamental processes of carbon and nitrogen cycling in agriculture, which will be used to develop and improve models of nutrient flows and GHG emissions in the livestock-cropping-system complex; 4) train highly qualified personnel and 5) facilitate communication and technology transfer between researchers, government, industry and producer groups interested in reducing GHG emissions from the dairy sector. We are taking an integrated approach so mitigation strategies will be evaluated collectively rather than in isolation. At each stage (livestock, manure, crops), we will measure the pertinent GHG emissions (CH_4, N_2O, NH_3) or sequestration (CO_2) to understand additive impacts (positive or negative) and determine the overall effect on emissions from the whole-farm. Currently, GHG emissions are being measured at two commercial dairy barns. Emissions from stored manure are being studied at one of the commercial farms, and in replicated meso-scale studies, where we can establish cause and effect relationships between BMPs and net GHG emissions. Soil emissions are being quantified from agronomic studies as well as field scale plots, as affected by application of manure from the commercial farms. Here we will provide an overview of this project and present the first year results focused on the cropping systems component of the project.

Materials and methods The overall effects of management changes on GHG and ammonia (NH_3) emissions from different cropping systems are being determined at the plot-scale and from 4-hectare fields. A plot-scale experiment at the Elora Research Station, Elora, Ontario, Canada, is investigating three liquid dairy manure application methods (surface application, incorporation following surface application, band injection), timing (fall vs. spring) and manure source (raw manure vs. digested manure) and their effect on emissions from corn. A plot-scale experiment at Campus d'Alfred Research Station, Alfred, Ontario, Canada, is examining GHG emissions from two soils (sandy loam vs. clay) receiving raw and digested manure in the fall or spring. The digested manure was obtained from anaerobic digesters located close to each experimental site. Raw manure was obtained from the same commercial farms where the anaerobic digester was installed. Manure was applied at a rate of approximately 150 kg N ha^{-1}. An unfertilized control and a plot receiving 150 kg N ha^{-1} of urea in the spring were also included at each site. At both sites GHG fluxes are measured approximately once a week year-round using non-flow-through non-steady-state chambers. In addition, half-hourly net ecosystem exchange (CO_2 fluxes) and N_2O fluxes are being measured from four 4-hectare fields at the Elora Research Station using a flux-gradient micrometeorological approach and a tunable diode laser trace gas analyzer. Two of the four plots are cropped with an annual (corn) and two of the plots are hayfields. Each set of plots (annuals vs. perennials) is receiving contrasting manure management practices. In the plots cropped with annuals, we are comparing timing of manure application (fall vs. spring). In the perennial plots we are comparing method of manure application (injected vs. surface applied) for a spring manure application date. Supporting data such as soil temperature and water content, soil nitrate and ammonium concentrations, and weather conditions are being collected.

Results Mean soil temperature at the time of manure application in November 2011 was ~5°C, and after spring application (April 24, 2012) was 7.7°C. Volumetric soil water content was approximately 38% in the fall, and 25% following manure application in the spring. At Elora, N_2O emissions from November to June were highest for the injected treatments, 3.5 and 5.3 kg N ha^{-1} for the fall and spring application dates, respectively. In contrast, emissions after incorporation and surface spreading were significantly lower and ranged from 0.9 to 2 kg N ha^{-1}. At the sandy loam site in Alfred, emissions were highest during the spring thaw that followed fall anaerobic digestate application. Micrometeorological N_2O fluxes were highest for the corn crop (~2.7 kg N ha^{-1}) compared to the hayfields where emissions were <0.6 kg N ha^{-1}. Emissions from November to end of February after fall manure application were twice as large as the plot that did not received any manure (13 vs. 6 g N ha^{-1} d^{-1} on average). Rates for both fall and spring treatments approximately doubled during the main spring thaw event at the end of March (30 vs. 14 g N ha^{-1} d^{-1}), respectively. This trend was reversed during the month after the manure application in May with the spring application treatment having the highest N_2O emission rate (31 g N ha^{-1} d^{-1}), compared to 7 g N ha^{-1} d^{-1} for the field that had received manure in the fall.

Conclusions Significant effects of manure application method and type were observed over the first year of the study, with emission increases associated with injection and application of digestate. At one site, timing of application (spring vs. fall) did not result in significant reduction in N_2O emissions due to a relatively mild winter, which induced moderate spring thaw emissions, and rainfall coinciding with the spring manure application. At the second site, with a colder winter and more snow, spring application resulted in substantially lower N_2O emissions compared to fall.

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Genomic selection for scarcely recorded environmentally important traits can be improved using predictor traits

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Introduction Feed-intake related traits in dairy cattle, such as dry matter intake (DMI), are genetically correlated with predicted methane emission (de Haas *et al.*, 2011). Long-term improvement of these traits can be achieved through genetic selection, however, collecting phenotypic information on the specific trait of interest is necessary. Obtaining phenotypic records on both DMI and methane emission require expensive measurements, and therefore, the data are often scarce. Genomic selection is suggested to be a promising selection tool to improve scarcely recorded traits, as it relaxes the necessity of extensive phenotyping (Calus *et al.*, 2013). Nevertheless, to perform genomic selection, a group of animals has to be phenotyped and genotyped to compose a so-called reference population. As for scarcely recorded traits the size of the reference population is often limited, ways to increase the accuracy of genomic selection, given small numbers of observations, are needed. One way to increase accuracy of genomic selection at reduced costs is to use easily recordable predictor traits in a multi-trait approach (Calus and Veerkamp, 2011). In this study, we investigated the impact of recording additional phenotypic observations for predictor traits on reference and evaluated animals on the accuracy of genomic breeding values for a scarcely recorded trait. For that purpose, we used DMI as the scarcely recorded trait, and fat-protein-corrected milk (FPCM) and live weight (LW) as the predictor traits.

Material and methods The animals originated from research farms in Ireland, United Kingdom, and the Netherlands. All 1 520 animals were genotyped with the Illumina BovineSNP50 BeadChip (Illumina Inc., San Diego, CA). In total, 869 DMI, 1 520 FPCM and 1 309 LW phenotypic records were available. Multi-trait genomic REML analyses were employed to estimate breeding values for DMI with information on the predictor available. Accuracies of breeding values were assessed through cross-validation (CV) using CV sets (n=7) defined by splitting the animals across genetic lines and management groups. Four reference populations were defined that differed by the number of traits for which phenotypic observations were available. Observations for the reference animals were available on: 1) DMI only; 2) DMI and FPCM; 3) DMI and LW; or 4) FPCM, LW, and DMI. Each of the CV sets was assumed to be a set of evaluated animals and was analysed four times considering that the animals have phenotypic information for: 1) none of the considered indicator traits, 2) FPCM, 3) LW or 4) FPCM and LW. Nine scenarios combining reference and evaluation sets were analysed. In the scenarios we considered the following situations. First, all reference populations were used to predict all evaluation animals without using any of their records on the predictor traits, to obtain a base line accuracy. Next, the reference population for which information on all traits was available was used to evaluate all possible evaluated sets of animals (i.e. no observations, FPCM, LW, and FPCM and LW). Additionally, the reference population with DMI and FPCM records was used to evaluate the CV sets with FPCM recorded, and the reference population with DMI and LW records was used to evaluate the CV sets with LW records.

Results When no additional traits were recorded for the evaluated animals, the maximum accuracy for DMI breeding values was 0.33. When the additional trait(s) were added, accuracies of breeding values increased from 0.33 to 0.50 when FPCM was added, and to 0.57 when LW was added. When both FPCM and LW were available for the evaluated animals the accuracy reached its maximum level of 0.63. Recording predictor traits only for the reference population alone did not increase DMI accuracy. Recording predictor traits for both the reference population and evaluated animals significantly increased DMI accuracy and removed bias observed when the evaluated animals had no observations.

Conclusions Using predictor traits can significantly increase the accuracy of genomic breeding values of scarcely recorded traits, as was shown here for the environmentally important trait DMI. This approach is successful only when predictor traits are recorded on both evaluated and reference animals.

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Measurement of methane in dairy cows via Photoacoustic Infrared Spectroscopy technique: Sources of variation in daily methane output

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Introduction Efforts to reduce the ecological foot print of milk production require a sound understanding of the sources of variation in enteric methane output of dairy cows. Particularly understanding the between and within-individual variations in methane output using a technique that is suitable for large scale measurement of methane (CH_4) is an essential requisite for developing genetic tools for its mitigation. In this study, a non-invasive Photoacoustic Infrared Spectroscopy (PAS) technique was used for measurement of methane from the breath sample of cows. The main objectives of the study were to identify the main sources of variation in daily methane output and its associations with intake, production and other functional variables in first-lactation Nordic Red cows.

Material and methods Records from fifty-four first-lactation Nordic Red cows from experimental dairy herd of the MTT Agrifood Research Finland were used in this study. The cows were managed similarly and fed on similar diet composed of silage and concentrate. Daily feed intake, milk yield traits and body weight were measured on each cow. Simultaneously, the methane output of cows was monitored continuously using F10 multi-gas analyzer (GASERA Ltd. Turku, Finland) that is based on Photoacoustic Infrared Spectroscopy technique (Negussie *et al.* 2012). Individual cow methane (CH_4), carbon dioxide (CO_2) and acetone outputs were measured from the breath sample of cows via sampling tubes of the analyzer fitted to two separate individual feeding kiosks. The feeding kiosks are visited by cows several times during the day to get their concentrate supplements and at each visit, breath of a cow was sampled and analyzed for the contents of the different gases and the ID, date, time and its measurements were recorded automatically. Measurements of the gasses were made alternatively from the two kiosks and every other minute a sample was measured from each kiosk resulting in 7 to 15 measurements recorded per cow per day. Repeated F10 measurements of the gases were used to calculate the daily mean CH_4:CO_2 ratio for each cow. The CH_4:CO_2 ratios were then used to estimate the daily methane output of cows (l/d) using the method by Madsen *et al.* (2010). Three-week data on daily feed intake, production and body weight records were combined with the corresponding estimates of daily methane output of each cow for statistical analysis. The data were analyzed fitting mixed linear model. The effects in the model included fixed effects of body weight (8 classes), milk yield (6 classes), TDM intake (8 classes), stage of lactation (9 classes), time of the day (23 classes), random animal and residual. The repeatability of daily methane output was estimated as a proportion of between-animal variation to total variation.

Results Feed intake and time of the day had highly significant ($P<0.001$) effects on estimated daily methane output of cows. Increase in the intake of fibrous feed such as silage increased the methane output. There was marked between-cow variation and the mean daily methane output ranged from 223 to 789 l/day with an average of 18.1l/kg milk, 23.2l/kg TDM intake and 0.85l/kg body weight of cows. The repeatability of daily methane output was 0.36(s.e. 0.23). During the day the time when CH_4 was measured showed high variability with three marked peaks observed at 08:00, 17:00 and 20:00 hrs.

Table 1 Effects of feed intake, production, body weight and time of day on estimated daily methane output (l/d) and its correlations with intake, production or body weight variables

Variables	P	Correlation
Silage DM intake(kg/d)	0.001	0.60
Concentrate DM intake(kg/d)	0.050	0.25
Total DM intake(kg/d)	0.001	0.55
Milk yield(kg/d)	0.010	0.17
Protein content (%)	0.050	0.13
Fat content (%)	0.010	0.25
Body weight(kg)	0.050	0.16
Time of day	0.001	0.32

Conclusions The large between-cow variability in estimated daily methane output indicate a possible genetic basis for the trait and selection for lower methane output could be one mitigation strategy. The higher s.e. for repeatability indicates the need for more measurements. Marked variability in methane output during the day suggest that description of daily methane output based on measurements taken at a particular time of the day may lead to a biased description the daily methane output profile in dairy cows.

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Effects of burnet(Sanguisorbba offinalis) extract on pathogenic microbial growth and rumen fermentation

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Introduction Antibiotics have been used to cure the animal disease and to increase the animal productivities. Especially, antibiotics can reduce methane production through modifying microbial population in the ruminants. But antibiotics abuse brought limitation of using antibiotics due to production of antibiotic-resistant bacteria. For that reason, many researchers try to study plant extracts as natural antibiotic additives in the animal feed. In this study, the effects burnet (*Sanguisorbba offinalis*) extract on pathogenic bacterial growth and rumen fermentation were examined to test the possible use of burnet extract as a feed supplement.

Material and methods Burnet extract were prepared by using methanol(1.5L/100g DM burnet) for 5hrs at 50℃. Salmonella enteritidis, Escherichia coli O157:H7, Clostridium perfringens, Staphylococcus aureus, Campylobacter jejuni and Bacillus cereus were prepared for paper disc method, Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) analysis. After each strain was incubated to 0.5A600, and diluted to $1.0X10^6$ CFU/ml for MIC and MBC. After 40 mg/ml of burnet extract was injected on the paper disc and this paper disc was incubated on the agar plate inoculating each strain for 24hrs at 37℃. The concentration of burnet extract is 0.125, 0.25, 0.5, 1, 2, 4, 6, 8 and 16 mg/ml for MIC and MBC test. Total Mixed Rations (TMR) for beef steer with iso-energy and iso-protein conditions were prepared using pure compounds including glucose, starch, casein, xylan and filter paper. Two hundred mg of mixed feeds and burnet extract (Control; 0, T1; 3.125, T2; 6.25, T3; 12.5, T4; 25 and T5; 50mg/L) were added to 50 ml of in vitro culture solution including rumen fluid and McDougall buffer, and then the bottles incubated for 24hrs at 39℃. Samples were collected after 0, 12 and 24 hours of incubation under anaerobic condition and dry matter (DM) digestibility, pH, methane production, ammonia, volatile fatty acid (VFA) concentrations and microbial populations were measured.

Results According to paper disc diffusion method, antimicrobial activities against *S. enteritidis, E.coli O157:H7, C. perfringens, S. aureus*, *C. jejuni* and *B. cereus* were detected with 0.5-16mg/ml concentration. MIC tests were ranged from 125μg/ml to 500μg/ml against pathogenic bacteria. The culture supernatant pH and DM digestibility were not affected by concentration of burnet extract ($p<0.05$). Whereas, culture head gas production were decreased with increased extract concentration. The extract concentrations over 6.25mg/L caused decrease in gas production per digested DM ($P < 0.05$). The comparative concentration of methane among total produced gas in treatment group was lower than that of control with significance ($P < 0.05$). Methane productions in 25mg/L and 50mg/L of extract treatment groups were lower than that of control group by 35% and 47% respectively. There were no significant differences among treatment in total VFA production and the molar ratio of acetate and propionate ($P > 0.05$).

Table 1 Culture head gas and methane production after 12 hours of incubation

	Control	T1	T2	T3	T4	T5
Gas(ml)	27.20±0.37	26.20±0.37	24.40±0.60	23.60±0.51	21.60±0.24	18.60±0.40
CH_4 (ml)	1.07±0.07	0.79±0.06	0.70±0.05	0.67±0.04	0.56±0.04	0.39±0.01
DM digestibility (%)	49.00±1.65	49.42±1.76	47.76±0.88	48.94±1.44	51.34±2.86	41.41±0.95
Gas(mM/gDM digested)	10.92±0.15	10.43±0.15	10.05±0.25	9.72±0.21	8.28±0.09	8.83±0.19
Fermentation efficiency (mg DM digested/ml gas)	3.60±0.05	3.76±0.54	3.92±0.10	4.16±0.09	4.76±0.05	4.46±0.09
CH_4(mM)	0.04±0.003	0.03±0.002	0.03±0.002	0.03±0.001	0.02±0.001	0.02±0.000
CH_4(mM/gDM incubated)	0.21^{a}	0.16^{b}	0.14^{bc}	0.13^{bc}	0.11^{cd}	0.08^{d}
CH_4(mM/g DM digested)	0.43^{a}	0.32^{b}	0.29^{bc}	0.28^{bc}	0.22^{cd}	0.19^{d}
CH_4(mmol/mol gas)	0.04±0.002	0.03±0.002	0.03±0.001	0.03±0.001	0.03±0.001	0.02±0.000
CH_4 decrease %	-	24%	28%	28%	35%	47%

Conclusions These results show that burnet (*Sanguisorbba offinalis*) extract have antibiotic effect on gram positive and negative bacteria. The methane production was decreased with burnet extract treatment even with slight decrease in DM digestibility at high dosage. Therefore burnet extract can add in the ruminant feed as natural plant extract additive for methane decreasing agent.

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Simulation of N_2O emission from Cropland using DailyDAYCENT

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Introduction At present, there are extensive discussions in the scientific community about the possible future range of greenhouse gas (GHG) emission from different anthropogenic sources (Forster *et al.*, 2007). Cropland contributes approximately 20% of the global anthropogenic GHG, of which nitrous oxide (N_2O) is the major one with the global warming potential of 298 times higher than that of carbon di-oxide. Over the last few decades several models have been developed to quantify the GHG emissions across different ecological and climatic zones. DailyDAYCENT is a terrestrial biogeochemical model which simulates the long-term dynamics of Carbon (C), Nitrogen (N), Phosphorus (P), and Sulfur (S) for different Plant-Soil Systems. The model includes plant growth with dynamic C allocation among plant components, soil organic matter decomposition and nutrient mineralization, and N_2O emissions from nitrification and denitrification. N_2O emissions are controlled by soil NH_4^+ and NO_3^-, water content, temperature, gas diffusivity, and labile C availability. Land management/disturbance events such as cultivation, water and nutrient additions, grazing, etc. can be readily implemented in the model. We have compared the simulated N_2O emission by DailyDAYCENT with the field N_2O emission estimate and studied the associated experimental parameter uncertainty on the simulation N_2O emission by DailyDAYCENT.

Materials and Methods We have used the experimental data from two arable cropland (Boxworth and Terrington) and grassland (Crichton and Rowden) sites (Table 1) in the United Kingdom to compare the simulated N_2O emission. N_2O emission data was collected at regular intervals from the field plots following the standard static closed chamber method for one year duration. The experiments were conducted with different types of N fertilizers and target application rates across the sites. At the arable cropland sites, nitrogen was applied as urea, urea ammonium nitrate (UAN) and ammonium nitrate; whereas, urea ammonium sulphate (UAS) was applied instead of UAN on the grassland sites. At the Crichton and Terrington sites nitrogen fertilizers were applied as 220 kg N ha^{-1} in three splits during the experimental period. However, at the Boxworth and Rowden sites nitrogen fertilizers were applied as 160 kg N ha^{-1} (Three splits) and 300 kg N ha^{-1} (four split) respectively.

Table 1 Soil and climatic data of different site

Site	Crop type	Bulk Density (Mg m^3)	pH	Sand (g 100 g^{-1} soil)	Silt (g 100 g^{-1} soil)	Clay (g 100 g^{-1} soil)	Ambient Temperature range	Precipitation (mm)
Boxworth	Winter wheat	1.18	8.5	19.4	35.9	44.5	-4.6 – 34.5°C	522.6
Terrington	Winter wheat	1.38	8.1	8.0	60.0	32.0	-3.6 – 30.6°C	724.0
Crichton	Grass	1.30	6.3	48.0	35.0	14.0	-4.2 – 27.4°C	1192.7
Rowden	Grass	1.20	6.1	21.0	47.0	32.0	-5.0 – 25.5°C	1022.8

The DailyDAYCENT model was initially parameterised to simulated N_2O emissions from each site, in an *ad hoc*, manner. Each soil and climate input has an associated uncertainty which can be expressed as a probability distribution function (PDF). Therefore to analyse the potential effects of these uncertainties on modelled N_2O emissions we assumed that each site value (Table 1) is the midpoint and there is a normal distribution of the input uncertainty we have simulated 5 step increase and/or decrease of each parameter from the site value. In each case a single parameter was changed and all other parameters were maintained at site value.

Results The seasonal cumulative simulated and observed N_2O emission is in the range of ±30% deviation from different treatments at the arable cropland sites. However, the difference was much higher (46%) in the ammonium nitrate applied treatment at the Boxworth site. Daily simulated N_2O emission is in the range of standard error of the observed values. The seasonal cumulative simulated and observed N_2O emission is in the range of ±25% deviation from different treatments at the grassland sites. Uncertainty in the bulk density of soil has much greater effect on N_2O emission at the Boxworth site while precipitation shows larger effect at the Terrington site. However, simulated uncertainty in the soil and climatic parameters does not reflect any significant change in the N_2O emission from the grassland sites.

Conclusion We will perform a monte carlo analysis using the PDF's of each input parameter, where we will simulate the N_2O emission using all possible combinations (with the PDF) of soil and climate parameters.

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Pilot-scale validation and comparison of mechanistic and empirical models of CH_4 emissions from slurry storages

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ntroduction Manure management is a source of methane (CH_4), an important greenhouse gas (GHG). The development of CH_4 mitigation strategies will help improve agricultural sustainability. One aspect of mitigation involves the development of models that accurately predict CH_4 emissions. The United States Environmental Protection Agency (USEPA 2011) describes an empirical model that is useful for performing national GHG inventories, for which the inputs are readily available data such as animal type, herd size, air temperature and manure management. Mechanistic models are, however, more appropriate for predicting CH_4 emissions in response to changes in management or climate (Kebreab *et al*. 2006). Although there have been substantial model development efforts, few studies have validated predicted emissions with measured data (VanderZaag *et al*. 2011). The objectives of this research were to (i) validate the USEPA model and a modified version of the mechanistic model described in Huang *et al*. (2010) with CH_4 emission data from pilot-scale storage tanks; and (ii) compare predictions from the empirical and mechanistic models.

Materials and Methods Data were collected at a site in Truro Nova Scotia, Canada. There were six pilot-scale slurry storage tanks (6.6 m^2 each). Each tank was filled with ~10.5 m^3 of dairy slurry with initial total solids (TS) contents ranging from 0.3-9.5% and stored undisturbed from May-20 through Nov-16, 2010. Each tank was permanently enclosed by a flow-through steady-state chamber. Each chamber was fitted with an exhaust venturi with a fan that continuously drew air through the system (~0.5 m^3 s^{-1}). There was a sprinkler inside each chamber that was used to simulate rainfall at a rate equal to the weekly normal for Truro NS. Inlet and outlet CH_4 concentrations were measured using a tunable diode laser trace gas analyser (TGA; Campbell Scientific Inc., Logan UT). There was a cup anemometer (Davis Instruments, Hayward CA) situated in each venturi for continuous air velocity, and thus chamber air flow rate monitoring. Manure and air temperatures were measured using copper/constantan thermocouples. Data were recorded using a CR5000 datalogger (Campbell Scientific Inc.). Slurry samples were characterized according to standard methods. Monthly CH_4 emissions were predicted using the USEPA inventory model assuming full volatile solids (VS) carry-over (Wood *et al*. 2012). Emissions were predicted using air temperatures and assuming the default ultimate CH_4 production potential (B_0) of 0.24 m^3 CH_4 kg^{-1} VS. A modified version of the Huang mechanistic model was used to predict CH_4 daily emissions. Carbon cycling was represented by 7 pools and 6 reactions mediated by 5 microbial groups. The model includes both aceticlastic and hydrogen/carbon dioxide methanogenic pathways. Volatile fatty acids and dissolved ammonia were considered inhibitors of methanogenesis.

Results For tanks with TS>1%, during the first and second mo of storage, monthly measured CH_4 emissions were lower than USEPA modelled emissions by 85-96% and 54-75%, respectively. The USEPA model did not predict the lag in emissions that was observed in the measured data. During 4-6 mo storage, the USEPA model always underestimated emissions. Often, for slurry storages, VS carry-over is not recommended (USEPA, 2011). These data, however, indicate that when using the USEPA approach to model slurry storages VS carry-over needs to be accounted for in some manner. The USEPA model performed better when integrated over the entire storage period, for which there was a linear relationship between predicted and measured emissions (slope = 2.1 s.e. = 0.24; intercept = -54.3; s.e. = 13.04). Since the data for some of the tanks were used to calibrate the mechanistic model, only the data for slurries with TS contents of 3.2, 5.8 and 8.2% were used for validation and comparison to the USEPA model. The mechanistic model predicted a lag in fluxes at the start of the storage period, prior to a peak in emissions. The predicted maximum fluxes in this first emission peak occurred within 7-14 d of the measured flux maxima. Errors in total CH_4 emissions predicted by the mechanistic model were -21.1%, -8.7% and 6.0% for slurries having TS contents of 3.2%, 5.8% and 8.2%, respectively. The USEPA model always predicted CH_4 emissions that were higher than those estimated by the mechanistic model. The relative percent differences between USEPA and mechanistic model emission predictions ranged from 19.9% to 37.3%.

Conclusions The USEPA model performed relatively well when integrating over the entire storage period, however, monthly emission estimates were less accurate. The mechanistic model was better at predicting fluxes over shorter timescales, and made more accurate total emission estimates. There was relatively good agreement between the USEPA and mechanistic model, which is encouraging from the perspective of advancing models that are suitable for national GHG inventories. There is a need for farm-scale field validations of the USEPA and mechanistic models to continually improve upon our abilities to develop mitigation strategies.

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Mitigation of ammonia emissions and improving animal welfare in pig housing by an optimized slatted floor

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Introduction Slatted floors are well established in the housing systems of pig production. The basic purpose of slatted floors is a controlled discharge of urine and feces into the manure canal beneath the pigs. Moreover they offer living space for the animals. The slot percentage of slatted floor is discussed controversially in Germany for reasons of animal welfare. A reduced slot percentage is supposed to reduce injuries of claws and legs. Typically the surface of the concrete floor is planar and has no slope. A novel design of slatted floor surface is expected to improve the discharge of urine. Even with a reduced slot percentage the passage of feces can still be realized due to a slope towards the slots (trapezoid-shaped surface). Consequently the cleanliness in the barn and the corrosive gas content in the stable and exhaust air is of interest. Therefore a comparative measurement of corrosive gases, particularly ammonia (NH_3), in the stable compartments equipped with a novel and a common slatted floor was carried out. The objective of this study is to assess the novel slatted floor and to investigate whether it leads to a reduction of emissions into the environment, improved air quality in the stable and in the end more appropriate conditions for the animals.

Material and Methods Measurements were performed in a fattening house for pigs at the agricultural center "Haus Düsse" in North Rhine-Westphalia, Germany. Each of the three different barn compartments was equipped with a different type of slatted floor. Ninety-Six hogs (Pietrain x (Landrace x Large White)) were reared per barn. During the measurement periods one barn was continuously equipped with the common concrete floor of 15 % perforation as a reference. The second barn was equipped with a concrete floor of 6 % perforation and a third barn with a perforation of 3 %. Gas concentrations in the different compartments were measured quasi continuously by using a photo acoustic field gas monitor (Lumasense Inc., Copenhagen, Denmark). The air samples were collected by vacuum pumps and pumped into plastic vessels. Out of these vessels the measuring point multiplexer coordinated the transmission of samples to the measuring device. To determine the emissions the air exchange rate was gathered by measurement fans. The daily temperature in the stable varied between 23.4°C and 29.3°C. Data were collected during the whole fattening period (105 days) and were recorded simultaneously. Every two weeks the level of contamination with feces in the compartments was recorded based on a standardized scoring system in order to draw inferences from contamination about the incurrence of corrosive gases like ammonia.

Results The contamination with feces in the barns with 3 % perforation was unsatisfactory because the drainage effect of the slot percentage in the floor ceased. Due to that the concentrations of NH_3 in this barn reached temporarily more than 100 ppm. Likewise the emission of NH_3 averaged 89.43 (±20.37) g NH_3 per livestock unit and day. On the contrary the contaminations in barns with 6% and 15% perforation were almost comparable to each other and much better than the contamination levels of the 3 % floor. The 6 % floor was more polluted than the 15% floor and emitted averagely 71.46 (±15.55) g NH_3 per livestock unit and day whereas the 15% floor emitted only 70.29 (±17.88) g NH_3 per livestock unit and day. Nevertheless there was no significant difference between these two concrete floors. However, the difference between the emission levels of these two floors and the 3% floor were significant ($p < 0.05$). Data were analysed using the statistical programme SPSS.

Conclusion A reduced slot percentage is in discussion to reduce injuries and improve animal welfare. The first results show that a highly reduced slot percentage (3%) alters the ammonia emission levels to unacceptable high concentrations in the barn. Furthermore the fouling in the compartments reached a high level.
The concrete floor with 6% and 15 % perforation showed acceptable cleanliness and comparable emission levels of NH_3. The premise of reduced ammonia levels compared to the common concrete floor due to the novel surface design could not be verified. Anyway it has to be assumed that a reduction of slots to 6% is a suitable solution to meet the requirements of animal welfare and climate control in the barn. In further measurements combinations of floors with 6 % and 3 % apertures as well as floors with 4.5 slot percentage (out of concrete and plastic) are under study. The investigations will show the practical relevance of the floor design and if a reduction to 4.5% apertures is feasible. Results of further measurement periods will be evaluated soon.

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Position monitoring of grazing cows for greenhouse gas emission measurements on pastures by micrometeorological methods

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Introduction Grasslands act as sinks and sources for greenhouse gases (GHG) and are, in conjunction with livestock production systems, responsible for a large share of agricultural GHG emissions. Ecosystem scale flux measurements (eddy covariance; EC) have been extensively used to investigate CO_2, CH_4, and N_2O exchange over different ecosystems and are becoming state of the art for animal grazing systems too. The advantage of EC flux measurements is the possibility of GHG emission monitoring under real grazing conditions on the pasture (in contrast to respiration chambers) with a high time resolution of about 30 min (in contrast to the SF_6 method). However, EC measurements represent a spatially integrated flux over an upwind area (the so-called footprint) in the order of 1000 m^2 containing a variable number of grazing animals. Thus a careful analysis of the footprint as a function of wind direction and wind speed (Schmid, 2002) is necessary. Recent studies using this method (e.g., Dengel *et al.*, 2011; Tallec *et al.*, 2012) lack data about the position of the animals relative to the flux footprint but stress the importance of this information. In our experiment we tested the applicability of an animal position monitoring system for EC flux measurements on a grazed pasture.

Material and methods The studied pasture (1 ha) at the Research Farm Agroscope ALP Posieux is located in the Central Plateau of Switzerland (46°46'N, 7°7'E) and is managed under a full-day grazing regime. During two days the positions of eight cows were monitored by commercial hiking GPS devices (BT-Q1000XT, Qstarz International Co., Taiwan) mounted at the animals neck and supplied with additional power from external batteries. Longitude and latitude were recorded at a rate of 1 Hz. The cows left the pasture two times a day for milking. The GPS sensor accuracy was tested by recording data for six hours by two devices placed at a fixed location side by side.

Results The difference in the position readings of the two fixed GPS sensors only varied between 0.13 and 1.98 m, and hence we consider the accuracy of the sensors to be about 2 m, which is much less than the typical extension of flux footprints. During grazing, the density distribution of the cow herd for an individual 30 minute interval (usual EC flux averaging time) shows that the cows were not uniformly distributed on the field nor in the footprint of the EC flux measurement (Fig. 1, left). For illustration the track of an exemplary single cow is indicated by the white solid line. Even during the entire 2-day grazing period, the spatial distribution of the cow herd (and also of individual cows) was not uniform (Fig. 1, right).

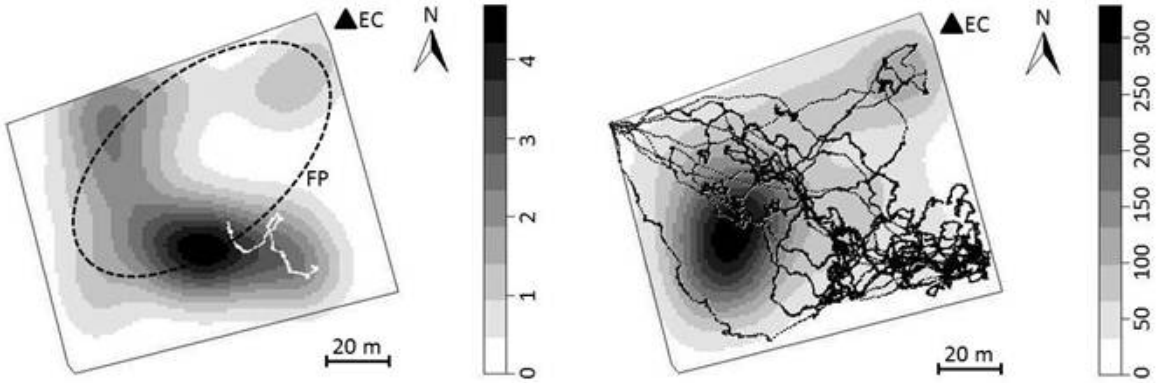

Figure 1 Density distribution of eight cows (gray shades, relative units) and track of an exemplary single cow (solid line) for an individual half-hour period (left panel) and for the entire 2-day grazing period (right panel). The triangle indicates the position of the EC measurement system and the dashed line represents a typical flux footprint (FP).

Conclusions The accuracy of the tested GPS devices is mainly determined by the number of satellites seen by the device and the atmospheric conditions. Despite the corresponding variations, the results indicates that the accuracy of the measured cow position is about 2 m and thus clearly sufficient for the localization of grazing animals within the EC flux footprint. With the data of the tested system, the relative contribution of the cows to measured GHG fluxes can be quantified. In this way the observed inhomogeneity of the animal distribution can be taken into account, which was not possible in previous studies.

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Methane and ammonia emissions from beef feeding in a U.S. southern High Plains region

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Introduction Concentrated animal feeding operations can be significant sources of methane and ammonia emitted to the atmosphere. Trace-gas emissions are difficult to evaluate accurately from animals, both under natural and animal feeding operations. However, studies have been successful using micrometeorological techniques including flux-gradient, integrated horizontal flux, and inverse dispersion analysis which avoid interference with emissions' sources and animal management. The purpose of this study was to simultaneously evaluate enteric methane and ammonia gas emissions from a beef-feeding operation.

Materials and methods The study was conducted at a commercial beef feeding operation in the semi-arid/arid southern High Plains. The animals (about 45,000 head) were housed in open pens and fed a grain-based diet. Necessary climatic and gas concentration measurements were made to determine trace-gas emissions using inverse dispersion analysis (Flesch *et al*., 2004). These measurements included open-path lasers (GasFinder, Boreal Laser Inc., Spruce Grove, AB) with measurement paths upwind and within the feedyard. Wind statistics were evaluated with a sonic anemometer (CSAT3, Campbell Scientific, Logan, UT) located above the feedyard. The commercial feedyard cooperatively provided monthly head counts, total feed, and diet composition for the seasons winter and summer, 2004, and spring, 2005.

Results Ammonia and methane concentrations were measured continuously for two weeks during winter, spring, and summer seasons. Figure 1 gives an example of measured concentrations and determined emission rates during summer. Daily ammonia concentrations and emission rates varied with time-of-day. Larger emissions occurred during midday due to physical and chemical effects of higher source concentrations (urea production), higher soil temperatures, and turbulence (windspeeds and unstable conditions). Larger concentrations occurred later in the study due to a rainfall and its effect on the dissociation of ammonium to ammonia at the soil surface. Daily methane concentration and emission rates showed little correlation with time of day; indeed, often larger emissions and concentrations were observed during nighttime. Careful observation of the varying methane fluxes indicated that increased methane flux was associated with cattle time-of-feeding. Harper *et al*. (1999) and others have shown increased enteric emissions from cows in pasture and feedlot conditions after feed consumption. Table 1 gives ammonia and methane emission rates for the three seasons studied. Ammonia emission rates were larger during spring and summer than winter due to climatic effects. Methane emissions are due to biological effects (enteric fermentation); however, emissions were higher in both summer and winter than spring suggesting a potential seasonal stress effect (due to the extremes of heat and cold). Average annual ammonia emission rates were not different from those of Todd *et al*. (2008) taken during the same seasons at the same feedyard using a different technology. Annual emissions of ammonia were 52 (± 12) % of input feed nitrogen and methane emissions were 1.6 (± 0.3) % of input feed dry matter.

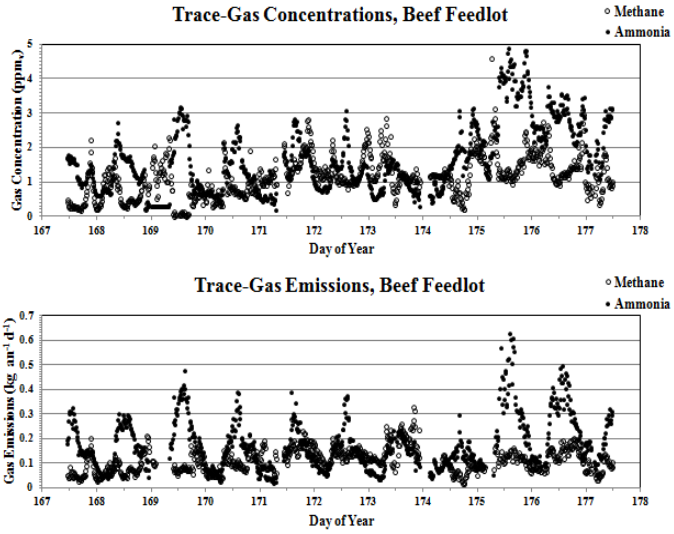

Figure 1 Methane and ammonia emission rates and concentrations during summertime at a beef feedlot in West Texas, 2004.

Table 1 Trace-gas emissions from a beef-feeding operation, West Texas, 2004-2005.

Season	CH_4 emissions (kg CH_4 an^{-1} d^{-1})	CH_4 StDev (kg CH_4 an^{-1} d^{-1})	NH_3 Emissions (kg NH_4 an^{-1} d^{-1})	NH_3 StDev (kg NH_4 an^{-1} d^{-1})
Winter	0.183	0.030	0.076	0.011
Spring	0.086	0.013	0.150	0.014
Summer	0.112	0.033	0.149	0.075

Conclusion Ammonia emissions were seasonal with larger emissions during warmer periods; however, methane emissions were smaller during reduced stress periods compared to higher heat/cold climatic conditions.

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N_2O emission during 15 days after turning of beef and dairy cattle manure in on-farm composting

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Introduction Aeration is one of the most important factors in on-farm manure composting because it can increase emissions of polluting gases such as nitrous oxide (N_2O), as response of nitrification and denitrification processes (El Kader *et al.*, 2007). Aeration is generally achieved by turning the manure heap periodically. The aim of the present study was to assess the effect of turning on N_2O emission during on-farm composting of beef and dairy cattle manure.

Material and methods A farm scale measurement trial was carried out from 10th April 2012 to 24th April 2012 in the North of Spain. Fresh straw-bedded manure from beef cattle (B) and dairy cattle (D) farms was removed from pens on December 2011 and formed into two heaps on land (Bd and Dd). For dairy cattle, effect of heap maturity was considered, including recently heaped manure (March 2012, Dm). Each heap covered and area of about 345 m^2 (3 m by 115 m) and was about 1.6 m high. Heaps were turned once with a tractor-turner on day 4 (13th April 2012). Gas measurements were collected daily on the first week when turning was carried out and once per week thereafter for 2 weeks. N_2O fluxes were measured using the closed chamber technique (diameter 20 cm, height 15 cm). The chambers were inserted into the manure to a depth of 3 cm, leaving a chamber volume of 3.8 l. Gas samples were taken 0, 20 and 50 min after chamber closure and collected in 9 ml gas vials. Concentration of N_2O was quantified by gas chromatography (GC-7890A, Agilent). As the increase in N_2O concentration into the chamber were generally linear (R^2>0.90) during the sampling period, emission rates were estimated as the slope of the linear regression between concentration and time and from the ratio between chamber volume and surface area (Abalos *et al.*, 2012). Manure temperature was recorded at 15 cm depth of the heap with thermocouples (TCdirect) and air temperature with a datalogger (HOBO U12-13). Unaltered manure samples were taken on day 4, before and after turning, to be analysed for density. Data were analyzed as repeated measurements according to the evolution of N_2O emission from each manure heap during measuring days by the Statistical Package for the Social Sciences 15.0 (SPSS Inc.).

Results After turning, Dm manure density decreased considerably (38%) with respect to that presented before turning (811.7 ± 57.1 g l^{-1}), while no density change was observed from Dd (915.9 ± 81.6 g l^{-1}) and Bd (767.4 ± 75.8 g l^{-1}). N_2O emission evolution from Dm manure was significantly different to that from Dd manure during the study, while almost negligible emission was observed from Bd (Figure 1). The highest N_2O emission was observed immediately after turning of Dm manure, with a peak of 80.75 ± 86.23 mg N_2O m^{-2} d^{-1}, followed by Dd on the following day with 21.39 ± 29.18 mg N_2O m^{-2} d^{-1}. During the following days after turning, Dm manure temperature increased considerably with respect to air temperature, coinciding with Webb *et al.* (2012), and N_2O emission from dairy cattle manure decreased. The accumulated N_2O emission during 8 measuring days was 157.65 mg N_2O m^{-2}, 52.38 mg N_2O m^{-2} and 8.68 mg N_2O m^{-2} from Dm, Dd and Bd, respectively.

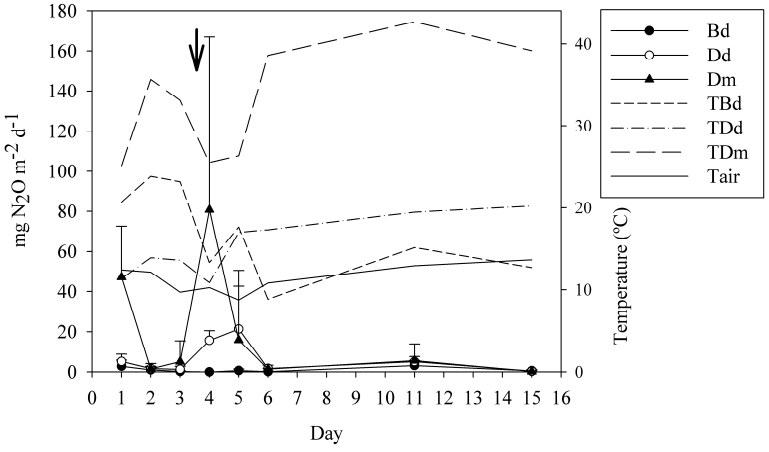

Figure 1 N_2O emission (mg N_2O m^{-2} d^{-1}) and temperature (ºC) during the study. Turning was carried out on day pointed by an arrow.

Conclusions These results show that turning could induce higher manure temperature and N_2O emission in dairy cattle manure. Higher maturity of dairy manure heap could lead to lower N_2O emission after turning.

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Pig slurry variability of commercial farms and its effect on CH_4 and NH_3 emissions

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Introduction Pig slurry composition plays a critical role on ammonia (NH_3) and methane (CH_4) emissions of pig production (Moset *et al*., 2012). It also determines the suitability of this by-product to be used as fertilizer or as biogas substrate. Slurry composition is, at the same time, heterogeneous, depending on a wide variety of factors including animal type, feeding, housing, management and environmental conditions. This work presents the results of the first study of a three year project, in which the potential emissions of NH_3 and CH_4 were determined and related to the variability found in slurry composition of commercial facilities.

Materials and Methods A total of 80 slurry samples were taken in two regions of Spain: Centre and East. Samples were distributed according to different animal categories: gestating sows (16), farrowing (16), weaners (16) and fatteners (32). One kg of feed and 5L of slurry were sampled for laboratory analysis. For each feed and slurry sample the following parameters were analysed: dry matter (DM), ash content, pH, electrical conductivity (EC), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and nitrogen linked to fibre (N-NDF). Furthermore, potential emissions of CH_4 and NH_3 were determined in laboratory. Potential CH_4 production (B_0) was measured by triplicate in 125 mL bottles for 100 days according to Verdenne *et a*l. (2007). Potential NH_3 emissions were determined daily during 15 days in 0.8L bottles. A constant flow of 1 L/min was exhausted from each bottle and passed through two consecutive impingers, following the methodology described by Pereira *et al*. (2012).

Results Table 1 shows the composition of feeds and slurries, as well as the potential NH_3 and CH_4 emission from slurry. A high variability was found, both within animal categories and regions and among them. The CP content of feed was not correlated with slurry N content, but was inversely correlated with total solids (TS) and volatile solids (VS) of slurry. The NH_3 emission depended on animal category ($P<0.05$) and was positively correlated with N-NH_3 ($r=0.78$; $P<0.01$), EC ($r=0.76$; $P<0.01$) and TS ($r=0.33$; $P<0.01$) of slurry. Potential CH_4 emission was negatively correlated ($P<0.01$) with fiber and lignin content of slurry (FND: r=-0.44; FAD: r=−0.56 y LAD: r=−0.65) and feedstuffs (FND: r=-0.32; FAD: r=−0.39 y LAD: r=−0.39).

Table 1 Chemical composition (average ± standard deviation) of commercial feedstuffs and slurries, and CH_4 and NH_3 emissions.

	Animal category				Region	
Feed	Gestation	Farrowing	Weaners	Fatteners	Center	East
DM (%)	89.6±1.65	89.4±1.57	89.9±1.54	89.9±1.45	90.0±1.64	89.5±1.36
Ash content (%DM)	6.26±1.04	6.55±0.56	5.90±0.49	5.34±0.60	5.80±0.73	6.06±0.91
CP (%DM)	15.0±1.48	17.9±1.55	19.0±1.51	17.2±1.59	17.1±1.92	17.4±2.18
NDF (%DM)	22.7±2.78	19.2±3.12	14.2±1.61	16.1±1.89	17.7±3.20	17.9±4.51
ADF (%DM)	8.51±1.77	6.63±1.89	3.85±0.69	5.37±1.24	5.70±1.72	6.32±2.52
ADL (%DM)	1.76±0.75	1.21±0.67	0.35±0.30	0.76±0.48	0.92±0.64	1.06±0.84
Slurry						
Total solids (%)	5.43±5.29	4.02±2.56	3.73±2.42	6.04±3.96	4.17±3.43	5.68±3.43
Volatile solids (%)	3.97±3.98	2.96±1.94	2.70±1.86	4.53±3.14	3.12±2.69	4.16±2.69
N (%DM)	11.5±6.30	11.0±5.79	11.3±4.68	11.6±5.94	11.8±4.79	11.0±4.79
NDF (%DM)	39.7±15.3	38.9±7.65	31.6±11.4	35.7±13.3	36.6±12.3	36.3±12.8
ADF (%DM)	20.7±9.29	19.7±4.81	13.5±5.84	18.2±8.68	18.1±7.02	18.1±8.64
ADL (%DM)	8.04±3.48	7.88±2.52	4.56±2.82	6.75±3.77	6.72±3.37	6.87±3.57
pH	7.77±0.14	7.60±0.33	7.35±0.57	7.44±0.37	7.34±0.45	7.71±0.45
EC (mS/cm)	22.0±8.16	15.6±5.90	21.2±10.5	28.4±14.2	21.3±11.9	23.7±11.3
N-NH_3 (%N)	67.3±14.4	62.4±14.0	63.0±12.1	68.8±13.6	62.5±13.1	68.8±13.4
NH_3 emission (mg/L/day)	***87.5±15.9***	***66.6±21.9***	***77.0±46.1***	***104±52***	***78.4±42.3***	***93.8±39.1***
B_0 (mL CH_4/g VS)	***295±146***	***223±77***	***387±157***	***321±145***	***303±139***	***309±148***

Conclusions A high variability was found in commercial feedstuffs and slurries, both within and among animal categories. NH_3 emission was correlated with certain slurry characteristics (TS, EC and N-NH_3). Potential CH_4 emission was negatively correlated with fiber and lignin contents of both slurries and feedstuffs.

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Data filtering in bLS calculation of emissions rates: improvements to increase data retention

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Introduction The bLS inverse dispersion technique is useful for measuring greenhouse gas (GHG) emissions. From the concentration rise downwind of a GHG source, and with the aid of a bLS dispersion model, one infers the GHG emission rate. This technique has been used to study emissions from cattle feedlots, barns, paddocks, waste lagoons, whole farms, etc. The advantages of bLS are the limited number of measurement requirements, and freedom to choose convenient measurement locations. However, bLS relies on wind conditions that meet accepted criteria, and this can result in a large fraction of unusable measurements. This can be a problem, particularly if the emission rate exhibits a temporal trend, as unusable data tends to be concentrated at certain times of the day (particularly night-time). The objective of this study was to examine the potential for more precise filtering criteria that would increase data retention.

Material and methods A tracer release study mimicked a square cattle paddock (1 ha). Methane was released from 10 point sources with a known emission rate Q. Open path methane lasers (GasFinder 2, Boreal Laser Inc., Edmonton, Canada) were placed to surround the paddock. The bLS technique (Flesch *et al*., 2004) gave the calculated emission rate Q_{bLS}. The ratio Q_{bLS}/Q gives bLS accuracy ($Q_{bLS}/Q = 1$ is perfect). A sonic anemometer gave the needed wind parameters: friction velocity u_*, Obukhov stability length L, surface roughness length, and wind direction. Air temperature T_{air} was measured at $z = 1$ and 3 m above ground. Over two weeks there were 196 release periods (15-min each), mostly in light wind conditions and night-time.

Results For all 196 periods the average $Q_{bLS}/Q = 1.33$, with a large standard deviation of 1.66. This is a disappointing result (33% over estimation), although the majority of these data would be rejected using typical filtering criteria. For a bLS application like ours, typical filtering criteria might require $u_* \geq 0.15$ m s^{-1} and $|L| \geq 5$ m, i.e, avoiding light winds and extreme atmospheric stratification. Using this filtering improves the average accuracy to $Q_{bLS}/Q = 1.00$, and dramatically reduces bLS variability (Table 1). However, the filtering results in the removal of 53% of our data.

The bLS calculation uses Monin-Obukhov similarity theory (MOST) to characterize atmospheric winds and turbulence, and inaccuracy in Q_{bLS} will occur when MOST is inaccurate. This often occurs in light winds or extreme stratification – and thus the reason for u_* and L filtering criteria. However, these criteria may be too coarse: rejecting periods accurately described by MOST. Because MOST makes predictions of the temperature difference between two heights (ΔT_{air}) based on u_* and L, a comparison between predicted and measured ΔT_{air} may provide an indication of MOST failure. The MOST formulae for ΔT_{air} can be found in standard textbooks (e.g., Garrett, 1992). We compare ΔT_{air} between $z = 1$ and 3 m, as measured and as calculated from MOST (ΔT_{meas} and ΔT_{model}). Based on this comparison, a new filtering criterion is imposed, where we accept only periods where $|\Delta T_{meas} - \Delta T_{model}| \leq 1$˚C. With this new criterion we can relax the u_* and L thresholds from (0.15 ms^{-1}, 5 m) to (0.05 ms^{-1}, 1 m) and still maintain bLS accuracy, with an average $Q_{bLS}/Q = 1.06$ (Table 1). The standard deviation of Q_{bLS}/Q also remains low with the new filtering. The benefit of using the ΔT_{air} criterion is that we have "recovered" more than half of the previously rejected emission data.

Table 1 Average and standard deviation of bLS accuracy, with the number of good observations, for different filtering criteria.

	No Filtering	Standard Filtering ($u_* \geq 0.15$ ms^{-1}, $\|L\| \geq 5$m)	New Filtering ($\|\Delta T_{meas} - \Delta T_{model}\| \leq 1$˚C, $u_* \geq 0.05$ ms^{-1}, $\|L\| \geq 1$ m)
Average Q_{bLS}/Q	1.33	1.00	1.06
Standard deviation Q_{bLS}/Q	1.66	0.22	0.30
Number of Observations	196	93	153

Conclusions The ΔT_{air} measurements allowed a useful filtering criterion for analysing bLS emission data. This criterion allowed a more precise identification of inaccurate measurement periods (compared with previous criteria) and resulted in significantly greater data retention. The measurement of ΔT_{air} at a field site will be simple and straightforward (shielded thermocouples), and we anticipate that future experiments will include such observations. This will be particularly helpful for identifying good night-time data, where light wind conditions have often meant a high level of data rejection.

