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IN VIVO METHANE DETERMINATION IN A SMALL CHAMBER SYSTEM

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SUMMARY

Confinement respiration chambers for gaseous exchange measurements are expensive and the aim of this work was to develop a small chamber system using material easily available in warehouse to measure methane (CH₄) released by sheep. The chambers (four) were adapted from metabolism cages commonly found in animal nutrition laboratories. Each chamber was 157 by 71 by 167 cm (volume 1.9 m³) and they were covered on its sides except the bottom with 7.7 m² polyethylene plastic, 0.3 mm thick. The chamber had one in-let opening (5 cm diameter) in the front and one out-let opening in the rear (5 cm diameter) in which an exhaust pump was connected to remove the inside air at a flow rate of 168 L/min (measured using an anemometer). The outgoing air was sampled into a balloon (coated with aluminium film) at 100 mL/min by using a peristaltic pump. Inside each chamber, a house-hold fan was fixed for circulating air so as to keep temperature and carbon dioxide at levels comfortable to the animal. Temperature and humidity was measured at regular interval using digital meters. The outflow air was also sampled at hourly interval with a 50 mL syringe and transferred to vacuntainer tubes to check if the balloons were able to hold the CH₄ and the values obtained by two methods are same. Methane was measured using a GC.

Methane output measured from the CH₄ levels in vacuntainers in which the gas was collected manually, when added up for 24 h, did not statistically differ from the CH₄ output using balloon samples (43.3 and 52.7 (s.e. = 7.88) ppm respectively). In this chamber set up, a known amount of CH₄ was released and recovery of 107 ± 9.8 % was obtained suggesting that the chamber could be used to measure CH₄ from small ruminant animals (sheep and goats). These chambers were used to measure the effect of coconut oil and a tannin-rich browse (72 g/kg condensed tannin; leucocyanidin equivalent) supplementation on CH₄ production.

Twelve Santa Ines sheep were allocated into three groups (liveweight = 47 ± 11.9 kg). All animals were fed a basal diet (Tifton-85 hay-*Cynodon sp.*, corn grain, soybean meal, cottonseed meal and mineral mixture). The control group (C) received only the basal diet and the other groups received on top of that 22 g/kg.day of coconut oil (T-OIL group) in the concentrate (2% in total diet) or 127 g/kg.day of *Mimosa caesalpineaeifolia* (T-MIM group) replacing the forage (24 % in total diet). The forage to concentrate ratio of diets were 69:31; 43:57 and 66:34 (C, T-OIL and T-MIM respectively) and dry matter intake differ ($P < 0.05$) among treatments (1.2, 0.8 and 1.3 (s.e. = 0.06) kg/day respectively for C, T-OIL and T-MIM). After 30 days of feeding the diets, the animals were individually kept in the chambers for measuring CH₄ production. The gas collections were done on two occasions (each of 18 to 22 h) for each sheep. Rumen fluid samples were collected at day 0 and day 28 and kept in a freezer. Methane released by C group was significantly ($P < 0.023$) higher (31 mL kg/DMI) than T-OIL (20 mL kg/DMI) or T-MIM (20 mL kg/DMI) (s.e. = 4.4). Protozoa numbers ($\times 10^5$) were lower ($P = 0.07$) in T-OIL and T-MIM than in C group (7.5, 8.5 and 10.7 mL⁻¹, respectively).

It is concluded that the small chamber system could identify the differences in CH₄ production *in vivo* and that coconut oil can reduce CH₄ release although its addition compromises animal performance. This preliminary study also shows that *M. caesalpineaeifolia* may have potential for reducing CH₄ emission from ruminants.

ABOVEGROUND CARBON STOCKS, NITROUS OXIDE AND METHANE FLUXES IN DIFFERENT LAND USE SYSTEMS IN THE PERUVIAN AMAZON

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SUMMARY

Currently there is general agreement, based on land use change data and atmospheric data, that the tropics are a net source of C to the atmosphere in the range of 1.1 to 2.1 Pg C/year (Houghton 1997). Sanchez et al. (1994) estimated total deforestation for over 627 million ha, or approximately 40% of the potential humid forest zone with 120 million ha of these lands subject to shifting cultivation or slash-and-burn agriculture.

Tropical forests are cleared for a variety of reasons that include logging, establishment of plantations and pastures, and slash-and-burn agriculture. The primary causes of deforestation differ by country even regions within country, but are usually associated with some form of slash-and-burn agriculture. Either as the primary driving force or as consequence of increases access to forest by logging operations and road construction. Farmers practicing slash-and-burn agriculture are clearing forest to produce food and seek improvement in their families' standards of living. In most cases, they are marginalized from society and government support programs and live in relatively poverty. Effort to reduce deforestation and greenhouse gas emissions resulting from deforestation must address these root causes.