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Comparison of traditional culturing and molecular biological techniques to study the changes in the rumen microbiome as affected by monensin using the rumen simulating technique (RUSITEC)

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Introduction Monensin has been widely used as a positive control in many *in vitro* simulations examining the effects of additives on methane (CH_4) production. Monensin has been shown to positively affect rumen fermentation by directing hydrogen into propionate production, reducing methanogenesis, reducing ruminal ammonia and affecting Gram positive bacteria (Van Nevel and Demeyer, 1977). Up until the late 1990's culturing methodologies were utilised to quantify changes in microbial populations using either roll tubes or most probable number (MPN) counts. However, molecular techniques have been developing at a rapid pace and offer a new toolkit in order to detect changes in the microbiome of the rumen. The aim of this study is to compare traditional culture methods with 16S rDNA based molecular techniques using the extensively studied methane inhibitor monensin in the rumen simulator RUSITEC.

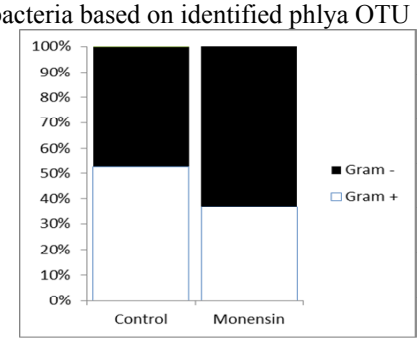
Materials and Methods Eight RUSITEC fermentation vessels (Czerkawski and Breckenridge, 1977) were randomly allocated to one of two experimental treatments; control (CON) or monensin (MON). Vessels were fed with 20 g/d of a standard hay/concentrate diet at 09:30 h with the treatment added directly to the vessel as either 1 ml ethanol or 1 ml ethanol containing 10 mg Na-monensin. Following a 14 d adaption period daily CH_4, VFA and ammonia production were recorded. The DMD was determined over 24 and 48 h. On d 19 approximately 1 g of residual feed was mixed with 20 ml of vessel contents, a 2 ml subsample withdrawn for protozoa counts and the remainder homogenised and serially diluted prior to inoculation in Hungate tubes for the enumeration of total bacteria, and methanogens using a 5 tube MPN assay. An additional sample of residual feed and vessel liquor were individually snap frozen in liquid N prior to DNA extraction. The MPN tubes were incubated for 21 d prior to scoring, tubes for methanogens were fed approximately 10 ml H_2 every 2d prior to determination of CH_4. The DNA was amplified using specific PCR primers for total bacteria and the methyl co-reductase A (mcrA) gene prior to T-RFLP analysis. Quantification of total bacteria was determined using qPCR. Samples of 16S ribosomal DNA for total bacteria and methanogens were amplified using PCR and sequenced using an Ion Torrent personal genome machine Data for fermentation parameters, methane production and microbial/protozoal numbers were analysed by ANOVA using Genstat. Fragment analysis was conducted by principal coordinate analysis (PCoA) using R. Sequence analysis and identification was determined using a R pipeline with reference to the NCBI database.

Results The effects of monensin on fermentation parameters, methane production and microbial numbers are presented in Table 1. Inclusion of monensin reduced daily acetate, ammonia and methane production and increased propionate production. There was no effect of treatment on culturable bacterial counts or total protozoa. There was a tendency (P=0.09) for samples from the MON treatment to have more bacterial DNA compared to CON. There was a difference (P=0.001) in PCoA for methanogens based on T-RFLP with no corresponding change in Shannon diversity. Analysis of bacterial phyla using operational taxonomic units (OTU) resulted in a reduction (P=0.034) in Gram positive bacteria for MON treated samples (Figure 1). Proteobacteria were increased (P=0.007) by MON treatment with a tendency (P=0.065) for a decrease in the proportion of Firmicutes.

Table 1 Effect of monensin on fermentation parameters, methane production and microbial numbers

	CON	MON	s.e.d.	P
Acetate, mM/d	13.5	10.2	0.76	0.005
Propionate, mM/d	5.9	9.0	0.49	<0.001
Ammonia, mM/d	1.2	0.4	0.03	<0.001
Methane, mM/d	3.5	2.6	0.30	0.024
Log_{10} MPN total bacteria, (back transformed mean /ml)	6.14 ($138x10^3$)	5.93 ($85x10^3$)	0.514	0.707
Solid associated bacterial DNA, ng/ul	5.0	11.4	3.17	0.090
MPN methanogens, /ml	$102x10^3$	$179x10^3$	$11.1x10^3$	0.511
Protozoa, /ml	2417	1975	537.2	0.442

Figure 1 Effect of treatment on Gram +/- bacteria based on identified phlya OTU



Conclusions The inclusion of 10 mg/d Na-monensin resulted in a shift in rumen fermentation as expected from acetate to propionate with a corresponding decrease in methane and ammonia. Differences could not be detected in microbial populations using culturing methodologies. However, molecular analysis using sequencing identified changes in key bacterial phyla.

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Effect of dietary forage to concentrate ratio and sunflower oil supplements on ruminal microbial communities in lactating dairy cows

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Introduction Ruminants are capable of utilizing fibrous feeds not digested by mono-gastrics and represent a valuable natural resource for meeting future increases in global food supply. Owing to concerns of increases in greenhouse gas emissions into the environment and potential effects on global warming, there is interest in developing production systems to lower methane emissions. Increases in the proportion of concentrates in the diet and fat supplements are known to alter the biohydrogenation of fatty acids and lower ruminal methanogenesis, but the underlying effects on rumen microbial ecology are not well established. The aim of this study was to evaluate rumen microbial diversity collected from an experiment with lactating cows examining the effects of changes in dietary forage:concentrate ratio and sunflower oil supplements on enteric methane production (Bayat *et al*., 2013).

Material and methods Four Finnish Ayrshire cows in mid lactation were used in a 4 x 4 Latin square to evaluate the effects of a high (H) or low (L) forage:concentrate ratio (65:35 vs 35:65) and either 0 (O) or 50 g/kg of sunflower oil (S) on ruminal microbial ecology. Rumen digesta samples (solids & liquid) were collected via the fistula at 09.00 h on d 22 and 15.00 h on d 24 of each 35 d experimental period. Total genomic DNA was extracted as described by Yu & Morrison (2004). The archaea diversity of rumen samples was evaluated by using T-RFLP technique. Ribosomal 16S DNA was amplified using the primer 21f (DeLong 1992) labelled with HEX and ARC915 labelled with VIC (Raskin *et al*., 1994). Forward and reverse fragments obtained after digestion were run in an Applied Biosystems® 3500xL Genetic Analyzer. The bacterial diversity was estimated by sequencing V2-V3 region of the bacterial 16S DNA on the 454 platform as described by Jami & Mizrahi (2012). Data quality control and analyses were performed using the QIIME pipeline (Caporaso *et al*., 2011). Effect of diet and oil supplement on rumen microbial communities was tested by ANOVA.

Results Archaea. Several T-RF fragments were present in all four feeding treatments with T-RF 187bp being the most abundant (45-82%). The amount of T-RF 187bp was significantly higher in cows fed low forage:concentrate diet, while high forage:concentrate diet considerably increased both T-RF 190bp and T-RF 192bp.
Bacteria. Firmicutes and Bacteroidetes were the predominant phyla, representing 77-93% of the sequences in all four diet treatments. Other less abundant phyla were Proteobacteria (0.8-14.4%), Fibrobacteres (0.07-1.2%), Actinobacteria (0.08-0.66%) and unidentified bacteria (5-8%). Decreases in ruminal methanogenesis on the low forage:concentrate treatment with oil supplement (Bayat *et al*., 2013) were associated with a significant increase in Bacteroidetes and significant decrease in Firmicutes. Proteobacteria was more abundant in cows fed low forage vs high forage diet but the changes were not significant. Effect of treatments on ruminal microbial communities is shown in Table 1.

Table 1 Effect of forage ratio and sunflower oil on the rumen microbial ecology

	P				P		
Bacteria phylum	Forage	Oil	Forage x Oil	Archaea T-RFs*	Forage	Oil	Forage x Oil
Proteobacteria	0.15	0.38	0.13	180bp	0.94	0.67	0.75
Firmicutes	0.01	0.62	0.06	187bp	0.05	0.53	0.68
Fibrobacteres	0.08	0.07	0.89	190bp	0.09	0.31	0.88
Bacteroidetes	0.02	0.08	0.12	192bp	0.006	0.71	0.16
Actinobacteria	0.22	0.86	0.39	540bp	0.60	0.57	0.74
Unidentified	0.36	0.79	0.15	860bp	0.13	0.19	0.71
				866bp	0.12	0.11	0.24

*Bands with a presence <2% did not show any significant P.

Conclusions Diet composition had a significant influence on the most abundant microbial taxa in ruminal digesta of lactating cows with evidence of an association between ruminal methane production and microbial composition.

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Quantification of methane emissions by dairy cows offered maize and grass silage-based diets in a cross-ventilated free-stall barn

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Introduction With a CO_2 equivalent of 25, methane (CH_4) is a major contributor to total agricultural greenhouse gas emissions. Extensive animal experimentation applying *in vivo* techniques to individual animals in respiration chambers and *in vitro* (e.g., gas production methods) has already been conducted to evaluate the potential of dietary changes and modifications to lowering CH_4 emissions in ruminants. However, both long-term assessments and measurements on herd or farm level are complex and hence still rarely reported. Therefore, the overall objective of this study was to perform a long-term measurement of CH_4 emissions in cross- ventilated free-stall barn to evaluate whether the type of forage in mixed diets fed to dairy cows has an influence on the amount of CH_4 emitted within the barn.

Material and methods All measurements were performed at the research facility "Haus Riswick" (Agricultural Chamber of North Rhine-Westphalia, Kleve, Germany). The cross-ventilated free-stall barn can be subdivided into three individually ventilated compartments with the help of specially designed air-tight plastic curtains. The air volume flow was estimated using the SF_6 tracer gas technique. The quantification of CH_4 concentration was done using photo-acoustic spectroscopy (Multigasmonitor; LumaSense Technologies, Frankfurt/Main, Germany) with 8 control points for each barn compartment. For the first 14 days, two groups, each consisting of 48 high-yielding dairy cows were fed a mixed basal diet as partially mixed ration (PMR) which contained the same proportion of grass and maize silage (each 380 g/kg dry matter [DM]). Additional concentrate in form of a pelleted dairy compound feed was offered to the cows according to production of energy-corrected milk (ECM) yield throughout the trial. Subsequently, for the next 80 days, the animals received either a PMR containing mainly grass silage (GRASS: 600 g grass silage/kg DM; 110 g maize silage/kg DM) or a diet containing mainly maize silage (MAIZE: 590 g maize silage/kg DM; 167 g grass silage/kg DM). The remainder of the DM of the PMR was a concentrate mix to ensure that both PMR were of similar protein value. The design was a two-period change-over design such that the diets were switched between the groups after 40 days. Following the separate feeding period the animals again received the diet with same proportions of grass and maize silage for 14 days. The measurements included analysis of the chemical composition of the diets and ingredients, daily individual feed intake, daily milk yield, body weight and gas concentration in the respective air space.

Results During the first 14 days of the trial, when cows were already assigned to their groups but still fed the same diet, the mean (± standard deviation indicating day to day variation of averages) DM intake (DMI) of the cows was 21.7 kg (1.2) for the GRASS and 22.3 kg (± 0.6) for the MAIZE cows. Mean daily CH_4 emissions were 324 (± 11) g/cow. In the second feeding period, when cows were fed the diets to which they were assigned, the mean DMI for the GRASS group was 20.0 kg (± 0.8) and 22.3 (± 0.7) kg for the MAIZE group. Methane emissions for GRASS cows averaged 360 (± 27) g/(cow x day) and 340 (± 22) g/(cow x day) for MAIZE cows. Methane emissions expressed as CH_4 g/kg DMI revealed slightly higher values for GRASS cows (18 ± 1 g/kg DMI) when compared to MAIZE cows (15 ± 1 g/kg DMI). During the periods in which both groups of cows received the same diet no difference was observed (16 ± 1 g/kg DMI for both groups). When CH_4 was expressed as g/kg ECM, GRASS cows showed numerically higher CH_4 values (12 ± 1 g/kg ECM) than MAIZE cows (10 ± 1 g/kg ECM): Again, both groups had similar values when they received the same diet (12 ± 1 g/kg ECM). A numerical difference between the groups was observed when CH_4 was expressed as g/kg NDFom (neutral detergent fibre, expressed inclusive residual ash) intake (GRASS: 42.2 ± 2.5 g/kg NDFom intake; MAIZE: 36.4 ± 1.5 g/kg NDFom intake). Whether or not these data indicate differences in fibre fermentability still needs to be studied.

Conclusions The results from this study indicate that estimating CH_4 emissions from dairy cows in a freely ventilated free-stall barn on herd or group level appears feasible. When compared to recent literature values based on individual animals, results seem plausible. As expected the group that received the maize silage-based diet emitted less CH_4 than the group receiving the diet based on grass silage. In future studies, the established technique will be used to examine other diet types including a variety of feed additives in regard to potential mitigation of greenhouse gas emissions by high-yielding dairy cows.

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The Nitrogen Footprint of EU27 food production

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Introduction About 15 million tons of nitrogen (Mt N) in biomass are grown every year on the agricultural land, and used as livestock feed, food, fibre of fuel. This is driven by a supply of nitrogen to agricultural land of 21.2 Mt N yr^{-1}, mainly in the form of mineral fertilisers (10.9 Mt N yr^{-1}) and the input of manure nitrogen (7.2 Mt N yr^{-1}). Only 2.3 Mt N yr^{-1} is consumed by European citizens, while more than 10 Mt N yr^{-1} is emitted from agricultural systems to atmosphere or hydrosphere in Europe. To understand the contribution of food products to environmental threats linked to emissions of reactive nitrogen (Nr) we have quantified the impact of EU27 food production as total emissions of Nr in the full production chain for main agricultural products.

Methods Quantification of nitrogen losses from the agricultural sector is done on the basis of a the agri-economic model CAPRI (Britz and Witzke, 2012) and the integrated environmental assessment model MITERRA-EUROPE (Velthof *et al.*, 2009). Both models follow a mass-flow approach representing the nitrogen cycle in agricultural systems. Also, LCA models are implemented in both the CAPRI model (Weiss and Leip, 2012) and the MITERRA model (Lesschen *et al.*, 2011). Thus, emission estimates for livestock products include also emissions from imported feed products. The LCA model applied considers also emissions related to the use of energy for on-farm operation or production of farm input. To assess how different diets or diet choices affect the losses of reactive nitrogen, we calculated these losses for the main twelve food commodity groups. These commodity groups cover about 95% of food products consumed in the EU-27. Six of these food commodity groups are plant-derived (cereals, potato, fruit and vegetables, sugar, vegetable oils and pulses) and six are from animals (dairy products, beef, pork, eggs, poultry meat, and sheep and goat meat). Emissions from fish and fish products are not simulated in the models used and have therefore not been included. Results are expressed as Nr emission intensities or total Nr emissions for each food product category.

Results Both models agree in the tendency of higher Nr losses from animal products compared to vegetal products by a factor of about 20, or – using a weighted average on the basis of domestic production – by a factor of 7.9 (CAPRI) or 7.4 (MITERRA). Moreover, also the ranking of the food commodity groups is consistent between the two models, with Nr emissions from ruminants being highest, followed by other meat products and eggs and dairy products having lowest emissions for the groups of animal products considered. Highest emissions in the group of crop products are from oils, followed by cereals, leguminous crops and sugars (with some differences in this sub-group between the models). Lowest emissions are calculated for fruits and vegetables and potatoes.

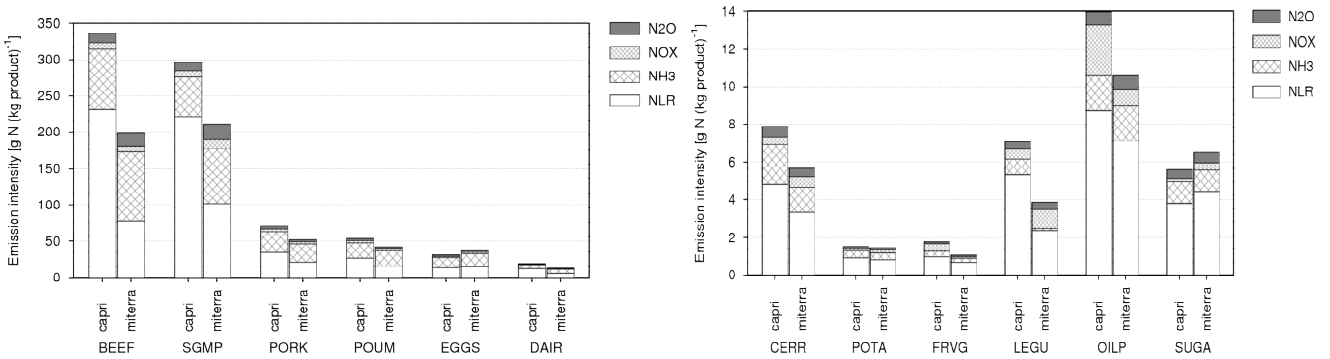

Figure 1 Emission intensities per food product for N_2O, NOx, NH_3 and N leaching and run-off (NLR) for the twelve main food commodity groups. The figures compare results from the CAPRI and the MITERRA-EUROPE models.

Conclusions Despite considerable uncertainty due to differences in model assumptions and data used, the nitrogen footprint of animal products is considerably higher than that of crop products. Corresponding values for nitrogen use efficiency are small for animal commodities (7-37%) and considerably higher for plant-based commodities (56-79%).

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Nitrous oxide emissions from arable soil following application of segregated and non-segregated pig manure and mineral fertilizer under different tillage systems

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Introduction Soil nitrous oxide (N_2O) emissions from application of animal manures and mineral fertilizer contribute to climate change worldwide. N_2O emissions depend on N fertilizer input, but may also be affected by the accompanied tillage method, as this alters the nitrogen and carbon dynamics in the soil (Choudary *et al.*, 2002). Although field scale measurements with different fertilizer and soil management regimes have been done to mitigate N_2O emission, little is known on the interaction between separated (or segregated) manure products and tillage regimes under arable farming. The objective of this work was to quantify soil N_2O emissions following application of segregated pig urine and faeces (i.e. kept separate under the housing system), liquid manure and mineral fertilizer under conventional and non-inversion tillage.

Material and methods Emission measurements were done on an experimental farm near Lelystad, the Netherlands during an experiment in potatoes (2011) and in sugar beets (2012). The soil type consisted of a marine clay loam soil. Prior to planting, segregated urine and liquid manure were injected whereas faeces were spread and incorporated into the soil in 2011 and injected in 2012 due to a higher water content. Mineral fertilizer (calcium ammonium nitrate) was applied by broadcasting. Application was aimed at 107 kg N ha^{-1} in 2011 and 140 kg N ha^{-1} in 2012. However, due to practical difficulties, N application deviated between treatments. A split plot statistical design was applied with three replicates (n = 3) of each fertilizer product under conventional and non-inversion tillage. Tillage method was not randomized. However, in the statistical analysis we assumed tillage method to be randomized over the plots. Additionally, we included a non-fertilized plot as a reference. N_2O measurements were done 23 times per year (3 measurements per plot) during 2011 and 2012. We used the closed chamber measuring approach. N_2O concentrations were measured using an Innova 1412 gas sampler. Measurements in potatoes included one replicate on top of the hill and two between hills. In potatoes and sugar beets, chambers randomly included crops later in the season. We calculated daily and cumulative N_2O emissions (linear interpolation between measurements) and final emission factors. Analysis of variance was performed on the data per plot. Pairwise t-tests were performed on the estimated means.

Results N_2O emissions increased after fertilizer application and altered slightly after harvest, ploughing and planting of the green manure crop (Figure and Table). Total N_2O-N emission ranged from 0.37 – 6.8 kg N_2O-N ha^{-1}. Segregated faeces showed the highest emission in 2011 (both absolute and relative), partly due to higher N application rates, whereas in 2012 this was relatively highest for liquid manure (ploughing) and urine (non-inversion till) most likely due to moist weather conditions after application of fertilizer.

Table 1 N application, N_2O-N emission and emission factors (means with different letters are significantly different within $P < 0.05$).

Soil		Application (kg N/ha)		Emission (kg N_2O-N/ha)				Factor (% of N applied)	
Management	Fertilizer	2011	2012	2011		2012		2011	2012
Non-inversion	Faeces	263	86	6.68	b	1.15	a	2.1	-0.5
	Urine	76	135	0.37	a	2.16	ab	-0.9	0.4
	Mineral fertilizer	107	89	0.74	a	1.52	ab	-0.3	0.0
	Liquid manure	175	133	1.50	a	1.49	ab	0.2	-0.1
Ploughing	Faeces	263	86	6.80	b	2.44	bc	2.2	1.0
	Urine	76	135	0.97	a	2.28	bc	-0.1	0.5
	Mineral fertilizer	107	89	2.15	a	1.16	a	1.0	-0.5
	Liquid manure	175	116	1.46	a	3.29	c	0.2	1.5
Reference	-	0	0	1.08	-	1.56	-	-	-

Conclusions N_2O-N emission following application of segregated pig urine and faeces, liquid manure, and mineral fertilizer ranged from -0.9 – 2.2% of applied N. Emission from application of segregated pig faeces was significantly higher than the other products in 2011, but not in 2012. Average N_2O-N emission under non-inversion tillage was lower compared to conventional tillage in both years. Results can be used for defining N_2O-N emission factors for arable farming under different fertilizer application and tillage strategies.

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Environmental and economic assessment of the utilization of algae by-products on Eastern Canadian dairy farms

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Introduction The increasing worldwide concern to find alternative sources of fuel that can help mitigate the environmental impact and counteract the competition with human and animal food associated with the production of ethanol lead us to the third generation of biofuels: algae based biofuels. Lately, studies have suggested that the algae by-product, after lipid extraction, could be a potential alternative as animal feed, but the environmental and economic impacts of its use have not been assessed. The aim of this study was to evaluate the environmental and economic impacts of using algae by-product on dairy farms in two regions of Eastern Canada.

Materials and methods First we estimated the value of the algae by-product (ABP) based on the composition of the microalgae *Nannochloropsis oculata* reported by Archibeque *et al*. (2009). These estimations were done either with the Petersen equation or the SESAME software (St-Pierre and Glamocic, 2000). Secondly, the whole-farm model N-CyCLES (Pellerin *et al.*, 2012) was used for the environmental and economic assessments. This model was developed to explore the change in economic performance of a dairy farm and to optimize the best management practices associated with maximizing income (minimizing cost) where ration formulations for the dairy herd, crop rotations choice, purchase of feed and fertilizers, and the allocation of manure to the land are considered as a single integrated unit of management. It evaluates the farm net income, the nitrogen (N) and phosphorus (P) balances and the greenhouse gas (GHG) emissions of the farm, reported as fat and protein corrected milk (CO_2 equivalents/kg FPCM/year) according to the International Dairy Federation. The analysis was done taking into account two typical production systems in Eastern Canada, one which includes corn grain production (CGP) and the other without corn grain production (WCGP). The characteristics of the two types of systems were a representative average of two climatic zones according to regional data. To feed the model, data from the last five years were used (2005-2009) based on regional sources. The two types of production systems were evaluated (maximizing for net income) without the inclusion (control) and with the inclusion of ABP.

Results The estimated value of ABP was 368.81$/t DM using Peterson equation with 172.69$/t DM for corn grain and 351.85$/t DM for soybean meal. The value estimated with SESAME, based on the prices and composition of 12 ingredients was lower at 257.42 $/t DM. In the production system CGP, the model did not include the ABP with the highest price calculated (table 1). For all other simulations, the farm net income was higher with the inclusion of ABP regardless the type of production system, whereas the P and N balances were slightly increased when the ABP was included compared to the control. The total GHG emissions were not affected by the inclusion of ABP in the diets. In the production system WCGP allocation of milk represented 75% of farm total GHG emission, cash crop and meat accounted for the remaining and in the production system CGP the allocation of milk was of 76%. In both productions systems the diets were reformulated when ABP was included mainly by reducing the amount of corn gluten meal. The crop rotation changed with the inclusion of ABP. Corn silage production was increased in both regions. As it is shown in table 1, the inclusion of ABP may be more interesting for the region WCGP, especially when the price of the ABP was $257.42.

Table 1 Net income, P and N balances and GHG emissions of dairy farms including algae by-product in the diets

	Without corn			With corn		
	Control	Sesame price	Petersen price	Control	Sesame price	Petersen price
ABP in the diets (kg/lactating cow/year)	0	1054	894	0	989	0
Farm net income ($/FPCM)	0.04	0.06	0.05	0.13	0.14	0.13
P balance (g/FPCM	1.34	1.44	1.44	0.40	0.54	0.40
N balance (g/FPCM)	27.3	27.6	27.6	18.2	18.6	18.2
Farm total GHG (kg CO_2e/kg FPCM)	1.37	1.37	1.37	1.31	1.31	1.31
GHG allocated to milk (kg CO_2e/kg FPCM)	1.03	1.02	1.02	1.00	1.00	1.00

Conclusions Inclusion of ABP in the diets increases the net income of dairy farms and has no impact on the GHG emissions of the farm. However, different factors such as the algae strain, growing conditions, cultivation and extraction, amongst others, may affect ABP composition leading to different results.

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Effect of essential oils on methanogenesis in the rumen of buffaloes

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Introduction The use of plant secondary metabolites as feed additive is an attractive and novel approach to improve livestock productivity under clean environment by controlling methanogenesis in the rumen. Essential oils, the fragrant part of some plants, are also classified as secondary metabolites and used as antimicrobials in cosmetics, as spices in the kitchen and also as a traditional medicine. In the authors' laboratory some essential oils have been tested *in vitro* and *in vivo* for inhibition of methanogenesis in the rumen and nutrient utilization in buffaloes.

Materials and methods *In vitro* screening of essential oils in graded levels were tested in *in vitro* gas production test (Menke and Steingass 1988) for their antimethanogenic activity. The substrate (200 mg) comprising of concentrate mixture and wheat straw in 50:50 or 30:50 ratio, was incubated in buffered buffalo rumen liquor. The concentrate mixture consisted of maize, 32; solvent extracted soybean meal, 20; wheat bran, 45; mineral mixture, 2; and salt, 1 parts. After 24h of incubation at 39^{o}C, methane production was estimated by gas liquid chromatography and the contents were used for *in vitro* true dry/organic matter digestibility (IVTD/IVTOMD). The selected oils (EO1 and EO2) were used as feed additive in adult buffaloes at the rate of 2 ml/head/d. *In vivo* methane emission from the animals was measured in an open circuit respiration chamber. The shifts in rumen microbial profile by inclusion of essential oils was done by Real Time-PCR. In another experiment, EO1 was fed to the growing buffalo calves (average body weight of 71.3 kg) divided into three groups : control, T1 with 0.01 ml and T2 with 0.02 ml/kg body weight for six months. The animals were fed on diet containing concentrate mixture and wheat straw in 1:1 ratio. The growing animals were fed a concentrate mixture of 20% CP to meet the requirement.

Results The inclusion of peppermint oil at 0.33 µl/ml caused a significant (P<0.001) depression in methane production with 9.9% reduction in IVTD (Agarwal *et al*., 2008), whereas inclusion of eucalyptus oil exhibited a similar methane reduction with a 22% reduction in IVTD at 1.0 µl/ml inclusion level (Kumar *et al*., 2009). Similarly, GO and CiBO showed the maximum, EO1 and EO2 the moderate and LGO the minimum reduction in methane production (Table 1). The EOs are more effective on high concentrate diet. Inclusion of GO and CiBO resulted in 38.49 and 34.88% methane inhibition on 50:50 (concentrate : roughage) diet, whereas the inhibition was only 19.49 and 22.50% on 30:50 (concentrate : roughage) diet. The results of *in vivo* feeding trials with Murrah buffaloes indicated that inclusion of EO1 and EO2 individually caused a significant decrease (P<0.01) of 20.65 and 23.2% in methane production with a depression in methanogens, ciliate protozoa and fungi populations in the rumen (Pawar, 2012). In growing Murrah buffalo calves the feeding of EO1 caused an increase in average body weight gain and improved feed conversion efficiency.

Table 1 *In vitro* screening of essential oils for methane production and effect on digestibility of feed

Essential oil (EO)	Level (µl/ml)	Methane (ml/g DDM/DOM)		IVTD/IVTOMD (g/g)		References
		Control	Treated	Control	Treated	
Mentha piperita (PO, Peppermint oil)[a]	0.33	54.70	43.83**	0.636	0.574**	Agarwal *et al.,* 2009
Eucalyptus globulus (EO, Eucalyptus oil)[a]	1.00	58.15	46.27*	0.609	0.473*	Kumar *et al.,* 2009
Allium sativum (GO, garlic oil)[b]	0.17	40.57	25.70**	0.613	0.595	Pawar, 2012
Cinnamomum zeylanicum (CiBO, Cinnamon bark oil)[b]	0.17	47.85	33.85**	0.629	0.579**	
Cymbopogon citratus (LGO, Lemon grass oil)	0.17	55.06	52.50*	0.568	0.552	
EO1[b]	0.17	47.92	39.86**	0.668	0.657	
EO2[b]	0.17	47.85	35.64**	0.629	0.622	

** P<0.001, * P<0.01, [a] DDM-Digested dry matter and IVTD ; [b] DOM-Digested organic matter and IVTOMD

Conclusions The essential oils inhibit methane production at low levels, but are sometimes accompanied with an adverse effect on digestibility of feed. Further *in vivo* trials might be essential before for their use as a feed additive is recommended.

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Metrological assessment of the absolute accuracy of methane emission measurements from livestock chambers in the UK

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Introduction A unique set of calibration experiments have been conducted to assess the absolute accuracy of methane emission measurements in livestock chambers at five different agricultural research facilities around the UK. In addition to validating the emission measurements from the individual facilities, this work also formally establishes the comparability of the measurements from different groups, giving confidence in the combination and comparison of different datasets from those groups. The validation campaigns were designed and run by scientists from the Environmental Measurements Groups at the National Physical Laboratory – the UK's National Measurement Institute.

Methodology As far as possible (given differences in facility engineering) a common approach was used for the validation work at each chamber facility. This consisted of:

- determination of a sensor calibration function by introduction of a series of calibration gas standards directly into the sensor inlet covering the applicable facility concentration range;
- determination of the ducting efficiency by introduction of a known mass emission rate into the duct that transports sample gas from the chamber to the sensor;
- determination of each chambers' capture efficiency by introduction of a controlled mass emission rate within the chamber;
- calculation of the overall calibration factor (one per chamber) combining the results from the above 3 determinations, and the total facility factor from the combination of the individual chamber results;
- derivation of the measurement uncertainties for each of the stages described above.

While the primary goal of the experiments was to determine the absolute accuracy of the emission measurements, this approach allowed the key components of each facility to be separately tested facilitating identification of the areas of greatest uncertainty and recommendations for future development.

The reference gases used for the work were common for all the validation experiments. A suite of seven methane standards with gravimetric traceability back to international standards (and the S.I.) were prepared specifically for this purpose to cover the range of concentrations observed by the different groups. These standards had an absolute concentration uncertainty of 0.45%-0.50% (k=2, 95% confidence) – this compares to a typical uncertainty of 2%-4% for the secondary standards available from the specialist gas suppliers that are generally used for regular span checks. In addition, the use of multiple concentrations, rather than a single span measurement, enabled sensor linearity to be assessed, and the calibration function to be determined across the required measurement range. An appropriate set of these standards, together with a high purity nitrogen zero gas (see below), was selected to match the requirements for each site.

The methane emission rate validation was carried out using a customised dynamic mixing source that can produce time-varying emission rates with calibrated mass-flow control. The dynamic mixing process combined different flows of Ultra High Purity (UHP) methane, with a minimum purity of 99.9995%, and BIP grade nitrogen, with hydrocarbon contamination of less than 50 ppb (expressed in methane equivalents). The mass flow control system was gravimetrically calibrated, using the appropriate gases, to determine the flows rates with an absolute uncertainty of within 0.5% (k=2, 95% confidence). The outputs from the mass flow controllers were blended to give a constant flow of gas with a controllable methane content. The methane emission levels were set at appropriate values to assess the chamber performances at typical operational levels.

In addition to the absolute calibration experiments a series of further tests were carried out to assess key aspects of the measurement system including sensor response time, chamber response time and concentration stability. Whilst these parameters do not always directly affect measurement accuracy they can influence experiment design and system optimisation.

Results and Conclusion The validation experiments have been successfully completed at all five chamber facilities. Calibration factors together with associated uncertainties have been derived for each facility, together with a number of other system parameters. This provides, for the first time, an assessment of the absolute accuracy of the methane emission measurement capabilities of a range of different facilities and chamber designs using a common metrological calibration procedure, and as a result establishes the comparability of methane emission measurements from the different groups.

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The effect of allocation methodology between milk and meat on the carbon footprint of a grass-based dairy production

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Introduction Following the FAO "Livestock Long Shadow" report there has been an increasing focus on quantifying greenhouse gas (GHG) emissions from milk and meat production. Consequently, there has been a widespread interest in determining the GHG emission from the life cycle (carbon footprint) of livestock products. The recent focus on product carbon footprints has resulted in a number of initiatives on developing standards to harmonise carbon footprint calculations (e.g. BSI, 2008). However, the standards do not agree on methods to allocate GHG emissions between co-products. Therefore, the purpose of this study was to assess the effect different allocation approaches have on the carbon footprint of a high performing grass-based dairy production system.

Materials and Method The carbon footprint of a high performing grass-based dairy production system was calculated using a dairy farm GHG model (O'Brien *et al*., 2011). The data used to model the grass-based system was based on the 5-yr research study of Horan *et al*. (2005) conducted at Teagasc, Moorepark dairy research centre. The study consisted of New Zealand Holstein Friesian cows that were turned out to grass in early February and housed in mid November. On average cows received 325 kg of concentrate DM and were stocked at 2.74 cows/ha with N fertiliser applied at 250 kg/ha. The GHG model was used to estimate all GHG emissions from the grass-based system: carbon dioxide (CO_2), nitrous oxide (N_2O), methane (CH_4) and F-gases. The model operates in combination with the Moorepark Dairy System Model (Shalloo *et al*., 2004), which provides the key parameters (e.g. feed rations) required for the GHG model to estimate emissions. The GHG model quantifies emissions of dairy systems using the life cycle assessment (LCA) method, which calculates emissions from all on and off-farm activities associated with milk production up to the point milk is sold from the farm e.g. includes emissions from the manufacture of purchased inputs such as fuel. The model estimates emissions in terms of their 100-yr global warming potentials (CO_2-eq), which on a weight basis relative to CO_2 was set to a factor of 25 for 1 kg of CH_4 and 298 for 1 kg of N_2O. The main output of the GHG model is a static account of dairy systems annual emissions, and the carbon footprint of milk production expressed per kg of energy corrected milk (ECM). In addition to producing milk, dairy farms also produce meat from culled cows and surplus calves. Therefore, GHG emissions should be distributed between these outputs. The following six approaches were used to allocate GHG emissions between milk and meat; 1) Milk –All emissions attributed to milk, 2) Mass – Mass of milk and meat, 3) Economic – Value of milk and meat, 4) Energy – Feed energy needed to produce milk and meat, 5) Emission – Emissions from surplus calves, dairy females <2 yrs and from finishing culled cows were attributed to meat with the rest assigned to milk, 6) System expansion - Assumes beef from milk production avoid emissions from alternative meat production. It was assumed that suckler beef was the alternative source of meat based on Cederberg and Stadig (2003)

Results and discussion Figure 1 shows, when the 100% allocation to milk scenario was excluded, that mass and economic allocation attributed the most GHG emissions directly to milk production (94-98%) followed by energy and emission approaches (87-88%). System expansion attributed the least emissions to milk production (59%), because the emissions associated with producing beef from the dairy herd (13.1 kg CO-eq/kg carcass weight (CW)) were significantly lower than the suckler herd (20.4 kg CO_2-eq/kg CW). The large differences between the results shows that comparisons of product carbon footprints are confounded when different allocation procedures are followed.

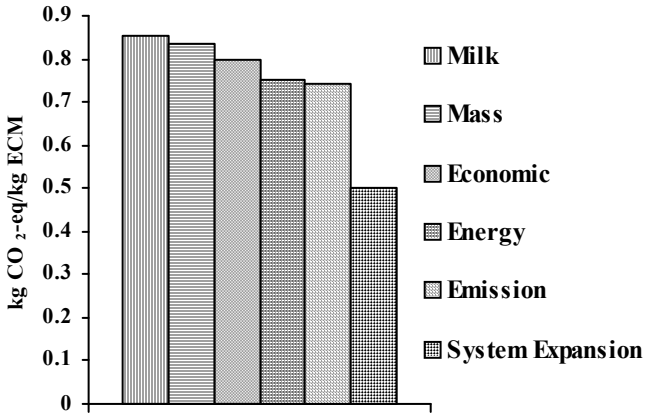

Figure 1 The effect of allocation method on the carbon footprint of a high performing grass-based dairy system

Conclusion This study shows that the carbon footprint of milk production cannot be compared when different allocation methods are used. Therefore, a single procedure needs to be agreed to facilitate a valid comparison of the carbon footprint of milk.

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Fuel consumption as a source of greenhouse gas emissions: off-road fuel consumption indicators for agricultural production in Belgium

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Introduction National governments are bound to report annual emissions, among which greenhouse gas (GHG) emissions, to the EU as part of the EU Climate treaty. Emissions are reported per industrial sector and often, subcategories are made such as off-road transport. Within this subcategory, the energy consumption and emissions by agriculture in Belgium are based on a 1994 calculation model that is no longer up-to-date as agricultural machinery has substantially changed with respect to both engine and operational efficiency. These innovations with a direct effect on the energy consumption and emissions require an update of the existing model. An emission model for mobile machinery during off-road use was established on request of the Flemish government, including data per Belgian region. The core of the emission model, in which exhaust and non-exhaust emissions are included, are the energy consumption data of the machines. This is also the subject of this paper. Detailed information about the fuel consumption in agriculture is an important part of LCA and carbon footprint studies that involve animal and plant production and products.

Material and methods The basis of the energy consumption model is a German study on the specific fuel demand of diesel engines in agriculture with respect to the tractor size, the type of trailed or tractor-mounted machine, the soil resistance, the field plot size, and the machine load (KTBL, 2005). For plant production, these factors are combined in the model per field operation to yield a field operation-specific partial fuel consumption (PFC), and this for each region (Flanders, Brussels, and Wallonia). The PFC's for all field operations that are involved in the cultivation of a crop are summed to yield the crop specific fuel consumption, here called a fuel consumption indicator (FCI in L/ha) for that crop. For animal production, the model uses a fixed number of hours of engine service for general tasks related to livestock farming. These FCI's have units of L/ farm. FCI's are always expressed on a year basis. The resulting amounts of fuel consumption are used for emission estimates on a regional (not farm) level, which implies the use of regionally adjusted weighing factors for farm-specific parameters in the model. These factors are machine size, soil resistance, and plot size. Agricultural machines were divided into 3 size classes: 13% small (35-69 pK), 85% medium (70 179 pK) and 2% large (>180 pK) tractors. Soil data were derived from the geological map of Belgium and subdivided into light, medium, and heavy soils. Data on the plot size were derived from national statistics. The energy consumption model includes stationary fuel consumption and fuel consumption for turning on the field and for transport to and from the field. A FCI was calculated for cereals, industrial crops, fodder, potatoes, dry harvested legumes, vegetables grown in open-air, temporary and permanent pastures, and fallow land. For livestock farming a FCI was calculated for cattle, pigs and poultry housing. When the FCI's for plant cultures and livestock are multiplied by respectively their total area under cultivation and the total number of farms, the absolute fuel consumption (L) of the plant cultures and livestock farming is known and can be further processed into corresponding GHG emissions.

Results Table 1 depicts FCI's for fodder crops and pasture. The fuel consumption of different field operations (PFC's) strongly differ. E.g. for forage maize cultivation in Flanders, ploughing (21.34 L/ha) and combine harvesting (23.75 L/ha) clearly are the most energy-intensive operations due to the soil resistance. In contrast, applying mineral fertiliser or spraying with a trailed machine barely consumes about 1 L/ha. Note that PCI's and PFC's are averages that apply to a farmer in general. The FCI's for livestock farming are currently under revision and will be presented at the conference. However, the energy consumption (FCI in L/farm) is expected to be considerably high because, although the number of hours of engine service is rather limited, it is continuously performed year-round.

Table 1 Fuel consumption indicators (FCI) for feedstock production in Belgium in L/ha

Feedstock	Flanders	Wallonia	Brussels
Forage maize	105.77	113.31	113.73
Fodder beets	130.42	133.67	140.32
Forage crops*	46.07	47.93	49.58
Pasture	54.06	57.33	56.03

*Clover, ryegrass, white mustard, cereal rye, and common vetch

Conclusions The respective FCI's are fairly similar for Flanders, Wallonia and Brussels. Within the FCI's for plant production, the fodder beets have the highest scores. The FCI for maize silage is lower, but still about 15 L/ha higher than the FCI for cereals (data not shown). The low FCI for pasture is due to the relatively small % of pastures that are actually mowed (70%) and of which the grass is ensilaged (56%) or pressed in bales (14%). Concerning livestock farming, the total off-road energy consumption is remarkably high (data not yet shown). In contrast to animal production with daily activities, the off-road energy consumption in feedstock production is spread over the year.

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The crude glycerin in diets for feedlot cattle can reduce emissions of methane and carbon dioxide

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Introduction The growing concern for renewable energy is of global interest, the production of biofuels has been the focus of much research. The use of biodiesel as a fuel provides environmental benefits for the entire planet, as it helps with the reduction of pollution and greenhouse gases. However biodiesel production generates large volumes of glycerin byproduct, which can be used as an alternative energy source in animal feed, particularly for ruminants, where glycerol is released directly for energy (Donkin, 2008). The objective of this study is to evaluate if the inclusion of crude glycerin in the diet of cattle can alter the production of carbon dioxide and methane during ruminal fermentation.

Material and methods The work was performed at the Laboratory of ingredients and Gases Pollutants Unit Animal Digestive and Metabolic Studies of the Department of Animal Science FCAV, UNESP, Jaboticabal, SP. We carried out an in vitro incubation of two diets used in finishing beef cattle, where one contained 15% glycerin (inclusion of replacing corn diet). The diets contained 30% of incubated corn silage and concentrate was composed of corn, soybean hulls, sunflower meal and absence or presence of glycerin. The quantification of gases, CO_2 and CH_4 was performed after 24 hours of incubation. After measuring the total gas produced, an aliquot was injected into a gas chromatograph Trace GC Ultra Thermo Scientific equipped with ionization detector flame, using argon as carrier gas with a flow rate of 25 mL / min and the oven temperature was 70 º C. Calibration was performed with a standard mixture of methane and carbon dioxide gases. The peak areas were integrated using software Chromquest 5.0. Six cattle, consuming a diet that contained the ingredients studied were donors of rumen fluid. Before feeding, ruminal fluid were collected, mixed and then placed in the fermenters, the ratio of 1.25 g DM/100 mL of rumen fluid and incubated at 39 º C for 24 hours. The study was a completely randomized design with 2 treatments, 24 repetitions and 6 blanks (tubes containing rumen fluid, but without feed). The fermenters contained only the white ruminal fluid sample without the ingredient. Analysis was performed by ANOVA and Tukey's test applied to 5% probability.

Results It can be observed that the inclusion of glycerin replacing corn decreased the production of gas during ruminal fermentation. The CH_4 production decreased approximately 39% while CO_2 reduced by 37% with the inclusion of crude glycerin in the diet. These reductions are very significant considering that number of animals confined in the world is increasing, as is the search for a sustainable animal production (Moss *et al*., 2000). The reduction of the production of these gases can be related to the rapid fermentation of glycerol in the rumen, which is fermented to propionate by reducing the elimination of gases analyzed and consequently increasing energy availability for the animal. According Alluwong *et al.* (2011) the quality of the foods to be inversely related CO_2 production e CH_4 thus the inclusion of 15% glycerol in beef cattle diets can reduce the production of gaseous CO_2 eCH_4 from rumen fermentation.

Table 1 Fermentation gas production "in vitro" diets

Production, mL/g MS	Treatments		
	control	glycerin	s.e.m.
CH_4	47.75	29.09	1.33
CO_2	198.15	123.47	5.99

Means followed by different letters differ by Tukey test at 5% probability.

Conclusions The crude glycerin can be an interesting source of energy for use in diets for beef cattle raised in feedlot, because it can reduce greenhouse gas emissions, reducing the environmental impact of creation. This increases the credibility of the use of biodiesel as an energy source, because besides being a renewable fuel, its main byproduct can be used as feed and reducing the environmental impact of production.

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Batfarm Software: A support tool for assessment of environmental strategies to mitigate gas emissions from intensive livestock operations (swine, poultry and cattle) in the Atlantic Region

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Introduction It is necessary to determine synergistic and appropriate mitigation options for individual farms in order to successfully and efficiently reduce environmental pollution from agriculture. Mitigation options should be assessed based on their abatement potential and cost-effectiveness along the entire production process. We present a novel tool to assess the mitigation potential of nitrous oxide (N_2O), methane (CH_4) and ammonia (NH_3) losses as a consequence of different strategies and techniques implemented on intensive cattle, pig and poultry farms. A preliminary case study result is shown.

Material and methods The modeling approach is an intermediate between traditional empirical and mechanistic models. Emissions and consumptions from different environmental mitigation strategies were reviewed and incorporated in the software data base. Some of these data came from on-farm measurements obtained in the regions of the Atlantic Area of Portugal, Spain, France, UK and Ireland. Default values that can be adjusted by the users, have been included to develop versatile and user-friendly software. Regionally specific input values for zootechnical data, climatic information and emission factors have been defined in order to reflect different climatic and production conditions within the Atlantic region. As a result, both nutrient balance and gaseous emissions are calculated throughout the different stages of the animal production system based on particular farm management as defined by the users. All calculations are performed on a cumulative monthly and annual basis. Housing stage: An animal nutrient balance is estimated using zootechnical and nutritional data. Gas emissions are calculated for different housing systems and manure management practices, including grazing periods. An example for a fattening pig farm (1000 places from 22 to 110 kg) in Spain under Continental Mediterranean conditions (mean temperature, 14ºC; annual rainfall, 354 mm) has been simulated. In this case study two scenarios are compared: a standard situation *vs* a situation where some BATs have been implemented (Tri-phase feeding + Weekly slurry removal). Storage: The calculations are based on emission factors and nutrient balance. The climatic conditions and the effects of covers and additives on nutrient and mass balance are also considered in the model for slurry storage. Treatment: Different treatment options are assessed resulting from the combination of mechanical phase separation techniques, aerobic treatment, anaerobic digestion, gravity decantation and composting. Landspreading: The climatic conditions, the application method, speed of incorporation into soil, and dry matter content in the manure (liquid manure only) are taken into account to calculate NH_3 emission following landspreading. Direct and indirect N_2O emissions are also estimated. An assessment of each stage on Faecal Indicators Organisms (FIOs) is also provided to the user, using a three-stage qualitative scale. To facilitate environmental strategies selection, users have the option of introducing the economical cost and they also can compare between two different situations.

Results Figure 1 shows the gaseous emission for the case study described. The model estimates a reduction both in total nutrients excreted and gaseous emission in the scenario where the BATs have been implemented. Protein and phosphorus consumption are reduced from 484 to 437 and from 15.7 to 13.6 g per kg of live weight produced, respectively. Although the volume of slurry removed from buildings is the same in both cases (1660 m^3/year), nutrient concentration is also lower in the situation where the BATs were implemented: from 5.8 to 5.3 g/kg in the case of nitrogen and from 1.5 to 1.2 g/kg in the case of phosphorus.

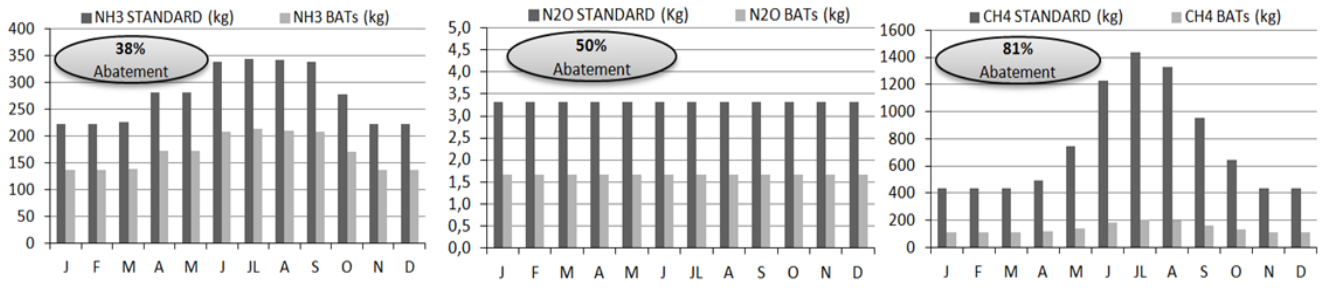

Figure 1 Gaseous emission pattern (NH_3, N_2O and CH_4) after the application of two abatement techniques at fattening pig housing.

Conclusions This work integrates existing information on the potential of different strategies and environmental techniques to affect gaseous and nutrient losses in livestock farms from the Atlantic region. The software enables simulation and comparison of the effects of different environmental techniques throughout all the farm process under specific management and climatic conditions. The software tool will facilitate the selection of the most suitable techniques for a particular situation. Further testing will be necessary to validate the results provided by this tool.

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The effect of tannin on In vitro digestibility, gas and methane production of six tropical browse species

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Introduction Recently there are numerous reports that have shown the reduction of methane production from ruminants due to inclusion of tannin in feeds. However, tannins from different plants exhibit variation in their effects even at the same concentration as evidenced by difference in magnitudes of gas production and true digestibility. This indicates that tannin from different plants might show different response in methane production. Therefore, this study aimed to investigate the effect of tannin from six tanniferous tropical browses on digestibility, gas production and calculated methane production under *in vitro* condition.

Materials and methods Fresh leaves of plant samples were collected, immediately frozen, dried in forced oven, and ground to pass a 1mm sieve in a Willey mill for this study. Neutral detergent fibre (NDF) and nitrogen were analyzed by standard procedures. Determinations of total phenols (TP) and total tannins (TT) were done based by Makker (2003). Rumen fluid was collected and prepared anaerobically using standard procedures while culture medium was prepared as described in Goering and Van soest (1970). A semi- automated system was used to measure gas production through *in vitro* incubation at 39°C, according to Theodorou *et al.* (1994). A 400 mg of respective feed sample (±400mg PEG) was weighed in to 120ml serum bottles, then 40 mL of rumen fluid + medium (15 rumen fluid and 25 ml of culture medium) was added under a stream of CO_2. Two replicates and four different runs were executed for every sample. The *in vitro* organic matter digestibility (IVOMD) was done according to Tilley and Terry (1963). Methane was calculated as described by Blummel *et al*. (1999a). Means were statistically analyzed using the 'GLM' option of SAS and differences among means were determined using Duncan's multiple-range test.

Results There was significant ($P<0.05$) variation in composition of OM (organic matter), CP, NDF, TP and TT among the plants due to inherent genetic differences. Tannin significantly ($P<0.05$) suppresses gas production, IVOMD, total VFA and methane as indicated by the result of inclusion of PEG. Lowest methane values were found with high tannin contents without inclusion of PEG, However; the ratio of IVOMD to Methane was negatively affected by tannin indicating compromised efficiency of tannin in ruminant feeding.