A partnership of scientists from several national and international institutions evaluated the above-and belowground carbon stocks in various land use systems at Yurimaguas and Ucayali benchmark sites in the humid tropics of Peru. Each evaluation was accomplished using the procedural guidelines developed by Tropical Soil Biology and Fertility (TSBF) and other partners for the alternatives to slash and burn program (ASB).

When forest is converted to agricultural uses, aboveground carbon stocks are considerably reduced (less dense and lower vegetation replaces woody species). As expected, managed forest and older natural fallows have the highest carbon contents. As fallows mature into secondary forest, they increase their carbon content. Among tree-based systems, the carbon content of perennial system is relatively high, ranging from 41 t/ha for oil palm plantation to 74 t/ha for rubber plantations (Ucayali). Carbon of multi-strata agroforestry systems (Yurimaguas) have a permanent under-story of a kudzu pasture, which increase the carbon stocks by 2-5 t/ha. Pastures contained the lowest quantities of carbon (2 t/ha).

In another study, monthly nitrous oxide and methane fluxes were measured in two cropping systems, three tree-based systems (include silvopastoral system) and 23-year secondary forest control. Average N₂O fluxes from the cropping systems were two to three times higher than the secondary forest control (9.1 μg N/m².h), while those of tree based-systems were similar to the secondary forest. Increased fluxes in the cropping system was attributed to N fertilization, while fluxes from the tree based-systems were related to litterfall N. Average CH₄ consumption was reduced by up to half that of secondary forest (-30.0 μg C/m².h) in the tree based-system and low input cropping system. There was net CH₄ production in the high input. This switch to net production was a result of increased bulk density and increased soil respiration resulting in anaerobic conditions.

Key words: contamination, three based-systems, silvopastoral, GHG emissions

EFFECTS OF SAKE YEAST FEEDING ON METHANE PRODUCTION, DIGESTIBILITY, ENERGY RETENTION AND RUMINAL FLUID OF WETHERS

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SUMMARY

It has been reported that some strains of *Saccharomyces cereviae* reduced methane production. In *Saccharomyces cereviae* sake yeast is more biological active than the other *Saccharomyces cereviaes*. So, in this study, effects of sake yeast feeding on the methane production, digestibility, energy retention and ruminal fluid of wethers were investigated. Three treatments: (i) sake yeast Kyoukai 7gou feeding (4 g/day), (ii) sake yeast Kyoukai-9gou feeding (4 g/day) and (iii) control were allotted 3 × 3 latin square using 3 cheviot wethers. Methane production was not changed by yeast feeding. By feeding sake yeast Kyoukai 7gou, the degradability of hemicellulose was slightly increased.

Efficiency of feed energy utilization was decreased by sake yeast kyoukai 9gou feeding. Total VFA concentration of ruminal fluid was decreased by sake yeast kyoukai 7gou feeding. Yeast feeding did not affect the ruminal pH, ammonia nitrogen content and rate of acetate/propionate. In this study, methane production and ruminal fluid characteristics were not changed by yeast feeding. As the reason for above phenomenon, the amount and form of yeast fed to wethers should be considered, furthermore, the survival of yeast in the rumen should be also considered.

INTRODUCTION

It was reported that *Saccharomyces cerevisiae* might stimulate ruminal propionic acid producing bacteria and increase propionic acid production from lactic acid (Nibist and Martin, 1991). Furthermore the addition of *Saccharomyces cerevisiae* enhanced hydrogen utilization of acetic acid producing bacteria (Chaucheyras 1995). So, it can be postulated that increment of metabolic hydrogen usage may suppress ruminal metanogenesis. For the effect of *Saccharomyces cerevisiae* on the ruminal methane production, Williams(1988) reported the depression of metanogenesis by *Saccharomyces cerevisiae*. In *Saccharomyces cerevisiae*, bread yeast and sake yeast are widely used and biological active (Inoue *et al.* 2000; Ando *et al.* 2005). It can be expected that the effect of sake yeast on ruminal methanogenesis may be strong. So, in this study effect of sake yeast on methane production, feed digestibility, energy retention, ruminal fluid of sake yeast fed wethers were investigated.

MATERIALS & METHODS

Sake yeast Kyoukai 7gou(K7) and Kyoukai 9gou(K9) were used in this study. One loopful of above yeast grown on YM-solid plate media was placed into 10 mL of YM Broth solution media. These solutions were incubated for 48 h at 30°C. These solution were placed into 100 mL of YM Broth solution media. Again these solution were incubated for 48h at 30°C. Yeast was obtained by centrifuge (400 rpm for 10min) and freeze dried. Sake yeast Kyoukai 7gou feeding (4 g/day), Sake yeast Kyoukai-9gou feeding(4 g/day) and control were allotted 3×3 latin square using 3 cheviot wethers fed timothy hay (40%), alfalfa hay (40%) and concentrate (20%). One period consisted of 7 days for adaptation, 5 days for faeces and urine and 1 day for measuring for gas metabolism. Gas metabolism was measured by using metabolism cage. Heat production was calculated by Browers's formula. Chemical composition of feed and faeces were measured by ordinal method. Nitrogen content of urine was measured by Kjeldal method. Energy content of feed, faeces and urine were measured by bomb calorie meter.