Table 1 Mean + s.e. chemical composition, TP and TT (g/Kg DM) of plants used in the study

Scientific names	OM	CP	NDF	TP	TT
Zizpus mucronata	840.4±5.2^{d}	243.3±2.3^{b}	339.2±6.5^{c}	79.6±1.0^{d}	11.4±0.6^{e}
Rhus Lancea	845.3±4.9^{c}	86.9±2.4^{f}	338.5±5.4^{c}	209.1±0.4^{a}	172±0.4^{a}
Olea europaea L	907.9±3.6^{a}	105.0±5.5^{e}	378.6±9.6^{b}	87.6±0.6^{c}	44.9±0.26^{c}
Melia azedarach	821.6±6.7^{e}	298.7±2.4^{a}	315.4±5.4^{d}	32.4±0.2^{e}	19.0±0.24^{d}
Peltophorum africanum Sond	865.4±10.4^{b}	171.7±3.6^{c}	303.7±1.3^{e}	206.7±0.2^{a}	113.7±0.4^{b}
Acacia nilotica	836.4±2.7^{d}	153.9±4.3^{d}	517.5±1.8^{a}	167.5±0.1^{b}	113.0±0.3^{b}

Table 2 IVOMD (%), 48hr GP (gas production), methane, individual and total VFA production from incubated feed samples. The % increase as the result of PEG inclusion was indicated in parenthesis.

Scientific name	IVOMD	48hr GP	Methane	Acetic	Propionic	Total VFA	IVOMD: Methane
Zizpus mucronata	39.4^{c}(93.4)	100.9^{a}(21)	33.2(2.8)	69.8(2.8)	18.5(1.6)	100.8(2.4)	1.17(93.2)
Rhus Lancea	40.4^{c}(94)	25.7^{f}(84.5)	20.9(51.2)	42.2(51.4)	9.65(31.6)	58.6(47.3)	1.12(89.3)
Olea europaea	52.6^{b}(57.9)	70.9^{c}(45.6)	21.7(3.4)	42.5(3.8)	9.5(31.2)	60.7(2.7)	1.47(56.4)
Melia azedarach	74.6^{a}(10.8)	87.9^{b}(7.2)	28.9(8.75)	58.1(8.4)	15.2(6)	85.5(8.1)	2.21(21.8)
Peltophorum africanum	34.4 ^{d}(88.4)	31.7^{e}(58)	22.8(13.5)	48.2(9.5)	52.8(14.8)	65.01(15.3)	0.98(90.8)
Acacia nilotica	41.9^{c}(81.1)	43.7^{d}(68.4)	20.4(23.1)	45(16)	14.3(-1.2)	64.7(13.9)	1.33(69.9)

Conclusion Multipurpose browse can effectively reduce methane production and as they are cost effective, can be used under small-holder farmers and pastoralists.

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Supplementing glycerol in forage diets increases in vitro methane production using a rumen simulation technique (RUSITEC)

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Introduction Glycerol from biodiesel production has been successfully included in diets of finishing ruminants as a replacement for cereal grains and subsequently its use in ruminant diets is likely to increase. Increases in propionate production when using glycerol as replacement of barley grain have not been associated with reductions in *in vivo* CH_4 production (Avila-Stagno *et al*., 2013). A possible cause for this lack of response is that the shift towards propionate fermentation is of sufficient magnitude given the propiogenic properties of barley grain. The effects of glycerol on CH_4 emissions from forage diets have not been assessed. Thus, this study was conducted to evaluate the effects of supplementing increasing concentrations of glycerol on fermentation characteristics of forage diets given that these diets are most commonly fed to the breeding and growing herds which account for more than 80% of total enteric CH_4 emissions within the beef production lifecycle.

Material and methods Four dietary treatments were replicated in two rumen simulation technique (RUSITEC) apparatuses (n=16 fermenters). The experimental period consisted of 15 d (8 d adaptation + 7 days sampling). The experimental treatments included brome hay, corn silage and glycerol in the following proportions: 1) control: 8.5 g hay + 1.5 g corn silage, 2) 8.5 g hay + 1.0 g corn silage + 0.5 g glycerol, 3) 8.5 g hay + 0.5 g corn silage + 1.0 g glycerol, and 4) 8.5 g hay + 1.5 g glycerol. Hay and silage were ground through a 4 mm screen. Glycerol was mixed with the hay proportionally for each treatment before filling the polyester bags (100 × 200 mm; pore size = 50 µm). Corn silage was incubated in separate bags (50 × 100 mm). Dry matter, NDF, ADF and CP disappearance at 48 h was determined daily from d 9 to 15. Fermentation gas was collected into vinyl urine collection bags connected to each fermenter. Just prior to feed-bag exchange, daily total gas production from each fermenter was determined by water displacement. Gas samples were taken from the septum of collection bags and transferred to evacuated 6.8-mL exetainers for CH_4 analysis. Fermenter pH was recorded daily at the time of feed-bag exchange and fluid samples were collected to determine VFA and ammonia concentrations.

Results The inclusion of glycerol in the substrates linearly increased DM disappearance from hay (P=0.01) and silage (P=0.02). Hay NDF (P=0.05) and ADF (P=0.02) disappearances were increased when glycerol was included at 15% of DM. Total gas production, CH_4 concentration and total CH_4 production per g DM incubated were linearly increased with glycerol inclusion in the substrates (Table 1). A linear reduction (P<0.01) in the proportion of acetate and a linear increase (P<0.01) in the proportion of propionate as a percentage of total VFA, resulted in linear reduction (P<0.01) in the acetate:propionate ratio with increasing glycerol. Glycerol donates electrons before entering the propionate fermentation pathway, thus increasing hydrogen available for CH_4 production. This study demonstrates that increases in propionate are not always associated with a reduction in CH_4, highlighting the complexity of rumen stoichiometry. The lack of effect in previous *in vivo* trials may be due to escape of glycerol from ruminal fermentation by direct absorption from the rumen or passage to lower gut, and not solely to a lack of an increase in propionate production.

Table 1 Effects of increasing concentrations of glycerol in the substrate on total gas and methane production in the RUSITEC.

	Glycerol concentration (% DM)					P			
Item	0 (Control)	5	10	15	s.e.m.	Treatment	Linear	Quadratic	0 vs. Glycerol
Hay DM loss, %	38.2a	37.7a	38.6a	40.5b	0.41	<0.01	<0.01	<0.01	0.11
Silage DM loss, %	51.0b	52.1b	56.2a	-	1.371	0.03	0.01	0.33	0.05
CH_4 concentration, %	1.1c	1.6b	1.8ab	2.0a	0.16	<0.01	<0.01	0.46	<0.01
Gas volume, mL	930	1015	1058	1056	57.1	0.21	-	-	-
Total CH_4, mg	7.5b	11.5ab	13.5a	15.3a	1.63	<0.01	<0.01	0.44	<0.01
CH_4, mg/g hay DMD	2.6b	3.6ab	4.5a	4.9a	0.58	0.02	<0.01	0.57	0.01
CH_4, mg/g DM incubated	0.8b	1.2ab	1.4a	1.6a	0.17	<0.01	<0.01	0.47	<0.01
Acetate (mmol/100 mmol)	56.3a	47.5b	42.3c	41.2c	0.63	<0.01	<0.01	<0.01	<0.01
Propionate (mmol/100 mmol)	21.8d	27.2c	31.3b	34.2a	0.50	<0.01	<0.01	0.02	<0.01
Acetate:propionate ratio	2.57a	1.76b	1.36c	1.21d	0.045	<0.00	<0.01	<0.01	<0.01

Least square means with different letter (a-d) within row differ P<0.05.

Conclusion Glycerol inclusion in forage diets increases DM disappearance from hay and increases production of CH_4 using a rumen simulation technique (RUSITEC).

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Impact of grazing on CO_2 fluxes of an intensively grazed grassland in Belgium

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Introduction To date, there are few studies assessing the impact of specific management events, particularly grazing, on carbon (C) and carbon dioxide (CO_2) fluxes in managed grasslands. Grazing effects are indeed difficult to discern. They vary with the stocking rate and the length of the grazing period. Moreover, they are often masked by environmental responses (Peichl *et al*., 2012). The aim of the present study was to assess the impact of grazing on the carbon balance of a Belgian grassland grazed by the Belgian Blue cattle.

Material and methods The research was run at the Dorinne terrestrial observatory (DTO), located in the Belgian Condroz (50° 18' 44'' N; 4° 58' 07'' E; 248 m asl.). The site is a permanent grassland of ca. 4.2 ha subjected to intensive management. The paddock is rotationally grazed by Belgian Blue cattle. Average stocking rate is around 2 livestock units per hectare (LU ha^{-1} y^{-1}). Grassland carbon budget (Net Biome Productivity, NBP) at the system boundaries is calculated from Net Ecosystem Exchange of CO_2 measured by eddy covariance by taking imports and exports of organic C and losses of carbon as methane (CH_4) into account. After 2 years of measurements (May 2010 - May 2012), the site was close to equilibrium (NBP = 23 ±34 g C m^{-2} y^{-1}). If management practices (harvest, fertilization and imports as supplementary feedings) and climate had a significant impact on C balance, the impact of grazing was uncertain, especially on CO_2 fluxes. To do this study, we analyzed the temporal evolution of gross maximal photosynthetic capacity GPP_{max}. This parameter was deduced from the response of daytime CO_2 fluxes to radiation over 5-day windows. We calculated GPP_{max} variation between the beginning and the end of grazing and non-grazing periods ($\Delta GPPmax$) and analyzed its dependence to stocking rate. In addition, a confinement experiment was carried out to analyze livestock contribution to Total Ecosystem Respiration. Each experiment extended over two days: the first day, cattle was confined in the footprint of the eddy covariance set-up (1.76 ha, 27 LU ha^{-1}) and the second day, it was removed from it. We compared filtered half-hourly data made at 24h intervals, in the presence or absence of cattle, considering that environmental conditions were equivalent (air temperature, wind speed, radiation and wind direction).

Results A significant decrease of GPP_{max} during grazing periods and a ΔGPP_{max} dependence on the average stocking rate allowed us to quantify the assimilation reduction due to grass consumption by cattle (results not shown). Moreover, the results of the confinement experience showed that CO_2 emission was significantly higher during cattle confinement (Figure 1). Livestock contribution estimation to CO_2 fluxes was estimated to be 6.6 µmol m^{-2} s^{-1}, which represented 0.14 µmol m^{-2} s^{-1} LU^{-1}.

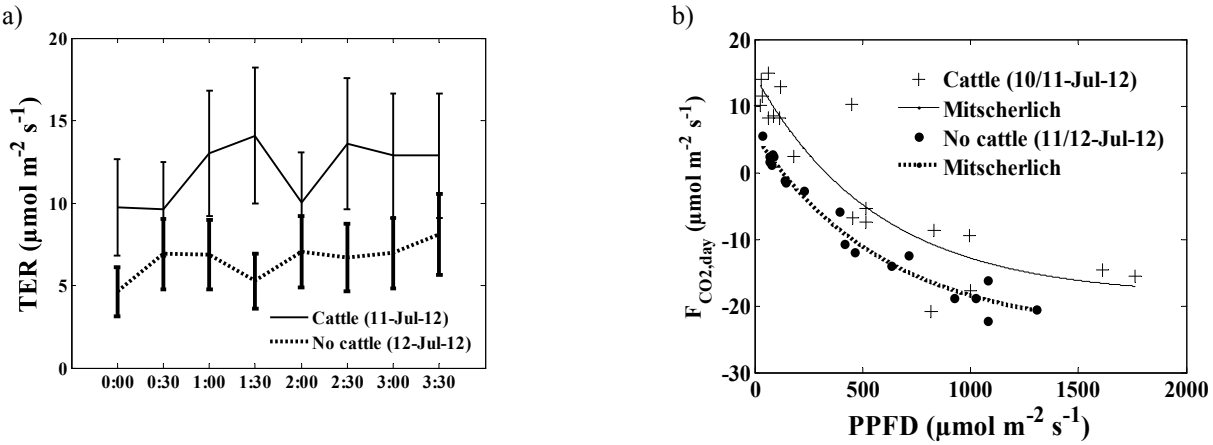

Figure 1 a) Nighttime CO_2 flux evolution and b) daytime CO_2 flux response to radiation of two successive days with or without cattle. Errors bars are the random error of measurement reported at a 95% confidence interval.

Conclusions We found a direct and an indirect impact of grazing on the CO_2 emissions by a meadow. The direct impact was an increase of CO_2 emissions in presence of cattle. It could be distinguished and quantified only thanks to the confinement experiment. The indirect impact was a decrease of photosynthetic capacity, due to consumption of vegetation. Discrimination of the latter impact from flux response to climate was possible only after gathering and treating two years of measurements taken under various climatic conditions.

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Assessing meteorological conditions effects on MIR predicted methane emissions of Holstein cows under a temperate environment

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Introduction Methane (CH_4) emissions produced from enteric fermentation by ruminants represent major losses of energy for dairy cows. These emissions contribute to global warming. Therefore, it appears necessary to develop different approaches to mitigate CH_4 emissions in order to improve the sustainability of dairy farming. CH_4 emissions could be partly influenced by the meteorological conditions as they vary according to the season (Lassey, 2007). Moreover, individual cows under high temperature could be partly responsible for these variations. The aim of this study was to assess the impact of meteorological conditions on CH_4 emissions of Holstein cows under a temperate environment.

Material and methods 257,635 milk mid-infrared (MIR) spectra collected between January 2007 and December 2010 in 983 herds by the Walloon Breeding Association (Ciney, Belgium) from 51,782 primiparous Holstein cows were used. CH_4 emissions values (g/day) were predicted based on the calibration equation developed by Vanlierde *et al*. (2013; R^2 of cross-validation=0.70) and applied to the recorded spectral data. Two traits were derived from these predictions: g CH_4 per day and g CH_4 per kg of fat and protein corrected milk yield (FPCM). Daily meteorological data from 4 public weather stations were available. Daily temperature humidity indices (THI) were computed as followed (NRC, 1971):

$$\mathbf{THI = (1.8 \times T_{db} + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T_{db} - 26)]}$$

where T_{db} was the dry bulb temperature and RH was the relative humidity. The mean daily THI of the previous 3 days before each test-day (TD) MIR CH_4 record was designed as the THI of reference for that TD. The mean distance between the weather reference station and the herd was of 25 km.
The following random regression TD model was developed in order to study the effect of THI on MIR CH_4 emission of cows:

$$\mathbf{y = Xb + Q_1(Wh + Zp + Za) + e}$$

where **y** was the vector of MIR CH_4 emissions (g of CH_4/day or g of CH_4/kg of FPCM), **b** was the vector of fixed effects (*i.e*., herd x TD, minor lactation stage, gestation stage x major lactation stage, and lactation stage x age at calving x season of calving), **h** was the vector of year of calving x herd (YxH) random regression coefficients, **p** was the vector of permanent environmental (PE) random regression coefficients, **a** was the vector of additive genetic random regression coefficients, and **e** was the vector of residuals, **X**, **W**, and **Z** were the incidence matrix, and $\mathbf{Q_1}$ was the covariate matrix for first-order Legendre polynomials related to THI. The model estimated the variance components using REML. The phenotypic expression of the different MIR CH_4 predictors was the consequence of general (intercept) and specific reaction to THI (slope) correlated values of YxH, PE, and genetic effects.

Results The mean THI was 50.83 (±10.31), mean MIR CH_4 (g/day) was 558.05 (±89.89) and mean MIR CH_4 (g/kg of FPCM) was 25.64 (±7.76). Table 1 shows the part of the variances associated to THI compared to variances not associated with (intercept effects).

Table 1 Variances associated to THI relative to the variances associated to the intercept effects.

Traits (N=257,635)	YxH	PE	Genetic
MIR CH_4 (g/day)	3.71	1.23	0.21
MIR CH_4 (g/kg of FPCM)	0.65	0.50	0.37

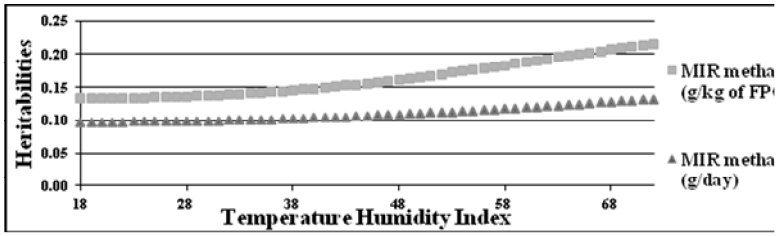

Figure 2 Heritabilities depending on THI.

The association between daily CH_4 emissions and THI scale was marked by changes of variances for the two traits with higher values of THI. As shown in Table 1, more important variations of PE and YxH variances were explained by a larger proportion of variances that was associated with THI. Moreover, heritabilities of the two CH_4 emissions predictors increased with THI, higher heritabilities being observed for MIR CH_4 (g/kg of FPCM; Figure 1).

Conclusions Results from this study showed that THI affects CH_4 emissions of dairy cows under a temperate environment. Large parts of environmental (PE and YxH) and, to a lesser extent, genetic variations were associated with THI.

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Yearly follow-up of methane turbulent exchange over an intensively grazed grassland in Belgium

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Introduction Methane emissions account for 8% of the EU-15 GHG emissions and livestock generates approximately half of these emissions (UNFCC, 2012). Recent technological advances in spectroscopy now permit methane flux measurement using eddy covariance. This method has numerous strengths. It can measure fluxes *in situ*, continuously and across broad areas. This provides information about meadow and cattle emission behavior throughout the year and across a broad range of climatic conditions. We will present here a one year monitoring of methane exchange between an intensively grazed meadow and the atmosphere obtained using the eddy-covariance method.

Material and methods Methane fluxes emitted by a grazed meadow were measured continuously from June 2012 to June 2013 at the Dorinne Terrestrial Observatory (50° 18' 44" N; 4° 58' 07" E; 248 m asl.) in Belgium. The site is an intensively pastured meadow of 4.2 ha managed according to the regional common practices where up to 30 Belgian Blue cows are grazing simultaneously. Flux measurements were made with the eddy covariance technique, using a fast CH_4 analyzer (Picarro G2311-f) and a sonic anemometer (Campbell Csat3). Carbon dioxide fluxes and various micro-meteorological and soil variables, biomass growth and stocking rate evolution were also measured at the site. Turbulent fluxes were calculated according to standard eddy covariance computation schemes (Aubinet *et al.*, 2012) using Eddysoft and were filtered for non-stationarity and for low friction velocity (u*) events.

Results Results presented here only concern the 2012 grazing season. During cow absence, the methane flux reaches 10.5 ± 24.9 nmol m^{-2} s^{-1} while it amounts to 111 ± 116 nmol m^{-2} s^{-1} when cows are present on the meadow. When cows were not present in the meadow, no relation was found between methane fluxes and soil temperature while a weak negative relation was found between methane fluxes and soil humidity at 5, 25 or 50 cm. No clear circadian evolution is observed, neither during grazing periods nor during cow free periods.

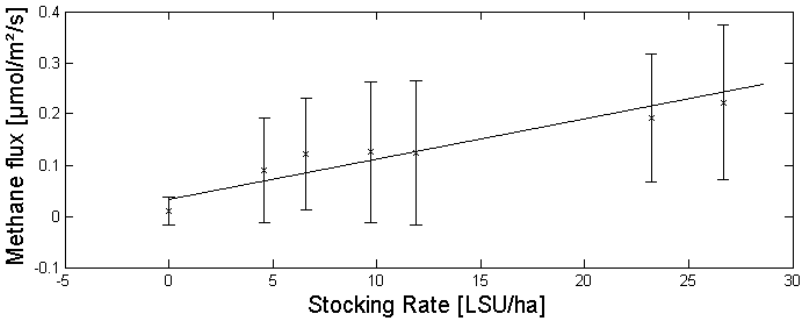

Figure 1 Methane fluxes above the Dorinne Terrestrial Observatory (grazed meadow) according to stocking rate with linear regression (Y=0.0085 X+0.0288).

Conclusions During cow presence periods, fluxes are highly variable, probably due to cow movements in and out the measurement footprint and cow digestion rhythm. However, when fluxes are integrated over large periods, methane emissions were found strongly related to cattle stocking rate with a slope of 7.34±0.78 mol CH_4 day^{-1} LSU^{-1}. Further developments are ongoing in order to improve cattle geo-localization through infra-red cameras and individual home-made GPS devices. The two systems will be compared in terms of cost, efficiency and ease of use.
Up to now, no soil methane absorption has been observed, the meadow behaving as a methane emitter, even in the absence of cows. Relation between soil methane emissions and environmental parameters is weak. No major environmental drivers are identified yet.

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Greenhouse gases emissions in Marandu grass fertilized with compost and biofertilizer of beef cattle excretes

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Introduction Global warming has intensified and the same is caused by the emission of greenhouse gases like carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) and livestock is responsible for much of these gases (IPCC, 2007). Brazil is a significant producer of cattle, so that livestock is a major source of emissions of greenhouse gases and the country lacks in studies regionalized to improve gas inventories. The use of fertilizers from organic sources could provide additional CO_2 emission (Frank; Liegig; Hanson, 2002), as well as providing methanogenic activity and a source of nitrogen providing N_2O emissions. This study aimed to evaluate the emissions of greenhouse gases in grassland of Marandu grass fertilized with compost and biofertilizer of beef cattle excretes.

Material and methods Experiment was conducted at the experimental farm of campus of Jaboticabal of the Universidade Estadual Paulista. The local soil is classified by Oxisol and climate as tropical with dry winter and rainy summer. The quantification of CH_4 and N_2O fluxes was conducted using the static chambers methodology where treatments were compost and biofertilizer of cattle being applied the equivalent of 80 g N per m^2 and control that received no fertilizer. Samples of gas were made during the morning and were followed by gas chromatographic determination. The evaluation period totaled 120 days and the total emission of the period was obtained by weighted average. Net emission of each treatment was obtained by discounting the emissions of control. Assessments of CO_2 emissions was accomplished through of closed gas flux system, a LI-COR model equipped with infrared gas analyzer and gas flux control unit was used to measure CO_2 evolving from the soil (LI-COR, Lincoln, NE). The uncertainty of the emissions obtained was as assessed using the standard error of the mean.

Results The availability of labile carbon after fertilizer application allowed net emission of CO_2. Moreover excellent oxidation conditions of Oxisol provided for methanotrophic bacteria sufficient oxygen for oxidation of CH_4 during the evaluation period, totaling negative CH_4 fluxes. N_2O fluxes were positive due to the higher availability of N of biofertilizer, taking the compost having main emissions. Net emission is shown in Table 1.

Table 1 Net emission of greenhouse gases as a function of biofertilizer and compost of cattle excretes in grass Marandu. In the last column observe the balance of the gases in CO_2 equivalent calculated on global warming power of each gas.

	CO_2	CH_4	N_2O	Balance in CO_2 eq
Treatments	g C-CO_2 m^{-2}	mg C-CH_4 m^{-2}	mg de N-N_2O m^{-2}	g CO_2eq
Biofertilizer	49.0 (13.2)*	-2367.4 (845.6)	0.6 (0.67)	-10.07
Compost	58.1 (5.8)	-4878.4 (2935.4)	4.2 (3.68)	-62.64

*In parentheses are the values of the standard error of the mean.

The compost presented higher oxidation of CH_4 due to a bigger amount of carbon and therefore energy source for the methanotrophic bacteria (Murrell; McDonald; Bourne, 1998). However, it was allowed higher CO_2 emissions. Although there N_2O and CO_2 emissions of CH_4 oxidation annulled these emissions.

Conclusions Fertilization of pastures of Marandu grass promoted CH_4 oxidation. The oxidation of CH_4 and CO_2 were higher the amount of carbon available. Gas emissions when evaluated together provided carbon sequestration system.

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Effectiveness of bacterial direct-fed microbials to reduce methane output in dairy cows

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Introduction The use of direct-fed microbials (DFM) is one possible option to reduce methane (CH_4) emission from ruminants (Martin *et al.*, 2010). A few *in vivo* studies were performed with yeasts but to date the use of bacterial DFM as modulators of rumen methanogenesis has never been explored. Recently we reported that the effectiveness of bacterial DFM to prevent acidosis in sheep depended on the ruminal fermentation patterns (Lettat *et al.,* 2012). The aim of this study was to assess the ability of bacterial DFM to reduce methanogenesis in dairy cows fed diets inducing contrasting fermentation patterns in the rumen.

Materials and methods Eight lactating Holstein cows fitted with ruminal cannulas were allocated to 2 groups of 4 animals fed twice daily either a high starch diet (HSD; 38%) or a low starch diet (LSD; 2%) in a 55:45 forage-to-concentrate ratio to promote different ruminal fermentation patterns. In each group, cows were randomly assigned to 4 treatments in a 4 × 4 Latin square design: control diet without DFM (C) or supplemented with *Propionibacterium* P63 (P), *Lactobacillus plantarum* strain 115 plus P (Lp+P), or *L. rhamnosus* strain 32 plus P (Lr+P). To ensure an entire consumption of the DFM, they were mixed with a small portion of concentrate and offered once daily before the morning feeding. Cows on the DFM treatments received 10^{10} CFU/d of each strain whereas control cows received only carrier (lactose). During each 1-month experimental period, daily intake, milk yield and methane production using the SF_6 tracer technique (Martin *et al.*, 2008) were measured on week 3, and ruminal fermentation end-products on week 4. Data were analysed using the MIXED procedure of SAS with diet (D), DFM treatment (DFM) and D×DFM as fixed effects, and animal nested within diet as random effect.

Results Two different ruminal fermentation pathways were successfully induced with the 2 diets as shown by the changes in volatile fatty acid profiles (acetate:propionate ratio = 2.42 and 3.78 for HSD and LSD, respectively; D effect; $P \leq 0.05$; data not shown). As expected, cows fed HSD diet produced less methane than those fed LSD diet (D effect; $P \leq 0.05$; Table 1). Irrespective of the diet, no significant effect of DFM was observed on intake and milk production. However, the D×DFM interaction was significant on CH_4 output expressed as g/d and g/kg of milk ($P = 0.06$ and $P = 0.05$; respectively), indicating that DFM effects on methanogenesis were dependent on the diet fed. For HSD diet, cows supplemented with Lp+P produced less CH_4 per kg of milk compared to control cows (-20%; $P < 0.05$). For LSD diet, CH_4 output were reduced in cows supplemented with Lr+P (-25%; $P < 0.05$). Association of *Propionibacterium* P63 with one of the two lactobacilli strains was more effective to reduce CH_4 output than *Propionibacterium* P63 alone.

Table 1 Effect of DFM supplementation to dairy cows on dry matter intake, milk production and methane output

	High starch diet (HSD)				Low starch diet (LSD)				Statistical effects[1]			
	C[2]	P	Lp+P	Lr+P	C	P	Lp+P	Lr+P	s.e.	D	DFM	D×DFM
DMI, kg/d	18.53	19.18	18.29	19.60	18.81	18.70	18.90	18.31	1.22	0.70	0.90	0.60
Milk, kg/d	25.03	25.66	24.20	26.36	23.63	22.50	22.80	23.15	1.62	0.30	0.60	0.70
CH_4, g/d	207^{AB}	228^{B}	157^{A}	236^{B}	315^{A}	301^{A}	303^{A}	236^{B}	31.2	0.05	0.50	0.06
CH_4, g/kg DMI	11.05	12.15	8.53	12.15	17.03	17.18	15.99	13.04	1.64	0.02	0.30	0.16
CH_4, g/kg milk	8.26^{b}	8.73^{b}	6.51^{c}	9.04^{a}	13.61^{a}	13.65^{a}	13.21^{a}	10.26^{b}	1.22	0.01	0.30	0.05

[1] The statistical effects are D, DFM and DxDFM for diet, DFM treatment and their interaction.
[2] The DFM treatments were C (control without probiotic), P (*Propionibacterium* P63), Lp+P (*L. plantarum* + P63) and Lr+P (*L. rhamnosus* + P63).
[a, b, A, B] Within row and diet, means that do not share a common superscript significantly differ for lowercase ($P \leq 0.05$) or for uppercase ($0.05 < P \leq 0.10$).

Conclusions We show that bacterial DFM (i.e., *Propionibacterium* P63 + *L. plantarum* 115 and *Propionibacterium* P63 + *L. rhamnosus* 32) effectively reduced methane output in dairy cows. This methane mitigation effect of the DFM was diet dependent. The mode of action of the bacterial DFM tested on rumen methanogenesis needs to be further investigated.

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Effect of Saponaria officinalis on rumen microbial populations, rumen fermentation and methane and ammonia production in dairy cows

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Introduction Plant bioactive components have the potential to modulate rumen microflora population, hence the study was conducted to determine whether feeding *Saponaria officinalis*, a rich source of triterpenoid saponins, altered rumen microbial populations, rumen fermentation, including methane and ammonia production. Mitigation of rumen methane and ammonia production has implications both for improvement of animal production efficiency and for decreased global environmental pollution. For the above purposes two experiments: *in vitro* with Rusitec system and *in vivo* with 4 fistulated dairy cows in 4 x 4 Latin square design were used.

Material and methods A rumen simulation technique (RUSITEC) apparatus with eight 1000 ml fermenters was used to investigate the effect of *Saponaria officinalis* powdered root in diets differing in source of dietary forage on ruminal fermentation, methane and ammonia production. Two diets were tested. The diets were the mixtures of forage to concentrate feeds (50:50% and 95:5%). Forage in the first diet was composed of maize silage whereas in the second mostly of grass silage and maize silage was added only to meet nutritional requirements. The diets were supplemented with 200, 400 and 600 mg of *Saponaria officinalis* powdered root. The experiment was conducted over four independent 10-day incubation periods with the last 5 days used for data collection. The pH and ammonia concentration were measured immediately after collection. Volatile fatty acids were determined by Varian Star CP 3800 gas chromatography. The population of bacteria was evaluated with Thoma counting chamber (Blau Brand®, Wertheim, Germany). Bacterial populations were assessed using real-time polymerase chain reaction and expressed as a percentage of bacterial 16S rRNA gene copies. Counts of protozoa (*i.e., Entodiniomorphs* and *Holotrichs*) were determined under a light microscope with defined volume of rumen fluid after incubation (Zeiss, Jena, Germany). The quantification of methanogens was carried out with the fluorescence *in situ* hybridization technique, according to Pers-Kamczyc *et al*. (2011). Fermentation gases were collected over 24 h in gas-tight bags and analyzed for the concentration of CH_4 by a SRI310 gas chromatograph. The *in vivo* experiments with four Polish Holstein-Friesian fistulated dairy cows lasted 24 days each (21 days of adaptation and 3 days of samplings). During *in vivo* experiment cows received a mixture (50:50%) of maize silage or grass silage and concentrate. The control diet was supplemented with 1, 2 and 3% of *Saponaria officinalis* powdered root. Diets were prepared daily and served as a total mixed ration (TMR, 20 kg dry matter). Before and 3 h and 6 h after morning feeding, rumen samples were collected to obtain the rumen microbial fermentation indicators. The rumen fluid was taken through the cannula, squeezed with the 4-layers cheese cloth and separated to the different vessels. The same methods as in *in vitro* experiment were used to determine the analyzed parameters *in vivo*.

Results As a result of *in vitro* experiment, it was found that only the higher doses (400 and 600 mg) of the experimental factors affected the processes occurring in the rumen fluid after incubation. It was also shown that the degree of influence of the experimental factor depends on the composition of the diet. The maize silage significantly inhibited rumen methane and ammonia production. Applications of 400 and 600 mg powdered root limited methane production (51 and 33%, respectively), and the concentration of ammonia (29 and 45%, respectively). Furthermore, it was observed that application of 400 mg *Saponaria officinalis* powdered root reduced total volatile fatty acids and decreased the ratio of acetic to propionic acid. Supplementation of *Saponaria officinalis* powdered root to grass silage diet resulted in a 28% reduction of ammonia concentration and increased concentration of propionic acid in the group with the highest powdered root dose. No effect of *Saponaria officinalis* powdered root on methane production when supplemented to grass silage diet. Some statistically significant effects of analysed factors were observed also in particular bacteria species tested *in vitro*. There was a reduction in the number of *P. ruminicola*, at the highest (600 mg) dose used, both when maize silage (from 1,87 x 10^7 in the control group to 9.40 x 10^6 copies of 16S rRNA gene/30 ng DNA) and grass silage (from 1.63 x 10^7 in the control group to 5.81 x 10^6 copies/30 ng DNA) were used. Similarly the number of *F. succinogenes* was limited at the highest *Saponaria officinalis* powdered root dose in both diets: maize silage (from 1.53 x 10^7 in the control group to 3.58 x 10^6 copies of 16S rRNA gene/30 ng DNA) and grass silage (from 6.81 x 10^7 in the control group to 3.90 x 10^6 copies/30 ng DNA). The data of real time PCR analyzes showed the reduction in the number of bacteria with the *Saponaria officinalis* addition compared to controls.The *in vivo* experiment partly confirm the potential of maize based diet supplemented with *Saponaria officinalis* powdered root on ammonia and methane production. Applications of 2 and 3% powdered root limited ammonia production (50 and 57%, respectively), and the protozoa population (43 and 50%, respectively).

Conclusions Results of *in vitro* study confirmed the thesis that the effect of feeding *Saponaria officinalis*, a rich source of triterpenoid saponins, depends on the dietary composition. Maize silage diets supplemented with *Saponaria officinalis* powdered root seemed to be more effective in modulation of rumen fermentation processes and hence in mitigation of methane and ammonia production. However, verification of *in vitro* results in dairy cows experiment only partly confirm the potential of maize based diet supplemented with *Saponaria officinalis* powdered root to mitigate rumen methane production. We can also conclude that the effect of *Saponaria officinalis* powdered root is dose dependent.

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Identifying relative importance of variables predicting GHG emissions from dairy farms of two German regions

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Introduction With approximately 89,000 dairy farms, milk production plays an important role in German agriculture. While achieving a viable income is the basic goal of most farmers, there is now an increasing focus on dairy farmers, by consumers and policy makers, to minimise the effects dairy farming has on climate change e.g. binding commitments to reduce GHG emissions. The aim of this study was to model GHG emissions of south and western German dairy farms and to identify those variables that have the highest impact on variation of predicted GHG emissions and other key farm indicators e.g. beef output and land use.

Material and Methods In this study we examined 27 dairy farms from south Germany (dual purpose Fleckvieh (FV) cows) and 26 dairy farms from west Germany (Holstein-Friesian (HF) cows) both feeding total mixed rations all year round. We used data from a farm accounting network that included economic data and information on production traits (e.g. milk yield, calving interval, replacement rate, feed use efficiency). Modelling of GHG emissions from all on and off farm sources associated with dairy farms up to the farm gate was based on international literature and databases (see Zehetmeier *et al*., 2012). We used stepwise multiple linear regression (SMLR) to identify those independent variables (table 1) which have the highest impact on variation of GHG emissions per kg milk and other key indicators of the dairy farms within a region. The additional farm indicators were beef output i.e. actual and potential beef output per farm (beef from culled cows and fattening of surplus calves outside the farm) and land use.

Results The results showed no statistically significant difference between the GHG intensity (kg of CO_2eq per kg milk) of south German FV dairy farms and west German HF dairy farms. A wide range in GHG intensity within the investigated regions was found (0.90-1.25 kg CO_2eq/kg milk for south German farms and 0.79-1.20 kg CO_2eq/kg milk for west German farms). The majority of the difference between the GHG emission intensity of farms within a region (67% for the south and 66% for the west) was explained by 4 key variables in the SMLR model. Outcomes of SMLR for all subset models showed that milk yield and replacement rate had the highest impact on variation of GHG intensity of dairy farms from south and west Germany. Milk yield and replacement rate were also identified as key variables influencing actual and potential beef output per kg milk, because a lower milk yield and higher replacement rate resulted in higher beef output per kg milk. No statistical difference between the regions and breeds was found in case of land use.

Table 1 Greenhouse gas (GHG) emissions in CO_2 equivalent (CO_2eq), beef output of 58 German dairy farms from different regions and breeds (Fleckvieh, FV; Holstein Friesian, HF)

GHG Emissions (kg CO_2eq/kg milk)	South (FV)	West (HF)	Beef output[1)] (kg beef/kg milk)	South (FV)	West (HF)
Mean (s.d.)	1.06 (0.11)	0.98 (0.12)		0.0427 (0.0057)	0.0227 (0.0027)
R^2	0.674	0.660		0.995	0.991
Relative importance of independent variables in the regression model (decomposition of R^2 in %)					
RR[2)]	33	27	Calving interval	12	16
DMI[3)]	9	22	Mortality	20	12
Ninput [4)]	1	16	RR[2)]	28	50
Milk yield [5)]	57	35	Milk yield [5)]	40	22

[1)]Actual and potential beef output = beef from culled cows and beef from fattening of surplus calves outside the farm; [2)]RR = Replacement rate (%); [3)]DMI = Dry matter intake per cow (kg DM/cow); [4)]Ninput = mineral Nitrogen (kg N/ha); [5)]kg milk/cow/year

Conclusions The analysis demonstrates that the variation of GHG intensity within region and breed was higher than between region and breed. This implies that the search for GHG abatement options for dairy farms should focus on variables influencing variation between farms instead of changing breed. However, it is important to note that abatement options that reduce the GHG intensity of dairy farms should not have a negative impact on beef output and land use (i.e. feed use efficiency). This is to avoid strategies that result in increased meat production from meat only systems, which could increase net GHG emissions. To investigate if the findings of this study also apply for a different feeding regime (grazing) a comparison with Irish dairy farms will be undertaken.

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Emissions of greenhouse gases in Marandu grass pastures fertilized with biofertilizer of poultry manure and with biofertilizer of swine manure in a tropical region of Brazil.

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Introduction The waste generated in the agricultural system has been treated and recycled by an appropriate process such as anaerobic digestion for use as fertilizer in different crops. The use of organic fertilizers may provide an additional emission of greenhouse gases (Frank *et al*., 2002). In Brazil, few evaluations of production of these gases due to the use of organic materials in the fertilization of pastures or crops were conducted. So this study aims to quantify the emissions of CO_2, CH_4 and N_2O according to the fertilization of the Marandu grass pastures with biofertilizer poultry manure and biofertilizer of swine manure, in the Brazilian state of São Paulo.

Material and methods The study was conducted at the Forage department of Faculdade de Ciências Agrárias e Veterinárias campus Jaboticabal of the Universidade Estadual Paulista. The local soil is classified as Oxisol and climate as tropical with dry winter and rainy summer. Treatments consisted subjecting the Marandu grass at a dose of 80 g N per m² using biofertilizer of poultry manure, biofertilizer of swine manure and a control treatment without any application of fertilizer. The quantification of CO_2 flux was conducted using a continuous flux chamber Model LI-8100 (LI-COR, Lincoln, NE) and N_2O and CH_4 fluxes were quantified using the static chambers methodology and were followed by gas chromatography determination. Net emissions from each treatment were obtained by discounting the emissions of control. The uncertainty of the emissions obtained were assessed using the standard error of the mean. The meteorological data were obtained from the Climatological Station Unesp.

Results Fertilization with biofertilizer of poultry manure showed largest fluxes throughout the evaluation period (Table 1). The largest fluxes were observed after rainfall events. After 60 days of fertilizer application the fluxes were equal ceasing the effect of the fertilizers.

Table 1 CO_2 fluxes due to the application of biofertilizer poultry and swine in Marandu grass pasture

Treatments	Low flux	High flux	Median	Standard error
	g C-CO_2 m^{-2} d^{-1}			
Biofertilizer poultry	2.54	4.70	3.74	± 0.12
Biofertilizer swine	2.31	4.58	2.96	± 0.11
Control	1.67	3.64	2.33	± 0.10

The biofertilizer of poultry presented higher emissions of CH_4. The biofertilizer swine showed oxidation in evaluations of CH_4 and N_2O. N_2O fluxes were negative on most events evaluated and positive until 3 days after treatment application. After 15 days fluxes related to treatment were equal to control, stopping the fertilization effect. The absence of rain in the last 60 days of evaluation conditions and good soil aeration can explain the consumption of N_2O found in treatments. Net emissions of CH_4 and N_2O are shown in Table 2.

Table 2 Net fluxes of CH_4 and N_2O in the fertilization of Marandu grass with biofertilizer obtained of poultry and swine manure.

Treatments	CH_4		N_2O	
	mg C- CH_4 m^{-2}	Standard error	mg N-N_2O m^{-2}	Standard error
Biofertilizer of poultry	831.2	± 126.1	0.03	±0.71
Biofertilizer of swine	-87.8	± 411.4	-2.96	±1.68

Conclusions The fertilization of pasture of Marandu grass provided oxidation of N_2O. Biofertilizers influence the fluxes of CO_2 up to 60 days after fertilization. More studies are needed to confirm these tendency.

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Effects of breed and forage type on methane emissions from hill replacement ewes

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Introduction Within the UK sheep industry, approximately 43% of breeding ewes are hill breed types. Unlike the lowland sector, where replacement ewes are sometimes bred as ewe lambs, breeding from hill replacement ewes rarely takes place until the ewes are approximately 18 months old, representing a significant overhead cost in terms of greenhouse gas emissions from hill sheep systems. Within the Tier 1 method of IPCC (2006), CH_4 emission factors for adult sheep are also applied to replacement ewes, despite their smaller body size, resulting in a significant overestimate of total greenhouse gas emissions. The aims were to investigate the effects of breed of hill replacement ewe and forage type on CH_4 emissions, and to establish CH_4 emission factors specific to growing hill ewes.

Material and methods Thirty six hill replacement ewes (18 pure Scottish Blackface and 18 Swaledale x Scottish Blackface (75:25)) aged approximately 12 months and weighing 42 ± 4.0 kg were allocated to 3 treatment groups balanced for breed and live weight. Each genotype was offered 3 forages *ad libitum*: fresh grass, grass nuts and grass silage. Grass nuts were sourced from a commercial supplier (Drygrass South Western Ltd, Burrington, UK). Fresh grass was harvested daily from the primary regrowth of a predominantly perennial ryegrass sward (16 May to 11 June 2012), while the grass silage was made from the 2nd harvest of perennial ryegrass ensiled with Ecosyl as an additive. The animals were individually housed and fed experimental diets for at least 14 days before being transferred to individual methane chambers for a further 4 days with feed intake, faecal and urine outputs collected and CH_4 emissions measured for the final 3 days. Live weight was measured at the beginning of the study and before entering and after leaving the chamber. Data were analysed in a 3 x 2 (diet x breed) factorial arrangement using REML (Genstat statistical package).

Results Effects of breed and forage type are shown in Table 1. There were no significant interactions between breed and forage type on any variable of live weight, DM intake and CH_4 emissions. Sheep offered grass nuts had higher DM intake ($P < 0.001$), live weight ($P < 0.01$) and CH_4 emissions as g/day ($P < 0.01$), but lower CH_4 output as a proportion of DM intake ($P < 0.01$) when compared to those given fresh grass and grass silage. CH_4 emissions as a proportion of live weight were similar between the 3 grass types. There were no significant differences between the 2 breeds in terms of DM intake, CH_4 emissions as g/day, or CH_4 emissions as a proportion of DM intake or live weight. However, Scottish Blackface ewes had higher live weights than British Swaledale x Blackface ($P < 0.01$). The statistical analysis using all data found that there was a significant relationship ($P < 0.001$) between CH_4 (g/d, y) and DM intake (kg/d, x), $y = 7.85 x + 4.74$, $R^2 = 0.70$ (Figure 1).

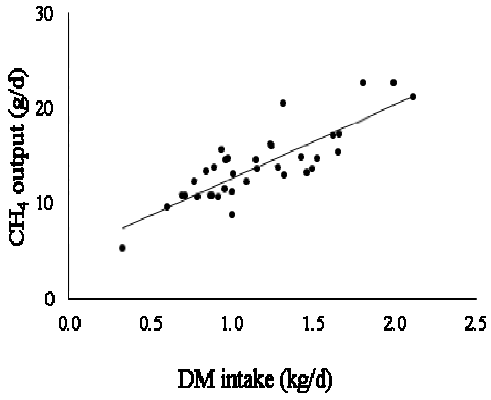

Figure 1. CH_4 emissions vs. DM intake

Table 1 Effects of breed and forage type on live weight, feed intake and CH_4 output in hill replacement ewes (mean (s.e.))

	Diet				Breed		
	Fresh grass	Grass nuts	Grass silage	P	British Swaledale x Blackface	Scottish Blackface	P
DM intake (kg/d)	0.96 (0.071)[b]	1.58 (0.068)[a]	0.88 (0.074)[b]	<.001	1.16 (0.060)	1.16 (0.060)	0.932
Live weight (kg)	43.0 (1.24)[b]	49.0 (1.19)[a]	42.7 (1.29)[b]	0.001	42.9 (1.04)[b]	47.0 (0.98)[a]	0.007
CH_4 (g/d)	12.9 (0.96)[b]	16.3 (0.92)[a]	12.0 (1.00)[b]	0.007	13.6 (0.81)	14.1 (0.77)	0.665
CH_4/DM intake (g/kg)	13.5 (0.52)[b]	10.3 (0.50)[a]	13.9 (0.54)[b]	<.001	12.2 (0.43)	12.6 (0.41)	0.518
CH_4/Live weight (g/kg)	0.30 (0.019)	0.34 (0.019)	0.28 (0.020)	0.118	0.31 (0.016)	0.30 (0.015)	0.599

[a,b] means within the same row under diet type with same superscripts are not significantly different ($P > 0.05$)

Conclusions Feeding grass nuts, rather than fresh grass and grass silage, can reduce enteric CH_4 emissions as a proportion of feed intake in sheep, while there is no difference in CH_4 emissions between Scottish Blackface and Swaledale x Scottish Blackface. Enteric CH_4 emissions from hill replacement ewes can be predicted from feed intake.

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Daily variation of methane emissions from a laying hen facility in Spain

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Introduction Literature on methane emissions from laying hen units is scarce. The main objective of this work was to study the hourly pattern of CH_4 emissions in a laying hen facility adapted to the welfare Directive (1999/74/EC) in Spain. In this study, the possible behavioural and management factors that could contribute to CH_4 emissions mitigation were also evaluated.

Material and methods The study was carried out in a commercial laying hen building with approximately 54,000 Lohmann-Brown hens. Animals were housed in a vertical tiered cage system adapted to Directive 1999/74/EC. Measurements were conducted from April to December 2012. Inlet and outlet air temperature and relative humidity were monitored and recorded every 15 min using data loggers (HOBO, U12-013).
Ventilation rate was measured following Calvet *et al.* (2010) methodology. The average percentage of operation of each fan was obtained every 5 minutes. An electronic data logger system converted every second the electric signal from each fan into digital data on fan status. Each fan was individually calibrated for airflow rate at different levels of pressure drop associated to each ventilation programme (2-31 Pa). The air was ducted 30 cm from each fan and the air velocity was measured at 25 different locations in the section using a hot wire anemometer (Testo 425). A ventilation performance curve was obtained according to the average values obtained from all fans. Pressure drop in the building was controlled and recorded every 5 min by a pressure drop meter (Veris PXU-05).
Methane concentrations were measured continuously by a Photoacoustic infrared gas analyser (INNOVA 1412). Air samples were taken from the air stream from 8 exhaust fans and from 4 outdoor points. Methane emission was determined by multiplying the housing ventilation rate times the difference in concentration between the inlet and outlet air.

Results are reported for two months differing in temperatures and thus, ventilation patterns: September, with an average outdoor temperature of 18.6ºC and December, with an average temperature of 11.3ºC. Ventilation rate was proportional to outdoor temperature ($R= 0.8$ $P<0.001$), being more than two fold higher in the warmer season with respect to the cold one, with 39.1 and 14.7 % of maximum ventilation rate respectively. Average CH_4 emission from the building did not differ significantly ($P>0.05$), with 4.3 and 3.8 kg CH_4/day for September and December respectively. Nevertheless, when analysing the daily variation of ventilation and methane emission, we observed a significant effect of the hour of the day ($P<0.05$) in both periods for both, ventilation and CH_4 emission. Daily differences were found to be higher during the warm period (Fig. 1), as also reported previously (Fabbri *et al*., 2007).

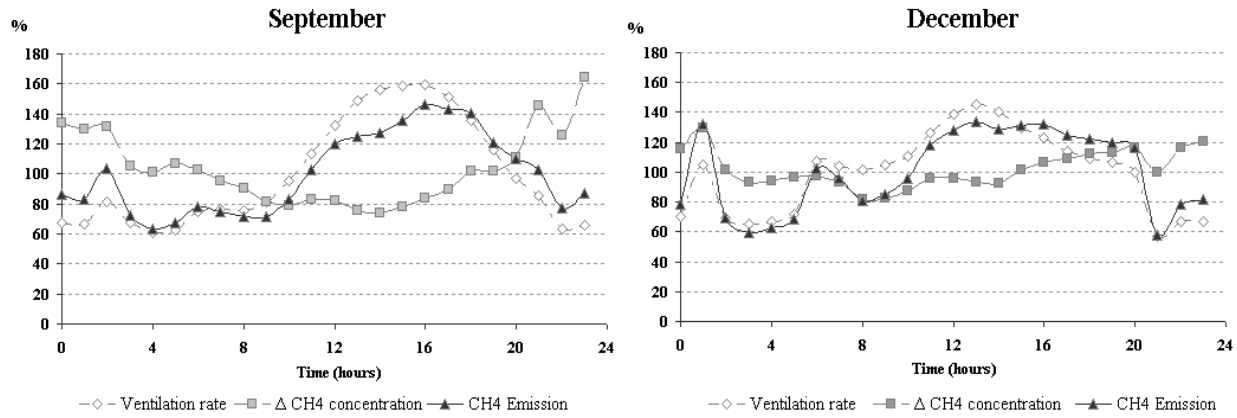

Figure 1 Average daily pattern (hourly average calculated as % of mean value for each day) for each month, of [○] ventilation rate, [■] Δ CH_4 concentration and [▲] CH_4 emission.

Conclusions CH_4 emissions significantly vary along the day, peaking from 12-19:00pm both, during a warm and cool period.

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GHG emissions from soil amended with cattle slurry: effects of soil application method and/or slurry pre-treatment.

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Introduction It is well known that cattle slurry application to soil led to a significant release of ammonia (NH_3) and may also induce a substantial increase of GHG emissions, namely methane (CH_4) and nitrous oxide (N_2O). Slurry injection or slurry pre-treatment by acidification or solid/liquid separation have been pointed out as efficient solutions to decrease NH_3 emissions but the effect of some of these solutions on GHG emissions is still not clear (Chadwick *et al*., 2011). Indeed, an efficient decrease of NH_3 emissions may result in "pollution swapping", namely an increase of N_2O emissions (Chadwick *et al*., 2011). On the other hand, some of these solutions imply a strong investment and cheapest solutions may lead to similar results in terms of GHG emissions reduction. In the present study, the impact on CH_4, N_2O and CO_2 emissions of untreated slurry injection in soil was compared with surface application of acidified slurry or liquid fraction followed or not by soil incorporation .

Material and methods A pot experiment was performed to grow oat on a sandy soil amended with treated and untreated cattle slurry. Pots were filled with 11kg of soil and an amendment equivalent to 150 kg N/ha was performed in each pot at the beginning of the experiment. The treatments (4 replicates) considered here were: slurry injected at 10 cm (SI), slurry (SSM) or liquid fraction obtained by centrifugation (LFSM) applied on soil surface followed by soil mobilization; liquid fraction applied on soil surface without mobilization (LFS); acidified slurry applied on soil surface with (ASSM) or without (ASS) mobilization; acidified liquid fraction applied on soil surface without mobilization (ALFS). Fluxes of N_2O, CH_4 and CO_2 were measured using the closed-chamber technique in conjunction with a trace gas analyser (TGA) (1412 Photoacoustic Field Gas-Monitor, Innova Air-Tech Instruments). For this, a PVC chamber was placed over each pot to form a gas-tight seal. The chamber was then connected to the TGA analyser via 5-mm outer-diameter nylon tubing. Changes in headspace gas concentrations measured at 1 min, 30 and 60 min after chamber closure were used for calculating gas fluxes. The statistical significance of the mean differences was determined by the Fisher's least significant difference (LSD) test at a 0.05 probability level.