RESULTS & DISCUSSION

Table1 shows the effect of sake yeast feeding on the methane production. There were no significant differences among treatments. Table 2 shows the effect of sake yeast feeding on the feed digestibility. There were no significant differences among treatments. But for hemicellulose digestibility, slightly lower value was observed in Kyoukai 7gou feeding than in controll. Table 3 shows the effect of sake yeast feeding on nitrogen retention. In Kyoukai 7gou feeding, urinary nitrogen content showed higher value than in control and retained nitrogen content showed lower value. Table 4 show the effect of sake yeast feeding on energy retention. In Kyoukai 7gou feeding, urinary and faeces energy and heat production showed higher value than in control and retained energy showed lower value. Table5 shows the effect of sake yeast feeding on ruminal fluid. There were no significant differences among treatments in all items. But for pH higher value was observed in yeast feeding than in control. For ammonia content higher value was observed in yeast feeding than in control. In this study yeast feeding did not affect the feed digestibility and methane production. These results did not support the report of

Ando (2005) and Williams (1988). As the reason for above phenomenon, the amount of yeast fed to wethers was comparatively smaller than former reports. At the same time, yeast used in this was freeze dried. So, the activity or survival of yeast in the rumen might be low.

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Table 1. Effect of Sake Yeast Feeding on Methane Production

	Controll	K-7	K-9
L	36.0	36.0	36.1
L/MBS	1.79	1.82	1.74
L/DM	34.7	36.1	35.1

Table 2. Effect of Sake Yeast Feeding on Feed Digestibility

	Controll	K-7	K-9
Dry Matter	65.5	65.1	64.1
Organic Matter	67.8	67.5	66.6
Crude Protein	69.9	70.3	67.9
Ether Extract	57.9	59.0	54.4
NDF	61.8	61.9	60.8
ADF	58.5	57.3	56.6
Hemicellose	67.7	70.0	68.2
Cellose	68.2	68.0	67.4
Energy	65.5	65.4	64.3

Table 3. Effect of Sake Yeast Feeding on Nitrogen Retention

	Controll	K-7	K-9
Intake	0.99	1.01	0.99
Fece	0.30	0.30	0.32
Urine	0.42 ^a	0.50 ^{ab}	0.54 ^b
Retained	0.27	0.21	0.13

g/1kgMBS/Day

Table 4. Effect of Sake Yeast Feeding on Energy Retention

	Controll	K-7	K-9
Intake	948.4	960.6	943.4
Fece	327.0	333.0	337.7
Urine	33.6	37.1	38.6
Methane	71.5	72.5	69.3
Heat	400.2	411.8	427.8
Retained	116.0	106.3	69.9

kJ/1kgMBS/Day

Table5. Effect of Sake Yeast Feeding on Ruminal Fluid

	Controll	K-7	K-9
pH	6.46	6.51	6.54
NH ₃ - N(mg/l)	16.1	16.3	17.2
Total VFA(mM)	139.0	124.6	130.7
C2 (mM)	103.9	94.1	97.7
C3 (mM)	20.5	17.8	19.4
C4 (mM)	11.4	10.1	10.9

AGRICULTURE AND GREENHOUSE GAS EMISSION CONCERNS IN PERU

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ABSTRACT

Placed over the Nazca plate and along the Andean mountain chain, Peru fronts naturally many climate change phenomena. The El Niño phenomenon and glacier top reductions appears as the most severe extreme changes. In the last 25 years, tropical Peruvian glaciers disappeared in 22% volume (7,000 million cubic meters). El Niño phenomenon has regularly and frequently occurred on Peruvian surface, one more severe than others, for instance in the year 1997-98 it generated economic lost equivalent to 4.5% of national gross domestic product. Heavy rainfalls, droughts, frost, healing, etc. are examples of extreme climate events. In the year 2004 alone the hard frost affected 52 provinces, leaving 360,000 homeless people, 66 frost killed children, 259,110 dead livestock, 1,362,695 frost affected livestock, disappearance of 216,756 ha of native grasslands, 34,948 ha of lost crops, and 111,890 ha unsown croplands.

Total Peruvian lands count for 128.5 million ha and it has 8 of 11 global climatic types. Of these lands 38 % of lands are rain forest, and 42 % are protected areas. Cropping and permanent crop lands represent only about 6 % of the total area. More than 15 million hectares (14 % of total surface area) is native grazing lands, pastures occupies 0.5 million hectares. Animal population (chiefly 'creole' is represented by sheep (10 million), cattle (4.5 million), horses and mules (2.2 million), goats (2.1 million) and South American camelids (3.5 million). All mostly distributed in the grazing lands of the provinces of Cajamarca, Junin, Ayacucho, Arequipa, Puno. These are the main grazing areas in Peru. These areas form part of the 13 priority Peruvian zones to be monitored for greenhouse gas emissions (GGE).