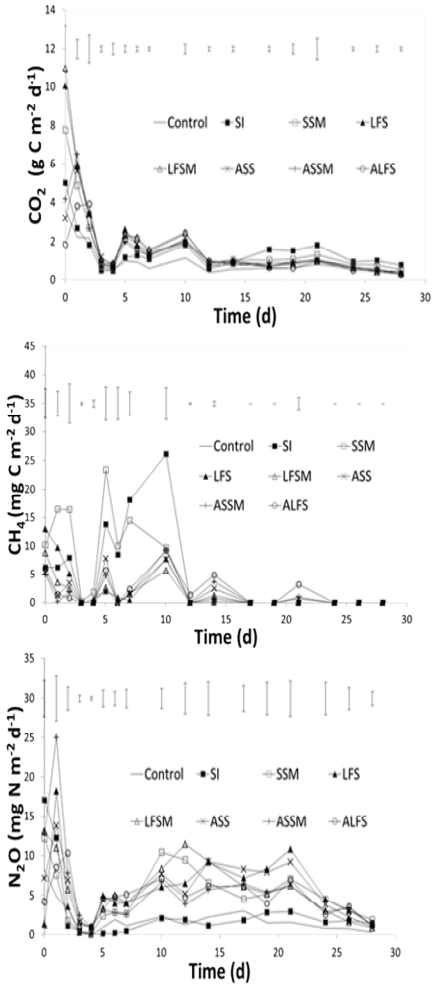

Figure 1 Average gas fluxes following application to soil of treated and untreated slurry (N = 4). Error bars represent the maximum value of standard error observed on each sampling day.

Results N_2O emissions varied widely between treatments all over the experiments (Figure 1) even if a similar pattern was observed. The lower N_2O emissions were observed from SI treatments with values similar to the control whereas the higher N_2O emissions were observed in LFS and ASS treatments. Considering the cumulated amount of N_2O released, no significant differences were observed between treatments (146 to 175 mg N_2O-N m^{-1}) except the SI (78 mg N_2O- N m^{-1}) which led to the lowest value. Methane emissions increased significantly in all amended treatments relative to control on day 0 with the highest increase observed in LFS. CH_4 emissions peaked on several times after day 3 but values remained low in most treatments except SSM and SI where CH_4 emissions remained higher than 8 mg CH_4-C m^{-2} d^{-1} between day 3 and day 10. More than 120 mg CH_4-C m^{-2} was released in SSM and SI against > 65 mg CH_4-C m^{-2} in all other amended treatments. An increase of CO_2 emissions relative to control was observed in all treatments except ASM, ASSM and ALFS. The cumulated amount of CO_2 released from ALFS (35 g CO_2-C m^{-2}) and ASSM (41 g CO_2-C m^{-2}) was significantly lower than from LFS (51 g CO_2-C m^{-2}) and SSM(50 g CO_2-C m^{-2}), respectively.

Conclusions Our results show that slurry injection is efficient to decrease N_2O emissions relative to the traditional surface application followed by mobilization (SSM) but it led to an increase of CH_4 emissions. Slurry acidification (ASM) resulted in similar CH_4 and N_2O emissions compared to SSM. LFSM treatment led to similar N_2O and lower CH_4 emissions compared to SSM. Hence, surface application of LF or acidified slurry appeared as a good alternative to slurry injection in order to simultaneously mitigate NH_3, N_2O and CH_4 emissions

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Aboveground biomass map to the Environmental Protection Area of Ibirapuitã – Brazil, calculated from satellite images

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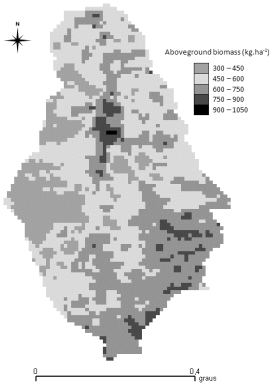
Introduction The Pampa biome has a unique kind of vegetation, characterized by C_3 and C_4 plants adapted to the transition from subtropical to temperate climates. The Pampa biome is situated in Brazil, Uruguay and Argentina and can broadly be classified as "grassland of the River Plate". The biome covers an area of approximately 700,000 km^2. The main economic activity in the region is cattle-raising based on the natural grasslands since the 18^{th} century. The monitoring of the aboveground biomass production of vegetation in this region will allow optimizing cattle-raising activity with the actual biomass production. By allowing adjustment of livestock numbers per area will enable the economic exploitation of these areas jointly with the conservation of natural vegetation and of the environment. The main objective of this study is the quantification of the aboveground biomass production for the natural grassland of the Pampa biome using NDVI data calculated from images acquired by SPOT-Vegetation instrument.

Material and methods The study area is located in the "Environmental Protection Area (EPA) of Ibirapuitã", Brazil, which is a region of 320,000 hectares. The objective of the EPA is to ensure the conservation of a significant portion of the biodiversity of the Pampa biome alongside other economic activities. For this study two datasets are necessary, namely "in situ" measurements of the amount of aboveground biomass ($kg.ha^{-1}$) and satellite data recorded by the Vegetation instrument onboard SPOT 4-5. The aboveground biomass dataset were collected in the field on a monthly basis during a 28 month period (August, 2001 to December, 2003).The plot where the aboveground biomass data was collected is situated at the following position: 30°06'S and 55°41'W. The NDVI data from the Vegetation instrument will be analyzed for the same months as "in situ" data of aboveground biomass are collected. We used the NDVI images, the so-called VGT-S10 products, which are a ten day Maximum Value Composite (MVC) synthesis for each pixel. In order to obtain a biomass map, the first step was to establish the relationship between the NDVI and aboveground biomass values collected in the field. This relationship can be expressed as an exponential equation using NDVI values as the independent variable. The next step is to calculate for each NDVI pixel the aboveground biomass values, using the equation established in the previous step.

Results The relationship between NDVI and aboveground biomass (AB) is represent by an exponential equation ($AB = 25.5e^{4.6NDVI}$) with a coefficient of determination (R^2) of 0.46. For this kind of vegetation (natural grasslands) one does not expected high correlations (Fonseca *et al*., 2007) because these are non-homogeneous areas, since the Pampa biome support very high levels of biodiversity (Overbeck *et al*., 2007). This behaviour is also observed for other non-homogeneous areas when the NDVI is used to estimate aboveground biomass (Wessels *et a*l., 2006). A sample for an aboveground biomass map is showed in Figure 1, calculated over the February, 2003 satellite image. The aboveground biomass values obtain from this map are consistent with the values collected at the field scale by other researchers.

Figure 1 Aboveground biomass map for EPA of Ibirapuitã – Brazil



Conclusions The data obtained from the Spot-Vegetation instrument can be used to derive estimates of aboveground biomass. The satellite observations can be converted into NDVI maps and this in turn, using an equation - mathematic model, can be converted into biomass. The results of the analysis presented here can be improved with a larger dataset of "*in situ*" measurements for different plots and collected over a longer period of time for analysis.

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Effects of type and level of starch in concentrate on methane emission in lactating dairy cows

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Introduction Starch is a major source of glucogenic energy for high-yielding dairy cows as well as an accessible source of fermentable energy for rumen microorganisms (Koenig *et al*., 2003). Compared with dietary fibre, starch may result in reduced methane (CH_4) production because fermentation of starch promotes production of propionate, which acts as a hydrogen sink (Bannink *et al*., 2006) and, unlike fibre, the rumen bypass fraction of starch is well digested in the small intestine without the production of VFA. However, measurements with regard to its effect on CH_4 emission in dairy cattle are scarce. The objective of this study was to determine the effects of type and level of dietary inclusion of maize starch on CH_4 emission in lactating cows.

Materials and methods Forty Holstein-Friesian dairy cows were selected and allocated to 10 blocks based on parity (mean ± s.d.: 2.9 ± 1.1 parity), days in milk (215 ± 89 DIM), and fat- and protein-corrected milk production (35.9 kg/d ± 9.5 FPCM). Cows within a block were assigned to one of four diets in a 2 × 2 factorial arrangement of treatments (2 types and 2 levels of starch) in a randomized complete block design. Treatment diets were composed of concentrate which contained either 27% (27) or 53% (53) starch on dry matter (DM) basis. The starch source was ground native maize starch (slow degradable, S) or ground gelatinized maize starch (fast degradable, F). Maize was exchanged with beet pulp and palm kernel expeller. Cows were fed a total mixed diet consisting of grass silage and concentrate at 60:40 ratio on DM basis. The experiment was conducted in 10 successive periods. Each experimental period consisted of 12 days of adaptation to the diets in tie-stalls, and 5 days in respiration chambers to evaluate methane production and animal performance. Two cows were housed per chamber, and the experimental unit for CH_4 and energy balance traits in the chambers thus consisted of a pair of cows. On day 10 and 11, rumen fluid was collected from fistulated cows (n =16) to determine VFA concentration and rumen pH at 0, 1, 2, 3, 4, 6 and 8 h after morning feeding. All other measurements and data collection were done during the days in the respiration chambers. Data were analysed by PROC MIXED model of SAS. The model included type, level, type × level interaction and respiration chamber as fixed effects, and period as random effect on CH_4 and energy balance traits; the model included block, respiration chamber, type, level, and type × level interaction as fixed effects on dry matter intake, milk yield and composition. Data for rumen pH and VFA were analysed as repeated measurements. Block, type, level, time, type × level and type × level × time interactions were included in the model as fixed effects, cow as random effect, and time as repeated measure.

Results Preliminary results are presented in Table 1. Dry matter intake (DMI) was higher for low starch compared with high starch, but type of starch did not affect DMI. Differences among treatments in milk yield or CH_4 production per kg of FPCM or per kg of DMI remained non-significant. Also milk fat and protein content were not affected by treatment (data not shown). Both total VFA concentration and molar proportion of propionate increased with fast degradable starch. Molar proportion of acetate tended to be affected by type of starch, but unaffected by level of starch. No significant differences were observed for butyrate molar proportion and rumen pH among treatments.

Table 1 Effect of type and level of starch in concentrate on DMI, milk yield, fermentation characteristics and methane emissions in lactating cows

Item	Treatment				s.e.	P		
	S27	S53	F27	F53		Type (T)	Level (L)	T × L
DMI (kg/d)	18.7	17.6	18.7	17.6	0.56	0.979	0.010	0.937
FPCM (kg/d)	28.0	25.2	27.0	26.9	1.21	0.643	0.106	0.149
CH_4 (g/kg of FPCM)	15.6	16.0	16.0	15.0	0.59	0.513	0.424	0.129
CH_4 (g/kg of DMI)	23.3	22.7	23.2	22.7	0.76	0.923	0.359	0.932
Total VFA (mM)	100.5	95.1	108.8	103.1	3.40	0.003	0.033	0.950
Acetate (%)	68.4	69.2	68.4	67.6	0.53	0.068	1.000	0.445
Propionate (%)	16.2	15.4	16.2	16.9	0.50	0.043	0.957	0.043
Butyrate (%)	11.6	11.2	11.1	12.0	0.30	0.553	0.355	0.089
Rumen pH	6.53	6.50	6.51	6.53	0.08	0.854	0.874	0.684

Conclusions Type and level of maize starch in the concentrate has no effect on CH_4 production per kg FPCM or per kg DMI, or on FPCM production.

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Methane emissions from different sheep breeds grazing tropical pastures with different growth habits

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Introduction Tropical pastures occupy almost two thirds of the arable land in the world, as well as a large part of Brazilian territory. However, very little is known about methane emissions from sheep grazed on tropical pastures. Sheep production present in almost all ecosystems in Brazil. This study aimed to compare the effect of different tropical forages, with different growth habits and leaf/stem ratio, combined with contrasting sheep breeds, on methane emissions.

Material and Methods The study was conducted in the subtropical climate region of Brazil (Latitude: 30 º 02'09 "S, Longitude: 51 º 01'18, 16" W). The experiment occupied an area of 1.2 hectares, subdivided in 0.2 hectare plots. The treatments were arranged in a ***randomized split plot design*** in blocks with three ***replications***, where the main plot was represented by different pasture species (*Cynodon spp.* cv. Tifton-85; and Aruana grass (*Panicum maximum* cv. IZ-5)) of different growth habits and structures, and the split- plot was composed of different breeds: Corriedale (breed of lower feed requirement used for wool and meat production) and Texel (breed of higher feed requirement used for meat production). Two methane emission assessments were carried out in March 2012. The pasture characteristics are shown in Table 1. The green leaf lamina allowance was standardized among treatments of 10 kg dry matter per 100 kg liveweight ha^{-1} day^{-1}. Samples were cut close to the ground for pasture structural characterization. The pasture quality samples were collected by hand plucking (Euclides, 1992). One Corriedale and one Texel lamb, weaned 3-4 months of age, per subplot were assessed during each period. The assessments were carried out employing the tracer sulfur hexafluoride (SF6), as described by Johnson *et al*. (1994). The concentrations of SF6 and CH4 were determined by gas chromatography. The analyses were performed using a Shimadzu gas chromatograph model 2014 'Greenhouse'. From the known rate of SF6 release in the rumen, and the concentrations of SF6 and CH4 in the samples collected, the methane released by each animal was calculated as follows: QCH4 QSF6 = x ((CH4 - CH4B) / (SF6 - SF6B)), where: QCH4 is the rate of methane emissions in g/day; QSF6 is the release rate of SF6 permeation capsule; CH4 and SF6 are the concentrations measured in the collecting duct (stainless steel cylinder); and CH4B and SF6B are the concentrations measured in the collector tube of the environment. The analyses of variance were performed using the PROC MIXED procedure to determine the effects of treatments on methane production by animals, using the statistical program SAS ® version 9.3 (Statistical Analysis System, Cary, North Carolina).

Table 1 Characteristics of different pasture species (*Cynodon spp.* cv. Tifton-85; and Aruana grass (*Panicum maximum* cv. IZ-5)

	Leaf:stem ratio	Crude Protein (g/kg)	NDF (g/kg)	NDA (g/hg)
Aruana grass	0. 36	1.7	6.1	3.3
Tifton-85	0.43	1.0	7.1	3.5

Results There was no effect of grass species or breed used on methane emissions (methane emissions per kg of body weight), as shown in Table 2. Despite the differences in the structure of the grasses and the animal genetics, animal diet selection may have played an important role in reducing the variability of the material consumed and therefore methane emissions, as sheep are known to be highly selective in terms of diet quality.

Table 2 Methane emission per kg of live weight of lambs grazing different tropical grasses in the Southern Region of Brazil

	Tifton-85	Aruana grass	P
Corriedale	1.04	1.01	0.9993
Texel	0.89	1.01	0.9623
P	0.9206	1.0000	

(standard error of the difference = 0.2407)

Conclusions This study showed that the structure and quality of tropical pasture, as well as sheep breed had little effect on methane emissions of grazing lambs. This result raises the hypothesis that genetic differences of plants and animals have little relevance on methane emission of sheep, when grazing tropical pastures, compared to animals that exhibit less diet selectives, such as cattle.

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Nitrous oxide emissions from different dairy cattle production systems with tropical pastures during the Brazilian spring

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Introduction The Brazilian production of milk is based on tropical pastures production systems. The direct recovery and adoption of intensive management of pastures have shown potential for mitigation of greenhouse gases (GHG) due to high biomass production of tropical grasses, which have high efficiency of N-fertilizer use, and consequent accumulation of soil organic matter. The aim of this study was to evaluate the impact of pasture management on the nitrous oxide emission. N2O emissions were measured in situ during 20 days, after grazing and fertilization. Daily rate and cumulative N2O emission during 28 days were compared between treatments. These results will be used by PECUS Research Network, a multi-institutional project conceived by EMBRAPA with the objective to obtain consistent data, using internationally accepted research protocols, in order to subsidize governmental policies and to contribute to the development of mitigation alternatives for GHG emissions.

Material and Methods The study was conducted at the experimental station of the Brazilian Agricultural Research Corporation (EMBRAPA), located in São Carlos, São Paulo state, in the southeast of Brazil. The experimental design was randomized blocks (n=2 areas per treatment) with repetitions (n=3 chambers per block). Three treatments were evaluated: two production systems - intensive irrigated pasture of *Panicum maximum* cv. Tanzânia overseeded with oat and ryegrass with high stocking rate (intensive), and degraded pasture composed of a mixture of pastures of *Cynodon nlemfuensis* and *Brachiaria decumbens,* (degraded) - and the native forest (Atlantic forest) near the experimental area (reference), representing the original atmospheric conditions of this site. The intensive system had 28 paddocks rotationally grazed with 1 day of occupation and 27 days of rest periods. The degraded system was managed under continuous stocking. The intensive system was established in 1992, receiving annual fertilizations and liming; the lowest dose of N-fertilizer used was 500 kg of N/ha per year. Three static chambers were allocated randomly in each block - after grazing and fertilization with 33 kg N/ha (20.05.20 + 6%S) in the pastures and simultaneously in the forest. Gases samples were collected during 20 days and N_2O emission accumulations calculated for 27 days. N_2O concentration was determined by gas chromatography. Climate conditions and soil characteristics were also evaluated. Data were analyzed using ANOVA and Tukey test for comparison of means (Silva and Azevedo, 2009).

Results Accumulated N_2O emissions (27 days) were different ($P < 0.01$) among treatments. The extensive system with degraded grassland and forest showed similar nitrous oxide emissions whereas the intensive system showed higher emission (Table 1) although representing only 0.01% of the N applied at the grazing cycle (33 kg/ha). In the intensive system there were two peaks of N_2O emission, after precipitation of 9.6 and 20.4 mm respectively (Figure 1, A).

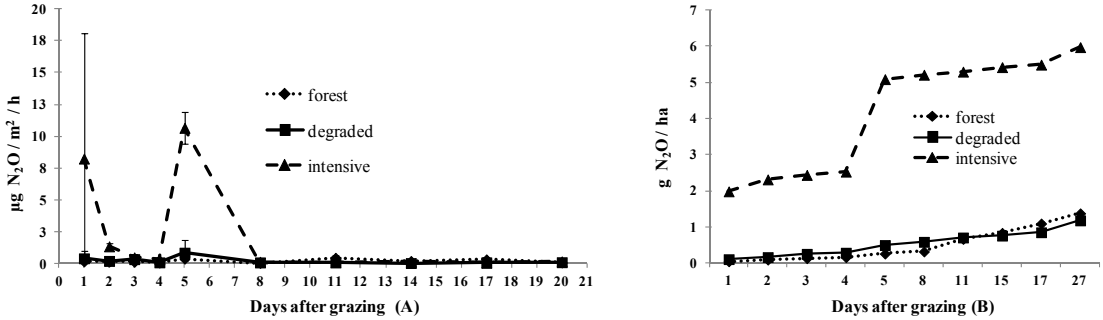

Figure 1 (A) Daily rate of nitrous oxide emission, (B) Accumulated nitrous oxide emissions.
Vertical bars in each evaluation of N_2O emission represent the standard deviation. Intensive = Intensive irrigated pasture with high stocking rate; degraded = Extensive system with degraded pasture; forest = Atlantic Forest.

Table 1 Nitrous oxide emission accumulated during 27 days (13/10/2012 a 08/11/2012).

treatment	forest	degraded	intensive
g N_2O/ha	1.375 b	1.180 b	5.973 a

Means followed by the same letter did not differ by Tukey test at 5% significance level. CV % = 35.95 (transformed data √x)

Conclusions The nitrous oxide emissions from intensive system, although greater compared with forest and extensive system, represented only 0.01% of the 33 kg N/ha applied in grazing cycle.

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In vitro measured enteric methane production in warmblood ponies receiving a roughage only or a roughage-concentrate diet

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Introduction Anthropogenic methane emission is an important contributor to greenhouse gases in animal agriculture. Although there is a large horse population worldwide, information about enteric methane emissions by these animals is scarce. Measurement of methane emission in ruminants and other animals may be performed with use of climate controlled respiration chambers (CRCs), which is considered the golden standard for performing metabolism measurements through indirect calorimetry. However, the use of respiration chambers is expensive and in addition, the confinement and isolation of animals may negatively influence the animal and possibly its digestive processes. *In vitro* gas production techniques provide the opportunity to yield information on fermentative degradation and methane production of dietary components without influencing the animal. The cumulative gas production technique (GPT) was originally developed to evaluate ruminant feedstuffs (Menke *et al.*, 1979), but has been adapted to study hindgut fermentation in horses, using faecal inoculum (Desrousseaux *et al.*, 2012). The main aim of the current study was to evaluate the effect of diet on the extent and kinetics of methane production in equines by gas production technique, using faecal inocula from ponies either receiving a roughage only (R) diet or a roughage plus concentrate (RC) diet. In addition, *in vitro* gas production parameters were compared to the methane production measured *in vivo* (Dansen *et al.*, 2013), to derive equations that predict *in vivo* methane production based on *in vitro* observations.

Material and methods Four mature warmblood pony geldings (BW 230 ± 10.5 kg; mean ± s.e.) were fed either a grass hay diet (R) (dry matter intake 5.0 ± 0.01 kg/d) or an isoenergetic mixed diet (RC), containing grass hay and concentrate, in a 50:50 ratio on energy basis (dry matter intake 3.7 ± 0.01 kg/d) in a cross-over design. The experiment involved 2 periods of 14 days each; 10 days of adaptation and 4 days where ponies were housed in CRCs. Freshly voided faeces from individual ponies, collected on the morning ponies were moved from the stables to the CRC's, was used as inoculant. Faecal inocula were prepared as described by Desrousseaux *et al.* (2012), following the method of Cone *et al.* (1996). Per inoculum 3 different substrates were tested in triplicate bottles; hay (H), concentrate (C) and a mix of hay and concentrate (H+C), with the same hay:concentrate ratio as used in the RC diet. Per bottle 500 mg of substrate was incubated with 60 mL buffered faecal inoculum. In addition, per pony blanks were included to test background fermentation by the inocula. Gas and methane production was determined for 72 h as described by Pellikaan *et al.* (2011), and data points were fitted by a biphasic and a monophasic non-linear model, respectively. After incubation, pH was measured and samples of fermentation fluid were stored at -20°C pending volatile fatty acid (VFA) analysis and ammonia measurement. The GLM procedure in SAS (Version 9.2, SAS Institute, Cary, NC, USA) was used to analyse the main effects of substrate (S), diet (D), period (Per), and pony (Po) on the fermentation kinetics. The statistical model used was $Y = \mu + S_i + D_j + Per_k + Po_l + (S \times D)_{ij} + \varepsilon_{ijkl}$. Post-hoc analyses were performed to determine differences between the sets of substrate×diet using Tukey multiple pairwise comparison test.

Results RC-inocula gave in general a higher (P=0.015) organic matter degradation compared to R-inocula (0.614 vs. 0.596, respectively), whilst maximum rates of gas production for the first (Rmax1) and second (Rmax2) phase were lower (P≤0.048) compared to R-inocula (Rmax1, 15.2 vs. 21.1 mL/h; Rmax2, 7.0 vs. 10.6 ml/h, respectively). Maximum rate of methane production was numerically lower for RC-inocula (P=0.115) compared to R-inocula (0.185 vs. 0.230 mL/h, respectively). For RC-inocula, substrate H yielded a higher methane production compared to H+C (P=0.041), or C (P=0.006). The higher (P<0.001) *in vitro* methane production in period 2 than in period 1 matched with the higher methane production data obtained *in vivo* in period 2 compared with period 1. Substrate had an effect on total VFA (P<0.001), the proportion of acetic acid (P=0.011), butyric acid (P=0.010), and branched chain VFA (BCVFA) proportion (P=0.001). Incubation of substrate C with R-inocula resulted in larger proportion of acetic acid (P=0.048) and smaller proportions of butyric acid (P=0.012) and BCVFA (P=0.010) compared to RC-inocula.

Conclusions The GPT showed that RC-inocula tended to a lower methane production compared to R-inocula, which matches with the results found *in vivo*. However, the effect of concentrate addition on methane production *in vivo* was more profound compared to the *in vitro* results. The higher methane production in period 2 was both found *in vitro* and *in vivo*. This indicates that GPT might be a useful tool to predict methane production in horses. However, the results obtained from this combined *in vivo* and *in vitro* trial will need additional investigations to be able to derive equations that are predictive for the *in vivo* methane production in horses.

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Ammonia volatilization of Marandu grass pastures by beef cattle excreta during the dry season in tropical region of Brazil

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Introduction Animal production systems can be a major source of ammonia (NH_3) to the atmosphere. Brazil has 220 million cattle and 200 million hectares under pasture (Ferraz and Felício, 2010). The nitrogen excretion by these animals enable the production of NH_3, this is an indirect source of emissions of nitrous oxide, a potent greenhouse gas. This study aimed to quantify the losses of nitrogen in the form of NH_3 in the faeces and urine of beef cattle on Marandu grass pasture in Northwest region of Brazilian State of Brazil.

Material and Methods The experiment was conducted at the Forage Sector of Campus of Jaboticabal of Universidade Estadual Paulista. The local soil is classified as an Oxissoil and tropical climate with dry winter and rainy summer. The quantification was performed using a open chamber that consists of a transparent polyethylene terephthalate (PET) bottle with bottom removed installed 10 cm above the soil. Inside each chamber had a 250 mm long wire designed with a hook to support it from the top of the bottle, and where basket on the bottom end to support a plastic jar that contained the acid solution where had a soaked foam. The foam absorbing the NH_3 volatilized. More information in Jantalia *et al*, 2012. The total nitrogen was measured by steam distillation. Treatments consisted of adding 1.5 L of urine, 1.5 kg of fresh faeces, 0.75l + 0.75 kg of urine and faeces, the control treatment not had addition of excreta. The loss of nitrogen through volatilization of NH_3 was expressed as a percentage and calculated the standard error. Volatilization of treatment minus control treatment was to allow only evaluate the effect of addition of excreta.

Results and Discussion The majority of the nitrogen loss occurred after the first 72 hours after treatment application. The volatilization of NH_3 faeces + urine and faeces were approximately 5%, 4 times lower than that proposed by IPCC (2006) for inventory purposes. Urine volatilization was 15.36%, 3 times higher than the other treatments, but still less than the factor of 20% proposed by the IPCC for bovine excreta. The percentage of N volatilized by the different treatments and the standard error are presented in Table 1.

Table 1 Percentage of N volatilized as NH_3 by excretes of beef cattle on Marandu grass pastures

Treatment	Median (%)	Standard error
Dung	4,98	± 1,73
Dung + urine	4,06	± 0,59
Urine	15,36	± 3,09

There is greater ammonium carbonate in urine, which results from hydrolysis of urea in the liquid phase. This in turn is not stable and dissociates into NH_3, for example, (Volk, 1959). When urine was mixed with faeces showed similar rates of volatilization of the faecal matter.

Conclusion The volatilization of N present in the urine of beef cattle faeces was higher than three of N present in the faeces or urine mixed faeces. Losses of N in the form of ammonia in this study were lower than proposed by the IPCC default factor for Brazil.

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Seasonal methane emissions from a beef cattle feedyard on the U.S. southern High Plains

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Introduction Enteric fermentation by ruminants is a significant source of methane (CH_4), with annual U.S emissions of 112 Tg (CO_2 equivalent), 22% of the U.S. agricultural greenhouse gas inventory (USDA, 2008). Beef cattle account for about 2/3 of enteric CH_4 emissions, mostly from the cow herd. As cattle production intensifies, a better understanding of CH_4 emissions from feedyards is needed to build more accurate emission inventories, help develop better predictive models, and meet potential regulatory requirements. Our objective was to quantify seasonal CH_4 emissions from cattle and corrals during winter and summer at a typical beef cattle feedyard on the southern High Plains of Texas, USA.

Material and methods Research was conducted at a commercial feedyard located in Deaf Smith county, Texas, during 32 days in winter and 44 days in early summer of 2010. Cattle ranged in weight from 250 to 500 kg and were housed in open corrals that covered 36.5 ha. Weight groups were randomly located in corral pens. Emissions were quantified using an inverse dispersion model (Windtrax 2.0.7.9, Thunderbeach Scientific, Nanaimo, BC, Canada). Windtrax requires measurements of background and source area CH_4 concentrations, wind data, and turbulence. Methane concentration was measured using open path lasers (Boreal Laser, Inc., Spruce Grove, AB, Canada). The background laser was located in a pasture 700 m southwest of the feedyard at a height of 1.0 m. The source area laser was deployed over the northeast quadrant of the feedyard at a height of 3.5 m. Both laser paths were 350 m long. A 3-axis sonic anemometer, located near the center of the feedyard, provided wind direction, wind speed and turbulence statistics. Input and Windtrax output data were filtered to meet quality assurance criteria of Flesch *et al.* (2005). Data were also filtered for wind direction to account for potential contamination of the background laser by the feedyard and of the feedyard laser by the water retention pond. Gaps in the data were filled using linear interpolation when the gap was 1 hr or less, and with the mean diel variation method (Falge *et al.*, 2001) for longer gaps. Further experimental details are reported in Todd *et al*. (2011).

Results Winter daily CH_4 loss was twice that in summer (Table 1). Hales *et al.* (2012), using respiration calorimetry, found daily enteric CH_4 losses of 7.9 to 12.9 g CH_4 kg^{-1} DMI. Per capita CH_4 emission rates reported in the literature for beef cattle fed concentrated diets ranged from 70 to 318 g animal $^{-1}$ d^{-1}, with our values near the middle of this range. The 24-h composites showed similar bimodal patterns, with magnitude of winter fluxes greater than summer fluxes (Fig. 1). Methane flux was lowest during early morning, then increased two to three hours after the first feeding of the day. Flux decreased during midday and then increased during the afternoon, reaching another peak in early evening following the last feeding. The evening peak was earlier in winter, presumably because cattle settled for the night earlier.

Table 1 Mean cattle populaton, dry matter intake (DMI), daily CH_4 loss and per capita CH_4 emission rate during winter and early summer, 2010 (day of year, DOY). Number in parentheses is standard deviation of mean daily loss.

Season	Population	DMI	Daily loss	Per capita emission rate
	animals	kg d^{-1}	g CH_4 kg^{-1} DMI d^{-1}	g CH_4 animal^{-1} d^{-1}
Winter (DOY 4 – 56)	9210	7.8	18.5(2.4)	145
Early summer (DOY 145 – 188)	13,250	8.5	9.3 (0.9)	79

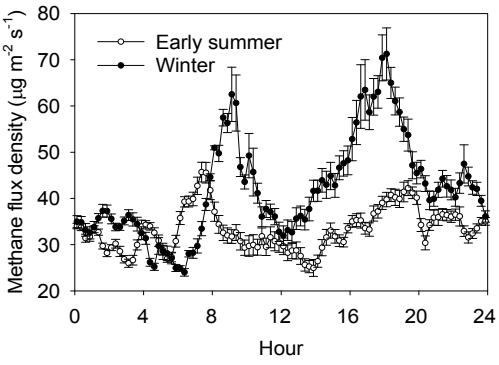

Figure 1. Composite diel methane flux density during winter and early summer. Error bars are standard error of the mean for a quarter hour flux.

Conclusions Methane loss normalized with DMI was double in winter compared with summer, but diel fluxes followed similar feeding-influenced patterns in both seasons. Annualized per capita CH_4 emission rate was 41 kg animal^{-1} yr^{-1}.

Acknowledgements Research supported by USDA CSREES Grant #TS2006-06009. Mention of trade names or commercial products is solely to provide specific information and does not imply recommendation or endorsement by USDA.

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Methane emissions by cattle as a function of forage allowance in south Brazilian natural grasslands

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Introduction Ruminant livestock are the single most important source of anthropogenic emissions of methane (CH_4) (Lassey 2007), accounting for about 28% of all anthropogenic CH_4 emission (Beauchemim *et al*., 2008). In Brazil, the livestock sector is responsible for most of the methane emissions from agricultural activities, where most of it originates in areas of extensive pastures. The intensity of CH_4 emission by ruminants is related to such factors as type of animal, food consumption level and forage digestibility (Johnson & Johnson, 1995). Grazing management is thus expected to have a strong potential effect on GHG emissions by ruminants. The objective of this study was to indentify the main source of variation of CH_4 emission by cows managed in natural pasture under continuous stocking.

Material and Methods We conducted the experiment at the Agronomical Experimental Station of the Federal University of the Rio Grande do Sul, South Brazil. The experiment, kept since 1986, involve continuous stocking of cows on natural grassland and under different forage allowances. Grazing pressure is adjusted monthly inside each of the five treatments to a forage allowance (in kg DM.ha^{-1} / kg LW.ha^{-1}) of 4, 8, 8-12, 12 and 16%. Forage allowance of the 8-12% treatment is 8% during spring and 12% during the rest of the year. The experimental design is a randomized complete block with two repetitions of each treatment. We estimated CH_4 emission from six cows per treatment (mean age 2.5 years) using the SF_6 marker technique. Methane emissions were collected over periods of 5 days for the summer, autumn and winter 2012.

Results The increased supply of forage resulted in a quadratic patterns of the CH_4 emissions per animal. During summer and autumn, the 12% forage allowance treatment showed higher emission rates, whereas all four other treatments resulted in relatively equal methane emission (Fig. 1). During winter, variations between and within treatments were much higher, with highest emission values observed for the treatment 8-12%. The overall mean seasonal CH_4 emission rate decreased from summer, autumn and finally winter.

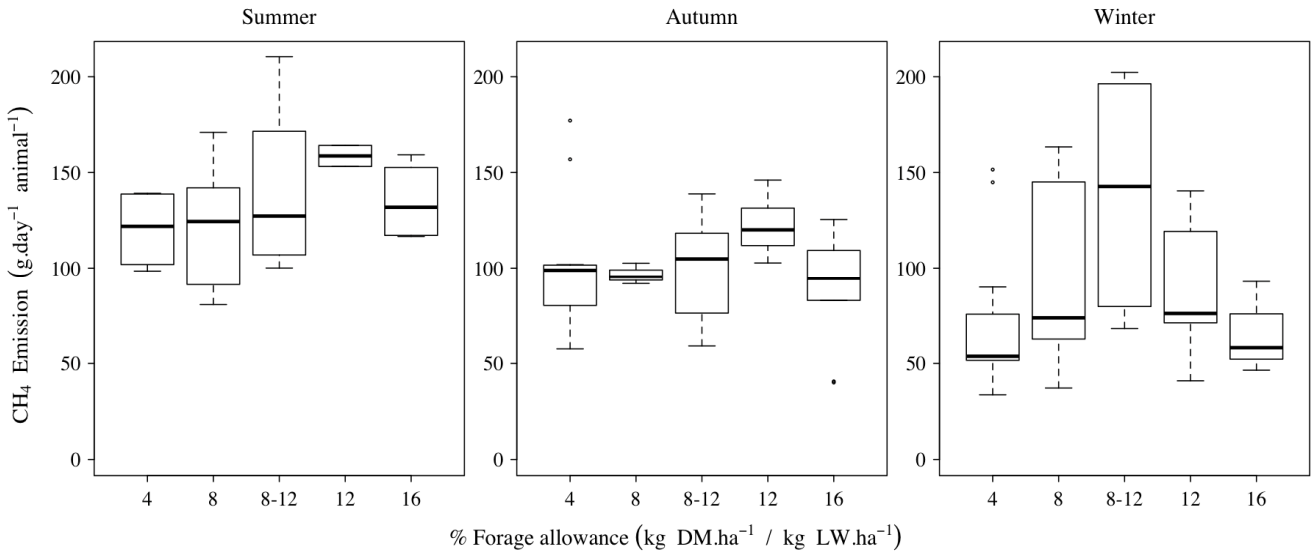

Figure 1 Methane emissions by cow managed under continuous stocking in natural grassland as a function of season and forage allowance. Horizontal lines are median values, boxes and dashed lines include respectively the central 50% and 95% of the distribution

Conclusions The management of forage availability and season alter the daily dry matter intake of animals as well as forage digestibility and these are probably the main factor affecting CH_4 emissions per animal in our natural grassland. However, it is remarkable that the lowest (4%) and highest (16%) forage allowance, whereas presenting very contrasting standing crop, species composition and forage quality, resulted in equal emissions per animal. The mechanisms leading to these similar emissions in two so contrasting vegetations are probably distinct. Examination of animal daily consumption and diet quality composition in the different treatments will likely help us to explain these results.

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Inter-annual variation in nitrous oxide emissions from white clover based grassland used for dairy production

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Introduction Nitrous oxide is a potent greenhouse gas and grasslands contributes 16 to 33% of global agricultural N_2O emissions (IPCC, 1995; deKlein *et al.*, 2008). Nitrous oxide is produced by multiple processes in soil which are affected by factors such as nitrate content, soil moisture, temperature, soil carbon, soil oxygen and pH. Changes in these controlling factors over time creates considerable variation in emissions of N_2O making it difficult to acquire accurate estimates of annual N_2O emissions. The objective of this study was to measure annual N_2O emissions from white clover based grassland and to assess annual variation in N_2O emissions.

Materials and Methods The study was conducted at Solohead Research Farm (52˚51'N, 08˚21'W). The soils have a clay loam texture and are seasonally wet. Nitrous oxide emissions from (i) white-clover based grazed pasture (WC) and (ii) white clover-ryegrass plots (WC_B: background N_2O emissions) were measured between November 2010 and November 2012. The WC consisted of three paddocks (1.6 to 2.07 ha) used primarily for grazing and occasionally cut for silage. Annual average stocking density was 2.35 cows ha^{-1} and annual fertilizer N input was 86 kg ha^{-1}. There were three WC_B (11 × 3m area) plots distributed across the farm which received no external input of N or grazing. Herbage on WC_B was harvested at monthly intervals and discarded. Nitrous oxide emission was measured using a static chamber method with five chambers per WC paddock and one per WC_B plot. The sampling strategy consisted of weekly gas sampling with increased frequency following N fertilization. Rainfall was recorded at the site. Data were analysed using analysis of variance on the PROC GLM statement of SAS. The model included year, treatment and the interaction between year and treatment as sources of variation.

Results and discussion There was relatively low annual rainfall (Fig 1a) in 2010/11 compared with 2011/12 and the ten year average previous to the study. With regard to N_2O emissions there was an interaction ($P<0.01$) between year and treatment. There was a difference ($P<0.001$) in N_2O emissions between WC and WC_B in 2010/11, whereas there was no difference between them in 2011/12. While there was no difference in emissions from WC_B between years, emissions from WC in 2010/11 was higher ($P<0.001$) than 2011/12 (Fig 1b). A prolonged period of N_2O peaks was associated with freeze thawing cycles in the winter. In addition relatively low rainfall creating aerobic soil conditions more favourable to partial denitrification, which led to the very large emission of N_2O in 2010/11. In contrast, a milder winter and saturated soil conditions under high rainfall in 2011/12 are likely to have caused the predominance of complete denitrification and hence lower N_2O emissions due to high water-filled pore space and hence anaerobic soil conditions.

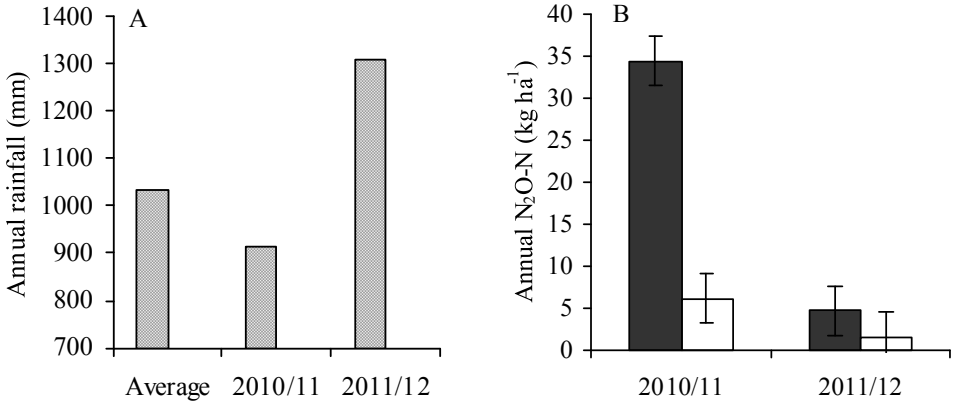

Figure 1 Annual rainfall (A) and annual N_2O emissions, from white clover based grazed pastures (WC ■) and background white clover plots (WC_B □), between 2010/11 and 2011/12 (B). Error bars represent the ± standard error of the mean.

Conclusion Differences between years in annual rainfall and winter soil temperatures impacted directly on soils conditions and hence soil biological and physical processes. The extent of these differences is reflected in the large variation in annual N_2O emissions between years. The sharp contrast in annual N_2O emissions found in this study also suggests the need for long term studies when quantifying annual N_2O emissions from grazed grassland.

Acknowledgements We acknowledge Legume Futures for financial funding.

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Estimation of greenhouse gas emissions in the agricultural sector: comparing different approaches

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Introduction Livestock farming has played an important role in global food production for centuries, but it has recently become a discussion issue due to its negative environmental implications, in particular to its role in climate change increase (Herrero *et al*. 2011). The contribution of livestock to greenhouse gases (GHG) emissions was estimated by FAO (2006) in 18% of total GHG emissions, mainly due to methane from enteric fermentation, nitrous oxide from manure management and, indirectly, carbon dioxide from land use changes. However, despite the acknowledged relevance of livestock in climate change related emissions, obtaining reliable estimations is not an easy task. Different sources provide highly varying estimates of livestock contribution to GHG emissions, ranging from 8 to 51 % and highly dependent on the accounting method (Herrero *et al*. 2011). The Intergovernmental Panel on Climate Change (IPCCC) is making an effort to develop harmonised and consistent methods, developing some guidelines for the countries under the UNFCCC and the Kyoto Protocol to carry out periodic national inventory reports. The first version of these guidelines was released in 1996, replaced in 2006 by a new version (IPCC 1997, 2006). In this study, we assess the relevance of these changes in the livestock emission estimation results, focusing on the EU countries. Overall, we will compare results of (i) official estimates as reported to UNFCCC; (ii) CAPRI calculation using IPCC 1997 plus parameters as reported by countries to UNFCCC; (iii) IPCC 1997 using default emission factors and parameters and (iv) IPCC 2006 using default emission factors and parameters.

Material and methods We calculate the GHG emissions from agriculture according to the following IPCC source categories: CH4 from enteric fermentation, CH_4 and N_2O from manure management and N_2O emissions from agricultural soils, following the different methodologies developed by IPCC 1997 and IPCC2006 guidelines, and IPCC 1997 while using country-specific parameters as reported by countries to the UNFCCC. The guidelines describe different levels of detail in calculations (tiers), which go together with the advice of using default or regionally specific parameters. In general, IPCC 2006 pushes towards higher tiers. Examples for differences include the definition of climatic zones, used to calculate feed intake and CH4 emissions from manure management. Regarding the estimation of net energy needs to calculate emissions from enteric fermentation, differences include coefficients for some livestock categories and for some feeding situations in the calculation of net energy for maintenance and net energy for activity; different equations for the calculation of net energy for pregnancy and net energy for growth; different equations for the calculation of the ratios net energy to digestible energy and net energy for growth to digestible energy, required to obtain a tier 2 emission factor. The calculation of the emission factor for methane from manure management is based in both cases on the daily volatile solid excretion per animal, which is calculated using different expressions in the two guidelines. Also for the calculation of direct N_2O emissions from manure management, equations provided are similar, but coefficients vary. The emissions factor to calculate direct N_2O emissions from agricultural soils has changed in 2006 version, compared to 1997 guidelines, as well as the conceptual N-flow model behind the equation. Finally, for indirect N_2O emissions, IPCC 2006 gives some detailed guidelines on the calculation procedures, while IPCC 1997 was mainly based on expert assessment. The GHG emissions calculations are performed using the agricultural sector model CAPRI (www.capri-model.org). CAPRI is a simulation model and also contains an ample database of the agricultural production sector, coming from international, national and regional data sources. The GHG emissions are currently calculated in CAPRI (Weiss and Leip 2012) based on a combination of approaches, basically following IPCC2006 guidelines for methane emissions (Tier 2 for cattle and Tier 1 for swine, poultry, sheep and goats), and IPCC complemented with the nitrogen-flow module developed in the MITERRA project for nitrogen emissions. The model is used to calculate livestock GHG emissions in the EU (26 member states) at a regional level (218 regions). Simulations are still on-going.

Results and conclusions We will present and discuss differences between calculations performed on the basis IPCC 1997 and IPCC 2006 guidelines in detail. Focus will be given to results at the national level, which are most relevant for the official greenhouse gas inventories. The results will be particularly significant, as for reporting for the first Kyoto commitment period (2008-2012) needs to be done on the basis of IPCC 1997 guidelines, but it is likely that the methodological requirements will be update for subsequent reporting periods to IPCC 2006. Our results will provide an important data base to assess the impact that such a methodological switch will likely bring. Furthermore, the data will be used to identify activity and/or parameters contributing largest to the overall uncertainty of the total GHG emission estimates.

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The environmental and economic impact of removing growth-enhancing technologies (GET) from United States beef production

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Introduction Beef industry sustainability is dependent upon balancing environmental impact, economic viability and social acceptability. The global beef industry faces a considerable challenge in producing sufficient meat to fulfill the needs of the growing population, which is predicted to increase from the current 7 billion people to over 9.5 billion by the year 2050. The FAO (2009) projects that the demand for animal-source foods will increase by 70% by 2050, therefore the livestock industry will be forced to produce more food using fewer resources as competition for land, water and energy intensifies. Improving productivity (growth rate and slaughter weight) within United States (U.S.) beef systems between 1977 and 2007 reduced resource input and waste output per kg of beef (Capper, 2011). To maintain the social license to operate in a demand-driven market where environmental impact is a significant concern, it is essential for beef producers to continue to improve productivity and to demonstrate the industry's commitment to sustainability. Consumers are concerned about the use of growth-enhancing technologies (GET) in animal production (Wandel and Bugge, 1997) and this has been used by some retail and food service outlets as a rationale for GET removal from the supply chain; yet little data is available upon the combined environmental and economic effects of GET use upon beef industry sustainability. The objective of this experiment was therefore to quantify the environmental and economic impact of withdrawing GET from the U.S. beef industry

Material and methods A deterministic environmental impact model (EIM) based on animal metabolism and nutrition was used to quantify resource inputs and waste outputs per 454 x 10^6 kg of beef. System boundaries extended from the manufacture of cropping inputs (fertilizers, pesticides) to the arrival of live animals at the slaughterhouse. The EIM encompassed four sub-systems: the beef population, the animal system (encompassing diet formulation), the cropping system and the transport system. The beef population contained cow-calf, stocker and feedlot systems as well as animal inputs from the dairy industry (surplus calves and cull dairy cattle). Production systems were modeled using management practices, population dynamics and production data characteristic of the U.S. beef industry. Diets were balanced for each group of cattle using AMTS Cattle Pro (2006) based on maintenance and production level (growth, lactation and/or pregnancy, where appropriate) and used to quantify resource (feed, energy, water and land) requirements and waste outputs (manure and greenhouse gases (GHG)). Two production systems were compared – one using GET (steroid implants, in-feed ionophores, in-feed hormones and beta-adrenergic agonists) where approved by the FDA at current adoption rates (CON); the other without GET use (NOT). Animal productivity gains from GET use were derived from AMTS Cattle Pro (2006) and peer-reviewed literature. The economic impact of GET withdrawal was calculated based on the cost of feed resources outputted from the EIM balanced against GET input costs (where appropriate) and applied as an economic tax to the beef industry. The tax was inputted into the CARD model (2011) to evaluate the global trade implications and carbon emissions resulting from GET withdrawal.

Results The average slaughter weight across all animal categories was 574 kg in the CON system compared to 521 kg in the NOT system. In combination with the increased number of days required to raise animals to slaughter weight (460 d in the CON system vs. 466 d in the NOT system) the reduction in slaughter weight conferred by withdrawing GET from U.S. beef production increased the size of the herd required to produce 454 x 10^6 kg beef by 385 x 10^3 animals (11.8%). Feedstuff and land use were increased by 2,830 x 10^3 t (10.6%) and 265 x 10^3 ha (10.0%) respectively by GET withdrawal, with 20,139 x 10^6 (4.2%) more liters of water being required to maintain beef production. Manure output increased by 1,799 x 10^3 t (10.1%) as a result of GET withdrawal, with an increase in GHG emissions of 714,515 t (9.8%) per 454 x 10^6 kg beef. The increased costs of U.S. beef produced without GET resulted in the effective implementation of an 8.2% tax on beef production, leading to reduced global trade and competitiveness and a projected 17.1% decrease in U.S beef production by the year 2023. To maintain worldwide beef supply it would therefore be necessary to increase beef production in other regions, with a projected cumulative increase in global carbon emissions of 3,147 x 10^6 t of CO_2-equivalent from 2009 to 2023 resulting from a combination of lower production efficiencies and deforestation.

Conclusions Withdrawing GET from U.S. beef production systems would reduce both the economic and environmental sustainability of the industry, with significant short-term and long-term consequences on a national and global scale. Further research is required to evaluate the relative social importance of the increase in environmental impact and economic cost conferred by GET withdrawal, against the consumer perception that beef produced without GET is a more desirable dietary choice.

Acknowledgments Authors gratefully acknowledge financial support for this study provided by the Sustainable Beef Research Center

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Continuous gaseous NH_3 and N_2O emission measurements from discrete experimental field treatments using scanning open-path FTIR

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Introduction An on-farm open-path experimental protocol has been developed for the continuous measurement of greenhouse gas emissions from field-scale treatments. The protocol uses a mono-static open path Fourier Transform Infrared (FTIR) Spectrometer scanning along five optical paths crossing two plot treatments to estimate the gaseous emissions from the two treatments. One-half hour gas emissions were determined using backward Lagrangian Stochastic (bLS) modelling.

Material and Methods Near-continuous scanning FTIR measurements of nitrous oxide (N_2O) and ammonia (NH3) concentrations and sonic anemometer measurements of turbulence were made over two fertilizer treatments at a commercial farm for 20 days in 2011 after a side dress NH_3 application. The crop was no-till maize. The treatments were 28% urea (UAN) at 180 kg/ha and 28% UAN at 180 kg/ha with Instinct® (a nitrogenase inhibitor of Dow Agroscience). It was expected that the two treatments would result in two distinct N_2O emission rates but identical NH_3 emission rates across treatments. The gaseous concentrations were measured continuously and sequentially along five optical paths varying in optical distance from 214 m to 480 m using a MIDAC FTIR spectrometer. Additional gas concentration measurements were made from air sampled at two additional locations at the field edge using a Teledyne gas filter correlation N_2O analyzer. Measurements of the atmospheric temperature, humidity, and pressure and three dimensional (3D) turbulence were also made. Quality assurance included comparisons of H_2O and N_2O concentrations form co-located point and line measurements. Emissions of NH_3 and N_2O were modelled using the WindTrax™ bLS model using as inputs all optical paths and the measured 3D turbulence information . Concentrations of NH_3, N_2O, CH_4, H_2O, and CO_2 were derived from the FTIR measurements using four programs: FTIR-FIT, IMACC IFSS, MIDAC AutoQuant®, and ThermoFisher Scientific TQS®. The N_2O concentrations were particularly difficult to extract from the spectra due to interferences of H_2O and CO_2. Comparisons of the N_2O concentrations measured by the gas filter correlation measurements and by FTIR spectra analysis of the nearest path to the gas filter correlation sampling inlet showed reasonable but not exact agreement. This was in part due to the difference in the air sampled at a point and integrated along a 200 m line. Comparisons between the H_2O concentrations measured at the automated weather station and by FTIR spectra analysis of the nearest path also showed reasonable agreement. The measurements were separated into three periods: 6-10 June, 13-17 June, and 20-24 June.