Peru is involved in the Global Climate Change (GCC) convention since 1993 and as a vulnerable country, has the agreement to survey and inventory the GGE along the countryside. The GGE inventory in Peru is those related directly to carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O). Data must be reported as emission source, time and spatial variations. Peru is not engaged to reduce gas emissions, but, it has the agreement to reduce emissions, it also can participate into the mitigation activities by means of the Mechanism Clean of Developing.

The national institutions involved in the GCC convention are represented in a technical committee formed within the National System of Environmental Management which it is chaired by the National Council of Environment. This council is conformed by thirteen institutions related to foundations, navy, education, natural resources evaluation, academy diplomatic, transport, mining sector, meteorological service, non-governmental organizations (NGOs) and presidential office. In the year 2002, this Peruvian group wrote the proposal of the National Strategy for Global Climate Change.

The Andean Countries Community stated that the Andean countries produce less than 2.5 % of global emissions, but it is mainly produced by logging and burning process in Bolivia, Ecuador and Peru. For example, Peru produces 0.4 % of the total GG emissions. Most of the Peruvian methane emissions (98 816 Gg, estimated for the year 1994) comes from pastoral livestock (60-70 %), land use changes (15-25 %) and waste management (15 %).

Pradel, *et al.* (2006) in La Encanada, Cajamarca, which examined the trade-offs between poverty alleviation and environmental contamination in the context of dairy production systems showed enteric methane emissions were higher than the total GG emissions from transport. The above study also showed that technology (base on improvement of feeding strategies) brought positive results in poverty alleviation, malnutrition reduction, improvement in household incomes, reduction in grasslands degradation and improved water management. Accurate GGE inventory activities and research of mitigation technologies requires international cooperation to build up our national capability to reduce the vulnerability areas.

In conclusion, Peru is a vulnerable country to climate change events. For example since Peruvian highlands concentrate 70 % of the world tropical glaciers will likely affect the water supply to 70 % of our population, and this may change climate conditions in the area.

The Peruvian network of interest on GG emissions from agriculture based at Universidad Nacional Agraria La Molina (Lima, Peru) is committed to engage in activities aiming at producing accurate inventories of GGE and researching mitigation technologies and considers that international cooperation is crucial to achieve these objectives.

EFFECTS OF SAKE YEASTS ON RUMEN METHANOGENESIS AND DIGESTIBILITY IN VITRO AND IN VIVO

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ABSTRACT

In experiment 1 (in vitro), the anaerobic incubations were performed for 24 h at 39°C using in vitro continuous methane quantification system attached 4 fomentor jars. Each jar contained 400 mL of artificial saliva and 400mL of strained rumen fluid. The treatments consisted of control (without yeast), Kyokai No.7, Kyokai No. 9 and baker's yeast. In the first trial, 0.08 g/800mL yeasts were added. In the second trial, the addition of Kyokai No.7 ranged from 0.08 g, 0.16 g, 0.24 g, 0.32 g to 0.4 g/800mL. In experiment 2 (in vivo), rumen cannulated three sheep were used in 3×3 Latin square design. Each animal was fed a basal diet consisting of 40% timothy hay, 40% alfalfa hay cube and 20% concentrate on a 55 g DM/kg^{0.75}BW. Treatments were arranged control (without yeast), 4 g/day of Kyokai No.7 and 4 g/day of Kyokai No.9. In Experiment 1 (trial 1), no significant differences in methane emission were observed in added yeast treatment. In Experiment 1 (trial 2), 0.4g of Kyokai No.7 decrease methane emission (P=0.05). In Experiment 2, no significant differences in methane emission were observed in Kyokai No. 7 and No.9. Kyokai No.7 tended to decrease propionate (P=0.10). No any positive effect was observed in supplementation of Kyokai No.9.

INTRODUCTION

Safe methods to control rumen methanogenesis have been developed from an aspect of GHG mitigation. Lila et al (2004) reported that yeast had a potential to reduce methanogenesis. On the contrary, Ando (2003) reported that yeast expedite rumen methanogenesis. Strong resistance of sake yeast to alcohol might be able to adapt its fermentative performance in rumen environment.

The present paper deals with the effects of Sake yeasts (Kyokai No.7 and No.9, Brewing Society of Japan) on rumen methanogenesis and digestibility *in vitro* and *in vivo*

MATERIALS & METHODS

In vitro (experiment 1)

The in vitro continuous incubation system was adopted according to Sar (2005). The 400 mL of incubation media of rumen fluid was collected from fistulated cows, and mixed with 400 mL of McDougall's artificial saliva. Four jars were aligned with control (without yeast), Kyokai No.7, Kyokai No. 9 and baker's yeast. For trial 1, 0.08 g/800 mL yeasts were added. For trial 2, Kyokai No.7 ranged from 0.08 g, 0.16 g, 0.24 g, 0.32g to 0.4 g/800 mL was used. Yeast was incubated with YM broth and used the suspension with H₂O purified.