Results Measurement problems identified included vibration of the FTIR and retro-reflectors due to wind, fluttering of leaves across the optical paths, variable terrain limiting optical path lengths, determination of background concentrations for emissions modelling, and overheating of the closed path gas analyser. Quality assurance protocols were developed to determine which FTIR spectra were valid. Invalid spectra resulted from low signal strength associated with the FTIR beam position drifting off the retro-reflector and partial or total blockage of the FTIR beam by the growing maize. The valid N_2O and NH_3 path concentrations were then combined into ½ hr mean values and merged with similarly-averaged turbulence measurements. Emission measurements were invalidated when the wind was from a direction in which the background concentrations could not be determined, when the wind speed was less than about 1.5 m/s, when the atmosphere was either very stable or very unstable, when there were missing paths in a given ½ h period, and when the five paths did not result in a computationally coherent set (possibly from analytic errors in the FTIR spectra analysis). Calculated negative N_2O emissions were probably due to spatially inconsistent N_2O path- integrated concentrations (PICs): often resulting in calculated emissions from the two treatments that were essentially mirror images of each other. The first measurement period had the greatest number of valid ½ h emission estimates and was the focus of further analysis. For the UAN-only treatment calculated N_2O emissions ranged up to 30 μg N_2O $m^{-2}s^{-1}$ while NH_3 emissions ranged up to 5 μg NH_3 $m^{-2}s^{-1}$, and for the UAN+instinct treatment calculated N_2O emissions ranged up to 4 μg N_2O $m^{-2}s^{-1}$ while NH_3 emissions ranged up to 4 NH_3 μg $m^{-2}s^{-1}$. High emission rates were associated with high winds and in general did not last for many hours during a day but did repeat for a number of sequential days. Maximum daily NH3 emissions declined daily through the week consistent with volatilization of the gas after the UAN application. The mean standard deviations (s.d.) of the modelled emission measurements during the first period were between 1.2 and 1.8 μg N_2O $m^{-2}s^{-1}$ and 0.3 and 0.7 μg NH_3 $m^{-2}s^{-1}$. Based on the nocturnal periods with negligible and steady emissions (+/- 2 μg N_2O $m^{-2}s^{-1}$ and 0.5 μg NH_3 $m^{-2}s^{-1}$) and the mean calculated emission s.d., the configuration and analysis method probably had a minimum detection limit (MDL) of about 1.5 μg N_2O $m^{-2}s^{-1}$ and 0.5 μg NH_3 $m^{-2}s^{-1}$.

Conclusions The use of the scanning open-path FTIR/ bLS protocol for the measurement of gaseous emissions from field treatments was demonstrated conclusively for NH_3 but not conclusively for N_2O. Substantial measurement errors in N_2O emissions still exist. Improvements on the N_2O emissions MDL are most likely to occur with improvements in the SNR of the FTIR spectral analysis.

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Effect of varying replacement rate on greenhouse gas emissions from dairying in Eastern Canada – Evaluation using farm based life cycle assessment

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Introduction In dairy systems, a major factor affecting farm profitability is the rate of replacement of milking cows. Reproductive failure, mastitis and conformation are primary reasons for culling; however, in addition to the economic cost of replacing dairy cows, maintaining a potentially large replacement herd may also have notable environmental consequences. This study assessed the effects of varying replacement rate on greenhouse gas (GHG) emissions from a typical dairy system in Eastern Canada.

Materials and methods A partial life cycle assessment (LCA) was conducted to estimate the effect of varying replacement rate on GHG emissions from a typical non-grazing dairy production system in Quebec. Additionally, as dairying generates both milk and meat, a number of methods of allocating emissions between these co-products were employed. The LCA was conducted according to McGeough *et al*. (2012), with the assessment considering 65 female Holstein calves, of which 60 heifers survived to first calving at 27 mo of age. Progeny were also included in the analysis, with bulls and heifers in excess of replacement requirements finished as grain-fed veal. All animals were housed indoors and fed forages and grains produced on-farm. Replacement rates were based on farm survey data compiled by Valacta (2012), the dairy production centre of expertise for Quebec and the Atlantic provinces. From these data, the mean, 10th and 90th percentile replacement rates were employed. Replacement rates were; a) 10th percentile: 50.8%, b) mean: 36.7%, and c) 90th percentile: 22.4%. Pre-farm gate GHG emissions and removals were quantified using Holos, a whole-farm model developed by Agriculture and Agri-Food Canada and based on IPCC Tier 2 methodology tuned to Canadian conditions. In addition to GHG generated on farm, emissions associated with the production of inputs used in the dairy system were also considered. Results are expressed as CO_2 equivalent, using the global warming potentials of the individual gases: CH_4 25; N_2O 298; and CO_2, 1. Various allocations of GHG emissions between meat and milk were considered: a) no allocation to meat, b) economic product value, c) dairy vs veal, and d) the International Dairy Federation (IDF) equation using feed energy demand for meat and milk production.

Results Decreasing dairy cow replacement rate, spanning the range observed on commercial farms, decreased the GHG intensity of milk production irrespective of allocation method (Table 1). The largest reduction (17.3%) was observed where there was no allocation to meat, with the lowest (4.3%) observed for allocation based on the IDF guidelines using the simulated farm meat to milk ratio. The relative proportion of the individual gases remained relatively unchanged irrespective of replacement rate (Figure 1). Decreasing replacement rate increased (18%) the milking and dry stock contribution to total GHG emissions (Figure 2.)

Table 1. Greenhouse gas emissions and land area required for feed supply as affected by varying dairy cow replacement rate

	Replacement rate, %					
	50.8		36.7		22.4	
GHG emissions, kg CO_2e	Milk[1]	Meat[2]	Milk[1]	Meat[2]	Milk[1]	Meat[2]
No allocation	1.10	0	0.97	0	0.91	0
Economic allocation	0.99	2.91	0.88	2.88	0.83	2.87
Dairy vs veal animal	1.07	1.94	0.95	1.94	0.89	1.94
IDF default[3]	0.94	4.20	0.83	4.52	0.78	4.73
IDF simulated[4]	0.70	10.57	0.68	9.34	0.67	8.78
Land area required for feed production, ha	49.3		62.5		72.6	

[1] kg fat and protein corrected milk, [2] kg beef carcass, [3]Using IDF default meat to milk ratio, [4]Using simulated farm meat to milk ratio

Figure 1. Relative contribution of GHG emissions as affected by replacement rate

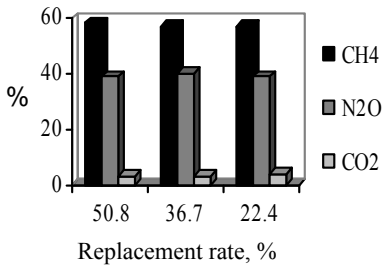
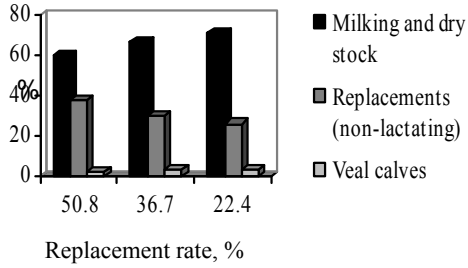


Figure 2. Contribution of the livestock groups to the total GHG emissions



Conclusions Reducing dairy cow replacement rate reduced the GHG footprint of milk production. This reduction may be also be additive when coupled with other mitigation strategies to further reduce the GHG intensity of milk production. Reducing dairy herd replacement rates would also be expected to improve farm profitability providing an economically attractive GHG mitigation strategy.

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Changes in methane emission due to species, maturity and legume proportion in grass:legume mixtures incubated in vitro of Colombian highland forages

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Introduction Kikuyu (*Pennisetum clandestinum*), ryegrass (*Lolium spp*) and clover (*Trifolium spp*) are the main forage species used for ruminant production in Colombian highlands. The tanniferous legume *Lotus uliginosus* has been recently introduced to this region. Literature suggests that methane emission by ruminants may vary due to species, maturity and proportion of the legume in grass:legume mixtures of the forage consumed. The objective of this work was to evaluate the effect of forage species, forage maturity and the proportion of a legume in a grass:legume mixtures on methane emissions of four forage species incubated *in vitro*. Knowing these differences may help to introduce on farm management practices that could lead to a reduction of methane emission by grazing ruminants in this region.

Materials and methods Four forage species, two grasses (*P. clandestinum* and *L. perenne*) and two legumes (*L. uliginosus and T. pratense*), were harvested in two different paddocks in highlands of Colombia (4°40'89''*N*, 74°13'13'' *W*; 2540 *mosl*). In a first experiment, forages were harvested at three different maturity stages (young, intermediate and mature) and freeze dried. In a second experiment, forages were harvested at an intermediate maturity stage, freeze dried and each of the grasses were mixed with each legume in three grass:legume proportions (90:10, 70:30, 50:50). *In vitro* 48 h gas production was measured and methane in gas determined by GC. Data were analyzed for each experiment as a randomized complete block (paddocks) design with a factorial arrangement using GLM procedure of SAS®.

Results Younger forages produced less methane (g/kg DOM) than more mature forages (Figure 1). On average *L. uliginosus* produced less methane than *L. perenne, T. pretense* or *P.clandestinum* (Figure 2). Increasing legume proportion in mixtures with *P. clandestinum* reduced methane production but this was not the case for *L. perenne* mixtures (Table 1).

Figure 1. ***In vitro*** **methane production (ml g^{-1} MO degradade) from spices with diferent mature stages**

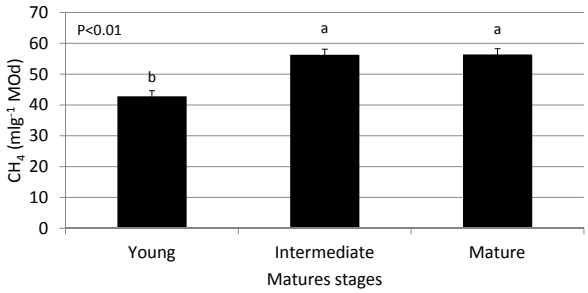

Figure 2. ***In vitro*** **methane production (ml g^{-1} MO degradade) from diferent spices of forages from Colombian highlands**

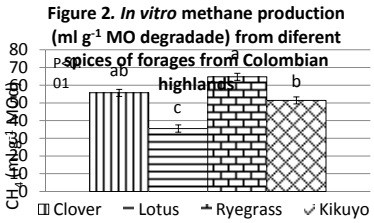

Table 1 *In vitro* methane production (ml g^{-1} MO degradade) in grass:legume mixtures

	Kikuyu		Ryegrass	
Gra:Leg	Clover	Lotus	Clover	Lotus
90:10	50.2[a]	44.5[a]	38.3	38.1
70:30	44.7[b]	40.1[b]	40.6	38.5
50:50	43.5[b]	37.1[c]	40.0	38.7
s.d.	3.6	3.7	1.2	0.3

[abc]. Means with different letter within a column differ ($p < 0.05$).

Conclusions Younger forages produced less methane *in vitro*. *P. clandestinum* grass produced less methane than *L. perenne* and the legume *L. uliginosus* produced the lowest methane of all forages evaluated. Increasing the proportion of legumes in a mixture with kikuyu grass reduced methane production but not with ryegrass.

Acknowledgments This work was possible due to the financial support of the Ministry of Agriculture and Rural Development (MADR) of Colombia.

Inclusion of lotus (Lotus uliginosus) reduce methane emission by rams fed kikuyu (Pennisetum clandestinum) grass hay

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Introduction In Colombian highlands, kikuyu (*Pennisetum clandestinum*) is the main forage used by grazing ruminants. Several studies suggest that adding a tanniferous legume to a grass basal diet may reduce methane emissions by ruminants. The objective of this experiment was to evaluate the effects of introducing the tanniferous legume hay (*Lotus uliginosus*) to a kikuyu hay basal diet fed to sheep.

Materials and methods Twelve indigenous breed growing rams, with an average weight of 23±2 kg, were assigned randomly to a 2x3 switchover design. Treatments consisted of 100 % kikuyu hay or 70% kikuyu hay 30% lotus hay (Table 1). Each of the three periods lasted 20 d, the first 15 days were an adaption to the diet and the last five days for measurements. Animals were allocated in metabolic cages, fed *ad libitum* once a day (8 AM) and had free access to water. Intake, fecal production and digestibility were determined for each ram during the last five days of each period. Methane production was measured for each treatment group (6 rams) in a poly-tunnel during the last three days of each period. ANOVA was performed using GLM procedure of SAS®. Multiple comparisons among treatment means were performed by Tukey´s method.

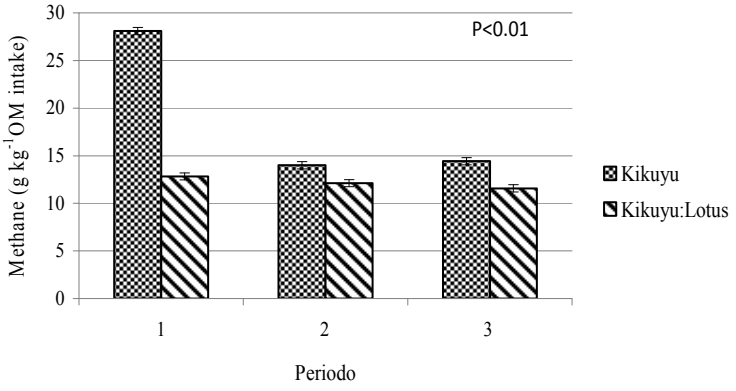
Results Organic matter (OM) intake increased by 23% ($p < 0.01$) when legume was included in the basal diet (418 vs. 514 g d^{-1}) but NDF intake was similar between treatments (332 vs. 360 g d^{-1}). When legume was included in the basal diet, OM digestibility tended ($p<0.1$) to increase by 2% (587 vs. 599 g kg^{-1}) and NDF digestibility decreased ($p< 0,05$) by 4.6% (615 vs. 587 g kg^{-1}). Legume addition reduced total methane production (51.6 vs. 43.1 g d^{-1}; $p<0.01$), methane per dry matter intake (DMI) (18.8 vs. 12.2 g kg^{-1} DMI; $p<0.01$), methane per degraded organic matter (DOM) (36.1 vs. 23.4 g kg^{-1} DOM; $p<0.01$) and methane per degraded neutral detergent fiber (DNDF) (43.5 vs. 33.9 g kg^{-1} DNDF; $p<0.01$). When legume was added there was a residual effect ($p<0.01$) for methane production between periods (Figure 1).

Table 1 Chemical composition of experimental diets

Composition	Kikuyu:lotus	Kikuyu
Crude protein, %	16.8	11.8
Ether extract, %	1.2	1.3
Neutral detergent fibre,%	61.2	69.9
Acid detergent fibre, %	31.3	31.6
Hemicellulose, %	29.9	38.4
Cellulose, %	23.7	27.8
Lignin, %	7.6	3.8
Ash, %	12.9	12.1
Condensed tannins, %	1.3	0
Organic matter, %	87.1	87.9
Energy (Kcal/g)	3.9	3.9

[1].100-(CP + NDF + Ash + EE + Condensed tannins)

Figure 1 Methane production (g kg^{-1} OM intake) by rams fed kikuyo hay with or without lotus



Conclusions Addition of 30% lotus hay to a kikuyu basal diet fed to rams increased total OM intake by 23% and decreased total methane emission by 16%. There was a residual effect of lotus inclusion on methane emissions suggesting a reduction in ruminal methanogenesis possibly due to condensed tannins in the lotus that could explain the more methane production of kikuyu in the first period. Lotus could be a suitable legume to reduce methane emissions in grazing systems in the highlands of Colombia.

Acknowledgments This experiment was possible due to the financial support of the Ministry of Agriculture and Rural development of Colombia (MADR).

The interplay of enteric methane emission, fiber intake, digesta passage time and digestibility in Holstein heifers fed a brown midrib or conventional corn silage

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Introduction Corn with the brown midrib (BMR) mutation has been well documented to have higher fibre degradability and will likely increase dry matter intake (DMI) of cows compared with those fed conventional corn silage (CCS). Besides improving feed efficiency, BMR fed cows may emit relatively less methane than CCS fed cows due to the higher neutral detergent fibre (NDF) quality. Only few reports exist on the effect of BMR on the actual enteric methane emissions or particle passage time. We aimed to perform a study combining all these key factors for assessing the potential of BMR to mitigate enteric methane emissions on a relative scale, using corn silage as major constituent of forage.

Material and methods Seven German Holstein heifers, with a body mass of 589 ± 47 kg (mean ± s.d.), were divided in two groups (BMR/CCS) and subjected to a change-over design with two 13-d experimental periods. Prior to the experiment, animals were kept on pasture supplemented with corn silage (30%). During a 3-wk pre-trial period, animals were gradually adapted to a corn silage proportion of 92% in the total mixed ration (TMR, 5% hay, 3% extracted soy meal). Experimental diets consisted of a TMR with either a brown midrib or conventional corn silage (four samples each due to the usage of several silage bales per crop and due to grouping and periods). Throughout the pre-trial and experimental period, animals were fed *ad libitum* twice daily. Food items offered and leftovers were quantified on a daily basis by weighing, and representative samples of them were stored frozen (−20 °C). On day 13 of the experimental period, 24-h measurements of individual methane production in the open-circuit calorimetric chambers was recorded as described by Derno *et al.* (2009). Solute (Co-EDTA) and particle (Cr-mordanted plant cell wall) passage markers were prepared according to Udén *et al.* (1980) and fed in the morning of day 8 of the experimental period before the regular feeding. Faecal (grab) samples were collected regularly from day 8 to 14 and stored frozen (−20 °C). Dry matter content of feed and faecal subsamples was determined by drying at 103 °C to constant weight. For nutrient analyses food and faecal samples were dried at 40 °C and 60 °C, respectively. Ground faecal samples were wet microwave digested and analysed by atomic absorption spectroscopy (Perkin Elmer AAS 3300, Ueberlingen, Germany) for cobalt and chromium concentrations. Average digesta passage time was calculated according to Thielemans *et al.* (1978). Apparent dry matter and nutrient digestibility were calculated after extrapolating the total amount of excreted faeces from the individually ingested (indigestible) acid detergent lignin (ADL offered –leftover). Results are shown as mean values. Statistical analysis was performed by the Student's (paired) *t*-test by using Systat 11 (Erkrath, Germany). The significance level was set to α=0.05.

Results Corn silage type had no effect (n=4, p=0.576) on NDF content of TMR, but as expected TMR on BMR basis contained less (n=4, 1.6 vs. 2.8 % DM, p=0.004) lignin than TMR on CCS basis. Accordingly, for the BMR-based TMR, the apparent digestibility of NDF was higher (n=7, 71 vs. 62 %, p=0.030) than for the CCS-based TMR. Corn silage type did not alter the average DMI during passage trials, neither on an absolute (kg, n=7, p=0.129) nor on a relative (g $kg^{-0.75}$ BM, p=0.125) scale. Accordingly, solute and particle passage times through the reticulo-rumen did not differ between diets (n=7, solute: p=0.217, particle: p=0.467), nor did the apparent digestibility of dry matter (n=7, p=0.173). During the 24-h measurement of methane emissions, absolute (L d^{-1}, n=7, p=0.110) and relative (n=7, L kg^{-1} DMI: p=0.657; L kg^{-1} digested DM: p=0.329; L kg^{-1} digested NDF: p=0.098) enteric methane production was not different between groups. During these 24-h, dry matter intake was on average higher (n=7, 8.7 vs. 7.4 kg, p=0.029) in BMR than CCS fed heifers.

Conclusions Although BMR was a major constituent of forage, enteric methane emission of BMR fed heifers did not differ from those fed a CCS-based TMR, neither on an absolute (per day) scale nor on a relative (per kg ingested DM, per kg digested DM, per kg digested NDF) scale. We conclude that BMR is not suitable for mitigating enteric methane emissions compared to CCS.

Acknowledgements The authors gratefully acknowledge the help of the involved staff at the FBN, IZW and FLI.

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Estimation of the inventory of enteric methane emissions produced by cattle grazing Pennisetum clandestinum, using in vitro cumulative gas production technique (IVGPT) in Antioquia-Colombia

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Introduction The production/emission of methane by domestic ruminants represents not only an economic problem but also an environmental one. However, despite some important advances, it remains a large gap in information related to the volume of emissions and the effectiveness of mitigation strategies, particularly in tropical regions where a high proportion of domestic ruminants are kept (Soliva and Hess, 2007). In Colombia the potential impact of its livestock in the global warming through methane gas emission is unknown by which measurement of methane emissions is required. This is one of the first approaches made in Colombia (South America) around this subject using this kind of methodology.

Material and methods Based on the inventory of cattle and milk production averages, five regions of the Antioquia Department (Colombia, South America) were selected. Four farms per region were included. In each one of the twenty farms were sampled pastures and concentrated supplements. Chemical composition (DM, OM, CP, NDF, ADF, ash, lignin and EE) was determined and *in vitro* incubations at 24 hours was performed (IVGPT) by using glass bottles of 110 mL added of 0.5 g of dry sample pasture or individual diets (pasture+concentrate in a 70:30 ratio) and ruminal fluid collected early in the morning from ruminally cannulated cows of the dairy farm "Paysandú" of the Universidad Nacional de Colombia. During the incubation process, bottles were continuously gassed with CO_2 and maintained at a constant temperature of 39 °C. After 24 h of *in vitro* incubation, the parameters measured were cumulative gas (mL), dry matter digestibility (%) -DMD, CH_4 production, and volatile fatty acids -VFA. Further tests were performed: voluntary forage intake of grazing animals by the agronomic direct method. Statistical analysis was carried out using the GLM procedure of SAS ® software (SAS Institute Inc., Cary, NC, USA). For IVGPT a complete randomized block design was performed for an experimental design with four replicates, where each repetition was constituted by a ruminal fluid. When a significant effect was found ($P < 0.05$), Tukey test was performed to compare averages. VFA concentrations (acetic, propionic, and butyric) and the concentrations of methane [CH_4] of the evaluated samples were determined by gas chromatography, using a Shimadzu model GC2014 with AFC [Advanced Control Flow] And APC [Advanced Pressure Control] and FID detector 2014. The injection for methane measurements was performed manually. Methane calculations were performed according to the methodology described by (Lopez and Newbold, 2007). The laboratory works were performed in the Laboratorio de Biotecnología Ruminal –BIORUM-, Departamento de Producción Animal, Universidad Nacional de Colombia –Sede Medellín.

Results The estimation of the enteric methane emissions produced by cattle in different regions of the department of Antioquia, Colombia, in dairy farms on which cattle are fed with *Pennisetum clandestinum*, was obtained from the number of animals in inventory and methane results found by region which reached a total volume of 55,31 Gg/100 kg bw/year. The results obtained using IVGPT are comparable with those reported internationally for other forages and in vivo techniques (see Table 1).

Table 1 Mean values of cumulative gas, dry mater digestibility -DMD, concentration of volatile fatty acids –VFA, and methane production after 24 hours of fermentation of diets from five regions of the Antioquia, Colombia.

	Region					
	1	2	3	4	5	P
Gas (mL)	112.9	118.7	112.2	109.7	115.3	N.S
DMD (%)	63.1	63.7	61.4	63.4	64.2	N.S
VFA Total (mmol/L)	17.5 c	21.25 a	20.2 ab	19.9 b	21.0 ab	<0.0001
Acetic (mmol/L)	10.5 c	13.23 a	12.1 b	11.9 b	12.6 ab	<0.0001
Propionic (mmol/L)	5.4	5.25	5.5	5.3	5.6	N.S
Butiric (mmol/L)	1.5 b	2.31 a	2.2 a	2.2 a	2.3 a	<0.0001
Acetic:Procionic	2.0 c	2.51 a	2.2 b	2.2 a	2.3 a	<0.0001
CH_4 g/100 bw/day	83.7 c	172.3 a	114.6 b	88.8 ab	112.1 cb	<0.0001

a-c Means with different letters between rows differ significantly ($P < 0.05$)

Conclusions The mean values of the main products of the IVGPT such as accumulated gas, DMD, and VFA´s are comparable with those reported to *Pennisetun clandestinum* grass in Colombia. Similarly, the volumes of methane recorded by means of IVGPT are comparables with those reported by scientific literature and it permits conclude that this technique is useful to estimate methane inventories or undertake actions to mitigate its emissions either our region or our country

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Use of acid soluble bio-organic substances extract as rabbits feed additive to reduce manure gases emission during storage

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Introduction Animals release a significant amount of acidifying and greenhouse gases (GHG) into the atmosphere, produced both directly from their metabolic processes and indirectly from manure storage and spread. Green compost extracts added to the feed may reduce gaseous emissions, modifying the gastro-intestinal environment and the faeces chemical composition (Islam *et al.*, 2005). Preliminary studies have shown as a green compost extract (acid soluble bio-organic substances or SBO), obtained by an experimental plant (Montoneri *et al.*, 2011), could have effect as feed additive (to 0.05 and 0.25% addition) on GHG (methane, CH_4; nitrous oxide, N_2O) and ammonia (NH_3) emissions during manure storage (Biagini *et al.*, 2012). The objective of this study was to evaluate the effect of SBO to higher concentration as feed additive to rabbit diet on growth performance, and GHG and NH_3 emissions from manure during storage.

Material and methods Three groups of 40 rabbits each were reared from 35 to 91 d of age under the same environmental conditions, fed iso-energetic (digestible energy 18.8 MJ kg^{-1} dry matter) and iso-proteic (crude protein 178 g kg^{-1} dry matter) diets (90% dry matter, DM) with different doses of SBO obtained from green pruning of gardens: absence (control group, D0), low (5 g kg^{-1} wet weight, D5), and high (10 g kg^{-1} wet weight, D10). During the experimental period (56 d), individual live weights and feed consumption were recorded weekly. To assess the effect of SBO addition to diets on CH_4, N_2O and NH_3 emissions from stored manure, during the last week of the rearing period faeces and urine excreted by 4 rabbits per group were collected separately during 6 consecutive days. After the collection period, faeces and urine of the same experimental group were accurately mixed in a ratio of 1:2 by fresh weight. Then, samples of 0.50 kg of each mix (manure) were placed in 1.5 L vessels (∅ 11.3 cm) and stored for a period of 19 d at room temperature (22.9±2.2 °C). Organic matter (OM) and N content of manure samples were analysed according to AOAC (2000). CH_4, N_2O and NH_3 emissions were measured by a dynamic chamber method using a gas trace analyser (1412 Photoacoustic Multi-gas Monitor, Innova Air Tech Instruments), following Dinuccio *et al.* (2008), in 8 sessions on 4 replicates per diet. Data were analysed by ANOVA (IBM SPSS Statistics 20.0) according to diet.

Results The SBO addition into diet did not affect individual live performances (weight gain 35 g/d and feed consumption 90 g/d as average). Likewise, there was no effect of SBO feed addition on the amount of faeces and urine (60 g/d and 90 g/d on average respectively) produced by each rabbit, and on the OM and N content of the manure (Table 1). Total CH_4 and N_2O emission from stored manures decreased as SBO level in the diet increased, however, no significant differences were observed with regard to total CH_4, N_2O and NH_3 emission from D5 and D10 treatments (Table 1).

Table 1 Manure samples composition and total emissions of CH_4, N_2O and NH_3 from the storage of manure from control (D0), low (D5) and high (D10) SBO content diets (mean ± s.d.).

	Diet			P
	D0	D5	D10	
OM (% DM manure)	88.8±3.8	86.9±2.3	83.6±3.6	0.16
N (% DM manure)	3.3±0.4	3.4±0.2	3.5±0.3	0.71
CH_4 (mg kg^{-1} wet manure)	380.7±114.4	300.3±67.2	246.8±81.8	0.16
N_2O (µg kg^{-1} wet manure)	8.8±3.1	5.5±1.0	6.7±1.6	0.13
NH_3 (mg kg^{-1} wet manure)	2986.9±689.3	3574.1±822.3	3301.7±1225.0	0.69

Conclusions The use of SBO as a feed additive do not affect animal performance and seems able to reduce GHG emissions (particularly CH_4 and N_2O) from rabbit manure to lowest doses than the tested ones in this trial (Biagini *et al*., 2012). This conclusion seems confirm the results obtained on *in vitro* swine intestinal fermentation (Montoneri E. personal communication). To estimate the effect of SBO addition to diets on gaseous emissions during the overall manure management, further experiments are currently in progress to evaluate the soil application stage.

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High partial feed conversion efficiency appears to be a persistent trait associated with reduction in selected measures of methane emissions in dairy cattle

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Introduction In the U.S., enteric CH_4 contributed 21% of anthropogenic CH_4 emission in 2010. Alongside dietary manipulations, exploiting among-animal variation in feed conversion efficiency (FCE) may offer possible CH_4 mitigation strategies (Martin *et al.*, 2010). In beef cattle, CH_4 emission was lower from animals with higher FCE, measured as residual feed intake (Hegarty *et al.*, 2007). Similarly, our preliminary short-term data suggested an inverse relationship between daily CH_4 emission and partial FCE for milk [pFCEmk, kg milk/kg dry matter intake (DMI)] (Arndt *et al.*, unpublished). This relationship could be explained by a number of factors including inherent differences in rumen pH and microbial population, rate of passage, digestibility of feed chemical fractions or post-absorptive nutrient utilization and partitioning. This experiment was designed to evaluate the medium-term persistency of FCE and related measures, to validate our preliminary CH_4 emission results, and to determine the relationship between CH_4 emission and selected measures of digestive and metabolic functions among cows with high and low pFCEmk.

Materials and methods The study was conducted at the U.S. Dairy Forage Research Center, Prairie du Sac, WI. First, only cows with more than 100 days in milk (DIM) were pre-selected (n=140). Then, pFCEmk was used as a criterion to select 16 cows grouped in eight pairs (high and low pFCEmk) based on parity and DIM fewer than 16 days apart. The DIM at the beginning of the trial ranged from 106 to 368. All cows were fed the same standard diet: 28.2% corn silage, 26.7% alfalfa silage, 23.2% high moisture corn, 7.1% distillers dried grains, 3.6% soybean meal, 8.8% roasted soybeans, and 2.4% vitamins and minerals on a DM basis. The diet contained 47.3% DM, and on a DM basis, 92.5% organic matter (OM), 16.2% crude protein, and 28.1% neutral detergent fibre (NDF). Body weight (BW), milk production and composition, samples of total mixed ration (TMR) and feed refusals, were collected over a 12-week period. The data were analysed as split-plot in time with repeated measures, cow pair and pFCEmk in the main plot, and week in the subplot. During a four-day CH_4 emission measurement period (collected on two pairs at a time as cows were placed individually in one of four air-flow-controlled chambers), TMR, feed refusals, and milk samples were collected daily and total faecal collection proceeded for the first three days in order to measure digestible OM intake (DOMI) and digestible NDF intake (DNDFI). In addition to evaluating pFCEmk, the NRC 2001 energy system was used to calculate daily expenditure of net energy for lactation (NE_L) in milk (NE_{milk}), BW gain (NE_{gain}), and maintenance (NE_{maint}) and to estimate pFCE for various measures of energy expenditure (see Table 1). Data were analysed as a randomized complete block with pairs as block and pFCEmk as treatment effect.

Results The effects of cow pFCEmk (Table 1) and week ($P < 0.01$) were significant but not the interaction of week by cow pFCEmk ($P = 0.17$). The high pFCEmk cows had greater milk somatic cell count (SCC, $P = 0.03$) than low pFCEmk cows. Other results are in Table 1.

Table 1 Performance, energy expenditures and CH_4 emissions from cows with low and high pFCEmk.

Parameter	Low	High	s.e.m[1]	P
pFCEmk, kg milk/kg DMI	0.99	1.77	0.11	<0.01
DMI, kg/d	19.9	23.7	0.80	<0.01
NE_{milk}, Mcal/d	14.7	28.8	1.88	<0.01
NE_{gain}, Mcal/d	3.47	1.96	0.78	0.22
NE_{maint}, Mcal/d	10.67	10.00	0.34	0.20
NE_{total}, Mcal/d[2]	28.86	40.55	2.06	<0.01
$pFCE_l$, Mcal/kg[3]	0.75	1.21	0.08	<0.01
$pFCE_{lgm}$, Mcal/kg [4]	1.46	1.71	0.08	<0.01
NE_{milk}/DOMI, Mcal/kg	1.12	1.88	0.13	<0.01
NE_{total}/DOMI, Mcal/kg	2.18	2.66	0.12	<0.01
CH_4, g/d	805	823	50.21	0.81
CH_4/NDFI, g/kg	147	125	6.19	0.03
CH_4/DNDFI, g/kg	293	275	16.47	0.42
CH_4/NE_{milk}, g/Mcal	64.3	29.1	na	<0.01
CH_4/NE_{total}, g/Mcal[1]	28.2	20.4	na	<0.01

[1]s.e.m. = standard error of the means, na = not applicable.
[2]$NE_{total} = NE_{milk} + NE_{gain} + NE_{maint}$.
[3]$pFCE_l$ = pFCE for milk energy (NE_{milk}/DMI).
[4]$pFCE_{lgm}$ = pFCE for total NE (NE_{total}/DMI).

Conclusion The relative difference between the low and high pFCEmk cow groups persisted over the duration of the study. The high pFCEmk cows had higher energy expenditures but were more efficient for total energy expenditure in milk, BW gain, and maintenance per unit of feed DM and digestible OM consumed. However, results suggested that energy expenditure by the immune system of cows with high SCC may have contributed to their lower pFCEmk. Contrary to preliminary results pFCEmk had no effect on daily CH_4 emission in this trial. However, similar CH_4/DNDFI warrant further investigation to evaluate if DNDFI could be used as a reliable predictor of CH_4 emissions across a range of diets. Further research should focus also on the relationship between pFCEmk and CH_4 emission, and the possibility of genetic selection of cows for greater energetic efficiency and lower CH_4 emissions.

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Methane production in warmblood ponies fed either a roughage only or a roughage plus concentrate diet

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Introduction Atmospheric methane concentrations have increased considerably since the pre-industrial era, mainly caused by anthropogenic contributions (Solomon *et al.*, 2007). For equines, enteric methane emissions are defined by the Tier 1 method and are estimated at 18 kg methane/head/year at live weight of 550 kg (IPCC, 2006). Reduction of methane emission from horses may be achieved by dietary interventions, e.g. by inclusion of concentrates in their diet. It is hypothesized that diets containing large amounts of concentrates with highly available starch sources may cause less methane production compared to diets containing mainly roughage, due to more enzymatic digestion pre-caecally and less fermentation in the hindgut. However, roughage rich rations are preferred from a health and welfare point of view (Elia *et al.*, 2010). Potential dietary interventions to reduce methane emission by horses may therefore conflict with optimization of the animals' health and welfare. Despite the large horse population worldwide, quantitative methane emission data on horses is scarce. The main objective of this study was to quantify methane production in warmblood ponies either receiving a roughage only (R) diet or a diet containing roughage plus concentrate (RC).

Material and methods In order to quantify methane production from warmblood ponies in relation to the energy intake of two different diets, a cross-over design with 2 treatments involving 4 mature warmblood pony geldings (BW 230 ± 10.5 kg; mean ± s.e.) was used. Ponies were fed at maintenance level with 2 iso-energetic diets (on NEm basis), either roughage only (R) (5.05 kg DM/day) or a combination of roughage and concentrate (RC) (2.52 kg DM hay/day plus 1.11 kg DM concentrate/day). For both diets the same grass hay was used (DM 898 g/kg; NEm 4.47 MJ/kg DM) and a commercial grain mix was used in the RC diet (DM 890 g/kg; NEm 9.64 MJ/kg DM). The experiment involved 2 periods of 14 days each; started with 10 days of adaptation, followed by 4 days of housing in large climate controlled respiration chambers (CRCs) (6m × 3m × 2m). Ponies were housed in pairs in CRCs and each chamber was fitted with a grid fence to separate the ponies, but the design was such to allow a pair to interact with each other. Oxygen (O_2) consumption, carbon dioxide (CO_2) production, and methane (CH_4) production were measured. Heat production (HP) was estimated by use of indirect calorimetry. Diet intake and faecal output were measured quantitatively to determine digestibility of the individual animals. Gaseous exchange measurements were averaged per period for each diet (i.e. 2 chambers as experimental units). Diet digestibility was determined for each individual pony (i.e. 4 individual ponies as experimental units). The General Linear Model (GLM) procedure in SAS (version 9.2, SAS Institute, Cary, NC, USA) was used to analyze both gaseous exchange and diet digestibility. The model for gaseous exchange included diet and period as fixed effects. Pony was included in this model for analysis of diet digestibility.

Results Ponies quickly adapted to housing in the respiration chambers, were apparently healthy during the whole experiment and no stereotypical behaviour was observed. Intake of NEm was equal for both rations (22.3 ± 0.07 MJ/day). Organic matter digestibility was higher (P = 0.006) for the RC diet compared with diet R (55.6 and 47.2%, respectively). Methane production (L/pony/day) was higher (P = 0.014) on the R diet (29.8 ± 0.11; mean ± s.e.) compared to the RC diet (23.2 ± 0.11). Methane production expressed in L/kg $BW^{0.75}$/day and as a percentage of DE was decreased for the RC-group by 21% (0.50 vs. 0.39 L/kg $BW^{0.75}$/day) (P = 0.064) and 12% (3.18 vs. 2.79 % of DE) (P = 0.113), respectively. Heat production, oxygen consumption and carbon dioxide production were not affected (P > 0.304) by diet. Diurnal patterns of CH_4 production and HP showed similar patterns for both diets. Methane production increased slightly after feeding and was lower for the RC diet at all timepoints throughout the day. For both diets heat production was higher post feeding than pre feeding and decreased again approximately 3 hours after feeding.

Conclusions Our results show that iso-energetic addition of concentrate to roughage diets reduces methane production in ponies. This confirms our hypothesis that diets containing larger amounts of concentrates with highly available starch sources cause less methane production compared to diets containing mainly roughage, due to more enzymatic digestion pre-caecally and less fermentation in the hindgut.

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Measurement and evaluation of enteric CH_4 emissions and variability in production environments

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Introduction Ruminant methane fluxes (J_{CH4}) are important components of global Greenhouse Gas flux (J_{GHG}) budgets. Understanding the variability of J_{CH4} among animals in production environments (PE) is essential for accurate J_{GHG} inventories and for evaluation of abatement options. However, measurement of J_{CH4} variability in PE is limited. The common methods, respiration chambers and SF_6, have been judged to be sub-optimal for accurate cost-effective monitoring of J_{CH4} in PE, therefore the development of new measurement methodologies is a high priority (Knapp *et al*, 2011). This paper reports J_{CH4} measurements from several PEs world-wide using a new standardized technique called GreenFeed (C-Lock Inc, Rapid City, South Dakota, USA) where J_{CH4} data are summarized, compared to literature values, and to estimate differences in J_{CH4} of individual animals a herd.

Methods and Results Data from six PEs were used including lactating dairy cows, beef cows, and beef heifers. All animals were fed ad-lib diets of pasture or a total mixed ration (TMR). For each PE, J_{CH4} data were collected for 2 week sampling periods when feeding practices were consistent. The herd averaged J_{CH4}, milk production, days in milk, weight (BW), and DMI were calculated along with the between animal coefficient of variance (CV_b, %). Early or late lactation cows were not included to facilitate comparisons across dairy PEs. The averaged within animal J_{CH4} coefficient of variance (CV_w, %) of the three day rolling averaged J_{CH4} was calculated. The herd averaged J_{CH4} by time of day was calculated and the maximum J_{CH4} hour was divided by the minimum J_{CH4} hour (H_dCH_4) to estimate diurnal J_{CH4} variability. A standard F-Test calculator (MedCalc Software bvba, Belgium) and $P < 0.05$ was used to determine significance of CV_b differences between PEs and between CV_b of J_{CH4} to CV_b in BW, milk, and DMI.

Type	Location	Breed, Type, DIM[1]	Diet	N[2]	J_{CH4} (g/d)	W[3] (kg)	Milk (l/d)	DMI (kg/d)	CV_b (%) J_{CH4}	BW	Milk	DMI	CV_w J_{CH4}	H_d CH_4
Milk	US, MI	H[4], L[5], 157	TMR	43	471	613	30	-	13	9	21		9	1.3
Milk	Sweden	SR[6], L, 120	TMR	24	457	630	28	20	10	11	18	12	7	1.2
Milk	NZ[7]	F[8], L, 93	Past[9]	15	356	429	23	-	14	11	12		7	1.5
Milk	US, NH	J_o[10], L, 215	Past	9	307	400	12	-	9	10	12		9	1.6
Beef	WA, US	A[11], Hr[12]	TMR	10	191	373	-	-	11	12	-		8	1.3
Beef	WA, US	A, C[13]	Past	7	305	701	-	-	11	9	-		8	1.2

[1]DIM = Average days in milk, [2]N = Number of animals, [3]W=Weight, [4]H = Holstein, [5]L = Lactating, [6]SR = Swedish Red, [7]NZ = New Zealand, [8]F = Friesian, [9]Past = Pasture, [10]J_o = Jersey organic farm, [11]A = Angus, [12]Hr = Beef Heifer, [13]C = Beef cow

The overall averaged J_{CH4} CV_b (12±s.d. 1.9) was consistent between PEs, and lower than reported by Granger *et al.* (2007) using ad-lib intakes (CV_b=18), but higher than Blaxter and Clapperton (1965) using constant intakes (CV_b =7). Comparing J_{CH4} CV_b across PEs, CV_b was not different between any two PEs ($P > 0.21$). The CV_b of J_{CH4} was not different than CV_b in BW in five of six PEs ($P > 0.38$). CV_b in milk was on average higher than CV_b in J_{CH4} (16 compared to 12) and higher in two of four PEs. In the PE where DMI was measured, the CV_b of J_{CH4} was not different than CV_b of DMI ($P = 0.38$). Overall, CV_b of J_{CH4} was greater than CV_w in all but one PE (CV_w=8.0±s.d. 0.9), indicating that high and low emitting animals may be identifiable. On average, 80% of the animals in a herd produce J_{CH4} values within ± 14% of the herd average, and the remainder were divided between high and low groups where the difference was 28%. It was noted the herd averaged J_{CH4} across PEs was strongly correlated with both milk and BW ($r = 0.94$, $r = 0.97$). Enteric carbon dioxide fluxes (J_{CO2}) were strongly correlated with J_{CH4} ($r = 0.97$, $P = 0.00$) and therefore findings for J_{CO2} are similar.

Conclusions There were not significant differences in J_{CH4} CV_b between PE regardless of animal type or feeding strategy because CV_b was relatively consistent and between 9 and 14 in all PEs studied. The CV_b of J_{CH4} was generally not different than CV_b in BW or DMI (where measured), and sometimes less than CV_b in milk. Individual animal differences in J_{CH4} are likely to be measurable, especially between the lowest and highest J_{CH4} animals in a herd. However, further study is required using longer-term sampling periods, consideration of J_{CH4} covariance with production variables, and to define long term ranking stability.

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Methane emissions of beef cattle in four Brazilian grazing systems

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Introduction The global demand for food will increase in the next decade (FAO, 2011) and Brazil plays an important role in this scenario, especially in animal protein supply. The challenge for future food production systems will be to reconcile the necessary increase in productivity, driven by increased demand, with more efficient production and distribution, reducing waste production while satisfying the growing concern for environmental sustainability. In a context of global economic crisis and food insecurity, the intensification of livestock production in tropical grazing areas should be based on the best use of the potential of pasture growth. The aim of the present study was to evaluate if different levels of intensification of grazing systems can be used as mitigation strategies for enteric methane emissions. These results are from the PECUS Research Network, a multi-institutional project conceived by EMBRAPA with the objective of obtaining the necessary data, using internationally accepted research protocols, to support governmental policies and to contribute to the development of mitigation alternatives for GHG emissions.

Material and methods The study was conducted at the experimental station of the Brazilian Agricultural Research Corporation (EMBRAPA), located in São Carlos, São Paulo state, in the southeast of Brazil. 24 Nellore steers, 12 months old and weighing 265.2±9.1kg in average, were distributed in four representative Brazilian grazing systems, in January 2012: irrigated pasture with high stocking rate (IHS) and dryland pasture with high stocking rate (DHS), covered by *Panicum maximum* since 2002; dryland pasture with moderate stocking rate (DMS) and degraded pasture (DP), covered by *Brachiaria decumbens* since 1996. HIS and DHS systems were composed of 12 paddocks each under rotational grazing with occupation period of 3 days and 33 days grazing intervals. The DMS system had 6 paddocks with occupation period of 6 days and 30 days grazing intervals. DP system was managed under continuous stocking. The plots were limed, corrected with superphosphate to achieve 20mg/dm^3 P and potassium chloride to achieve 4% K in cation exchange capacity. Top-dressing fertilization with nitrogen was applied at the rate of 600 kg N/ha.year in HIS, 400 kg N/ha.year in DHS and 200 kg N/ha.year in DMS. Degraded pasture was not fertilized. The complete experimental period will be from January 2012 to August 2013. Animals will be kept in the same grazing systems from weaning until slaughter and will be evaluated for performance, growth efficiency, carcass and meat quality. During the experimental period emissions of CH_4 and N_2O, as well as the carbon incorporation in soils, will be evaluated once in each season, providing the GHG balance of the four systems. This abstract shows the Spring season methane collection that occurred in October 2012, in the end of the dry season, using the SF_6 tracer technique (Johnson *et al*., 1994). Animals were dosed with permeation tubes with an average load of 1423.3 ± 67.6 mg of SF_6 and average emission rate of 1.20±0.35 mg/d. Each animal received two permeation tubes five days before the start of the collections. Samples were collected every 24 hours for five consecutive days. Gases were analyzed on a Shimadzu GC 2014. Data were analysed using GLM procedure of SAS and averages were compared with Tukey test. Treatment differences were considered significant at $P < 0.05$.

Results Methane emissions, per animal per day, were higher ($P < 0.05$) in the intensive and irrigated system - HIS - but this system also allowed higher ($P < 0.05$) live weight gain (LWG) and stocking rate than the others (Table1). Considering the stocking rates, the HIS system emitted four and a half times more methane than the degraded pasture, but the production of body weight was also seven times greater. Methane emissions per LWG per hectare were not significantly different.

Table 1 Different production systems effects on methane yields

	Production systems					
	HIS	DHS	DMS	DP	s.e.d	P
Methane emission (gCH_4/d)	211.9^a	163.6^{ab}	165.6^{ab}	119.1^b	9.4	0.0033
Live weight (LW; kg)	434^a	410^a	420^a	352^b	7.6	<0.0001
Daily gain (LWG; g/d)	552.5^a	338.0^b	243.8^b	307.4^b	35.3	0.0015
Stocking rate (AU/ha)	3.73^a	1.53^b	0.90^d	1.17^c	0.24	<0.0001
Methane emission (gCH_4/ha)	777.1^a	227.7^b	139.8^b	109.5^b	64.3	<0.0001
Daily gain (gLW/d.ha)	1992.2^a	515.8^b	221.4^b	361.7^b	165.8	<0.0001
Methane emission (gCH_4/gLWG.ha)	0.479^{ab}	0.449^{ab}	0.765^a	0.306^b	0.058	0.0247

Conclusions Considering the end of the dry season, with limitations of sunlight, temperature and rain, improved pastures did not express their full production potential, but the HIS system partially benefitted by irrigation, allowed higher daily gain per hectare. Although there is a higher demand for inputs in the more intensive systems, one hectare of well managed pasture can substitute seven hectares of degraded pasture, producing meat with the same methane emission.

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Effects of monensin, fumaric acid, and glycerin in methane emissions from beef cattle

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Introduction The rate of accumulation of methane in the atmosphere from enteric fermentation in cattle, has an important role in the greenhouse effect and thus in the global warming. Additionally, the emission of methane gas disrupts the energy efficiency of the substrates fermented in the rumen. Understanding the effect of the diet on enteric methane emissions could help to identify strategies to reduce emissions of greenhouse gases. Therefore, the main objective of the present investigation is to determine the effect of including some nutritional additives such as monensin, fumaric acid and glycerol in the diet of cattle fed with *Pennisetum clandestinum*, in order to mitigate the methane production and also measures others parameters by means of the *in vitro* of rumen fermentation technique.

Material and methods We conducted a test crop of rumen microorganisms not renewed (CNRMR) of short duration, using the technique of gas and following the protocol described by Theodorou *et al*. (1994). The ruminal fluid was extracted from 4 cannulated cows the rumen that were consuming *Pennisetum clandestinum*. Incubation was performed control treatment was only fodder (*Pennisetum clandestinum*) and the inclusion of additives were performed as percentage of dry matter consumed. Thus, the treatments 1, 2 and 3 corresponded to monensin in levels 0.2, 0.4 and 0.6% respectively, treatments 4, 5 and 6 corresponded to fumaric acid at levels 5, 10 and 15% respectively and the treatments 7, 8 and 9 corresponded to the powdered glycerin levels on 7, 14 and 21%. The parameters measured during the ruminal fermentation test were gas production (ml), dry matter digestibility (%), ammonium (ppm) and methane (ml). The gas production was measured after 48 hours using a pressure transducer to measure the pressure inside the flask and calculate the volume of gas generated. Subsequently, a graduated syringe was used to take a sample of gas from the fermentation product in each vial and injected into vacutainers (with vacuum) to 10 ml, to determine the concentration of methane (CH4) by gas chromatography. After 48 hours of fermentation, the bottles were opened to sample and measure rumen fluid by ammonium selective ammonia electrode. Finally, the content of each bottle was filtered through crucibles, provided with a porous plate (No. 1) and was calculated digestibility of dry matter through the difference between the incubated and the obtained post-incubation. Statistical analysis of the data was generated using the GLM procedure of SAS ® software (SAS Institute Inc., Cary, NC, USA). A complete block randomized design was performed with four replicates, where each repetition is constituted by a ruminal fluid and nested the levels in the additives. Differences between treatments were declared significant if $p < 0.05$ and Tukey's least significant honest difference was used to compare means.

Results The more affection additive methane production was monensin but it also affected the dry matter digestibility (DMD), the production of gas (Gas) and the availability of ammonium. Glycerin did not affect the emission of methane and the dry matter digestibility and it increased availability of ammonium and gas production, compared to the control. Fumaric acid increased emissions and the production of methane gas, didn't generate any differences in the dry matter digestibility and decreased availability of ammonium, compared to the control.

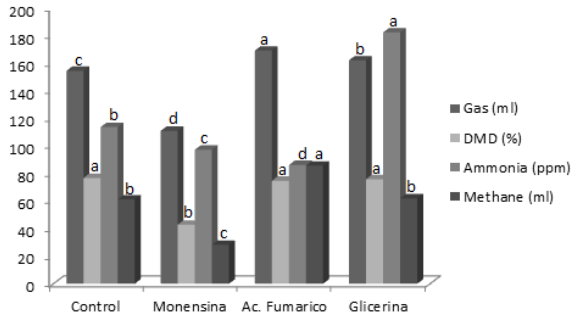

Figure 1 Ruminal fermentation parameters after 48 hours, using monensin, fumaric acid and glycerin based on a grass (*Pennisetum clandestinum*) diet.

Conclusions Monensin is the only evaluated additive that generates a clear decrease in methane production. Fumaric acid generates the highest gas production and it also generates increased production of methane and glycerin does not affect the production of methane, but if gas production increases without affecting the dry matter digestibility. It is necessary to evaluate the parameters of ruminal fermentation in other times, in order to trace if the effect continues thru the time.

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Methane emissions by Holstein and Holstein x Jersey crossbreed lactating cows in two Brazilian grazing systems

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Introduction Global demand for food will increase in the next decade (FAO, 2011) and Brazil plays an important role in this scenario, especially in animal production. The challenge for food production systems in the future will be to reconcile the necessary increase in productivity, driven by increased demand, with the growing concern for environmental sustainability. In a context of global economic crisis and food insecurity, the intensification of dairy production in tropical grassland areas should focus on the efficiency of pasture utilization. The aim of the present study was to evaluate methane emissions from pure Holstein and ½Jersey½Holstein high producing lactating cows in two different grazing systems. This trial was part of PECUS Research Network, a multi-institutional project conceived by EMBRAPA with the objective of obtaining the necessary data, using internationally accepted research protocols, to subsidize governmental policies and contribute to the development of mitigation alternatives for GHG emissions.

Material and methods The study was conducted at EMBRAPA's (Brazilian Agricultural Research Corporation) experimental station, located in São Paulo state, Southeast of Brazil. Treatments were a combination of two factors, two breeds (Holstein - HOL and ½Jersey½Holstein - JH) and two grazing conditions systems: extensively grazed pastures with low stocking rate (ELS) or irrigated pastures under intensive management and high stocking rate (IHS). Pastures in the ELS system were composed mainly of *Brachiaria decumbens* and *Cynodon nlenfuensis* (10.5% CP; 49.8% DMD) while the IHS system was composed of 27 paddocks of *Panicum maximum* cv Tanzania (21.0% CP; 62.8% DMD) overseeded with oat and ryegrass. Forage availability were 22.2 and 37.2 kg DM/AU respectively for ELS and HIS. A total of 24 dairy cows were used (2 breeds x 2 systems x 3 animals per paddock x 2 replicates), grouped according to age, stage of lactation and level of milk production. Cows were kept at pasture and supplemented with minerals and concentrates in accordance with milk yield (1 kg of concentrate/3 kg of milk produced). IHS pasture was rotationally managed and both IHS and ELS were managed under variable stocking rate ("put-and-take"). Forage production and animal performance variables were measured in order to subsidize environmental, technical and economic assessments. Methane emission evaluation took place in August 2012, in the middle of the dry season, using the SF_6 tracer technique (Johnson *et al*., 1994). Each animal received two permeation tubes (average load of 1428.6±50.5 mg of SF_6 and average emission rate of 1.25±0.14 mg/d) five days before collection. Samples were collected every 24 hours for five consecutive days. Gases were analyzed on a Shimadzu GC 2014. Data were analysed using GLM procedure of SAS and averages were compared with Tukey test. Treatment differences were considered significant at $P < 0.05$.

Results No interactions were observed between breed and grazing system. Pure Holstein cows were heavier than ½Jersey½Holstein (P<0.05), but milk production and methane yield per kilogram of milk were similar (Table1). Methane emissions in Litres/day determined in this experiment (614±133L/d) were higher than indicated by Boadi *et al*. (2004) in a review on methane mitigation strategies in dairy cows (552±65L/d) but milk production in this trial was also higher (34.7±5.3 vs. 30.6±8.1L/d). As a consequence methane yield in Litres per kilogram of milk was lower in this experiment for both breeds (average of 17.8±2.9 vs. 19.3±5.3L/kg milk).

Table 1 Different breeds and grazing systems effects on methane yields

	Breed				Grazing system			
	HOL	JH	s.e.d	P	IHS	ELS	s.e.d	P
Methane emission (g/d)	469.4	411.4	19.5	0.1187	476.7	404.1	19.5	0.0550
Methane emission (L/d)	654.7	573.8	27.2	0.1187	664.8	563.6	27.2	0.0550
Cow weight (kg)	570[a]	496[b]	12.9	0.0031	530	536	12.9	0.7979
Milk production (kg/d)	36.0	33.3	1.1	0.2264	35.5	33.9	1.1	0.4797
Methane (L/kg milk)	18.3	17.3	0.6	0.4180	18.75	16.9	0.6	0.1290

Conclusions Despite being lighter than pure Holsteins, crossbreed ½Jersey½Holstein cows can produce the same amount of milk as pure Holsteins, regardless of grazing system. Efficiency of milk production can be a mitigation strategy because less methane is emitted per Litre of milk, but the treatments evaluated in this research in the dry season could not confirm that.

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Greenhouse gas emissions from pen surfaces at Texas High Plains beef feedyards during Fall

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Introduction The US beef industry produces nearly 11.3 billion kg of beef per year, contributing $35 billion to the economy. At the same time, the livestock industry is responsible for 198 Tg of carbon dioxide equivalents (CO_2-eq) annually which is 3.4% of the total national greenhouse gas (GHG) emissions (Stackhouse *et al*., 2011). The Texas Panhandle is the biggest cattle feeding area within the US contributing 42% of the national beef production. Approximately, 5 million tonnes of manure is produced each year in beef cattle feedyards in the region leading to environmental pollution including GHGs (Sweeten *et al*., 2012). Little information exists on the GHG emissions from feedyards and accurate methods are required to estimate GHG emissions from feedyards under High Plains' conditions. The objective of this experiment was to estimate the emission rates (ERs) of methane (CH_4) and nitrous oxide (N_2O) from pen surfaces under the region's typical seasonal conditions.

Materials and methods Fall GHG emission sampling studies were conducted in two typical Texas High Plains open beef feedyards (Feedyard-A and Feedyard-C) between October and December 2012. Three, 1-week (5-day) studies were conducted at Feedyard-C, and one, 1-week study was conducted at Feedyard-A. The manure pack at both feedyards would be considered quite dry with only 275 and 420 mm of precipitation being recorded at Feedyards A and C respectively from 1 January to 31 October with 101 and 135 mm respectively between 1 August and 31 October. Non-flow-through, non-steady-state (NFT-NSS) chambers (Hutchinson and Mosier, 1979) were used to sample GHG emissions from the feedyard pen surfaces. Greenhouse gas sampling measurements for all studies were performed starting at 1200 h central US time. Ten chamber bases were installed in a recently emptied pen in two rows on a Friday afternoon and the GHG measurements were conducted from following Monday to Friday for each study. Air samples from the headspace of each chamber were drawn with a plastic syringe at 0, 10, 20, and 30 minutes after the chamber cap was placed on the steel base. Then the samples were injected into pre-evacuated exetainer® glass vials (a total of 40 vials/day) and the vials were transported to the laboratory for immediate analysis. Analysis of GHG concentrations was conducted on a Varian 450 gas chromatograph (with a CombiPal autosampler) equipped with a flame ionization detector (FID) for CH_4 and an electron capture detector (ECD) for N_2O. A total of 800 samples (600 Feedyard-C and 200 Feedyard-A) were analyzed. Methane and N_2O emission fluxes were determined by fitting a quadratic regression equation to emitted concentrations at 0, 10, 20, and 30 minutes and solving for the slope at time zero. Temperature data were acquired from pen surface using a handheld, infrared thermometer and the manure pack (continuously logged) by installing thermistor-based, temperature sensors at 50 mm depth.

Results Emissions fluxes of CH_4 and N_2O are compiled in Table 1. There was considerable spatial variability in emission rates within each pen evidenced in the results. Nitrous oxide emissions tended to be higher at sampling positions near the feed bunk, the water trough and on the edges of manure mounds. Whereas, CH_4 emission rates were higher from areas where the manure pack was deeper and/or moister including manure mounds and wet patches. The N_2O emission data indicate a large variation between the two feedyards. The variation could result from the different manure management practices at the feedyards with Feedyard-A harvesting manure from the pens twice per year whereas manure removal was performed annually at Feedyard-C.

Table 1 Methane and N_2O fluxes (mg/m^2 per hour) during four study periods at Feedyards A and C

	Feedyard A		Feedyard C					
	5-9 November		21-25 October		26-30 November		10-14 December	
	N_2O Flux	CH_4 Flux	N_2O Flux	CH_4 Flux	N_2O Flux	CH_4 Flux	N_2O Flux	CH_4 Flux
Avg.	14.56	5.3	0.29	9.9	0.18	1.1	0.16	2.7
s.d.	40.59	3.4	0.56	10.1	0.23	1.4	0.39	7.2

Conclusions The CH_4 emission rate from the manure pack at Feedyard- A was 5.3 mg/m^2 per hour while at Feedyard-C emission rates averaged 4.6 mg/m^2 per hour over the three study periods. Nitrous oxide emission rates were much more variable with the average emission rate at Feedyard-A being 14.56 mg/m^2 per hour while it at Feedyard-C, it averaged 0.21 mg/m^2 per hour. Both CH_4 and N_2O emissions were generally temperature dependent and decreased with decrease in manure pack temperature.

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Methane production of goats during feed restriction and refeeding

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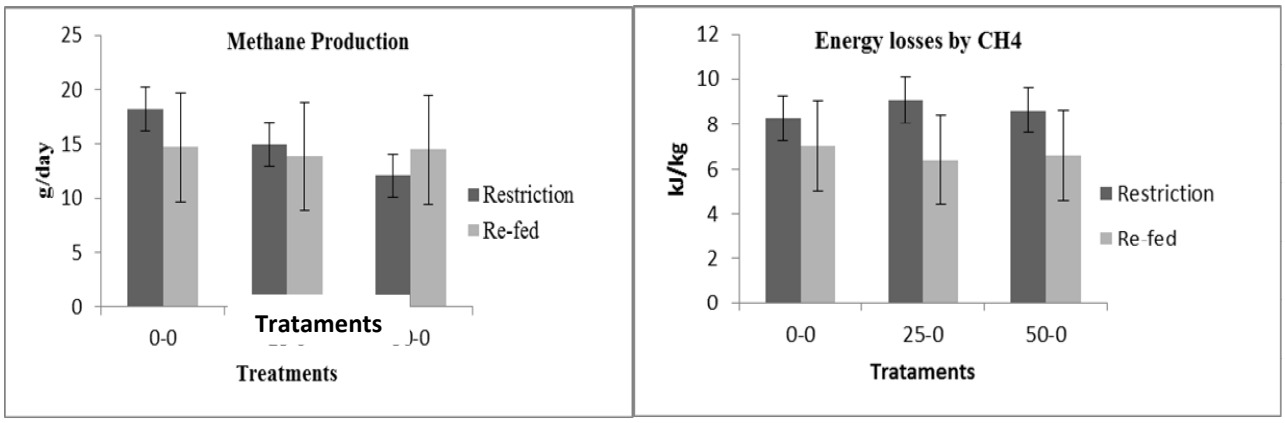
Introduction Goats have the ability to adapt to periods of poor nutrition in both for medium or long term. Microbial fermentation of feed in the rumen results in enteric CH_4 production that represents energetic losses for the animal (Beauchemin and McGinn, 2006), however the amount of feed consumed is assumed to affect emissions levels of CH_4 (Johnson and Johnson 1995). Based on these assumptions, this study aimed to evaluate the effect of feed restriction and refeeding on methane production and energetic efficiency.

Material and methods A total of 15 Nubian goats, non-pregnant and non-lactating, were randomly distributed into five groups (blocks) of three animals. In the first period, each goat within the group was allocated to one of treatments: ad libitum (T0), and intake reduced by 25% (T25) and by 50% (T50) of ad libitum intake. In the second period all animals were fed ad libitum. Goats were housed in individual metabolic cages and the diet was equal for all treatments, consisted of 47% corn hay and 53% concentrate (15 % crude protein, 16.5 MJ/kg GE on fresh weight), calculated according to NRC (2007) to meet their growth requirements During a digestion trial, feed intake, feed refusals, faeces and urine were collected during 6-d after 20-d adaptation period. Aggregated samples were dried and gross energy was determined using a Parr calorimeter. The measurements of methane emission were performed using the sulphur hexafluoride (SF_6) tracer technique and analyzes were conducted at Embrapa Environment (CNPMA). The experimental design was a randomized complete block design using mixed models with fixed effect of treatment and random effect of blocks and error, using the MIXED procedure of SAS (version 9.2).

Results Figure 1 reveals that the animals fed ad libitum had greater CH_4 production (g/day), compared to those subjected to 25% and 50% restriction. When re-fed, goats subjected to 50% feed restriction showed a slight increase in CH_4 production, whereas no differences in CH_4 production were observed for goats subjected to 25% feed restriction. Dry matter intake (DMI) between first and second period of goats fed ad libitum (737.6 ± 98.08 and 691.75 ± 98.08, respectively) and of goat subjected to 25% feed restriction (550.7 ± 98.08 and 748.09 ± 98.08, respectively) did not differ ($P < 0.05$). DMI of goats subjected to 50% feed restriction in the first period (384.9 ± 98.08) was less than that on re-feeding (742.5 ± 98.08).

Figures 1 and 2- CH4 production (g / day) and methane energy losses were expressed as a proportion of gross energy intake in animals submitted to feed restriction, followed by a re-feeding.



Conclusions Goats subjected to a severe restriction and then re-fed showed only a slight increase in amount of CH_4 (g/day), but animals fed moderate restrictions, when re-fed, do not exhibit a significant increase in the CH4 production. The results support the view that under-fed and re-fed goats are more energy efficient and overall may have reduced methane yields as a result.

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Methane and ammonia emissions from a lagoon with fermented cattle slurry determined by Open Path FTIR and a micrometeorological model

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Introduction Slurry from livestock husbandry is often stored for several months prior to field application. Due to their surface characteristics uncovered storage tanks and in particular lagoons pose a source of potentially high emissions. However, due to methodical difficulties there is still only limited data available about these emissions. More and more precise data is required to assess the CO_2 footprint of animal husbandry and for national greenhouse gas inventories.
We determined methane (CH_4) and ammonia (NH_3) emissions from a storage lagoon containing dairy cattle slurry treated by anaerobic digestion in two measurement campaigns during the winter season using optical remote sensing and a micrometeorological dispersion model.

Material and methods CH_4 and NH_3 concentrations were measured by Open Path Fourier Transform InfraRed spectroscopy (OP FTIR) with an optical path length of c. 45 m downwind to a lagoon of c. 1250 m^2 (c. 42 x 30 m). The lagoon was filled with c. 1000 m^3 (first measurement campaign) or c. 3000 m^3 (second campaign) dairy cattle slurry which was treated by anaerobical fermentation for c. 35 days prior to storage. Wind data were collected using a 3D ultrasonic anemometer in the middle of the optical measuring path. All measurements were done in 1.3 m height. Positions of instrumentation and the lagoon were determined by high precision GPS (SAPOS®; < 1.5 cm). Trace gas concentrations were retrieved from collected FTIR spectra using the software MALT (Griffith, 1996) considering actual air pressure and temperature. Trace gas fluxes were estimated by the software WindTrax (Thunder Beach Scientific, Edmonton, Alberta, Canada). This software uses a backward Lagrangian stochastic dispersion model (bLS; Flesch *et al*., 1995) to estimate emission rates from a known source area based on the measured concentrations and wind data by simulating the displacement of 50,000 particles through the measuring path.
To validate this approach, a tracer release experiment was carried out. Nitrous oxide (N_2O) was released across the surface of a lagoon in a regular pattern via a tubing system at constant rates and the recovery rate by the bLS model was determined.

Results Detected CH_4 emission rates from the lagoon were 48 (first campaign) and 34 kg per day (second campaign). NH_3 emission rates were 150 and 170 g per day.
In the N_2O tracer experiment a very good recovery rate higher than 95% was achieved, i.e. the discrepancy between released and recovered N_2O was less than 5%.

Conclusions These results show that high CH_4 emissions can occur during slurry storage in lagoons, which impacts the CO_2 footprint of animal husbandry and connected biogas production. NH_3 emissions were found to be relatively low during the winter season.
In the tracer experiment the very good N_2O recovery rate proved our methodical approach to be viable for this kind of measurements.

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Influence of meteorological variables on methane emission associated to metabolic heat production and latent heat loss in lambs

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Introduction Enteric fermentation is one of the main sources of methane emissions (CH_4). Environmental conditions (air temperature, relative humidity and solar radiation) can influence the emission of CH_4. The study of CH_4 emission associated to the metabolic heat production of lambs can provide valuable information to this scenario. The aim of the present study was to correlate the CH_4 emission of lambs with the metabolic heat production and latent heat loss from respiration in tropical conditions.

Material and methods Corriedale lambs (n=10; five males and five females) were allocated in a 10 x 10 latin square (10 animals in 10 different schedules), and each animal was evaluated through an hour per day. The animals were housed during the experimental period, receiving no direct solar radiation. The measurements were done from 8:00 to 18:00. Air and black globe temperatures, relative humidity, partial vapour pressure and the saturation pressure were continuously monitored by a datalogger (Hobo, Onset). Solar radiation was observed through a pyranometer (Kipp & Zonen). Metabolic heat production (M, W m^{-2}) was measured through indirect calorimetry, using the Calorimetry Set from the Animal Biometeorology Laboratory (Faculty of Agrarian and Veterinarian Sciences, UNESP, Jaboticabal, Brazil). According to Kleiber (1972), metabolic heat production can be obtained by the difference between O_2 and CO_2 concentrations from inspired and expired air, which were measured by the Metabolic System for Large Animals, Sable System) and by the Physiology System (respirometry and metabolic, ADInstruments). These systems were connected to a facial mask adapted for lambs. Latent heat loss (E_R, W m^{-2}) from the respiratory tract was determined as described by Maia *et al*. (2005). The enteric emission of CH_4 (E_{CH4}, L h^{-1}) was monitored by the methane analyzer (Sable Systems). Data was submitted to analysis of variance using the least square method, and the adjusted means were compared by Tukey's test ($p<0.05$). Statistical analysis considered the effects of gender, animal, day of measurement, schedules of evaluation and its interaction.

Results Metabolic heat production differed ($p<0.05$) between 8:00 to 10:00 and the other evaluated schedules, except the last schedule, with the highest mean, and from 12:00 to 14:00 it was observed the lower metabolic heat production, and the lower CH_4 emission. These results show a positive relation among metabolism and enteric CH_4 emission. A correlation between enteric CH_4 emission and production of CO_2 (r=0.845) and consumption of O_2 (r=-0.75) was observed. The emission occurs as peaks through the day, being more frequent in the first schedules of evaluation, lower from 11:00 to 15:00 and raising with a higher intensity after 17:00.

Figure 1 Variation in the enteric methane emission (L h^{-1}) of lambs through one day of evaluation

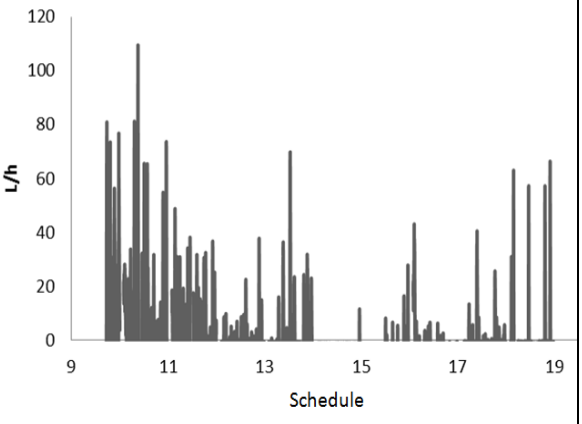

Table 1 Adjusted means to the metabolic heat production (M), emission of enteric methane (E_{CH4}), and latente heat loss in the respiratory tract (E_R) for Corriedale lambs

	M (W m^{-2})	E_{CH4} (L h^{-1})	E_R (W m^{-2})
General means	116.77±6.00	2.61±0.52	27.01±1.38
Schedules			
08:00<hour<=09:00	187.83±5,90^{a}	8.54±0.16^{a}	18.30±2.09^{d}
09:00<hour<=10:00	171.72±5,31^{a}	8.06±0.17^{a}	21.90±2.32^{b}
10:00<hour<=11:00	143.89±5,42^{b}	4.03±0.13^{b}	24.95±1.65^{c}
11:00<hour<=12:00	131.63±5,04^{c}	0.82±0.19^{e}	31.56+2.06^{b}
12:00<hour<=13:00	115.74±7,68^{cd}	2.38±0.17^{c}	21.76±2.13^{c}
13:00<hour<=14:00	108.20±5,90^{d}	0.07±0.16^{g}	33.46 ±2.09^{b}
14:00<hour<=15:00	140.66±5,90^{b}	0.50±0.16^{f}	43.68 ±2.09^{a}
15:00<hour<=16:00	177.60±5,90^{a}	1.66±0.16^{d}	31.77 ±2.09^{a}
16:00<hour<=17:00	123.89±5,90^{c}	0.88±0.16^{e}	17.68 ±2.09^{d}
17:00<hour<=18:00	18 4.36±5,90^{a}	1.59±0.16^{d}	24.81 ±2.09^{c}

Means with same letter in the column do not differ (P>0.05), Tukey's test

Conclusions Enteric methane emission in lambs can be related to metabolic heat production. The higher emissions of CH_4 is observed in the morning, and comparative results with animals exposed to direct solar radiation will be later discussed in future studies.

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Screening of plant secondary metabolites and their interactions on ruminal metabolism

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Introduction Increasing research on natural products that are both safe for the animal and humans, particularly regarding their effect on rumen fermentation and methanogenesis has been reported (Bodas *et al*, 2012). However, scarce information is available on the dose-window of plant secondary metabolites (PSM) in complex microbial ecosystems like the rumen. Further, literature is available on the effects of plant essential oils or mixtures of the latter, but effects cannot be assigned to one particular component. The main objective of this study was to screen pure PSM and their combinations on rumen fermentation and biohydrogenation (BH) *in vitro*.

Materials and Methods Thirty-five pure PSM and their combinations were screened for their effect on rumen fermentation and BH *in vitro*. These PSM have been selected (based on literature data) and/or isolated from mixed essential oil extracts of alpine vegetation in Southern Italy (Falchero *et al*, 2008). The experimental design was set-up using the D-optimality criterion, where 180 different dose combinations of the 35 PSM and 12 control-flasks (no PSM added) were included and equally divided over 3 runs. This design was used in 2 experiments using substrates representative of concentrate rich diets (Experiment 1, with glucose) or of fiber rich diets (Experiment 2, with cellulose). In both experiments, each incubation flask contained a 50:50 mixture of linseed and sunflower oil (20 mg) as an external polyunsaturated fatty acid (FA) source, straw (75 mg) and glucose or cellulose (120 mg) as well as 15 PSM, except for the control-flasks. In Experiment 1, PSM and their combinations were tested at 3 different doses: 1, 10 and 100 mg/L media, whereas in Experiment 2, only 2 doses were tested: 1 and 10 mg/L media as in the first experiment the highest dose (100 mg/L media) resulted in a general rumen metabolism suppression. After 24h *in vitro* incubations at 39°C (Vlaeminck *et al*, 2008), samples for volatile fatty acids (VFA; Van Ranst *et al*, 2010) and long chain FA analysis (Boeckaert *et al*, 2007) were prepared. Results are expressed as estimates of increase or decrease relative to the control.

Results The effect of PSM and combinations thereof on methanogenesis was indirectly assessed based on the changes caused on the A/P ratio, as it reflects both the reduced methane production and the direction of H_2 from methane to propionate (Van Nevel *et al*., 1974). Our criterion to consider a PSM or a combination thereof as an effective methane and/or BH inhibitor required an A/P ratio reduction by 1 unit or more and/or ruminal BH by 10% or more, without decreasing total VFA production by more than 100 µmol, i.e. a reduction of the VFA production of 18% at the maximum. Results of Experiment 1, showed limited effects of PSM or their combinations on the A/P ratio and ruminal BH of both C18:2 n-6 and C18:3 n-3, except for limonene+hexanol, that decreased both the A/P ratio (by more than 1 unit, s.e. 0.076) and ruminal BH (by more than 10%, s.e. 0.035) at the lowest dose (1 mg/L media). Production of total VFA was significantly increased (by more than 200 µmol) in the presence of 14 of the 35 PSM and 10 combinations of PSM, whereas 15 of the 35 PSM and 11 combinations of the latter decreased total VFA production significantly (by more than 120 µmol) when supplemented at the lowest dose (1 mg/L media). Increasing doses carvacrol+farnesol, eugenol+guaiacol and eugenol+cinnamaldehyde decreased A/P ratio and ruminal BH. Production of total VFA was significantly increased (by more than 220 µmol) only in the presence of 4 combinations of PSM, whereas only 3 of the 35PSM caused significantly decreased total VFA production (by more than 260 µmol) at 10 mg/L media. In Experiment 2, the lowest dose (1 mg/L media) resulted in very limited effects on ruminal fermentation and BH. Production of total VFA was significantly increased (by more than 100 µmol) and A/P ratio was decreased (by more than 1 unit) in the presence of allyl mercaptan (s.e. 40.0 & 0.246 for total VFA and A/P ratio, respectively), heptanal (s.e. 30.7 & 0.135) and penta-3-ol (s.e. 19.5 & 0.177) at 10 mg/L media. However, effects of the latter on ruminal BH were minimal. On the other hand, cinnamaldehyde decreased ruminal BH by more than 10% (s.e. 0.023) but increased A/P ratio (s.e. 0.236) and total VFA production (s.e. 21.4). Addition of thymol+farnesol, farnesol+phytol, eugenol+carvone at 10 mg/L media decreased A/P and increased total VFA significantly, without significant effects on ruminal BH.

Conclusions Effects of PSM or their combinations on rumen BH and acetate to propionate ratio seem to be stronger when using substrates representative of concentrate rich diets compared to fiber rich diets.

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Life-cycle environmental benefits derived from immunological castration of pigs as compared to physical castration: from a global perspective to a United States specific model

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Introduction As the world's population continues to grow, global meat consumption will also increase. There is pressure from all sectors of society to produce food more sustainably. The demand for more meat must be met by using fewer resources while simultaneously mitigating the environmental burden. This will mean further intensification and industrialization of livestock production and adoption of technology that improves production efficiencies while also accounting for animal welfare issues. Improvest® is a product approved for use in 63 countries that reduces boar taint and eliminates the need for physical castration. The immunological product works with the pig's immune system. The mode of action is referred to as immunological castration. Boars are able to grow to their full potential with all the inherent advantages of intact males; improved feed conversion, less manure production and carcasses with a greater percentage of lean meat than barrows. Improved production efficiency and resource savings provides significant life cycle environmental benefits.

Materials and methods In the period 2009-2011, a global study was conducted using reliable life cycle burden data collected from modern farms with intensive pig production where pigs were Physically Castrated (PC) and compared to data collected from the same or similar farms in the same countries where pigs were Immunologically Castrated (IC). The study was conducted using Life Cycle Assessment (LCA) ISO compliant guidelines. The LCA boundaries included: 1) Upstream processes, including suppliers of the immunological product manufacturing level; 2) Core processes including gate to gate manufacturing of two doses on the immunological product and 3) Downstream processes, including the use phase of the product, pork production and abattoirs where the final product (pork) is processed. The functional unit here is 1 kg live weight of pig ready for harvest and the unit mass of the final product (1 kg pig carcass after dressing) **[1]**. Data was collected by direct interviews in modern farms and abattoirs in many countries at the global level and an Environmental Product Declaration was published in early 2012 **[2]**. In 2012, after the introduction of the immunological product in the United States (US), the global LCA model was adapted to the USA specific inputs, including cultivation yields for feed production, feed composition including ration specifics such as inclusion level of dried distillers grain solubles, manure management practices and manure composition, all according to the University of Arkansas LCA model **[3]**. This led to a country specific "Americanized" LCA model that released new results which emphasize the immunological products added value and benefits in terms of environmental sustainability.

Results The GWP (Global Warming Potential) contributions for 2 doses of immunological product manufacturing are negligible and represented only 0.01% of the total GWP for one kg of pig live weight. The main contributions to the GWP are related to the production of feed given to pigs and pig manure management. The US LCA model key input was feed conversion and came from results from 8 trials conducted for product approval; the improvement in feed conversion for IC pigs compared to PC pigs was 8.4% which resulted in feed savings of 26 kg/pig. Based on USDA statistics for crop yields during the years 2009 – 2011 (2012 yield data was excluded due to drought conditions), a land savings (devoted to crop production) of 31 m2/pig is realized. Reduction in pig manure, in the absence of direct measurement, was assumed to be directly proportional to the reduction in feed intake. Method of castration results for GWP are shown in Table 1. IC pigs had a 6% lower GWP than PC pigs when comparing live weight results and 8.0% for carcass weight results.

Table 1 Summary of carbon footprint results of PC pigs compared to IC pigs

	PC pigs (kg CO2e)	IC pigs (kg CO2e)
GWP per kg live weight	3.71	3.48
GWP per kg carcass	4.95	4.55

Conclusions Overall, taking into account an average of 124 kg of pig live weight at harvest, the use of the immunological product over the baseline scenario of physical castration results in a reduction of GWP of about 28 kg CO_2 equivalents per pig. If only 33% of the 53.3M male pigs (2011 data) raised annually in the US were immunologically castrated, that is equivalent to approximately 500,000 mt of avoided GHG emissions per year, equivalent to removing emissions of 103,536 passenger vehicles /year or the carbon sequestered by 164,850 hectares of pine forests.

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Farm-based greenhouse gas accounting for livestock production systems in Germany

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Introduction In terms of climate change mitigation, a number of measures are available to dairy farm managers to reduce emissions from their holdings. Since 1990, the number of dairy cows has been decreasing in Germany. A further reduction in the total number of dairy cows is difficult to realize without cuts in the consumption of dairy products and beef. A particularly effective measure to mitigate greenhouse gases is the efficient use of nitrogen through an adequate feeding of dairy cows based on their specific requirements, and an optimized fertilization of crops according to their needs of nitrogen. However, there are no simple solutions to mitigate greenhouse gas emissions from dairy production systems, as a number of interactions have to be considered. The objective of the present study was to describe the C and N mass flow and to calculate GHG emissions of a representative German dairy farm. Sensitivity analyses were performed to assess the environmental sustainability of mitigation measures.

Material and methods A farm-based greenhouse gas accounting tool was used to derive specific emissions from the farm. The calculations were realized. For each production sector, i.e. crop production, dairy farming and biogas production, a separate model was developed in MS Excel 2010®. For each model, the C and N inputs, transformations and outputs are calculated to describe the C and N mass flow and account greenhouse gases. Disaggregated data for machinery, equipment, materials, requirements and consumptions (e.g. energy, fuel, and feed) were taken from the KTBL database to describe production steps and processes in each production sector. In a next step, the models were interfaced with each other. Different mitigation options, alone and in combination, were implemented to assess their effect on total greenhouse gas emissions. Mitigation measures that were considered for the model calculations are summarized in Table 1. Outputs were related to a constant amount of milk of 6.000 kg milk.

Table 1 Mitigation options considered in the calculations

Factor	Standard option	Mitigation option
Milk yield cow^{-1} year^{-1}	6.000 kg	10.000 kg
Manure storage	Open store	Use of slurry in biogas plant with sealed store Power and heat production
Manure application (N management)	Slurry application: - Broadcast on grassland and arable farm land - Incorporation within 4 hours (arable farm land) N surplus (mineral fertilizer): 50 kg/ha	Slurry application: - Injection on grassland - Broadcast with immediate incorporation on arable farm land N surplus (mineral fertilizer): 20 kg/ha

In the context of an increase in the milk yield per cow and year, other interactions than environmental effects have to be considered. These include, among others, the production of beef and the land use. In Germany, nearly 70% of beef originates from dairy production (Weiß and Kohlmüller, 2010). An extension of the system to account for a constant beef output was therefore performed.

Results The highest reduction potential for greenhouse gas emissions per production unit was realized by an increase in milk yield per cow and year (-32%), since emissions from livestock husbandry, manure storage and crop production are reduced. Fewer animals are needed to produce the same milk output and enteric methane emissions are reduced. At the same time, the slurry amount is lower and therefore, emissions from storing and applying slurry are reduced. The use of slurry in a biogas plant reduces CH_4 and N_2O emissions from storing the manure and energy from fossil sources is saved. Total emissions are reduced by 22%. If the N management in crop production is optimized, N losses through NH_3 are reduced. The amount of mineral fertilizer needed decreases, leading to additional reductions in emissions from fertilizer production and N_2O emissions. The overall emission reduction of an optimized N management amounts to 8%. As could be expected, a combination of all mitigation options leads to the highest emission reduction (-53%). An extension of the system to account for a constant beef output partly compensates the mitigation effect of an increased milk yield because additional emissions are produced from suckler cow herds.

Conclusions and outlook Effective manure management along the complete production process and enhanced livestock performance are crucial factors for mitigating GHG emissions from livestock production. Setting up whole farm balances helps identify the most relevant measures for effective mitigation strategies in farming systems. The model will be further extended to account for costs and benefits to evaluate the economic efficiency of relevant mitigation strategies.

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Estimation of carbon footprint of a typical cow-calf operation in Florida

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Introduction Human's influence in the enhanced greenhouse gas effect has been widely acknowledged, and agriculture has an important part in it. Therefore, it is important to analyze and quantify greenhouse gases (GHG) sources in activities such as cow-calf production, which are very common in Florida. GHG emissions from animal production systems can have several sources, including enteric fermentation, animal's waste, fertilizers, lime and fossil fuels. It is crucial to have a better understanding of models currently used to estimate these emissions. The Fourier Amplitude Sensitivity Test (FAST) can be use do perform a sensitivity analysis of models, showing the influence of inputs in the output's variance. The objective of this study was to calculate the carbon footprint of a typical cow-calf operation in Florida, the Buck Island Ranch (BIR) and to make a sensitivity analysis of the IPCC (2006) model for methane (CH_4) emissions from enteric fermentation from beef cattle under two feeding situations: pasture and feedlot.

Material and methods Data from BIR was analyzed using the methodology from the Intergovernmental Panel on Climate Change (IPCC, 2006) along with the Inventory of Greenhouse Gas Emissions and Sinks: 1990 – 2006 to estimate GHG emissions. The model evaluated in this project is the one for estimating CH_4 emissions from enteric fermentation from beef cattle. Three inputs were evaluated: average daily gain (ADG, kg $head^{-1}$ day^{-1}), digestible energy (DE, %) and methane conversion rate (Ym, %); ranges used were 0.06 to 0.70 (Sollenberger *et al*., 1997), 55 to 75 and 5.5 to 7.5 (IPCC, 2006) for grazing conditions and 0.49 to1.14 (Philips *et al*, 2006), 75 to 85 and 2 to 4 (IPCC, 2006) for feedlot conditions, respectively. FAST analysis was performed in the R Data Analysis Software.

Results Results show that Buck Island Ranch produces an average of 11,733.4 tons of carbon dioxide equivalent/year. The larger GHG source is enteric fermentation, responsible for around 59% of total emissions, followed by livestock waste with 23% of emissions. The other 18% of total GHG emitted is related other sources, like fertilizer and lime applications (3.8% and 5.6%, respectively), tractor operations (1.6%) and pasture burning (1.4%). Emissions do not vary much between years because the most important source (enteric fermentation) is influenced mainly by the number of animals and feed quality, which do not vary significantly with time. Variation in the emissions is mostly related to fertilizer and lime applications. Figure 1 displays the results obtained by the sensitivity analysis. For estimations of animals in both feedlots and pasture the main factor affecting methane emissions is the weight gain, showing that animal performance is the most important input influencing results in this model. However, when the simulation is made for animals in feedlots the Ym is also greatly responsible for methane emissions estimated. Different from that, for animals on pasture the Ym has less influence in the simulation's output than DE.

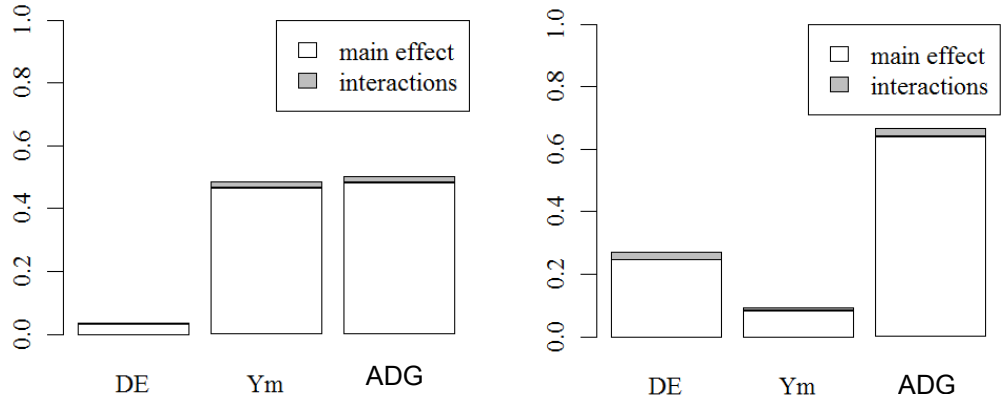

Figure 1 Fourier Amplitude Sensitivity Analysis for animals on feedlot (left) and pasture (right) conditions.

Conclusions Enteric fermentation is the main component of the carbon footprint of a typical cow-calf operation in Florida. The output of the model used for estimating methane emissions from enteric fermentation in cattle is mostly influenced by ADG, a measurement of animal performance.

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Estimations of the potential methane production in cows from smallholder dairy farms located in Central Mexico based on the in vitro analysis of feed ingredients

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Introduction Around 30% of the milk production in Mexico is provided by smallholder dairy farms, which are characterized by relative low rates of production and efficiency due to the use of traditional methods of animal husbandry, which includes inadequate ration formulation and feeding programs associated with the use of low quality ingredients that leads to increments in greenhouse gases emissions, mainly methane, from enteric fermentation. The objective of the study was to evaluate the chemical composition, the volatile fatty acid concentrations and to estimate the methane production in feed ingredients and the impact of using feed ingredients with high and low methane emission potential on diet formulation in smallholder dairy farms located in Central Mexico.

Material and methods A survey of feeding systems, dietary feed ingredient composition and feed allowance per animal per day was carried out in 20 smallholder dairy farms in the state of Queretaro, located in Central Mexico. During the visits, samples of feed ingredients used for diet formulations were taken for laboratory analysis with the aim to know the nutrient adequacy of the diets being fed. The analyzed ingredients were: Alfalfa (n = 17), corn silage, (n = 5), distillers dried grains with solubles (DDGS, n = 5), grass hay (n = 5), sorghum stover (n = 5), corn stover (n = 5), poultry manure (n = 5) and commercial dairy feeds (n = 11). Dry matter (DM, %), in vitro digestibility of DM (DMivD, %), gross energy (GE, kcal/ kg), ADF (%), NDF (%) and acetic (Aca, mmol), propionic (Pra, mmol) and butyric acid (Bua, mmol) were determined. The ratio Aca + Bua / Pra, and methane production were estimated (by stoichiometry of volatile fatty acids, mmol). Subsequently, methane estimates were used to calculate the total methane production by cow in five dairy farms according to the ingredient composition of the diet and the amount ingested of each ingredient per day, using the minimum and maximum methane estimations within each feed ingredient. Then, low and high methane producing diets were compared among farms. Data of chemical composition and total methane production/kg DM intake/cow/day were subjected to analysis of variance using the GLM procedures of SAS.

Results The DMivD was lowest for grass hay and sorghum stover and highest for concentrated feeds and DDGS ($P < 0.01$). The GE content was lowest in sorghum stover and highest in DDGS ($P < 0.01$). The ADF was lowest in grass hay and corn stover and highest for DDGS and concentrated feeds ($P < 0.01$). The minimum quadratic means and minimum and maximum values of Aca, Pra, Bua, Aca+Bua/Pra and methane are shown in Table 1. The Aca concentration was lowest in corn silage and DDGS, intermediate for concentrated feeds and higher for the rest of the feed ingredients ($P < 0.01$). The Pra was lower in all ingredients except for corn silage ($P < 0.01$). Bua was lowest in alfalfa and DDGS and highest in corn silage ($P < 0.01$). The ratio Aca + Bua / Pra was lower for corn silage, corn stover, poultry manure and concentrated feeds and higher for the rest of the ingredients ($P < 0.05$). Methane production was lower for corn silage than for the rest of the ingredients ($P < 0.01$). Estimates of methane production based on the use of feed ingredients with low and high potential of methane production (29.02 vs 33.65, mmol/ kg of DM intake/cow /d) indicate that the difference in emissions was 16% higher ($P < 0.01$) when ingredients with high potential of methane production were used.

Conclusions

The *in vitro* test and estimations of methane production of feed ingredients indicate that the combination of concentrated feeds and corn silage had the lower potential of methane emissions, but, it should be kept in mind that: 1) there was a large variation within each feed ingredient regarding the nutritional quality and methane production, such that factors associated to low methane production of the ingredient should be identified, and 2) the results are based on *in vitro* analyses, which should be verify in *in vivo* animal trials.

Table 1 Means, minimum and maximum values of Aca, Pra, Bua, Aca+Bua/Pra and methane in feed ingredients.

	Aca, mmol		Para, mmol		Bua, mmol		Aca+Bua/Pra		Methane, mmol	
	Mean	Min-max	Mean	Min-max	Mean	Min-max	Mean	Min-max	Mean	Min-max
Alfalfa	77.7^{ab}	70.2-84.2	16.5^{a}	11.9-22.2	5.8^{a}	4.4-9.1	5.3^{a}	3.5-7.4	32.7^{a}	28.7-36.2
Corn silage	58.3^{d}	29.8-83.6	36.5^{b}	12.2-67.1	5.2^{a}	3.2-9.8	3.5^{b}	3.9-7.2	18.3^{b}	29.8-35.9
DDGS	62.3^{cd}	43.4-74.5	16.6^{a}	13.2-22.2	21.1^{b}	8.9-43.4	5.3^{a}	3.5-6.6	31.9^{a}	28.5-34.0
Grass hay	76.7^{b}	73.4-83.5	16.9^{a}	12.4-19.6	6.4^{ac}	4.0-7.6	5.1^{a}	4.1-7.0	32.4^{a}	30.4-35.8
Sorghum stover	75.9^{b}	73.1-83.7	16.1^{a}	11.8-17.9	8.0^{ac}	4.5-9.2	5.4^{a}	4.6-7.5	32.9^{a}	31.6-36.3
Corn stover	71.9^{b}	64.3-77.8	18.9^{a}	14.1-24.7	9.2^{ac}	4.3-15.3	4.5^{ab}	3.0-6.1	30.8^{a}	26.5-34.4
Poultry manure	74.0^{b}	68.9-77.5	18.6^{a}	16.6-20.4	7.4^{ac}	5.9-10.7	4.4^{ab}	3.9-5.0	31.2^{a}	29.7-32.7
Conc. feeds	66.8^{bc}	54.6-71.2	21.5^{a}	19.4-25.6	11.7^{c}	7.6-19.8	3.7^{b}	2.9-4.2	28.8^{a}	25.5-30.5

a–d Means in the same column with different superscript letters differ ($P < 0.01$).

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In-vivo effects of natural additives on ruminant methanogenesis, a meta-analysis approach

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Introduction Mitigation of methane (CH4) represents not only an environmental interest for the planet but also a nutritional interest for ruminants (Martin *et al*., 2010). The use of feed additives was developed to improve ruminant performances and they are also tested to reduce CH4 emission. A review (Benchaar *et al*. 2011) described the potential use of feed additives as CH4 mitigation strategies in ruminants The aim of this study was to quantitatively evaluate the effect of natural additives containing tannins, saponins and essential oils on in vivo methanogenesis. The objectives were: 1 / quantify the impact of natural additives on CH4 emissions in ruminants using published data, and for those additives reducing CH4, 2 / evaluate their impact on animal performances.

Material and methods A meta-analysis statistical approach (Sauvant *et al*., 2008) was used to compare the effect on methane emissions of different additives (tannins, saponins and essential oils) supplemented to ruminants. A quantitative review was performed on available published data (Web of Science, CAB ...) that reported both criteria, on the same animal, of dry matter intake, CH4 emissions, digestibility parameters, feed chemical composition and additives content (secondary metabolites of plant extracts) in the diet. For this purpose, a database was compiled from studies from literature. The main factors tested in statistical analyses (Proc GLM, Minitab 2007) were crude protein content (CP) of the diet, OM total tract digestibility, level of intake (DMI), animal species, secondary compound's content of the diet and study effect.

Results: The database contained 32 publications and 108 treatments on the effect of natural additives on CH4, where 19, 12 and 6 publications tested the effect of tannins, saponins and essential oil, respectively. Additives 'dose and source were variable, they were included in forage, concentrate or given as feed supplements and fed to different animal species (cow, sheep and goat). Means (± s.d.) of some variables in our database are presented for each additive and ruminant species (table 1). The effect of additive family and animal species were included in the statistical analysis but failed to be significant, in our study. The secondary metabolites plant content was a significant factor explaining CH4 variation in our database. Furthermore, CP content of the diet was also a significant factor, decreasing root mean standard error (RMSE) and enhancing the relationship between CH4 and secondary metabolites plant content (equation 1).

- **CH_4** (g/kg DMI) = 34.4(±3.72) – 0.07 (0.011) **Secondary metabolites** (g/kg DMI) –0.1 (0.02) **CP**(g/kg DMI)
- n= 85 treatments, n=32 trials, RMSE=1.6 g/kg DMI, R^2 adjust.=87.7% $P<0.05$ **(equation 1)**

Our meta-design, which was not well balanced, could not allow us to compare the 3 additives within the same study. Only 12 publications have tested the effect of plant extracts on CH4 and animal performances (50% on milk production and 50% on meat production). This may explain why we did not observe any relationship between CH4 decrease caused by additive supplementation and animal performances.

Table 1 CP, secondary metabolites, DM intake, OM digestibility and CH4, of the database (mean ± (s.d.)).

	Saponin			Tannin			Essential oil		
	cow	sheep	goat	cow	sheep	goat	cow	sheep	goat
CP(g/kg DMI)	161 (13.2)	133.3 (24.1)	-	186 (45.7)	164 (69.7)	126 (14.5)	151 (13.4)	149.5 (44.4)	-
DMI (kg/BW0.75)	139 (47.3)	66.6 (13.2)	-	109 (41.9)	63.3 (17.9)	52.5 (7.9)	119.1 (27.8)	58.6 (23.16)	-
Secondary metabolites (g/kg DM)	5.5 (4.93)	5.0 (9.90)	-	11.9 (12.27)	9.73 (17.15)	81 (74.5)	6.5 (7.99)	0.6 (0.92)	-
OM total tract digestibility (%)	-	65.1 (7.9)	-	79.3 (2.91)	64.9 (7.57)	60.6 (8.02)	-	67.5 (5.68)	-
CH4 (g/kg DM)	19.0 (2.88)	18.2 (3.16)	-	19.4 (5.55)	17.8 (4.33)	16.6 (5.97)	21.4 (6.02)	19.6 (6.16)	-

Conclusions These results show that secondary metabolites supplementation decreased CH4 and this may be explained by antimicrobial properties of the additives (Doreau *et al*. 2011). An additional decrease was observed when there was an increase of CP content in the diet, after additive supplementation. However, in our study, there was no significant relationship between CH4 decrease and meat or milk production.

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Effects of feeding a natural biopolymer (chitosan) on methane emissions in beef cattle

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Introduction Greenhouse gas (GHG) emissions by the U.S. livestock sector reached 211.7 million metric tons of CO_2-equivalents in 2010, which is equal to almost half of the total GHG emissions of Spain in a year. From a production standpoint, methane production can account for 5 to 12% of dietary energy losses in beef cattle. As a result, recent research efforts are concentrating on strategies to mitigate GHG emissions in beef production systems. Recent in vitro experiments indicate that the natural biopolymer chitosan could modify ruminal fermentation toward energetically more efficient routes including a decrease in methane production (Goiri *et al*., 2010). The objective of this study was to evaluate the potential of the natural biopolymer chitosan as a feed additive to mitigate in vivo methane emissions in beef cattle.

Materials and methods Twenty four crossbred heifers (BW = 252 ± 24 Kg) were used in a randomized block design replicated in two periods. Heifers were stratified by weight and randomly assigned to 12 pens (2 heifers/pen). Pens were then randomly assigned to one of six treatments in a 2 × 3 factorial arrangement. Factors included diet [high concentrate (HC) or low concentrate (LC), including 85% or 36% concentrate on a DM basis, respectively] and either 0.0, 0.5 or 1% of chitosan dietary inclusion on a DM basis. Chitosan (90% deacetylation degree) was mixed with the diet using a premix included at 2% of the diet DM. Heifers were housed in the University of Florida-Feed Efficiency Facility in Marianna, FL, USA. Diets were offered ad libitum and intake was recorded by a GrowSafe system (GrowSafe Systems, Airdrie, Alberta, Canada). Heifers were adapted to their dietary treatments for at least 14 d before measuring CH_4 emissions using the SF_6 tracer technique (Johnson *et al*., 1994) for 5 consecutive d in each sampling period. Data were analyzed using the MIXED procedure of SAS (SAS Institute, Cary, NC) using heifer as the experimental unit and including the fixed effects of diet and chitosan inclusion level, and the random effect of period. Orthogonal polynomial contrasts were conducted to determine linear and quadratic effects of chitosan inclusion level.

Results No effects ($P > 0.10$) of chitosan or chitosan × diet interaction were found for methane emissions or dry matter intake. A diet effect ($P < 0.01$) was found for methane emissions expressed as g/d or relative to metabolic body weight or feed intake. Heifers consuming a LC diet produced 130 g/d of methane vs. 45 g/d in the HC diet. When adjusted for intake, heifers produced 7.1 and 18.2 g of methane/kg of dry matter consumed in HC, and LC, respectively.

Table 1 Effects of feeding increasing levels of chitosan on methane emissions in growing beef heifers

	High concentrate diet			Low concentrate diet						
	Chitosan inclusion, % of diet DM			Chitosan inclusion, % of diet DM				P [1]		
Item	0	0.5	1.0	0	0.5	1.0	s.e.m.[2]	DIET	CHIT	CHIT × DIET
DMI[3], kg/d	8.6	8.1	9.4	8.5	8.9	8.1	0.57	0.63	0.91	0.22
CH_4 emissions, g/d	51	42	41	121	115	155	54.8	<0.001	0.71	0.61
CH_4 emissions, g/kg DMI	8.4	6.5	6.5	18.0	15.3	21.4	4.53	0.005	0.78	0.77
CH_4 emissions, g/kg MBW[4]	0.61	0.52	0.52	1.50	1.35	1.83	0.355	<0.001	0.79	0.75

[1]Observed significance levels for the main effects of: DIET = diet (n = 24 heifers/mean), CHIT = chitosan inclusion level (n = 16 heifers/treatment), CHIT x DIET = interaction between chitosan inclusion level and diet.
[2]Standard error of the mean, n = 8 heifers/treatment.
[3]DMI = Dry matter intake average during the methane collection period.
[4]MBW = Metabolic body weight.

Conclusions The addition of up to 1 % of chitosan (DM basis) did not affect methane emissions in growing heifers consuming high- or low-concentrate diets. Heifers consuming a diet comprised of 36% concentrate produced 2.9 times more methane per day than those fed an 85% concentrate diet. However, when expressed as methane produced per kg of dry matter consumed, heifers consuming a 36% concentrate diet produced 2.6 times more methane than those consuming an 85% concentrate diet.

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Environmental impacts of feed additives used in dairy production systems using LCA approach

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Introduction The reduction of enteric CH4 in ruminant production represents both an environmental and a nutritional interest. The use of feed additives is an alternative that is being explored for reducing CH4 emissions from ruminants (Martin *et al*. 2010). Production and use of feed additives, however, generate also GHG emissions and consumes resources that should be accounted for when assessing the environmental impact of their application. Through the FP7 project SMEthane, we had access to enteric CH4 emission measurement from *in vivo* experiments and to information on the industrial production processes of two plant additives. The objective of this work was to give a more holistic vision of the results obtained on animals in SMEthane trials by assessing environmental impacts at the whole farm scale.

Material and methods Two reference systems, one with 11% of silage maize in the forage area (FA, T10%) and one with 33% of silage maize in the FA (T30%) were based on the work of Nguyen (2012). Eight virtual farms with the same usable agricultural area (55 ha) and the same total milk production (250 000 L of fat-and-protein-corrected milk) were then simulated. For additive 1 (Ad1) that was only given to cull cows, we created four sub-systems, two within each reference system in which the additive in one case decreased CH4/kg DMI by 20.4% (Ad1 case 1) and in the second case it decreased CH4 to the same degree and increased feed intake by 15 % (Ad1 case 2). For additive 2 (Ad2) that was given to producing and cull dairy cows, the simulation was made on the two reference systems. The additive 2 increased by 4.8 % CH4 emissions by kg DMI (Ad2). For both additives, CH4 emission and intake data were based on SMEthane trials. The environmental impacts (climate change, eutrophication, total cumulative energy demand, acidification and land occupation) of the studied systems were calculated by the life cycle assessment (LCA) method (ISO 2006).