In vivo (experiment 2)

Rumen cannulated three sheep were used in 3×3 Latin square design. Each animal was fed a basal diet consisting of 40% timothy hay, 40% alfalfa hay cube and 20% concentrate on a 55g DM/kg^{0.75}BW. Treatments were arranged control (without yeast), 4 g/day of Kyokai No.7 and 4 g/day of Kyokai No.9. Yeast was incubated as same as experiment 1, but used the freeze dried one suspended with skim milk.

RESULTS & DISCUSSION

In Experiment 1 (trial 1), no significant differences in methane emission and VFA contents were observed in yeast treatments (Table1). For trial 2, 0.4 g of Kyokai No.7 mitigated methane emission (P<0.05), which might be correlated to A/P ratio in the rumen (P=0.8). In Experiment 2, however, no significant differences in in vivo methane emission were observed in Kyokai No. 7 and No. 9. Kyokai No.7 tended to decrease propionate (P=0.1) (Table 2). In addition, Kyokai No.9 decreased CP digestibility (P<0.05) (Table 3).

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Table 1. Effects of kyokai7 (k-7), kyokai9 (k-9) and baker's yeast (Bak) on *in vitro* ruminal fermentation
Mean values within a row with different superscripts significantly different ($P < 0.05$)
Values are means ($n = 4$) of 6 sampling times (0, 2, 4, 6, 8, 10, 12 and 24 h), except mean values for methane production is means ($n = 4$) of 24 h incubation

Fermentation parameters	Yeast in trial 1					Yeast in trial 2						
	No-additives				s.e.m.	K-7					s.e.m.	
	Control	k-7	k-9	Bak		Control	0.08g	0.16g	0.24g	0.32g		0.4g
Total VFA (mmol/L) ^A	91.44	97.80	97.01	92.32	11.68	64.50	56.38	47.32	59.51	55.71	54.18	10.42
Acetate (mmol/L)	69.39	73.84	72.24	69.75	7.02	48.52	42.41	36.96	43.28	40.14	40.12	7.68
Propionate (mmol/L)	12.96	14.08	14.96	13.27	3.04	9.26	8.05	7.33	8.81	8.10	8.08	1.50
A/P ratio	5.80	5.69	5.25	5.73	1.04	5.51	5.45	5.33	5.06	5.16	5.19	0.40
NH ₃ -N (mg/L)	60.08	70.86	62.21	60.70	8.25	92.51	79.62	81.19	88.42	87.35	90.67	10.03
Methane production (mL)	32.08	30.51	30.16	32.51	3.37	31.93 ^a	29.84 ^{ab}	28.56 ^{ab}	29.71 ^{ab}	29.77 ^{ab}	25.18 ^b	3.92

^AVFA, volatile fatty acids.

Table 2. Effects of kyokai7 (k-7) and kyokai9 (k-9) on ruminal fermentation in sheep fed on timothy hay, alfalfa hay cube and concentrate (4:4:2, on a DM basis)

Values are means ($n = 3$) of 6 sampling times (0, 1, 2, 3, 6 and 8 h), except mean values for methane production is means ($n = 3$) of 24 h

Fermentation parameters	Treatment ^A			
	No-additives control	k-7	Yeast k-9	s.e.m.
Total VFA (mmol/L)	139.0	124.6	130.7	9.92
Acetic (mmol/L)	103.9	94.1	97.7	7.40
Propionate (mmol/L)	20.5	17.8	19.4	1.55
A/P ration	5.12	5.37	5.10	0.20
NH ₃ -N (mg/L)	228.2	207.6	207.2	30.72
Methane production (L/kg DM intake)	34.7	36.1	35.1	4.56

^ATreatments were: control; kyokai7 4 g/day; kyokai9 4 g/day.

Table 3. Effects of kyokai7 (k-7) and kyokai9 (k-9) on apparent digestibility of nutrients in sheep fed on timothy hay, alfalfa hay cube and concentrate (4:4:2, on a DM basis)

Mean values within a row with different superscript letters differ ($P < 0.05$).
All values are means of three animals (i.e. 3 observations)

	Treatments ^A			s.e.m.
	control	k-7	k-9	
Dry matter (%)	65.5	65.1	64.1	2.92
Organic matter (%)	67.8	67.5	66.6	2.93
Crude protein (%)	69.9 ^a	70.3 ^a	67.9 ^b	4.24
Neutral detergent fibre (%)	61.8	61.9	60.8	4.34
Acid detergent fibre (%)	58.5	57.3	56.6	3.90

^ATreatments were: control; kyokai7 4 g/day; kyokai9 4 g/day.

MEASURING THE METHANE EMITTED BY GRAZING CATTLE OF THE ARGENTINIAN BEEF SYSTEM

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SUMMARY

We report the first measurements of the methane emitted by cattle of the Argentinian beef system, one of the largest in the world. Special care was devoted to keep the animals under the production conditions typical of a commercial farm of the main livestock region of the country (the Pampa), characterized by free grazing with a tendency toward the use of zonal grazing in paddocks of some ha. Therefore, we applied the SF₆ tracer technique (Johnson et al, 1994) to 20 young Aberdeen Angus steers (weighing about 300 kg) randomly chosen in the farm. In a first experiment (December 2005) they were divided in two groups: one grazed in a range land paddock (RL) and the other in a sown paddock (SP). In a second experiment (February-March 2006) all the steers grazed in a single large sown paddock. The liveweight (LW) of the animals and its daily change (LWG) were followed during the research. We left the herbage allowance and quality to change in space and in time as it is normal during a commercial growing process. The prices paid for working under production conditions were the difficult in defining a representative average value of the dry matter digestibility (DE) in such large and rather heterogenous paddocks. The values given in the Table 1 are averages over 6 collected samples in each paddock. Probably as a consequence, we observe a considerable inter-animal and inter-day variability of the methane emission rate. Another hindrance was a low collection efficiency of gas samples. In fact, although 20 steers were loaded with enteric SF₆ permeation tubes, the total number of gas samples successfully collected and analyzed was 76 over a total of 17 field work days. DE and the net liveweight gain (NLWG = 0.96 LWG kg/day) varied significantly during the field work and from paddock to paddock. In spite of these changes, the average daily methane emissions per head E_m did not depart very much from 9.5 MJ (≈170 g/day). However, larger variations were observed in the NLWG/ E_m and in the methane yield Y_m, given by the ratio between the energy content of the methane emission to the gross energy intake GE. On turn, GE was calculated using the energy requirement model recommended by the IPCC (IPCC, 1996 b). The results are shown in Table 1.

The work was also useful to evaluate some technical features of importance in making the technique suitable for growing steers under the extensive condition prevailing in the Argentinian beef system. Specifically, for future work we decided to use 500 cm³ stainless steel collecting vessels and to develop inflow regulators which allow the extension of the collecting time to 5 days.

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Table 1. Summary of the results

	Experiment 1 - RL		Experiment 1 - SP		Experiment 2
	No. of days:	8-14	15-22	8-14	15-22
NLW = 0.96 x LW	277	286	277	288	324
NLWG	1.901	0.394	1.670	1.190	0.989
E _m (MJ)	9.0	9.3	9.1	10.2	9.0
NLWG/kg CH ₄	11.78	2.36	10.18	6.47	6.07
DE (%)	55.7	49.2	67.1	62.5	63.0
GE (MJ)	260.5	166.0	159.4	157.1	154.4
Y _m	0.035	0.056	0.057	0.065	0.058

A SIMPLE TECHNIQUE FOR MEASURING ENTERIC METHANE EMISSIONS IN COWS

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INTRODUCTION

Methane (CH₄) is a potent gas, when considering its contribution to global warming. There are different sources of CH₄, ruminant eructation being one of them. In order to measure the amount of CH₄ produced by animals through this mechanism, a standard technique using SF₆ has been developed (Johnson and Johnson, 1995; Ulyatt *et al.* 1999). Though very accurate, it results very expensive and not too easily applied under field conditions. Therefore, a trial was performed for determining the amount of methane (CH₄) emitted during a 24-h period utilizing a simple gas collection technique.

MATERIALS & METHODS

Animal and experimental site

A 6-year old, 550 kg body weight, dry Holstein cow, was utilized at was the Pathobiology Institute Experimental Unit, INTA Castelar, during March 2007.

Experimental model description

For the experimental model, the technique was developed according to the following procedure: On the animal standing in a head-gate, following a 24-h fasting period, a 2-cm diameter rumen fistula was done under local anesthesia, in the left abdominal cavity, below the first lumbar vertebra transverse apophysis. On day 15 after surgery, the animal was ready to be included in the trial, with a 3-week adjustment period and 1-week measurements. The cow was fed a diet consisting of an *ad libitum* alfalfa and bromegrass (*Bromus unioloides*) pasture, in a grazing system for the 3-week period. During the measuring week, the gas produced in the rumen was collected and its CH₄ concentration was determined.