Results Climate change impact (Fig. 1) as well as other environmental impacts of the systems supplemented with additives decreased less than 1% as compared to the reference systems. The effect of Ad 1 on the environmental impacts of the farm was very low. This can be explained by the fact that Ad 1 was only given to cull cows, which represented one third of the herd, and only during 2 months of fattening. Ad 2 increased climate change impact by up to 2.5% and other environmental impacts were also negatively affected. The effect of Ad 2 on the environmental impacts of the T10% system was lower than those of the T30%. In contrast, additives intrinsically contributed to less than 0.1% for most environmental impacts studied, except for the energy demand of Ad 2. The contribution of Ad 2 that was 7.9 and 11.9% for T10% and T30%, respectively.

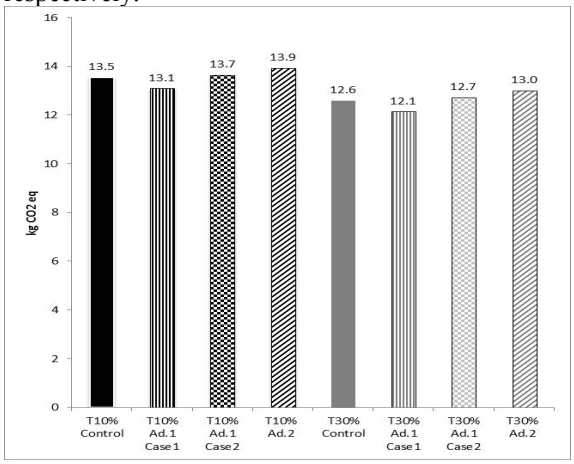

Figure1 Climate Change impact of two plant additives (Ad 1 and 2) for 1 kg of carcass weight of cull cow in eight virtual farms

Conclusions The additives tested in this work were either supplied to a small numbers of cows in the herd or they were marginally effective in reducing enteric methane emissions. Consequently, their supplementation did not affect farms' environmental impacts. The results also showed a weak environmental cost to produce additives, meaning that they have to be effective at reducing enteric methane and if they should be given to the majority of animals in order to reduce the environmental impact of the farm. Further work is needed to assess additives effects in beef production system.

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Variation in methane emission from dairy cows and beef heifers measured in open-circuit chambers with an infrared laser spectrometer

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Introduction Methane emitted in ruminant husbandry accounts for about one quarter of all anthropogenic CH_4 emissions. This makes CH_4 from ruminants a target for abatement measures, which have been rather unsuccessful so far (Beauchemin *et al.*, 2008). The controlled conditions in open-circuit chambers provide an ideal platform for testing mitigation strategies which show lasting effects. During four trial periods the past 18 months, we have collected methane emission data of 16 dairy cows (Holstein Friesian) who were fed typical Flemish rations and 18 beef heifers (Belgian Blue) all fed with the same finishing diet. The aim of the present work was to analyse the between animal variation in methane emission.

Material and methods The monitoring system with six individual open-circuit chambers was designed to accurately measure methane, carbon dioxide, nitrous oxide and ammonia emissions, and to allow feeding and milking, and collect faeces and urine separately (Peiren and De Campeneere, 2013). The system was successfully used for the long term trials in the SMEthane project (Yanez-Ruiz *et al.*, 2013). Each trial started with an adaptation period of one month for adaptation to the standard ration. This was followed by a control period of two weeks with a restricted feed intake at 95% of *ad libitum* feed intake to avoid leftovers. At the end of the second week cows entered the chambers to measure the methane production individually from Tuesday morning till Friday morning. The gas concentrations were measured with an infrared laser optical-feedback cavity-enhanced absorption spectrometer (OFCEAS). The air from all chambers (at each of the six outlets) and the ambient air (two, near the air inlets) were continuously sampled and a gas switching device delivered a sample stream to the gas analyser at intervals of 180 seconds. The last 60 seconds were used to calculate the emissions. The chambers operated at an airflow between 300 and 500 m^3 /h. The dairy cows (n = 16) were fed and milked twice daily, with concurrent removal of faeces and urine. Three rations were used which differed in the proportions of maize silage, grass silage, pressed beet pulp, concentrate, rapeseed and soybean meal. The beef heifers were fed in the morning, with concurrent removal of faeces and urine. Their ration was a finishing diet with maize silage and concentrate (50:50 on DM). During the trial we recorded other parameters such as feed intake, milk production, milk composition, body weight, etc. Statistical analysis was performed with SAS.

Results Although the measurement of the other gases are necessary for durable long-term strategies only the methane emissions of the control measurements are presented here (Table 1). There was no significant difference between the dairy cow rations ($P = 0.58$) . The correlation ($P < 0.01$) between the mean methane emission and mean DMI was $r = 0.75$. No significant correlation was found between methane emission and milk production or body weight. The methane emission between animals with the same ration and DMI was significantly different ($P < 0.05$), with coefficients of variations of more than 10%. Variations between three consecutive days for the same animal ranged from 1 to 20%. Because of these large animal variations, a sufficient number of animals per treatment should be used. The within-day variation indicated that most methane was emitted in the first two hours after feeding, while minimal concentrations were measured before feeding. The correlation between the mean methane emission and mean DMI for beef heifers was $r = 0.71$. The variation of methane emissions between the heifers with the same ration and DMI ranged from 12 to 16%. Variations between three consecutive days in the same animal ranged from 1 to 23%.

Table 1 Cow performance and methane emissions (mean, standard deviation, minimum, maximum and coefficient of variance)

	Dairy cows (n = 16)					Beef heifers (n = 18)				
	Mean	s.d.	min.	max.	c.v.	Mean	s.d.	min.	max.	c.v.
Dry matter intake (kg/d)	17.4	2.5	14.8	21.4	13.5	8.5	0.6	6.8	9.3	6.7
Body weight (kg)	620	65	508	700	8.9	538	86	412	696	16.0
Milk production (kg/d)	24.0	4.3	19.1	34.9	18.0	-	-	-	-	-
Methane emission (g/d)	301	43	206	382	14.2	144	26	95	188	18.0

Conclusion Our system is adequate to monitor methane emissions. Because methane emissions showed a large between-animal variation, we recommend including sufficient animals per treatment.

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Exploring relationships among selected animal performance measurements, methane emission, and ammonia emission

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Introduction Enteric methane (CH_4) emission and CH_4 emission from manure of ruminant livestock are major contributors to anthropogenic greenhouse gases (GHG) emission in many countries. Similarly, livestock manure is an important source of undesirable atmospheric ammonia (NH_3). Identifying and quantifying relationships between animal performance and emission of CH_4 and NH_3 are critical first steps in the development of emission models that may be used to evaluate GHG mitigation strategies. The modelling effort of Dijkstra *et al* (2011) suggested that mitigation options aimed at reducing urinary nitrogen excretion (the main source of manure NH_3) may result in elevated CH_4 emission levels, depending on neutral detergent fiber (NDF) level. Thus, our objective was to summarize a series of trials conducted in our laboratory to explore responses associated to CH_4 and manure NH_3 emission from high producing lactating dairy cows fed typical diets of the Midwest of the United States.

Material and methods Data was obtained from three experiments (Arndt *et al*. 2010; Aguerre *et al*. 2011ab) comprising 12 dietary treatment means (4 replicates per treatment) with data of diet composition, animal performance and CH_4 and NH_3 emission. Animal performance and gas emission measurements were conducted in four air-flow controlled chambers, each constructed to house four cows in a modified tie-stall barn. Chamber was the experimental unit, but for convenience of data interpretation, all results were expressed on a per-day and per-cow basis. Air samples entering and exiting each chamber were analyzed for CH_4 and NH_3 concentration with a photo-acoustic gas monitor (Innova Model 1412). Forage to concentrate ratio of dietary treatments, fed as total mixed rations, ranged from 47:53 to 68:32 (DM basis). Alfalfa silage to corn silage (the only forages fed) ratio ranged from 20:80 to 80:20 (DM basis). Values for dietary chemical composition ranged from 157 to 181 g/kg for CP, 271 to 383 g/kg for NDF and 200 to 290 g/kg for starch. Average DMI ranged from 20.0 to 29.5 kg/day, energy correct milk yield (ECM) ranged from 36.1 to 45.6 kg/day, ECM/DMI ranged from 1.53 to 1.82, intake N ranged from 498 to 757 g/day, CH_4 emission ranged from 538 to 764 g/day, and NH_3 emission ranged from 13 to 57 g/d. Pearson correlation coefficients were used to determine associations between CH_4 emission (g/day), DMI (kg/day), ECM (kg/day), NDF intake (NDFI, kg/day), and ECM/DMI, and the association between NH_3 emission (g/day), intake N (g/day), milk N (g/day), milk N/ intake N, milk urea-N (MUN; mg/dl) and CH_4 emission . The PROC REG procedure of SAS (Statistical Analysis Systems Institute, 2001) was used to assess the linear or quadratic relationships among the following variables: CH_4 emission, DMI, NDFI, and ECM/DMI and between NH_3 emission, intake N, and milk N/ intake N.

Results Table 1 summarizes correlations among measured responses and CH_4 emission of the 12 dietary treatments. Methane emission was most highly correlated with ECM/DMI (r = -0.78), but was also significantly correlated with DMI and NDFI. Furthermore, the relationship between ECM/DMI and CH_4 emission was quadratic ($y=-5907x^2 + 19386x +15153$, $R^2 = 0.87$) with a maximum emission at 1.64 suggesting a reduction in DM digestibility at higher efficiencies due to faster feed passage rate. A quadratic relationship was also observed between DMI, NDFI and CH_4 emission ($R^2 = 0.75$ and 0.66, respectively). Ammonia emission was highly correlated with milk N/ intake N (r = -0.85, P<0.01), but was also correlated with N intake (r = 0.80, P<0.01). Interestingly, MUN was weakly correlated with intake N (r = 0.13) and NH_3 emission (r = 0.39). The relationship between NH_3 emission and intake N was linear (y = 0.14x-57.90, R^2=0.64), but the relationship between NH_3 emission and milk N/ intake N was quadratic ($y = -0.92x^2 + 45.53x - 506.95$, R^2=0.80). Also, there was a correlation (r=0.68) between CH_4 and NH_3 emission.

Table 1 Correlation coefficients among CH_4 and measured responses.

Item	CH_4	DMI	NDFI	ECM	ECM/DMI
CH_4	1.00				
DMI	0.70*	1.00			
NDFI	0.68*	0.90**	1.00		
ECM	0.66*	0.98**	-0.83**	1.00	
ECM/DMI	-0.78**	0.97**	-0.90**	-0.90**	1.00

* P < 0.05 significance level for testing H_0: r = 0.

Conclusions Results from this study corroborate previous findings of positive relationships between CH_4 emission, DMI, NDFI and ECM. Results also suggest that animal selection based on feed and N utilization efficiencies may be important tools for mitigating CH_4 and NH_3 emissions. This study captured only a few of the likely important animal factors that control CH_4 and NH_3 emission. Although results should be confirmed, our analyses suggest that dietary strategies to abate either CH_4 or NH_3 will also reduce the other. Future research should address the impact of rumen environment and manure chemical composition on CH_4 and NH_3 emission.

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An examination of the ranking of methane emissions measurements from growing beef heifers fed different forage diets over time

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Introduction It has been proposed that one method by which enteric methane (CH_4) emissions may be reduced is through genetic selection of animals. A limitation to examination of this option has been the lack of ability to measure CH_4 emissions from a large number of animals over a long period of time and across varying diets. The objective of this work was to examine emissions from a population of heifers across a production year and different diets as they increase in size and dry matter intake.

Materials and methods Methane emissions were measured from six mature cows using the sulfur hexafluoride (SF_6) technique (Johnson *et al.*, 1994) concurrently with the GreenFeed™ (GF) system (Nelson *et al*., 2012; C-Lock, Inc., Rapid City, SD). The gas chromatograph used for sulfur hexafluoride (SF_6) technique was calibrated daily with standard CH_4 and SF_6 gases and daily CH_4 emissions were calculated. GreenFeed sensors were calibrated at regular intervals with zero and span gases. Raw outputs for gas concentrations, air flow, and climate conditions from GF were converted with known factors. From these data, an algorithm was constructed using the lowest CH_4 concentration in the rolling 30-min time period surrounding any single 1-sec data point subtracted from that point to account for background CH_4 level. Average daily emissions were then tabulated for each animal when raw outputs from the carbon dioxide and head proximity sensors were 150% and 200% above the median raw output for each over an entire day, respectively. Any visit that was less than 1 min in duration was omitted from analysis.
The algorithm described above was then used to determine CH_4 emissions from 18 growing beef heifers fed different diets during periods in April, July, and September (Table 1). Methane emissions data were blocked by period and analysed as a completely randomized block with repeated measures design (SAS 9.2). Animals were also ranked by average period emissions as low to high emitters. These data were analysed using a Friedman t-test to examine whether the rankings were consistent across periods.

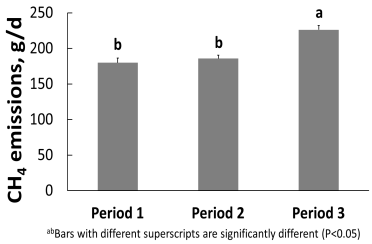
Results Methane emissions were similar in Periods 1 and 2 and lower ($P<0.05$) than emissions in Period 3 (Figure 1) when heifers were grazing an irrigated alfalfa/grass pasture. CH_4 emissions within Periods 1 & 2 varied significantly ($P<0.05$) from day-to-day and most likely reflected variation in daily intake by the animal (Figure 2). Daily emissions in Period 3 were less variable and reflect more consistent intake of high quality, plentiful pasture. Friedman's test determined that there was not a significant change ($P=0.8778$) in rank when heifers were consuming the different diets.

Table 1 Diets fed to heifers in periods 1, 2, and 3

Period	Month	Diet	Age (mo)
1	April 2012	70% alfalfa/grass hay 30% bluegrass straw	12
2	July 2012	Pasture – native grasses	15
3	September 2012	Pasture – irrigated alfalfa/grass	17

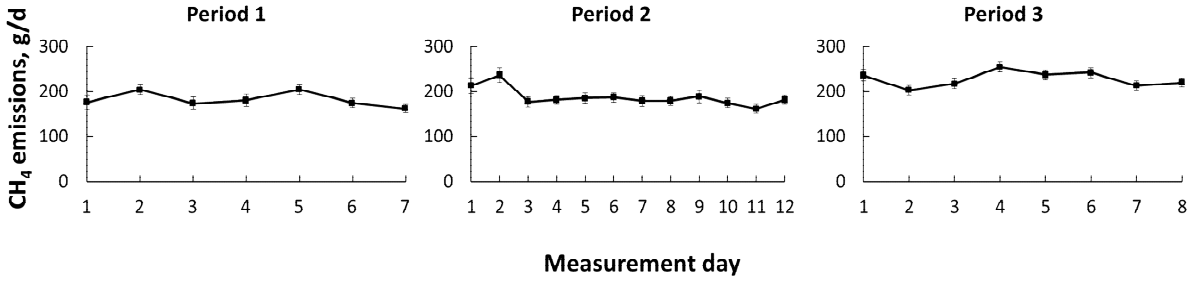
[ab]LSMeans with different superscripts are significantly different ($P<0.05$)

Figure 1 Average daily CH_4



[ab]Bars with different superscripts are significantly different (P<0.05)

Figure 2 An example of average daily CH_4 emissions for each period (LSMeans ± s.e.)



Conclusions These data indicate that the ranking of animals for CH_4 emissions remains relatively constant across time and different forage types. Additional measurements should be made to determine how consistent the ranking on forage diets relates to diets containing higher levels of concentrate.

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Enteric methane emissions from sheep on pearl millet pastures fertilized with different nitrogen doses

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Introduction The emission of greenhouse gases (GHG) from livestock is one of the main concerns in a global scale, because of the increasing world demand for food and mitigation of GHG emissions, simultaneously. Studies conducted in Europe concluded the use of nitrogen in pastures increases stocking rate and the soil emissions of N_2O (nitrous oxide) and animal CH_4 emissions per unit area as well. However, those emissions were compensated by the great atmospheric carbon influx into the soil, meaning these grazing lands can potentially mitigate the greenhouse effect (Soussana *et al*., 2007). Notwithstanding, this potential is very dependent on grazing management, and data on enteric methane emissions from animals grazing tropical pastures are very scarce. In this context, the hypothesis tested was that nitrogen fertilization causes less CH_4 emissions per unit animal production providing higher levels of herbage allowance are available..

Material and methods The experimental design was a randomized completely block, with three replicates. Experimental units were constituted of 12 paddocks, area varying between 800 m² and 1200 m² according to N doses. Pasture used was pearl millet (*Penissetum americanum* (L.) Leeke) sown in a no-tillage system. The experimental period was 70 days of pasture use, from February to April 2011. Three tester animals were used in each experimental unit, summing 36 Texel ram lambs ageing 5 months. Animals were grouped according to weight as blocking criteria. Average live weight was: 20 ± 1.6 kg. Treatments were nitrogen levels (50; 100; 200 and 400 kg N/ha) applied in one dose using urea. The grazing method was continuous stocking with variable stocking rate (put-and-take), so pasture structure was intended to be similar (30 cm sward height) among treatments. The organic matter intake (OMI) was estimated by fecal nitrogen. The sulfur hexafluoride (SF_6) technique was used for two sampling periods of five consecutive days each to quantify daily methane (CH_4) production. The pasture crude protein contents (g kg DM^{-1}) were 235; 262; 280 and 294, and neutral detergent fiber (g kg DM^{-1}) were 592; 556; 550; 553 for the nitrogen application rates of 50, 100, 200 and 400 kg ha^{-1}, respectively. Data collected were submitted to variance and regression analysis at a significance of 5%. Treatment and blocking effects were considered in the model. The means were compared by the Tukey test at a significance of 5%. Linear and non-linear regressions were tested, being the best model chosen based on R^2 and significance level ($P<0.05$). The statistical package used was SAS version 8.02 (SAS Institute Inc., Cary, NC, USA, 2002).

Results Nitrogen fertilization levels did not affect ($P>0.05$) the variables presented on Table 1. On average, animals emitted 16.3 g of methane per kg of organic matter ingested. The gross energy intake to methane conversion factor, estimated by IPCC for sheep ageing one year is 4.5% (IPCC, 2006). Values of gross energy ingested and converted to methane (methane yield) in this study were on average 5%, agreeing with results found in the literature.

Table 1 CH_4 emissions per kg of organic matter taken in (CH_4CMO) and methane yield by sheep on pearl millet pastures.

Variables	Nitrogen fertilization levels, kg ha^{-1}				Mean ± S.E[1]	P
	50	100	200	400		
CH_4CMO, g/kg	17.3	17.4	14.6	15.4	16.3 ± 0.89	0.2287
Methane yield % CEB	5.55	5.39	4.58	4.79	5.08± 0.33	0.2194

[1] Standard error

Conclusions Methane emissions, expressed as grams of methane per kilogram of organic matter ingested, as well as the conversion of energy consumed into methane, did not present differences related to the levels of nitrogen fertilization, even at non limiting herbage allowances.

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Latent heat loss and physiological variables associated to enteric methane production in sheep

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Introduction Agricultural activity generates greenhouse gases, mainly through enteric fermentation (eructation) in ruminants. This emission can be influenced by the environmental and physiological conditions. This study aimed to relate daily methane emission in sheep with latent heat loss under tropical conditions.

Materials and methods Five Corriedale wethers were used in a 5 x 5 Latin square design trial. Each period was of 5 days with 5 sampling periods each day: 1= 8-10h, 2= 10-12h, 3= 12-14h, 4= 14-16h, 5= 16-18h. Various meterological parameters were recorded throughout the study. Sheep were sheltered from direct solar radiation throughout the study. The diet was composed of corn silage and concentrate (corn and soybean meal). Feed and water were offered *ad libitum*, except during the data collection. The methane emission was determined by indirect calorimetry using a facial mask developed in the Laboratório de Biometeorologia Animal (Animal Biometeorology Laboratory), at UNESP Jaboticabal, SP, Brazil. This mask isolated only the animal's muzzle, avoiding atmospheric and breathed air mixing. The inspired and expired air samples were analyzed continuously during data collection. The CH_4 concetration was analyzed using a methane analyzer (MA-10 Sable of Methane Analyzer System) at the Field System for Large Ruminants facility. The latent heat flux was estimated by the ventilation index product of the difference in the humidity content in the expired and inspired air (Maia *et al*, 2005). This product was divided by the corporal surface area of each wether. The respiratory volume ($V_{RC,}$ $L.s^{-1}$) as well the respiratory frequency ($F_{R,}$ $resp.m^{-1}$) were determined by Spirometer (model ML141, AD Instruments). The rectal temperature ($T_{R,}$ °C) was continuously measured during the data collecting using a rectal sensor (MLT 1404, AD Instrument). The air temperature ($T_{A,}$ °C) and the relative humidity (U_R, %) were measured through a HOBO data logger at regular intervals of five minutes. The short wave solar radiation (R_{UV}, $W\ m^{-2}$) was measured using a Pyranometer CMP-22 (Kipp&Zonen) at ten minute intervals. Data were submitted to a variance analysis using the minimal squares method. Means were adjusted and compared using the Tukey test ($P<0.05$). The program "Statistical Analyses System" (SAS) was used to analyze data using mixed models with main effects and regressions.

Results The period 3 sampling time (12-14h) had higher mean enteric methane emission (0.83 +0.19 $L\ h^{-1}$) compared to the periods 1 and 2 (Table 1). There was no correlation between methane emission and the meteorological variables, probably because sheep were sheltered from direct solar radiation. Methane production was related to heat production and metabolic rate. Thus latent heat loss was increased by respiratory evaporation (Table 1). There was also an elevation in the period 3 respiratory frequency. The respiratory volume and the respiratory frequency had a similar distribution throughout the day. The physiological variables may have some influence on the methane emission. There was a positive correlation between F_R and the respiratory volume (0.847). It means that the elevation in the respiratory frequency was followed by a respiratory volume increasing during each respiration cycle. The rectal temperature was not related to the meteorological conditions, as the animals attempted to maintain homeostasis.

Table 1 Minimum-mean square of methane emission (E_{CH4}), respiratory evaporation (E_R), respiratory volume, respiratory rate, rectal temperature, air temperature, air humidity and short-wave solar radiation

	E_{CH4} ($L\ h^{-1}$)	E_R ($W\ m^{-2}$)	V_{RC} ($L\ s^{-1}$)	F_R ($resp\ min^{-1}$)	T_R (°C)	T_A (°C)	U_R (%)	R_{UV} ($W\ m^{-2}$)
Means	0.48±017	12.49±2.04	1.01±0.075	135.84±4.81	39.43±0.26	27.72	52.55	464.58
Periods								
1	$0.12±0.15^{b}$	$8.94±2.71^{bc}$	$0.76±0.087^{b}$	$130.02±4.27^{ab}$	$39.54±0.050^{a}$	25.93±0.27	57.62±1.43	516.50±8.73
2	$0.27±0.16^{b}$	$2.28±2.30^{c}$	$0.85±0.064^{b}$	$127.49±4.49^{b}$	$39.66±0.037^{a}$	28.87±0.11	50.67±0.61	707.71±27.16
3	$0.83±0.19^{a}$	$22.14±2.30^{a}$	$1.25±0.081^{a}$	$140.87±5.17^{ab}$	$39.59±0.049^{a}$	28.39±0.17	52.53±0.72	362.20±10.60
4	$0.59±0.19^{ab}$	$15.62±1.51^{a}$	$1.20±0.073^{a}$	$144.76±5.13^{a}$	$39.73±0.045^{a}$	27.84±0.49	51.38±1.95	454.47±17.77
5	$0.59±0.18^{ab}$	$9.93±1.45^{b}$	$0.99±0.071^{ab}$	$136.04±5.01^{ab}$	$39.65±0.042^{a}$	27.09±0.46	51.93±1.89	196.67±8.57

Means with same letter in the column do not differ ($P>0.05$), Tukey's test

Conclusions There was no correlation between the physiological and meteorological variables and the methane emission. This may be because sheep were maintained under sheltered conditions.

Acknowledgements The authors gratefully acknowledge funding from FAPESP.

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Methane emissions during growing and finishing phases of Belgian blue double-muscled bulls change with diet

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Introduction Methane (CH_4) emission by animals is a major concern regarding environmental impact of agricultural ruminant based sector. However, referential values and opportunities to reduce them are still required.

Material and methods Belgian blue double-muscled bulls (BBdm) were sorted on the basis of their initial weight to set up three groups of nine animals with low intra-group weight variation. Within each groups, animals were allocated in three subgroups of three. Three diet types (Table 1), AR (mainly concentrates), MR and MT (both based on maize silage) were randomly assigned to one of each subgroup within each group. For AR and MR the concentrates included heat treated linseed products called respectively NUTEX from Dumoulin SA, Seilles, Belgium and LINEX from NV Aveve, Merksem, Belgium. BBdm were raised in two phases (PI and PII) according to animal weight (initial weight PI: 414±29; PII: 528±35 kg/head) without change in subgroups diet type but well in diet composition to fit bulls requirements.

Table 1 Diet composition (g/kg DM as theoretically proposed)

	PI							PII						
	Maize silage	Cereal straw	Concentrate	VEVI[1]	CP[2]	Fat	Cel[3]	Maize silage	Cereal straw	Concentrate	VEVI	CP	Fat	Cel
AR	0	87	913	1054	187	50	144	0	90	910	1193	173	61	148
MR	501	28	471	993	191	53	167	288	40	672	1138	175	62	149
MT	502	28	470	970	191	31	189	288	40	672	1053	173	31	179

[1]: Net energy concentration available for the animal (Dutch system), [2]: Crude protein concentration, [3]: Cellulose concentration

BBdm body weight gain (BWg) and dry matter intake (DMI) were measured over periods of 64±2 and 70±2 days in PI and PII respectively. For the whole experiment, the animals were raised by subgroups in common barns except for two weeks, at the middle of each phase, during which the subgroups of the same group were moved simultaneously to three fully strawed experimental barns. The experimental barns (Mathot *et al.*, 2012) were equipped to work as dynamic chambers with air CH_4 concentrations measured thanks to photoacoustic multi-gas devices (Lumasense Technologies SA, Ballerup, Denmark). In experimental barns, CH_4 emissions were recorded during 5 to 6 days. CH_4 emitted by the manures accumulated in barns were measured during 24 hours after the removal of the BBdm of the experimental barns and were discounted from the total emissions from the barn using trapezoidal rule (day 0=0, last day: measured emissions from manure) to obtain emissions by BBdm alone. CH_4 emissions were reported on daily gain calculated on the whole experimental phases and CH_4 emissions per DMI with DMI of the days with CH_4 emissions measurements. For each phase and subgroup, mean values of CH_4 emissions and BWg were used in ANOVA2 scheme with groups of animals as random factor and the diets as fixed factor followed by multiple comparisons according to TukeyHSD.

Results Diets influenced bulls performances but also methane emissions (up to – 40% compared to MT, Table2). Methane emissions were low in PI (AR: 36±5, MR: 49±5 and MT: 55±4 CH_4 energy/gross energy intake (kJ/MJ) and in PII (AR: 55±6, MR: 40±4 and MT: 54±4 CH_4 energy/gross energy intake (kJ/MJ). In PI, the lowest emissions were observed for AR followed by MR. In PII, the lowest emissions were observed for MR followed by MT if expressed by g/kg DMI and by AR if expressed by g/kg BWg.

Table 2 Mean± s.d. methane emissions from BBdm

	PI				PII			
	DMI/BWg (kg/kg)	BWg (kg/bull/day)	CH_4 (g/kg DMI)	(g/kg BWg)	DMI/BWg (kg/kg)	BWg (kg/bull/day)	CH_4 (g/kg DMI)	(g/kg BWg)
AR	4.85±0.39^{a}	1.80±0.14^{a}	12.1±1.8^{a}	60±14^{a}	5.81±0.23^{a}	1.35±0.20^{a}	18.2±2.0^{b}	109±8^{b}
MR	4.71±0.16^{a}	1.78±0.28^{a}	16.0±1.7^{ab}	77±7^{ab}	5.36±0.63^{a}	1.58±0.21^{a}	13.0±1.4a	73±16^{a}
MT	5.28±0.74^{a}	1.65±0.23^{a}	18.0±1.3^{b}	97±19^{b}	6.61±0.38^{b}	1.40±0.17^{a}	17.5±1.2^{b}	119±8^{b}

Conclusions Methane emissions from BBdm can be strongly influenced by the diet like by including heat treated linseed.

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Annual variation of soil CO_2 emission in a tropical grassland with different grazing intensities

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Introduction Soil CO_2 emission (ECO2) has large variability due to climatic conditions, especially due to the changes of soil temperature (Tsoil) and soil moisture. The management of the pasture in a livestock system can also affect the emissions due to the interference of the plant and animal in soil physical, chemical and biological properties. Considering the importance of understanding the greenhouse gases balance in livestock production, the aim of this study was to quantify the annual variation of ECO2, considering both to auto- and heterotrophic respiration, and its relations to Tsoil and precipitation (PP) in an area with Marandu-grass pasture submitted to different grazing intensities.

Material and methods The experiment was conducted in an area with Marandu-grass pasture for the last ten years located at UNESP (Jaboticabal, SP, Brazil). The pasture has been submitted to continuous stocking with different grazing intensities defined by three heights of the pasture (15, 25 and 35 cm). One ECO2 evaluation was carried out on morning (8-10 am) each fifteen-day period, during the year 2012, being recorded with a portable LI-COR system (LI-8100, Lincoln, NE, USA) which was placed on PVC soil collars previously inserted at a depth of 3 cm into soil, between plants. Tsoil was monitored by using a 20 cm depth with a thermistor based probe inserted into the soil. PP was registered by a weather station around one kilometre far from studied site. Data were analysed as a time repeated measurements with grazing intensity, period and interaction as sources of variation in variance analysis using proc mixed from SAS. Tukeys's test was used for mean comparison. A linear regression was adjusted to ECO2 and Tsoil.

Results ECO2 mean value was 4.47 µmol CO_2 m^{-2} s^{-1} with lowest value (0.23 µmol CO_2 m^{-2} s^{-1}) in winter (dry season) and maximum (14.75 µmol CO_2 m^{-2} s^{-1}) in summer (rainy season). The effect of grazing intensity in ECO2 was significant ($P < 0.004$). In pasture having height of 35 cm ECO2 and Tsoil were lower than in the other heights, with annual mean of 4.73 µmolCO_2 m^{-2} s^{-1}, while the areas of 15 and 25 cm of height emitted 5.29 and 5.17 µmol CO_2 m^{-2} s^{-1}, respectively. In smaller grazing intensity sites, the plant leaf area cover was greater in relation to other treatments, which results in smaller values of Tsoil and consequently in ECO2 (Epron *et al*., 2006). Another explanation may be the fact that under lower grazing intensities, plant metabolism is reduced in comparison to higher grazing intensities where the plant growth and tillering is stimulated due to grazing, which could be reflected in higher root respiration. The differences of emissions along the year occurred both in accord variations in Tsoil and PP events. The relation between ECO2 and Tsoil was linear and significant ($P<0.0001$), being explained by ECO2 = -7.60 (±1.55) + 0.59 (±0.07) * Tsoil, with R = 0.70. The ECO2 mean value between April and September (3.19 µmol CO_2 m^{-2} s^{-1}) is lower than emissions observed in the January-March and in October-December periods (mean ≈ 7 µmol CO_2 m^{-2} s^{-1}). As the rain reduced in March (29.3 mm), the effects on ECO2 are observed in April, with values remaining low until Tsoil begins to increase later in October, but ECO2 reaches maximum values in December, when both PP and Tsoil increased.

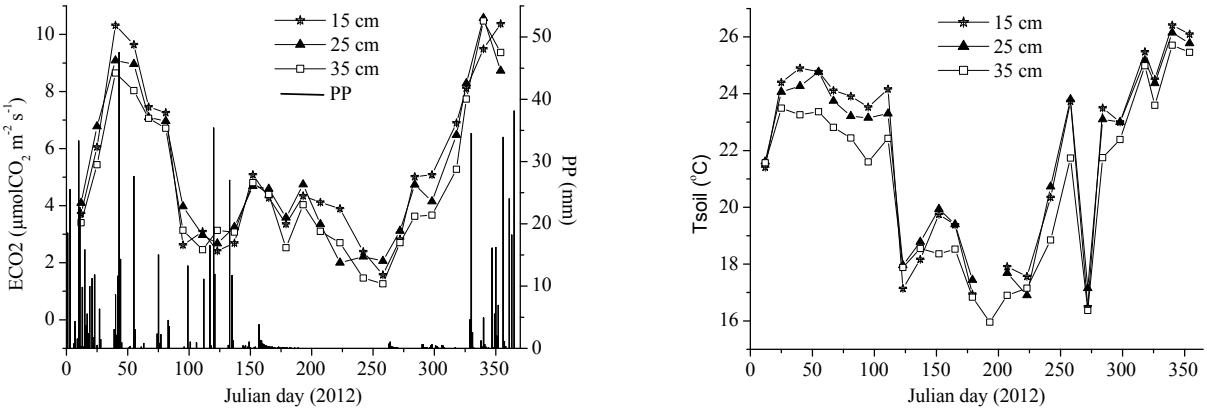

Figure 1 Soil CO_2 emission and soil temperature in different grazing intensities along 2012

Conclusions Both management and climatic condition affect soil CO_2 emission. Emissions are lower when the plant covers more the soil, as observed in smaller grazing intensity, where the soil consequently achieve lower temperature, which reflects in lower emissions. Both soil temperature and precipitation affects the variations of soil CO_2 emission along the year.

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Determination of methane production on grass fed goats

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Introduction Methane emissions from cattle are already known, but for goats that value is still estimated (IPCC, 2006). Around the world, the number of goats is estimated on 910 million head, mostly are raised on pasture. To measure the methane production from animals under grazing conditions, the sulfur hexafluoride (SF_6) tracer technique is considered appropriate for cattle.
This study aimed to adapt the SF_6 technique to use in small ruminants, and measure the methane (CH_4) emission in goats under grazing conditions.

Material and methods Two experiments were performed. The first was to adapt the SF_6 tracer technique, described by Johnson *et al*. (1994) for measure in goats and the second had the objective of quantifying the emission of CH_4 from these animals. The first study we used one adult male weighing 78.5 kg, under generous grazing conditions to test adaptations. The major adjustments were: making collector cylinders with a capacity of 2 liters, although smaller, and the change of position from the neck to the back of the animal with the aid of an apparatus similar to a saddle, so not to adversely affect the movements and access to food (Figure 1). Another important test was related to the emission rate of tracer gas capsules, on which were evaluated, in the animal, two emission rates (622 and 1217 ng / min.). For each emission rate, four samples of methane eructed by the animals were collected. Concentrations of CH_4 and SF_6 in the collection cylinders were measured by gas chromatography, for such the cylinders were positively pressurized with N_2 and connected directly into the chromatograph (Agilent ® Model 6890). From the known rate of clearance of the tracer gas in the rumen, and concentrations of methane and trace gas in gas samples captured, the methane release by the animals were calculated. In order to quantify the methane emissions the second trial was initiated with five goats, adult females of the Anglo Nubian breed, with an average weight of 54.4 kg under intermittent stocking, grazing for 11 hours in areas formed with *Panicum maximum* cv. Tanzania, with *ad libitum* access to water and mineral salt, being gathered into the fold at night.

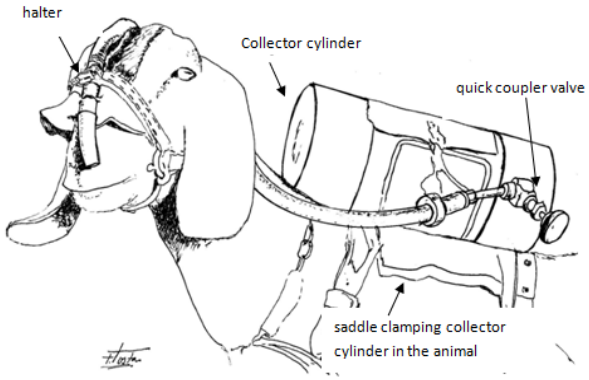

Figure 1. Goats with halter and methane collector cylinder

Based on the results of the first experiment, the capsules introduced into the rumen were in the range of 500 to 1,000 ng/min. emission of SF_6. The pre evacuated collector cylinders (-12.00 to -12.60 psi) were placed in the animals at 7am before releasing them in the grazing area and changed every 24 hours. The collections were made for six consecutive days. The statistical analyses in experiment 1 was the Pearson's correlation between CH_4 and CF_6 concentration, and in experiment 2 the GLM procedure was used to test the model, and the Student Newman Keuls Test to analyze the averages considering 5% of probability.

Results In experiment 1, the average CH_4 emission was 1.4 ± 0.2 and 1.2 ± 0.1 g/day/metabolic body weight (body weight$^{0.75}$) for emission rates 622 and 1217 ng/min. respectively. Highest correlation was observed between the concentrations of SF_6 and CH_4 in the samples taken when the animal received the capsule with emission of 622 ng/min. (r = 0.99) when compared with the rate of 1217 ng/min (r = 0.41). In the second experiment the average CH_4 emission was 17.59 g/day/animal or 0.88 g/day/ metabolic weight for the goats used in the experiment, corresponding to 0.91 g/day or 5.3 kg/year in 40 kg goats.

Conclusions After adjustments, the SF_6 methodology was considered adequate for measuring methane emission on grass fed goats. The emission of methane per kg of metabolic body weight of 0.91 g/day or 5.3 kg/year for 40 kg goats can be used as reference in grazing goats. This value is close to the one estimated by IPCC of 5 kg/year.

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Influence of difference source of the fat on animal performance and methane emission

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Introdcution Methane emissions from ruminant livestock have increased fivefold over the last century (Johnson *et al*., 2000) and now constitute ~15% of global CH4 emissions (McAllister *et al*., 1996). However, improving forage quality (i.e. increasing dietary starch content) through the supplementation of alternative forages, has the potential to reduce CH4 emissions per kg animal product as a result of increased diet digestibility and a shortened duration of feeding (Beauchemin *et al*., 2009). Dietary strategies such as, use of the sources lipids have been successful in manipulating methanogenesis, at least in the short term, through either i) the direct inhibition of methanogens, ii) reducing the production of hydrogen in the rumen or iii) providing alternative sinks for the disposal of hydrogen (Beauchemin *et al*., 2009). This trial aimed to evaluate the effects of lipid supplementation on performance and methane emission of beef cattle grazing *Brachiaria brizantha* cv. Marandu pasture during the summer.

Material and methods The experiment was conducted at Animal Science Department of the São Paulo State University, Campus Jaboticabal, during 84 days in a 6.0 ha area divided in 6 paddocks, using 18 Nellore young bulls in continuous stocking rate grazing system. Lipid sources supplements evaluated were: soybean grain, fat-protected with calcium salts, and control. Animals were supplemented daily, 0.5 g/kg body weight, between 11:00 am to 14:00 pm to minimize the effects of supplement on grazing behavior. All supplements presented the same content of crude protein, and total digestible nutrition (TDN), 260 g/ kg DM, and 980 g/kg DM, respectively. Control treatment consisted of mineral salt. . The experiment was analyzed by a complete randomized design with tree treatments and two replications (paddocks) with six animals in each one. The CH4 emissions were evaluated using the SF6 tracer gas technique. Five consecutive gas samples were collected in 24-h intervals from each animal. Data were analyzed using the GLM procedure of SAS. Characteristics evaluated in the study were CH4 emission expressed in kg of CH4 emitted per year (kg CH4.yr-1), gram of CH4 emitted per day (g CH4.d-1), gram of CH4 emitted per day per kg of metabolic BW (g CH4.d.MBW-1), gram of CH4 emitted per day of weight gain and animal performance.

Results The use of different lipid sources showed no statistical difference (P> 0.01) over the variables analyzed. However, supplementation improved animal performance increasing at ~ 400 g / day. This difference in weight gain of the animal will decrease the residence time the animal of this system, which in turn reflects on positive results in lowering total emissions of greenhouse gases.

Table 1 Effect the sources of the fat methane emission

Tratament	Variable			
	g CH4.d-1	Kg CH4.yr-1	g CH4.d.MBW-1	g WG
Control	89.5a	32.6a	1.2a	631.7b
Protect fat	97.3a	35.5a	1.2a	1008.3a
Soybean grain	96.0a	35.0a	1.2a	963.3a

Conclusions The use of sources of lipid supplementation in Nellore maintained in Brachiaria brizantha did not alter the production of methane but improved animal performance.

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Methane and carbon dioxide emissions from Tibetan sheep grazing system in Qinghai-Tibet Plateau alpine meadow

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Introduction On the Qinghai-Tibet plateau, grasslands cover 1.5×10^6 km^2 and consist of 35% nearly of the total plateau area (Hafner *et al*., 2012). The grasslands of the Qinghai-Tibet are the world's largest high altitude pasture area and animal husbandry represents the traditional land use in this region (Wang *et al*., 2005). Grazing could be a certain factor for a C sink and source switch (Wang *et al*.,2005). So, study on C dynamics of the Qinghai-Tibet Plateau under different stocking rates is critical to understand the regional and global C budget.

Material and methods The study was located in the Maqu County, Gansu, China (35°58′N, 101°53′E, 3,650 ma.s.l.). There is no absolute frost-free period. Study site has a mean annual temperature of 1.2 □ and mean annual precipitation is 620 mm, with 85 % falling during the growing season from May to September. Grassland of the study site belongs to alpine meadow. Two stocking rates were concerned in the study, 8 sheep/ha (8 Tibetan sheep, 1 ha plot) and 16 sheep/ha (8 Tibetan sheep, 0.5 ha plot) and all the plots have 3 replicates. CH_4 and CO_2 flux was measured by the static chamber method in May, July and December in 2011. Three chamber anchors were fixed into the soil in each plot 24 h prior to the flux measurement to maintain balance of the top soil. Daily CH_4 and CO_2 flux from each anchor was measured at 10:00 and 16:30 for 7 consecutive days (present the mean flux of the month) on 10-16 May, 12–18 July and 11–17 December. The significance of differences between the carbon emissions from grazing grassland with different stocking were obtained by using one-way ANOVA (Spss 13.0).

Results The flux of CH_4 was minus in all the three season we determined under either lower of higher stocking rate (Fig.1). The highest daily absorption of CH_4 (-0.27 mg/m^2/d and -0.18 mg/m^2/d, respectively) in alpine meadow was in the turning green season (May) and the lowest was in December (-0.04 mg/m^2/d) when the stocking rate was 8 sheep/ha but that was found in July (-0.02 mg/m^2/d)when the stocking rate was higher (16 sheep/ha). For CO_2, it presents the similar changes. Higher stocking rate results in higher CO_2 emissions. The highest daily emission was found in July (71 mg/m^2/d and 97 mg/m^2/d) and the lowest one was in December (5.7 mg/m^2/d and 2.0 mg/m^2/d).

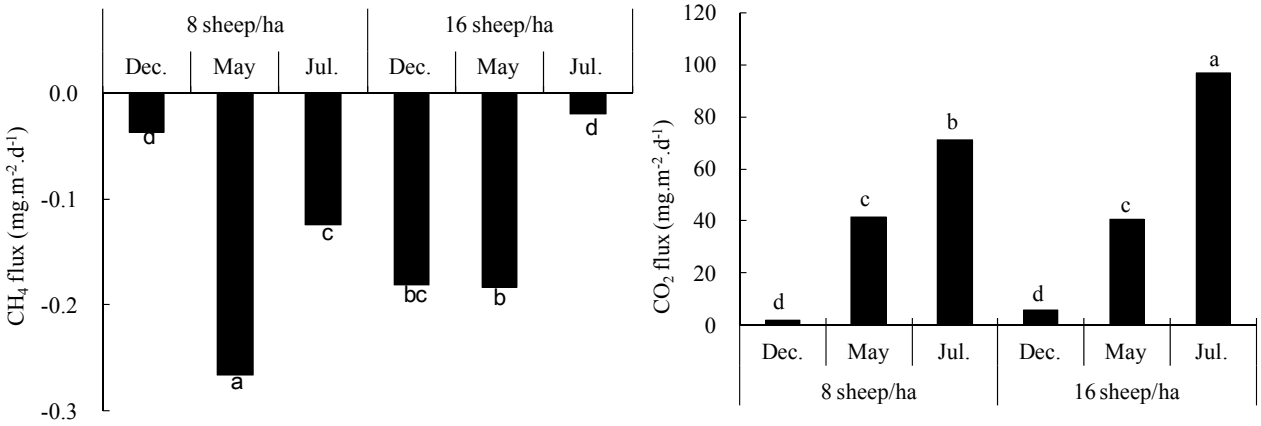

Figure 1 CH_4 and CO_2 emissions from the grazing alpine meadow in Qinghai-Tibet Plateau

Conclusions Grazing alpine meadow was the sink of CH_4 and moderate stocking rate could enhance the ability of absorbing CH_4 in Qinghai-Tibet Plateau. Higher stocking rate will activate the alpine meadow fluxing more CO_2.

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Potential of milk fatty acids as biomarkers for effectiveness of methane mitigating additives in dairy cattle under similar conditions

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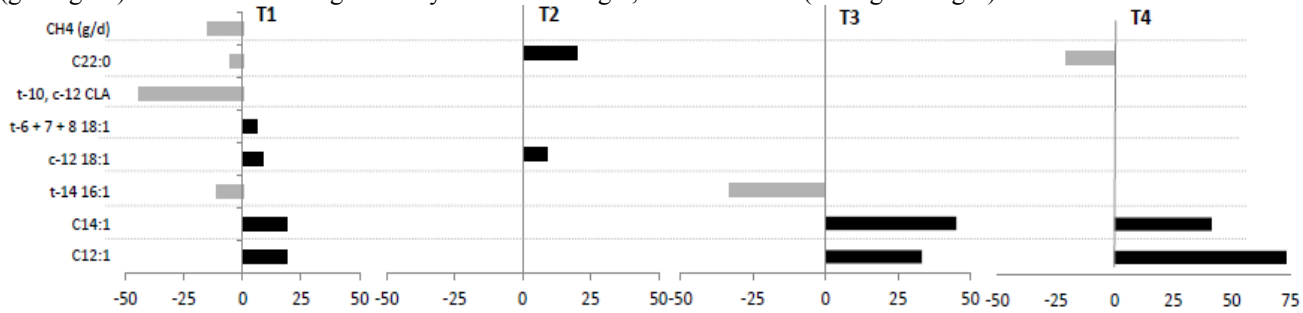
Introduction Recently, milk fatty acid (MFA) profiles gained interest as biomarkers for enteric methane (CH_4) production, as milk fat contains a rich spectrum of fatty acids originating from several processes, some of which reflect rumen metabolism. However, between previous studies (e.g. Chilliard *et al*., 2010; Dijkstra *et al*., 2011; Mohammed *et al*., 2011) little coincidence exists in the MFA identified with the strongest relationships with CH_4. This lack of conformity might be related to the particular conditions of each experiment. In this study we identified the changes in individual MFA for 4 dietary treatments: two essential oils (T1 and T3) and two organosulphourous compounds (T2 and T4) tested under similar conditions and how their variations relate with CH_4 production.

Material and methods Each treatment was tested with four dairy cows in a trial lasting 8 weeks. The cows had an average bodyweight of 620 kg and were 262 days in milk at the beginning of the trials. The diet contained grass silage, maize silage, concentrate and soybean meal for all treatments. For T3, rapeseed cake and sugar beet pulp were also included. After two weeks, enteric CH_4 was measured by keeping the cows in individual open circuit chambers (Peiren and De Campeneere, 2012), with this measurement being considered as control. Various dietary additives were then added during a period of six weeks, with measurements and samplings 2, 4 and 6 weeks after the start of the administration. Each measuring period lasted from Tuesday morning until Friday morning. A small negative atmospheric pressure was generated inside the chamber to avoid leakage of air to the outside. Samples of air were continuously taken from just before the inlet of 2 of the chambers and from the outlet of each chamber. Each channel was attached to a gas switching device, which then connects to the multigas analyzer determining CH_4 every second. Each channel was monitored during 180 seconds. The average of the last 60 seconds was used as a data point. Inside the chambers, cows were fed, milked and cleaned twice a day at 0730 and 1730 h. Milk samples were analyzed for FA analysis by GC after extraction and methylation as described by Vlaeminck *et al*. (2005). The data were analyzed using the MIXED procedure of SAS with repeated measurements in time and using an autoregressive covariance structure. Orthogonal contrasts were used to find differences between the control and the average of the supplementation period (2, 4 and 6 weeks after administration). Significances were declared at $P<0.05$ and tendencies at $P<0.1$.

Results Methane production decreased only for one of the dietary treatments (T1) (Figure 1), which coincided with changes in seven MFA, showing either decreasing (C22:0, *t*-10, *c*-12 CLA and *t*-14 C16:1) or increasing concentrations (*t*-6+7+8 C18:1, *c*-12 C18:1, C14:1 and C12:1) in milk fat. However, either T2, T3 and/or T4 also induced changes in most of these MFA, except for *t*-10, *c*-12 CLA and *t*-6+7+8 C18:1 without modifying CH_4 production Furthermore, other MFA which did not change in T1 showed differences in the other treatments (not shown in Figure 1).

Figure 1 Relative changes as compared with a control period without additive administration in CH_4 (g/d) and MFA (g/100g fat) when administering a dietary additive during 2, 4 and 6 weeks (average changes)



MFA presented in the figure are those changing ($P<0.05$) in T1 where CH_4 production changed

Conclusions Concomitant and exclusive changes in both CH_4 and MFA were observed for a limited number of MFA only (*t*-10, *c*-12 CLA and *t*-6+7+8 C18:1).

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Methane and hydrogen emissions from finishing cattle fed either a forage- or concentrate-based diets

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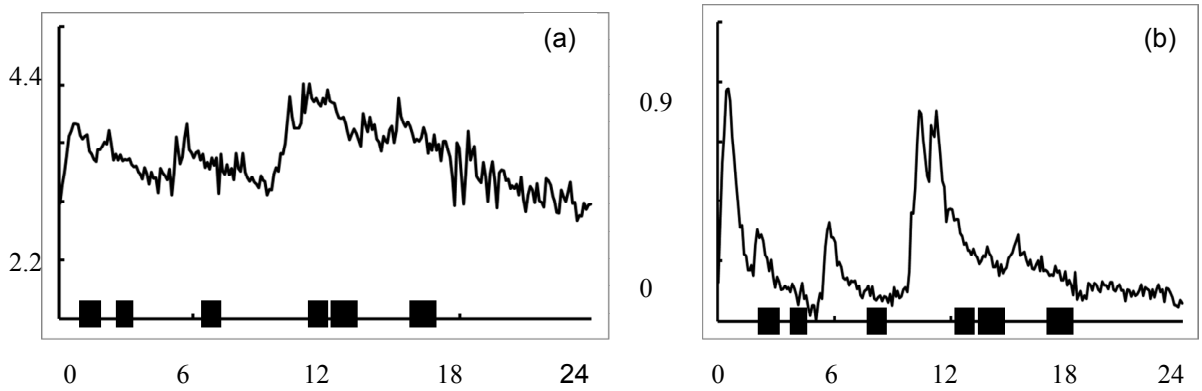
Introduction Carbon dioxide (CO_2) and hydrogen (H_2) are the main gaseous end-products of the fermentation of feed organic matter in the rumen. These gases are the principal substrates for the formation of methane (CH_4) by the methanogenic archaea. Other reactions, such as the formation of succinate, also consume H_2 in a group of reactions known as interspecies H_2 transfer. This process is not 100% efficient as small quantities of H_2 gas and therefore energy are emitted from the rumen. There is little information on the extent of H_2 emissions and how these are affected by diet. Here, we report a large-scale comparison of CH_4 and H_2 emissions from forage-based or concentrate-based (barley beef) diets fed to finishing beef cattle.