Collection system

The collection system consisted of different parts: (i) a 9 mm external diameter and 2 mm thick plastic tube, perforated in its closed end, and fixed to a circular 10 cm diameter rubber patch; (ii) an Intertech® anesthetic one-way valve (SMITHS INDUSTRIES, USA) to obtain a system, in which the gas flowed freely from the rumen during its contractions, but it could not return; (iii) a 350-L capacity thick airtight polyethylene bag; (iv) silicon tubes to connect the different above described components. To fix the system, the holed portion of the perforated device was introduced in the rumen dorsal sac through the fistula. The rubber patch was stuck to the skin with contact cement, providing a hermetic closure. This system had been previously tested to determine the lack of esophageal gas losses during eructation, by means of a tube introduced via the nose into the cervical esophagus. The animal entered the head-gate at 11:00 am, immediately after grazing *ad libitum*. The gas was collected over a 24-h period, during which the animal was unfed.

In order to measure the volume, a spirometer (SPIROBANK; Italy) was utilized. It allowed the determination of the volume accumulated, by means of its flow meter.

Sampling and determination of methane concentration

To determine methane concentrations, 4 samples were taken over the 24-h period, at 1400, 2000, 0200 and 0800 hours. At sampling time, the gas produced in the rumen was deviated to 5-L capacity Tedlar® gas sampling bags (ICON, Argentina). The gas was then analyzed at the Gasses Detector Laboratory, INTI, by means of a commercial gas detector calibrated by the gas dilution system with mass flux controllers.

PRELIMINARY RESULTS

The total amount of gas produced over three non-consecutive 24-h periods is presented in Table 1.

Average total daily gas volume was 911.7 ± 50.3 L. Methane concentrations (L/L) ranged between 20 and 32%. The amounts of CH₄, based on the percentage of the gas obtained at the different sampling times, represented 247 L/day. These preliminary results indicate that this is a promising technique to be utilized under field conditions, since the CH₄ levels obtained here are consisting with values reported in the literature (Johnson and Johnson, 1995).

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Table 1. Total gas volume, methane concentration and methane produced by a 550 kg dry Holstein cows during three non-consecutive 24-h periods

Period	Total gas volume (L/day)	Methane percentage (L/L)				Average	CH ₄ volume (L/day)
		1400	2000	0200	0800		
1	965	31	27	26	22	26.5	255.7
2	865	31	30	29	20	27.5	237.9
3	905	30	29	28	23	27.5	248.9

BIOCHEMICAL ASPECTS OF MITIGATION OF METHANE EMISSION IN ATMOSPHERE BY RUMINANTS

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SUMMARY

The problem of abatement of methane emission by rumen microorganisms of ruminants in atmosphere includes two aspects. First, the ruminants provide with gases approximately 20 % of total quantity of methane, which is issued in atmosphere from various sources; second, with methane in ruminants is lost about 12 % of forages energy. Hence, the first aspect of problem has particular ecological importance that is caused by influence of methane on greenhouse effect on the planet; the second aspect has economic importance that is connected with losses of energy of forages. Therefore, searches of ways of mitigation of methane production in rumen of ruminants are actual. To modulators of intensity of redox reactions belong some carboxylic acids, especially nonsaturated acids, which may accept hydrogen, necessary in reactions of reduction of CO₂ to CH₄. Therefore, our researches were directed on finding out of some carboxylic acids effect on the production of methane by cattle rumen microorganisms. For the estimation of influence of these compounds on vital activity of rumen microorganisms, simultaneously the intensity of formation volatile fatty acids in incubation medium was studied.

The samples of rumen liquid from three steers were incubated with nutrient medium in anaerobic conditions during 24 hours without (control) and with (experimental) addition of:

- sodium acrylate, oleate, citrate, fumarate, α -ketoglutarate, lactate, malate, pyruvate, formiate, acetate, propionate and butyrate (10 mmol/L);
- ethanol or methanol (10 mmol/L);
- amino acids (Ala, Arg, Asp, Val, His, Gly, Glu, Ile, Leu, Lys, Met, Pro, Ser, Tyr, Thr, Trp, Phe, Cys, Asn, Gln) together or separately (10 mmol/L).

After incubation, in gas phase of samples the amount of produced methane was determined and in incubatory medium – the contents of volatile fatty acids.

It was established, that carboxylic acids unequally influenced the intensity of methanogenesis in cattle rumen. So, the most expressed inhibitory effect on this process revealed nonsaturated acids, and particularly oleic acid, addition of which reduced methanogenesis on 33%, and also acrylic and fumaric acids (on 19 and 14% accordingly). The attention attracts also the decrease of methanogenesis in presence of α -ketoglutaric acid (on 18%), whereas another ketoacid – pyruvic acid reveals smaller inhibitory effect. Formic, acetic, and propionic acids, on the contrary, stimulate formation of methane in rumen liquid (accordingly on 20, 13 and 8%). Obviously, they also, though in a smaller degree than CO₂, may be its precursors. Other acids don't show significant effect on methanogenesis in the rumen.