Material and Methods Aberdeen Angus x Limousin (AA x LIM) or Limousin x Aberdeen Angus (LIM x AA) cross-bred steers (n=72) were fed complete diets (g/kg, dry matter (DM) basis) consisting of either 480 forage: 520 concentrate (F) or 75 forage: 925 concentrate (C), respectively. Diets were offered *ad libitum* to steers once daily. The experiment was a 2 x 2 factorial arrangement of breed and diet. Prior to chamber measurements, feed intake and live-weight gain (LWG) had been measured for 8 weeks. Steers were allocated to respiration chambers using a replicated (3 times) randomised block design so that allocation was balanced for live-weight, breed and diet. Six indirect open-circuit respiration chambers were used with gas production being recorded for the last 48 h of a 72 h measurement period. Samples of inlet air were also taken for measurement of ambient gas concentrations. Gas concentrations (10 samples per h for each chamber outlet and ambient air) were measured by infrared absorption (CH_4) or by chemical sensor (H_2), (MGA3000, ADC Ltd., Hoddesdon, UK) and gas production measured as the difference between outlet and ambient. Dry air flow, corrected to standard temperature and pressure, was calculated for each individual record. Daily gas production was calculated as the average of individual values and converted to a mass basis. Measurements were not made on one steer because of illness and data were rejected from three steers because an air leak rendered data unreliable. Data were analysed using Genstat using linear mixed models where the factors were the 2 x 2 arrangement of breed and diet, block, chamber and week of experiment. Data are reported as means and standard error of difference.

Results CH_4 production was greater (P<0.001) for diet F than diet C on both a total daily basis (205 v 144 g/d, s.e.d. 12.6) or per kg DM intake (21.8 v 13.6 g/kg DM intake s.e.d. 1.20) basis. The amounts of H_2 produced were less than CH_4 but also greater on diet F than C whether on a daily (2.2 v 2.1 g/day; s.e.d. 0.10, P<0.05) or DM intake (0.24 v 0.20 g/kg DM intake, s.e.d. 0.019, P<0.001) basis. On a molar basis, daily production of H_2 relative to CH_4 production (mol/mol, 0.088 v 0.120, s.e.d. 0.0136 P<0.001) was lower on diet F than diet C.

Figure 1 Example of changes in (a) CH_4 and (b) H_2 concentrations (µmol/l) over 24 h (from a single steer). Solid bars denote times when feed consumed.



Both CH_4 and in particular H_2 concentrations increased after feed was consumed (Figure 1). Whereas median H_2 concentrations (µmol/l) were marginally greater for diet F than C (0.24 vs 0.22 s.e.d. 0.015, P=0.07), the frequency of H_2 concentrations greater than 0.22 µmol/l above median values (0.080 vs 0.041; F v C; s.e.d 0.0245, P=0.011) was higher for diet F than diet C.

Conclusions The amounts of H_2 produced were, on a molar basis, approximately 0.1 of the amounts of CH_4. H_2 emissions were greater from animals receiving the higher forage diet. This suggests that the H_2-consuming reactions of interspecies H_2 transfer, including methanogenesis, are more easily saturated with a forage-containing diet than a high-concentrate diet.

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Farm scale greenhouse gas accounting as basis for emissions trading or financial support

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Introduction Agriculture in the EU is a significant source of greenhouse gas (GHG) emissions, being responsible for about 9 percent of total GHG emissions. However, these emissions include several gases (methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2)) and a range of sources that are only partly under farm management control. CO_2 is released largely from microbial decay or burning of plant litter and soil organic matter. CH_4 is produced when organic materials decompose under anoxic conditions, notably from fermentative digestion by ruminant livestock, stored manures, wetlands and rice grown under flooded conditions. N_2O is produced by the microbial transformations of nitrogen (N) in soils and manures, and is often enhanced where available N exceeds plant requirements, especially under wet conditions. These greenhouse gas emissions may be reduced by lowering input intensities and thus often also farm productivity, through adoption of the efficiency measures that increase production per unit emissions, or through changes in land use, farm management or technologies that target reduction in GHG emissions. Different farm types may have very different options for emissions reductions depending on farm type, farm structure, agroecological conditions, infrastructure and available financing. The usual practice within the Common Agricultural Policy (CAP) in terms of incentivizing GHG emissions in agriculture is to support certain practices or technologies based on a list of approved measures. Whereas such a list in most cases would lead to emissions reductions, the costs may be relatively high, since the measures are not targeted local conditions, and also such a generalized list leaves little room for innovations in the sector to further reduce emissions. The emissions from the energy sector in the EU is regulated through the Emissions Trading System (ETS), which despite current problems with CO_2 quota prices may be an efficient system for ensuring emissions reductions. The question therefore arises whether the emissions from agricultural sector could be regulated the ETS (Brandt and Svendsen, 2011), or whether other incentives that take a whole-farm perspective could be introduced based on a farm level GHG accounting system that also accounts for measures taken to reduce emissions.

Materials and methods We suggest a farm level GHG accounting system that is largely based on an emission factor approach by including available data on farm activities that describes flows of carbon and nitrogen on the farm and gives a characterization of the environmental and management conditions that affect emissions. This may be based on existing farm-scale GHG accounting tools such as the CoolFarm tool (Hillier *et al*., 2011). However, such a tool would need to be locally adapted to estimate emissions under the regionally specific conditions and to properly represent effects of emissions reduction measures.

Results Our analysis shows that most of the data needed to calculate farm level GHG emissions are already recorded in farm-scale or national databases in many European countries, in particular for the larger and more professional farms. The challenge therefore is to develop a system that links these data and to certify a system that also allows new technologies and management techniques to be included in terms of their effects on emissions. Additional challenges in terms of monitoring and control procedure could be included under current control procedures that aim at. Calculations for farm types in Denmark demonstrate a realistic potential for GHG emissions reductions of 20-40 % provided farms are allowed to locally optimize their actions.

Conclusions We consider it possible to develop a farm scale GHG accounting system that would allow estimation of GHG emissions on specific farms that would also include the effects on management measures and technologies adopted to reduce emissions. In the longer term this may allow agriculture to participate in an emissions trading system, whereas in the shorter term such a system could be supported through environmental subsidies under the EU CAP.

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Nutritive value and in vitro methane emission of concentrate feeds in buffalo inoculums

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Introduction Feed ingredients viz. brans, cereal grains, chunies/husks and oil cakes are the main component of concentrate mixture fed to livestock in India. These feeds differ in their chemical constituents and degradability/digestibility depending on their origin, processing and chemical/physical properties. Feed ingredient should be adequate in readily fermentable protein and carbohydrate fraction to support microbial growth for efficient rumen fermentation (Nocek and Russell 1988) to minimize the methane production from diets as it causes 4-12 % loss of dietary energy. The aim of study was to screen feeds for chemical makeup, degradability and CH_4 production to formulate low CH_4 emission diets.

Materials and methods Samples of protein (mustard seed cake-MSC, cotton seed cake-CSC, groundnut cake-GNC and coconut cake-CNC) and energy feeds (wheat bran-WB, barley grain- BG, oat grain-OG, maize rain-MG, wheat grain-WG, gram husk with broken seed (gram chuni)-GC, rice bran-RB) were evaluated for proximate constituents, cell wall polysaccharides, in vitro dry matter digestibility-IVDMD, protein fractions and carbohydrate fractions as per Cornell Net Carbohydrate and Protein sytem- CNCPS following standard methods. Air-dry feeds (1.0 g) were incubated (quadruplets) at 39 ^{0}C in 150 ml serum bottles inoculated with 8 ml rumen inoculums collected from 2 buffaloes fed wheat straw-concentrate diet (65:35) and gas was measured at different hours using pressure transducer (Theodorou *et al*.1994), while CH_4 in gas was measured by gas chromatograph using FID. Methane measured was related with total gas to estimate concentration (Tavendale *et al*., 2005). Gross energy (GE) of feeds was measured with bomb calorimeter using benzoic acid as standard.

Results Crude protein and EE were higher in oil cakes than grains and brans. RB had higher cell wall polysaccharides than other feeds. In oil cakes NDF, ADF, cellulose and lignin contents ranged between 26.4-59.5, 22.3- 36.5, 12.3-26.1 and 3.9-10.0 %, respectively. Neutral detergent insoluble protein-NDIP was highest in CNC, while acid detergent insoluble protein-ADIP was highest in CSC. In energy feeds NDIN and ADIN contents were higher in GC and RB. P_A contents were highest in MSC (37.49) against lowest in CNC (0.47%). Wheat bran had highest contents of P_A. Unavailable protein (P_C) was almost two times more in CSC and CNC than MSC and GNC. Slow degradable protein (P_{B3}) was more in CNC than other cakes. GC and RB had higher contents of P_C than grains. Rapidly degradable protein (P_{B1}) was highest in OG (35.61) and lowest in BG (13.4 %). Carbohydrates were ($P<0.05$) higher in grains and brans than oil cakes. Non structural carbohydrate-NSC was highest in WG (54.8 %) and lowest in RB (3.61). Insoluble carbohydrate fraction (C_C) was ($P<0.05$) higher in RB and GNC than grains. Rapidly degradable carbohydrate (C_A) was lowest in RB against higher in MSC and WG. Gram chuni and CNC had higher contents of slowly degradable carbohydrate fraction (C_{B2}) than lowest in MSC and GNC. Energy contents were lowest in GC and highest in GNC, while IVDMD of WG and MG was highest against lowest of GC and RB. Gas production (ml/g) at different periods of incubation was higher from WG, MG and OG than oil cakes, while the lowest gas production was recorded from RB. Methane production (ml/g) was highest from WG and lower from GC and RB. CH_4 concentration and maximum from WG. CH_4 production was lower from MG (22.2) and higher from WG (35.4 g/kg DDM). Loss of feed energy as CH_4 was minimum from MG (5.98) and maximum from GC (12.2%).

Table 1 Gas and methane production (ml/g), gross energy (Kcal/g), IVDMD (%) and methane % energy of feeds

Feeds	MSC	CSC	GNC	WB1	WB2	BG	OG	MG	WG	RB	GC	CNC	s. e. m.
Gas 12 h	64.5	62.8	65.5	67.8	72	67.8	74	71	75	61.5	60.5	64	0.703
CH_4 12 h	19.5	12.5	15.4	18.1	21.2	18.6	19	13.2	23.2	9.08	6.69	15.5	0.697
Gas 24 h	114	110	114	120	122	121	126	125	130	106	114	115	10.31
CH_4 24 h	37.9	23.5	27.7	36	38.1	35.9	32	26.2	44.3	15.7	17.3	30.6	1.24
Gas 48 h	160	157	158	164	166	169	174	174	179	151	173	166	1.207
CH_4 48 h	53.7	38	40.6	50.1	52.3	53.2	45.6	39.1	62.3	24.2	38.7	51.8	1.464
IVDMD	79.9	56.9	74.2	73.4	82.4	77.8	69.8	89.2	92.4	38.6	34.5	87.8	2.73
CH_4 g/kg DDM	34.3	31.3	27.4	35. 5	34.9	34.7	32.9	22.2	35.4	29.4	36.1	25.1	0.67
Gross energy	5.09	5.1	5.25	4.47	4.59	4.56	4.33	4.79	4.32	4.26	3.79	4.44	
CH_4 %GE	8.68	7.9	6.74	10.1	9.79	9.81	9.8	5.98	10.6	8.9	12.2	7.3	

Conclusions Methane production (g/kg DDM) and loss of energy as methane was less from GNC, MG and CNC. Feed ingredients with less methane emission and medium to high degradability can be utilised to formulate low methane production diets for ruminants.

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Methane from ewes and steers measured with the Laser Methane Detector correlates with open-circuit respiration chambers measurements

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Introduction Ruminants contribute significantly to global anthropogenic methane (CH_4) emissions. As most CH_4 produced by ruminants is excreted by breathing and eructation, the Laser Methane Detector (LMD) has been proposed as a method to characterise enteric CH_4 emissions from animals in their natural environment. The LMD is a hand-held gas detector that measures CH_4 concentrations in the air between LMD and the mouth and nostril areas of an animal during short periods of time. This work aimed to validate LMD-CH_4 measurements against respiration chamber measurements.

Materials and Methods The LMD was used to measure CH_4 concentration (ppm/metre) from the exhaled air of 24 lactating Scottish Mule ewes (Blue-Faced Leicester x Scottish Blackface). Measurements were taken once a week during 4 weeks. Each day, LMD measurements were made during five 2-min periods per ewe at 1000, 1100, 1300, 1400 and 1500 h (P1 to P5, respectively). Ewes were fed twice a day with alfalfa pellets. The LMD was also used to measure CH_4 in 72 finishing Aberdeen Angus x Limousin steers fed *ad-libitum* with complete diets of either 48:52 (F) or 8:92 (C) forage to concentrate ratio (DM basis). LMD measurements were made once daily during a 4 min period for 3 days while steers were in training pens, prior to chamber measurements. The LMD was used at 1 metre distance from the animal in both species. The following week ewes or steers entered to open-circuit respiration chambers (ewes in pairs) to quantify their daily CH_4 output (chamber-CH_4, g/d) during 48 h. LMD outputs consisted of periodic events of high CH_4 concentrations superimposed on a background of oscillating lower CH_4 concentrations. The high CH_4 events were attributed to eructation and the lower background CH_4 to respiration. By fitting a double normal distribution to the dataset, a threshold of 99 % of probability of the lower distribution was used to separate respiration from eructation events. The LMD data was analysed as a series of point measurements, and peaks and troughs were also identified. Mean values as well as the sum and number of observations were considered for further analysis of the overall, respiration and eructation events. Distributions fitting, correlations and stepwise selection of Generalized linear models were performed in GenStat (11th version).

Results In ewes, chamber-CH_4 (y) was predicted best by DM intake (DMI, kg/d) and the number of eructation points (NE) in P2: y = -15.1 + 0.008*DMI + 0.493*NE (AdjR^2 = 0.92, P<0.001; Figure 1a). This model showed an improvement from the relationship between chamber-CH_4 and DMI only (AdjR^2 = 0.79, P<0.001), and chamber-CH_4 and LMD alone (AdjR^2 = 0.89, P<0.001). In finishing steers, there was a significant relationship between observed chamber-CH_4 (y) and predicted CH_4 with the model containing Diet, DMI, body weight (BW, kg), and number of respiration peaks (NRP): y = 335 + 44.9*Diet + 9.37*DMI - 0.359*BW - 0.165*NRP (AdjR^2 = 0.52, P<0.001; Figure 1b). In this case, LMD together with DMI and BW improved the prediction in comparison to chamber-CH_4 and DMI alone (P=0.971); DMI, BW and Diet alone (AdjR^2 = 0.48, P<0.001) and chamber-CH_4 and LMD alone (AdjR^2 = 0.31, P<0.001).

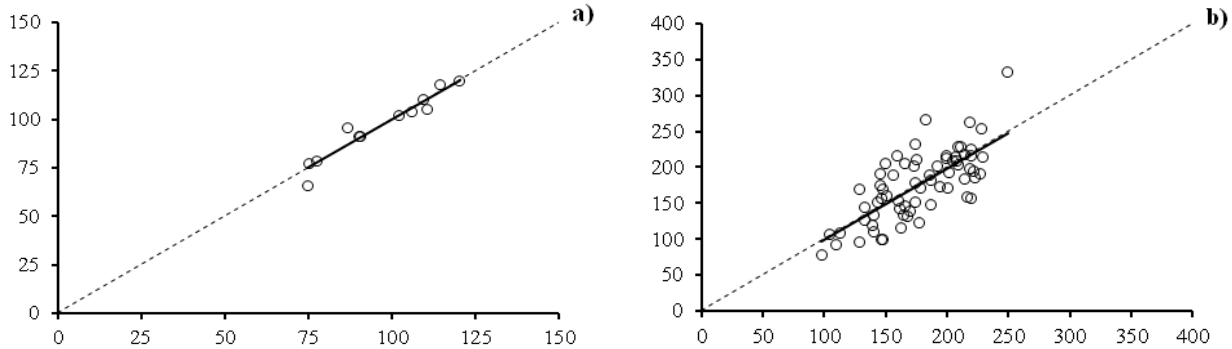

Figure 1 Relationship between observed CH_4 from respiration chambers (y axis, g/d) and predicted CH_4 (x axis) from (a) lactating ewes (n=12) with the model y = -15.1 + 0.008*DMI + 0.493*number of eructation points, and (b) finishing steers (n=67) with the model y = 335 + 44.9*Diet + 9.37*DMI - 0.359*BW - 0.165*number of respiration peaks. Dashed line denotes 45 degree line.

Conclusions The LMD has the potential to provide detailed information about how CH_4 is released by ruminants over short periods of time. High correlations between LMD and chamber measures were observed only after separating eructation and respiration events. LMD alone showed potential to predict CH_4. However adding information of animal intake and body weight into a model including LMD measures resulted in the best prediction of CH_4 data from respiration chambers. Further assessment of the LMD should be performed in relation to animal feeding behaviour and physiology in order to validate the assumptions of eructation and respiration CH_4 levels and better investigate its application for CH_4 mitigation testing.

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Representative stratified surveys on livestock and manure management as a valuable tool to assess the temporal development of livestock emissions

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Introduction Under the Gothenborg Protocol countries are obliged to reduce the emissions of different air pollutants to a set target and to report on the compliance. For example, Switzerland had a target to reduce ammonia (NH_3) emissions by 13% between 1990 and 2010. To reliably assess the development of emissions the emission inventory also has to take into account changes in farm management. Such changes therefore have to be reliably recognized and documented. Expert judgement is not sufficient for this because no expert can reliably and reproducibly assess small changes over a longer range of time and in consideration of the large variability of farming practice. In Switzerland such activity data is therefore compiled by regular surveys that consider a representative stratified sample of farms which are then used for emission inventory modelling calculations. So far this approach was only used for the NH_3 emission inventory. With some additional parameters it could also be used for greenhouse gases.

Material and methods In the framework of NH_3 emission inventory work, stratified surveys on farm and manure management were conducted in Switzerland by means of mail surveys for 2002, 2007 and 2010, respectively. A stratified random sample of 3877 (5.8% of all farms), 6565 (10.6%) and 6351 (10.8%) farms was used in the survey of 2002, 2007 and 2010, respectively. Farm classes were defined for the stratification accounting for three geographical regions, three altitude zones (valley, hill, mountain) and five farm types (arable farms, cattle farms, pig or poultry farms, mixed farms, other farms). A return rate of about 50% was achieved. Tests for plausibility and procedures for correction were established for non-biunique and missing entries in the questionnaire. Ammonia emissions were then calculated for each farm participating in the survey using the N flow model AGRAMMON (Kupper *et al*. 2010). Average emission factors (housing, storage, application, grazing) for different livestock categories and farm classes where determined for up-scaling national emissions.

Selected results

- The time dairy cows were grazed increased from 724 h a^{-1} (1990) to 1575 h a^{-1} (2002) and 1595 h a^{-1} (2010). An increase was observed for other cattle categories as well, though less pronounced.
- Between 2002 and 2010 the average amount of concentrate fed per dairy cow increased from 1.3 to 1.7 kg per day during summer feeding and from 2.1 to 2.4 kg during winter feeding. The percentage of dairy cows receiving maize silage during summer feeding increased from 25 to 40%.
- Between 2002 and 2010 the share of dairy cows kept in loose housing systems increased from 6 to 48%. Tied housings decreased accordingly. Although less prominent, a similar evolution occurred for heifers and suckling cows.
- Between 2002 and 2010 the share of dairy cows producing only slurry (no solid manure) increased from 46 to 53%. The share of beef cattle producing only slurry decreased from 32 to 19%.
- Special animal-friendly housing systems for fattening pigs including a multi-area pen with a littered area and an outside yard were not operated in 1990 but used for 50 to 60% of the animals in 2010.
- The storage volume for slurry increased by about 30% between 1990 and 2010. While the share of covered slurry stores remained close to 90%, solid covers slightly decreased until 2010 and were replaced by other covers, i.e. perforated covers.
- The mixing frequency in slurry stores decreased. While 60% mixed more than 1x/month in 2002 it was 29% in 2010.
- Low emission spreading systems for slurry were used for 25% of the slurry volume in 2010. Band spreading using a trailing hose was the prevalent technology used.

Conclusions

The three surveys show a considerable development of farming practice over recent years. In general, the results seemed to be plausible. They provide a deepened insight into livestock and manure management. Although the regular surveys require a large investment, they yield valuable data on the development of farming practice and its impacts on various issues such as gaseous emissions. By slightly extending the survey to further relevant management parameters, it could yield valuable data for emission inventory monitoring of other gaseous emissions like methane and nitrous oxide. A careful assessment should be conducted to identify the necessary parameters. Ideally, the different gases to be monitored should then be calculated with the same model to allow a more comprehensive assessment of emissions and emission mitigation options. This would need an extension of AGRAMMON or other models.

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Methane emissions from growing beef cattle grazing semi-natural upland and improved lowland pastures

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Introduction Methane (CH_4) is a by-product of the digestive process in cattle and contributes significantly to the greenhouse gas emissions attributable to agriculture. Grazed grass is a relatively cheap and intrinsically nutritious feed for ruminants but herbage species and quality vary with short-term and long-term pasture management and with land type, potentially impacting on animal performance and CH_4 production (Hart *et al.* 2009). The aim of the current study was to measure the performance and estimate daily CH_4 emissions and forage intakes of beef cattle grazing one of two contrasting pastures; semi-natural upland and improved lowland.

Materials and methods Forty eight spring-born (2010) beef cattle [24 Holstein Friesian steers (HFS); 14 Charolais x steers (CHM) and 10 Charolais x heifers (CHF)] of mean initial live weight (LW) 407 (s.d. 29), 469 (s.d. 36) and 422 (s.d. 50) respectively, were allocated to one of the two pasture types (n = 24 each) according to LW, age, breed and gender. The cattle on each pasture were assigned to four paddocks (two groups of 6 HFS; one group of 7 CHM; one group of 5 CHF). The semi-natural upland site (SNU) was at Glenwherry, Co. Antrim, UK, (Latitude: 54.849353^0, Longitude: -5.993700^0; altitude: 312m) at which paddocks were each 3.6ha of botanically diverse grassland. The improved lowland site (IL) was at Hillsborough, Co. Down UK, (Latitude: 54.451906^0, Longitude: -6.075110^0; altitude: 112m) where the paddocks were each 0.9ha and predominantly comprised of *Lolium perenne*. The cattle grazed these pastures from 01/06/11 to 29/09/11 and LW, CH_4 emissions and dry matter intakes (DMI) were estimated between 20June – 1 July (P1), 18-29 July (P2) and 15-26 August (P3). Methane emissions were estimated using the SF_6 technique of Johnson *et al. (*1994). To enable this, a pre-calibrated, slow-release brass permeation tube containing SF_6 (mean release rate: 2.64 mg/d; s.d. 0.596) was inserted into the rumen of each animal on 26 May 2011. DMI was estimated by the long-chain *n*-alkanes method of Dove *et al. (*1986) which required cattle to be intra-ruminally dosed with 2x (500mg) boluses of C_{32} alkanes once daily from days 1-11 of each period. Forage samples (200g fresh wt), closely representative of the herbage grazed, were collected between days 5 and 10 from each paddock in each period. Faeces samples (at least 100g) were taken *per rectum* from all cattle (concurrent with C_{32} dosing) between days 8 and 11. Faeces samples were bulked for each animal for all 5 days of collection in each period. The experimental design was a factorial of 2 (pasture types) x 4 (breeds/gender) x 3 (period). Data were statistically analysed using a REML (GenStat 15.1.) repeated measures model, with breed and gender fitted as random effects. The correlation between time points was accounted for using an autoregressive model of order 1. Gender was fitted as a main fixed effect together with the main effects and all interactions of site x breed x time.

Results Cattle grazing IL pasture emitted 15% more CH_4, had 25% higher LWG and 9% higher DMI but produced 17% less CH_4 per kg LWG than the cattle grazing SNU. The 7% more CH_4 emitted by the IL cattle was not significantly higher than those on SNU.

Table 1 Estimated CH_4 outputs, DMI and liveweight gain (LWG) of growing cattle grazing SNU or IL pasture.

	IL	SNU	s.e.d.	P
CH_4 (g/d)	197.9	168.8	6.193	<0.001
LWG (kg/d)	0.89	0.64	0.042	<0.001
CH_4 (g/d) per kg LWG	229.2	276.7	16.37	<0.01
DMI (kg/d)	9.6	8.7	0.196	<0.001
CH_4(g/d) per kg DMI	21.15	19.80	0.785	n.s.

Conclusions The greater DMI and LWG of cattle grazed on the lowland (IL) site in the current study than of those grazed on the SNU) site reflected the poorer nutritive value of the latter pasture. The more efficient use of the higher quality IL resulted in less CH_4 per kg of LWG than in similar cattle grazing SNU. The similar CH_4 emissions per kg DMI between the two pasture types follows a similar pattern for emission from cattle fed either a high or low digestibility grass diet under a zero-grazing protocol (Hart *et al*. 2009). It is likely that while the SNU cattle emitted less CH_4, they would take longer to finish, hence off-setting this advantage; however the contribution of grazing cattle on SNU as an ecosystem management aid i.e. promoting biodiversity (Dawson *et al.* 2011) needs to be considered when evaluating the role of grazing on semi-natural or unimproved grassland.

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Measurement of methane from finishing cattle fed either a forage-based or high concentrate diet from both feeder-mounted samplers and respiration chambers

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Introduction Although measurement of methane (CH_4) using respiration chambers is accepted as the most accurate means of estimating CH_4 emissions from ruminant livestock, limitations are the relatively low throughput of measurements possible and the need to confine animals individually. In a beef enterprise, capturing breath samples when cattle are feeding and measuring breath CH_4 concentrations may provide a more cost-effective alternative means of estimating individual CH_4 emissions. As a first step in validating the use of feeder-mounted samplers, simultaneous measurements of CH_4 emissions were made using respiration chambers and feeder-mounted hoods located within the chambers.

Material and Methods Aberdeen Angus x Limousin (AA x LIM) or Limousin x Aberdeen Angus (LIM x AA) cross-bred steers (n=72) were fed complete diets (g/kg, dry matter (DM) basis) consisting of either 480 forage: 520 concentrate (F) or 75 forage: 925 concentrate (C) respectively. Diets were offered *ad libitum* to steers once daily. The experiment was a 2 x 2 factorial arrangement of breed and diet. Steers were allocated to respiration chambers using a replicated (3 times) randomised block design so that allocation was balanced for live-weight, breed and diet. Six indirect open-circuit respiration chambers were used, with gas production being recorded for the last 48 h of a 72 h measurement period. Within each chamber, the feeder was mounted on a weigh cell to allow continuous recording of feed intake. The feeder was enclosed within a clear polycarbonate hood through which air was continuously extracted by a fan at known flow. Air was sampled for gas analysis from the hood. Gas concentrations (10 per h for each chamber and ambient air and 30 per h for each feeder-mounted hood) were measured by infrared absorption for CH_4 and carbon dioxide (CO_2; MGA3000, ADC Ltd., Hoddesdon, UK). Dry air flow, corrected to standard temperature and pressure was calculated for each individual chamber record and daily CH_4 production was calculated as the average of individual values and converted to a mass basis. For feeder-mounted hoods, valid records were defined as records where data from feeder weigh cells and increased CO_2 concentration both indicated the presence of the steer within the hood. Valid values for each steer were corrected for background chamber CH_4 concentrations and the mean of these values multiplied by air flow to give estimates of CH_4 production. There were valid data for 55 steers. Data were analysed using Genstat using linear mixed models where the factors were the 2 x 2 arrangement of breed and diet, block, chamber and week of experiment. Comparisons between hood and chamber measurements were made by simple correlation, and multiple regression was used to explore relationships between hood CH_4 and other variables.

Results Chamber CH_4 production was greater ($P<0.001$) for diet F than C on both a total (205 v 144 g/d, s.e.d. 12.6) or per kg DM intake (21.8 v 13.6 g/kg DM intake s.e.d. 1.20) base. Feeder-mounted hood CH_4 (l/h) was also greater for diet F than diet C (2.65 v 1.62, s.e.d. 0.21, $P<0.001$) and there was a positive correlation (Figure 1, $r^2 = 0.47$; $P<0.001$) between daily CH_4 output from chambers and feeder-mounted hood estimates. There were also a significant relationship between feeder-mounted hood CH_4 (y) and diet, dry matter intake and methane output: y = -0.94 + 0.58 * diet + 0.0058 * methane output (g/d) + 0.104 * DM intake (kg/day), $r^2 = 0.59$; $P<0.001$.

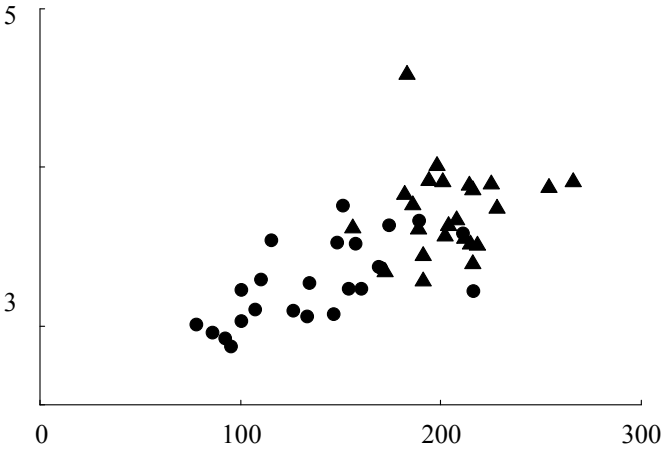

Figure 1 Relationship between daily CH_4 output (x, g/day) from respiration chambers and CH_4 measured in feeder-mounted hoods (y, l/h). Circles denote concentrate diet; triangles, forage diet

Conclusions The measured increases in hood CH_4 concentrations over chamber background agreed well with daily CH_4 outputs and displayed the expected relationships with diets fed and DM consumption. Research is currently underway to establish the relationship between measurements of CH_4 from feeder-mounted hoods made in an open shed and measurements made on respiration chambers.

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Quantifying variation in methane emission between cows by use of IR-technique

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Introduction Methane (CH_4) occurs in the rumen as an end-product from the degradation of feed, particularly fibre. Microbial populations, predominantly methanogens, in the rumen synthesize CH_4 in a way to keep the partial pressure of hydrogen low to allow an efficient anaerobic fermentation. It is known that there is a variation in enteric CH_4 emissions between cows. Variation is due to both animal and dietary factors. Variations between cows could make it possible to mitigate enteric methane production by the use of genetic selection. Use of animal variation requires a better understanding of the biological basis in order not to impair the cow's unique ability to digest fiber. An earlier study by Danielsson *et al.* (2012) confirmed on a small number of cows that the total number of methanogens does not correlate with the CH_4 yield, it is rather depending on specific species of methanogens that occur in the rumen. Based on that study, this study will further investigate the relationship of the rumen microbial population, ruminal factors and CH_4 produced in a larger population of cows. In this study direct measurements on lactating cows are performed with an aim to rank cows as high or low emitters and relate that to their ability of utilize fiber. Analyzes of the microbial composition of rumen fluid will be included as a way to increase the understanding of the differences.

Material and methods The study is performed at the Swedish Livestock Research Centre at Lövsta Uppsala from October 2012 until May 2013. In total 80 dairy cows (40 Swedish red and 40 Holsteins) in mid-lactation are included, approximately 40 % primiparous cows and 60 % multiparous. Cows are housed indoors in an automatic milking system. Silage and concentrate is fed in a 60:40 proportion to all cows throughout the period. Feeding levels is based on calculations of the individual energy requirements of each cow according to the Swedish standards for dairy cows. Each cow is included in the study during 90 days in the period 90 to 180 days post parturition. Three specific sampling weeks take place during the period, the first occur around 90 days, the second around 135 days and the third around 180 days of lactation. During the sampling weeks; samples of milk, faeces and rumen fluid are collected for further analyses. Milk will be analyzed for composition and fatty acid profile. Acid insoluble ash (AIA) in feed and faecal samples is used as an internal marker for the estimation of the apparent in vivo digestibility. Rumen fluid is sampled by a stomach tubing method described by Shingfield *et al.* (2002). Microbial population analyzes will be performed on the rumen fluid. Feed intake and milk production is recorded automatically on a daily basis. Methane is monitored continuously during the whole 90 days period. IR-equipments (Garnsworthy *et al*., 2012) for CH_4 measurement are placed in the concentrate feeder of the milking robot and in two concentrate feeders outside the robot. Automatically measurements occur each time a cow comes in to the robot for milking and when the cow comes into one of the concentrate feeders.

Results The aim of these studies is to get a better understanding of the variation among cows in enteric CH_4 production over the lactation. Results will be linked to the results from Danielsson *et al.* (2012), where the relation between CH_4 production and total number of methanogens were not correlated. No significant difference ($P<0.05$) were seen in mean CH_4 yields between the two diets with different forage/concentrate proportions 900/100 and 500/500, 16.9 and 20.2 g/kg dry matter intake, respectively, the opposite were shown in total number of methanogens which were significantly higher in the 500/500 diet ($P<0.0004$). Further, individual cows responded different to the diet, which strengthen the theory of the variation between cows. We are complementary interested in investigating the relationships between methane production and fiber digestibility. Possibilities to use fatty acid profile in milk as a proxy for methane production will also be part of the study. Some preliminary results will be presented in our poster.

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Use of autofeeders vs bunk feeding systems affects feed intake and efficiency but not carcass attributes of feedlot cattle

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Introduction Estimates of total methane emissions of feedlot-fed cattle are dependent on measurements of feed intake and efficiency, which are usually recorded on individual animals using auto feeding (AF) systems, such as that used by the Australian Beef CRC (Robinson and Oddy, 2004). Analyses showed a tendency for feed intake to be reduced if more than 12 animals are housed in the same AF pen, which contains a single auto feeder (Robinson, 2005). With average feeding times of up to 2 hrs per head per day for some groups (Robinson *et al*., 1997) the AF is occupied for a considerable proportion of the day and night. It is therefore important to know how intake and efficiency of animals in AF systems compares to those of similar cattle in pens with conventional bunk feeders.

Material and methods Composite breed steers (Brahman-based, n=384; initial LW = 414 kg) were introduced to the feedlot and adapted to a diet based on cracked barley (70.3%) and maize silage (8.5%) with an NIR derived ME content of 13.1 MJ/kg DM. Cattle started their finisher diet on day 20, in 14 AF and 18 bunk pens, with 12 steers per pen and one of 4 treatments applied to the feed in each pen – nitrate supplementation (0.95% or 1.90% NO_3 as calcium nitrate in DM), or urea (0.50% or 1.0% in DM). Further details and discussion of treatment effects are provided by Hegarty *et al*. (2013). A few steers originally allocated to AF pens that did not learn to use the auto feeders within 2 days were moved to bunk pens of the same treatment, resulting in an average of 13.7 steers per bunk pen and 10 steers per AF pen. All weigh scales used to measure feed delivery and refusals were cross checked to ensure consistency. The statistical model for growth rates and carcass characteristics was based on individual animal data, with random pen and animal effects. For feed intake data, the analyses used individual data for animals in AF pens and means for bunk pens, with separate residual variance terms for individual and aggregate animal data as well as random pen effects. These models were checked by comparison with analyses of means for both AF and bunk pens which led to almost identical conclusions.

Results As shown in Table 1, steers in the AF pens had similar initial weights, no reduction in weight gain, and similar scan and carcass fat measurements to steers in the bunk pens. However, their DMI was significantly lower ($P < 0.001$), especially in the latter half of the finishing period ($P = 0.006$) resulting in 7.6% greater FCE over the 90 days of feed intake measurement ($P < 0.001$).

Table 1 Least square means, differences and P values for comparisons of AF vs bunk pens

Weights, Feed Intake and Efficiency					Scan and Carcass Characteristics				
	AF	Bunk	s.e.d	P		AF	Bunk	s.e.d	P
Initial weight (kg, day 1)	414.7	413.5	2.68	0.655	Scan rump fat (mm)	12.59	12.08	0.326	0.117
Final live weight (kg)	595.8	588.3	4.37	0.086	Scan rib fat (mm)	7.40	7.50	0.173	0.578
Weight gain (kg/day)	1.68	1.62	0.042	0.138	Scan IMF%	5.20	5.16	0.064	0.506
DMI (kg, days 20-109)	10.47	11.00	0.150	< 0.001	Live muscle score	7.55	7.40	0.252	0.575
FCE (kg/kg)	0.159	0.148	0.003	< 0.001	Carc. rump fat (mm)	12.8	13.6	0.536	0.105
DMI (kg, days 20-64)	10.21	10.59	0.220	0.093	Carc. rib fat (mm)	6.25	6.38	0.361	0.718
DMI (kg, days 65-109)	10.80	11.36	0.204	0.006	MSA marble score	338.8	344.2	7.73	0.482
Carcass weight (kg)	333.6	328.0	3.15	0.078	Ausmeat marble score	0.961	1.084	0.081	0.127

Day 1 = 1 June 2012. Final live weight, ultrasound scans and live muscle scores recorded on day 110/111; slaughter was on day 126. Carcass characteristics were adjusted to the mean carcass weight of 330 kg. Initial weight was fitted as a covariate for all other traits (except weight gain) to allow for differences in weights of the steers before starting the trial.

Conclusions Experiments that measure feed intake, efficiency and methane emissions of individual animals may change animal behaviour in ways that alter feed intake and efficiency and therefore emissions. Restricting feed intake can improve efficiency, but often at the expense of reduced weight gain (Murphy and Loerch, 1994). Animals in AF pens may have to wait for access to feed, resulting in a small reduction in feed intake. The resultant 7.6% increase in FCE seems comparable to the effects of some treatments that might be used to improve efficiency. Future research and greenhouse accounting procedures may need to consider such effects.

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Quantification of ammonia emissions and greenhouse gas concentrations from a naturally ventilated dairy housing with an outdoor-exercise area

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Introduction One goal of the "Agricultural Climate Strategy" in Switzerland is a more than one third reduction in greenhouse gas GHG emissions from agriculture by 2050 compared to 1990 level (Wiedemar and Felder 2011). Additionally, NH_3 emissions need to be reduced by approximately 40% according to the Agricultural Environmental Targets (BAFU and BLW 2008). The NH_3 and GHG reduction potential of mitigation measures, which can be implemented in Swiss dairy housing systems, is currently not known. We present a dual tracer-ratio method in combination with continuous NH_3 emission measurements and analysis of GHG concentrations as a tool to evaluate housing systems and quantify reduction potentials.

Materials and methods Emission measurements in naturally ventilated housing systems are challenging from both a methodological and conceptual point of view as air flow rates are difficult to quantify. The Research Station Agroscope Reckenholz-Taenikon ART and Empa have developed a tracer-ratio method with two tracer gases (SF_6 and SF_5CF_3) to determine NH_3 emissions from natural ventilated housings and from area sources such as outdoor-exercise areas (Schrade *et al*. 2012). This technique allows quantification of emissions from two separate areas and modelling of their source intensities. The diluted tracer gases are continuously dosed directly to the emitting aisles via a pipeline system with so-called critical capillaries. For representative sampling of tracer and target gases a gas-collection system consisting of Teflon tubes with critical orifices is mounted 3 m above the ground. Tracer gas concentrations are analysed simultaneously on a gas chromatography system (GC-ECD, Varian) while NH_3 was quantified with a commercial photoacoustic analyzer (TGA-310, Omnisens). The measurements presented below were carried out in both summer and winter season in a naturally ventilated loose housing with cubicles (40 cows), solid floor surfaces and outdoor-exercise area alongside the housing. As part of these studies indicative measurements of greenhouse gases (CH_4, N_2O, CO_2) were taken on three days during the summer measuring period. The samples were collected in one sampling line inside the housing and in one sampling line in the outdoor-exercise area in Tedlar™ bags (40 l) integrating over 24 hours. Bags with unpolluted ambient air (background) were sampled over one day upwind of the housing. Samples were analyzed in the laboratory for N_2O and CH_4 by a quantum cascade laser absorption spectrometer (QCLAS) and for CO_2 by a non-dispersive infrared (NDIR) analyzer.

Results The NH_3 emissions showed a strong seasonal dependence with daily average values between 38.9 to 48.3 g LU^{-1} d^{-1} in summer and 13.2 to 15.5 g LU^{-1} d^{-1} in winter. The outside temperature varied between 7 to 25 °C in summer and 1 to 9 °C in winter season. Mixing ratios of N_2O and CO_2 inside the housing and in the outdoor-exercise area were close to background concentrations (Fig. 1 a and b). CH_4 concentrations in the housing and outdoor exercise area were increased as compared to ambient air. Differences between housing and the exercise area were highest for day 2 and might be due to strong rainfall events (Fig. 1 c).

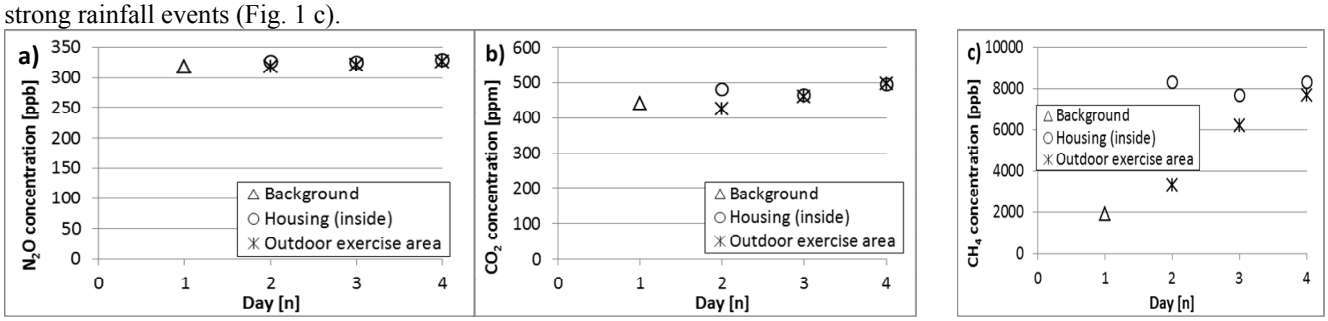

Figure 1 Mixing ratios of N_2O (a), CO_2 (b) and CH_4 (c) in the ambient air (background), inside the housing and in the outdoor-exercise area during the summer measuring period.

Outlook An experimental housing for two dairy herds (reference group and mitigation measures group) will be built and provide opportunity for the progressive development and comparative assessment of reduction strategies (housing concept, feeding stalls, exercise area design, manure removal, feed ration etc.) and quantification of their reduction potential. Emission measurements will be based on the described dual tracer-ratio method which will allow to disentangle and compare emissions from the "reference housing" and the tested measures. Tracer gases, NH_3 and the GHG CO_2, CH_4 and N_2O will be analysed in real-time by GC-ECD and laser spectroscopy. These studies will be important steps in the reduction of NH_3 and greenhouse gas emissions and the attainment of climate change objectives as well as environmental NH_3 emission targets. This approach will improve the sustainability and carbon footprint of dairy products at the milk production stage.

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Comparison of biomarker and molecular biological methods for estimating methanogen abundance

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Introduction The method of choice for estimating methanogen abundance in samples derived from the ruminant digestive tract is quantitative real-time PCR (qPCR). Primers to quantify these methanogens have been based on different sections of a number of genes, including the methanogen 16S rRNA (*rrs*) gene and the methyl-coenzyme M reductase α-subunit gene (*mcr*A). The different primers have been used with a wide range of sample types and results expressed in a number of different ways, resulting in considerable confusion. Archaeol is a promising alternative marker for methanogen abundance, including in ruminant digesta. It is a membrane lipid ubiquitous in methanogenic Archaea that can be quantified by gas chromatography-mass spectrometry (GC-MS). The suitability of archaeol as a molecular proxy for methanogen abundance has not yet been assessed. The aim of this experiment was to compare total methanogen populations estimated using the new archaeol approach with estimates based on qPCR.

Materials and methods Rumen fluid samples were selected from a previous study (McGeough *et al*., 2010). There were three samples each from four forage-based dietary treatments. Rumen fluid was lyophilized over 2 d prior to archaeol analysis according to the methods outlined by McCartney *et al*. (2013). Genomic DNA was isolated from the ruminal fluid using the established bead beating method. Specific primer sets and probes were used to detect dominant methanogen species *M. ruminantium, M. smithii, M. stadtmanae* and total methanogen populations, along with a prokaryote reference gene. qPCR was performed using either SYBR green chemistry or FAM dye. Methanogens were quantified relative to the prokaryote *rrs* gene using the equation: $\Delta Ct = 2^{-(Ct\ methanogen - Ct\ reference)} \times 10^6$. ΔCt values were then expressed on a sample DM basis. Relationships between the various estimates of methanogen abundance (based on qPCR and archaeol) were made using simple linear regression (one outlier was identified on the basis of Cook's test and excluded from subsequent analysis).

Results and Discussion The relationships between qPCR-based estimates of methanogen abundance (ΔCt relative units, DM basis) and archaeol (mg/kg DM) are presented as a correlation matrix in Figure 1. The different techniques resulted in quite different ranking of methanogen abundance, and there was considerable variability in the relationships between estimates. This may have been the result of: amplification bias of the total methanogen primers, the absence of *mcr*A in some methanogen species, and the (albeit negligible) presence of non-methanogenic Archaea. Problems of primer specificity and/or low abundance of individual species help to explain weak relationships between *rrs* estimates. The strongest relationship was between estimates based on archaeol and the *rrs* gene using primers specific for *M. ruminantium,* which is widely regarded as the most abundant methanogen in the rumen. This suggests there is less variation associated with *M. ruminantum* estimates than *rrs* total methanogen estimates, perhaps as a result of primer specificity problems with universal methanogen primers. Furthermore, the data imply that universal methanogen primers for *mcr*A and *rrs* genes preferentially amplified *M. smithii*.

RRS	r = 0.82 P = 0.002				
RUM	r = 0.31 P = 0.351	r = 0.71 P = 0.015			
SMI	r = 0.91 P < 0.001	r = 0.83 P = 0.001	r = 0.44 P = 0.175		
STAD	r = 0.36 P = 0.281	r = 0.45 P = 0.164	r = 0.64 P = 0.033	r = 0.48 P = 0.138	
AR	r = 0.53 P = 0.097	r = 0.72 P = 0.013	r = 0.87 P < 0.001	r = 0.57 P = 0.068	r = 0.61 P = 0.046
	MCRA	TM-R	RUM	SMI	STAD

Figure 1 Correlation matrix showing the relationships between archaeol concentration (mg/kg DM) and the abundance of total methanogens/dominant methanogen species (ΔCt relative units, DM basis) in rumen fluid. (RRS) *rrs*: total methanogens, (RUM) *rrs: M. ruminantium*, (SMI) *rrs: M. smithii*, (STAD) *rrs: M. stadtmanae*, (AR) archaeol, (MCRA) *mcr*A: total methanogens.

Conclusions The relationships between total methanogen abundance estimates based on archaeol and qPCR were variable. The universal methanogen primers for *mcr*A and *rrs* genes appeared to preferentially amplify genes from *M. smithii.* Archaeol had the strongest relationship with the dominant rumen methanogen *M. ruminantium*. Archaeol analysis was a useful adjunct to molecular biology methods; it seems that a good specific primer for *M. ruminantium* is more useful than a biased primer for total methanogens.

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What affects CH_4/CO_2 ratio in cow's breath

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Introduction Enteric methane from livestock is a major contributor to anthropogenic methane production. Indirect calorimetry is the golden standard, but it is very resource demanding when many animals are to be assessed. Madsen *et al.* (2010) suggested CO_2 to be used as an internal marker combined with measurement of the CH_4/CO_2 ratio to estimate the enteric CH_4 production. This seems to be a promising way to measure the enteric methane production on many animals in a short time, but it is important to know what affects the CH_4/CO_2 ratio. The aim of this study was to analyse how animal characteristics and feed parameters affect the CH_4/CO_2 ratio of dairy cows on data obtained from respiration chambers.

Material and methods A data set with 157 observations on lactating dairy cows from 8 different experiments covering 30 different diets was used. All data was obtained from the respiration chambers at Aarhus University. The ratio between CH_4 and CO_2 was calculated from the mean daily excretion of CH_4 and CO_2 (Hellwing *et al.*, 2012). For each animal 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, 3.14 MJ/kg), production of CH_4 (L/d) and CO_2 (L/d), loss of methane energy as % of gross energy (CH4%) and concentration of crude protein (CP), crude fat (FAT) and carbohydrate (CHO) in the diet dry matter was compiled. Data was analysed with PROC STEPWISE in SAS with the stepwise procedure. Variables entered the model if $P<0.15$ and were omitted again if $P>0.15$.

Results and discussion The CH_4/CO_2 ratios varied between 0.053 and 0.105 with a mean of 0.084±0.008 (s.d.). The daily consumption of O_2 was not included in the data analysis, as the correlation coefficient between O_2 and CO_2 was 0.95. Other variables were also correlated but kept in the analysis to explain variance. The stepwise analysis included CH4%, DMI, CO_2, CH_4, FAT, CHO and DIM in the model. Mean, standard deviation, parameter estimate, P, partial and model R^2 are given in Table 1 for all variables explaining more than 1% of the variation in the CH_4/CO_2 ratio.

Table 1 Mean, standard deviation (s.d.), parameter estimate, P, Partial and model R-square for variables that explained more than 1% of the variation in CO_2/CH_4 ratio.

Variable	Mean±s.d.	Estimate	P	Partial R-square	Model R-Square
Intercept		0.042	<0.001		
CH4% [% of GEI]	6.20±0.8	0.005	<0.001	0.54	0.54
DMI [kg/d]	19.6±2.7	-0.001	<0.001	0.06	0.60
CO_2 [L/d]	6856±922	-0.00001	<0.001	0.36	0.96
CH_4 [L/d]	577±95	0.00009	<0.001	0.03	0.99

Table 1 shows that CH4% explained 54% of the variation in the ratio between CH_4/CO_2. Daily CO_2 production explained 36% of the variation, and DMI and daily CH_4 explained 6% and 3% of the variation, respectively. The parameter estimates for CH4% and daily CH_4 production were positive and were negative for DMI and CO_2. The partial R^2 of 54% for CH4% demonstrated the strong relationship between CH4% and the CH_4/CO_2 ratio. This showed that cows with a high loss of gross energy as methane also had a high CH_4/CO_2 ratio. The correlation coefficients between CO_2 and CH_4, DMI and CO_2, and DMI and CH_4 were 0.85, 0.79 and 0.69, respectively. Even though these correlation coefficients were relatively high, all three variables helped to explain the variation in CH_4/CO_2 ratios. Notably both daily production of CO_2 and CH_4 were included in the model to explain the variation in CH_4/CO_2 ratio. The metabolism of protein, fat and carbohydrates produces different amounts of CO_2 and CH_4, and thereby affects the CO_2/CH_4 relationship differently. Moreover, more active animals produce more CO_2 and therefor have a lower CO_2/CH_4 ratio. The CH_4 production from the rumen was affected by the fermentation pattern of e.g. carbohydrates. Four out of the five highest ratios between CH_4/CO_2 measured were on diets with a high sugar concentration (240 g/kg DM). The ratio between CH_4/CO_2 is the final result of the different metabolic processes both in the rumen and the body.

Conclusions It is concluded that CH4% explained 54% of the ratio between CH_4 and CO_2 which indicates that cows with high CH_4/CO_2 ratios also have the highest loss of methane in % of gross energy. Daily CO_2 production, DMI and daily CH_4 production explained additional 36%, 6% and 3% of the total variation.

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