The added alcohols unequally affected on intensity of methanogenesis in the rumen. Under the influence of ethanol some inhibition of this process was observed, whereas methanol, on the contrary, intensified its activity. Probably, the last may be the precursor of methane in cattle rumen. The added mixture of amino acids and also separate of them showed marked effect on methane production. So, in particular, under the action of mixture of amino acids, necessary for protein molecules synthesis, the considerable increase of methanogenesis intensity was observed, in comparison with intensity of this process by addition only urea as source of nitrogen (on 40%). After addition of separate amino acids to medium, the activatory effect had only threonine (the increase of methane production averaged 32%). Some of amino acids, on the contrary, inhibited methanogenesis. In particular, significant inhibitory effect revealed cysteine. As to mechanisms, which underlie their action, so the activatory influence of threonine probably is connected with the participation of that amino acid as compound of the cofactor of one of final reactions of the CO₂ reduction to methane – mercaptoheptanoylthreonine phosphatase. The inhibition of methanogenesis by some amino acids has not direct explanation. Probably, in this case takes place the mediated action of products of these compounds metabolism, which influence the specific reactions in cells of microorganisms, stimulating or inhibiting growth of separate groups of bacteria.

All of used carboxylic acids, alcohols or amino acids don't markedly influence the level of volatile fatty acids in the incubatory medium.

INFLUENCE OF MILK PRODUCTION LEVEL ON METHANE EMISSION FROM DAIRY CATTLE

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ABSTRACT

Methane emission from dairy cattle is influenced from the milk yield and the service life of the cows. Increasing milk yields usually involve decreasing service lives. Calculations were done for milk yields between 4000 and 12,000 kg FCM/cow.year and a service life of the dairy cows between 15 and 48 months. The results showed that methane emission per produced milk is decreasing with increasing milk yield which overcompensate the effect of decreasing service life. Under current circumstances methane emission related to German milk production is in the range of 26 g CH₄/kg.FCM.

INTRODUCTION

Methane, emitted from enteric fermentation and from manure, is the most relevant greenhouse gas from animal agriculture. Several investigations focus on possible abatement techniques in animal nutrition as well as manure management.

With the investigations reported below, the influence of animal performance, as a key parameter of the procedure of milk production, on specific methane emission per produced milk was analysed. The starting thesis was: higher milk yields result in a higher feeding efficiency, because of a decreasing share of maintenance. In this way, lower specific emissions per production unit on the one hand side, and a lower service life of the dairy cows and thus an increasing share of reproduction on the other hand side occur. The question was which of both effects dominates, and how methane emission per produced milk is influenced if reproduction is considered.

MATERIALS & METHODS

Calculations were based on the 'Calculations of Emissions from German Agriculture – National Emission Inventory Report (NIR) 2006 for 2004' (Daemmgen *et al.* 2005) following the methodology of the IPCC Guidelines (IPCC 1997), with methane emission from calves, heifers and dairy cows, as well as appropriate milk yield as main parameters.

Methane emission from enteric fermentation was studied on the basis of the energy and feed requirements of the animals known from animal nutritional science. Gross energy (GE) was calculated from net energy assuming a feed digestibility of 66% for all milk yield levels. A constant feed digestibility was used because it was assumed that a higher digestibility of diets for higher yielding animals is compensated by a lower digestibility because of an increasing feed intake. Methane emission from enteric fermentation was estimated using a conversion rate of 0.06 MJ MJ⁻¹ GE⁻¹.

Methane emission from manure management was studied by calculating the amounts of volatile solids (VS) excreted on the basis of the gross energy. A maximum methane producing capacity of 0.24 m³ CH₄/kg.VS, and a methane conversion factor of 0.1 kg C/kg.VS (liquid manure) were assumed.

Calculations were done for milk yields between 4,000 and 12,000 kg FCM/cow.year and a service life of the dairy cows between 15 and 48 months. Different assumptions were done on the relationship between the milk yield and the service life of the dairy cows. For reproduction – calve and heifer rearing – two years were assumed.

RESULTS & DISCUSSION

The calculations showed that the milk yield influence methane emission much stronger than the service life of the dairy cows (Fig. 1). With an increasing milk yield its influence is decreasing. For an increase of the milk yield from 8,000 to 10,000 kg FCM/cow.year e.g. a reduction of methane emission of nearly 2 g kg⁻¹ FCM⁻¹ can be estimated, whereas for an increase from 10,000 to 12,000 kg FCM/cow.year only about 1 g CH₄/kg.FCM less can be expected. To reach such a decrease of 1 g CH₄/kg.FCM within a milk yield level by increasing the service life an increase in the range of 8 months would be necessary.

The mean annual milk yields in Germany were 5,625 kg (5757 kg FCM)/cow in the year 1997 and 6,563 kg (6727 kg FCM)/cow in 2004 (Stat. Bundesamt 2005). If these milk yields would be related to a service life of 36 and 30 months, the methane emissions were 26.9 and 25.9 g CH₄/kg.FCM respectively (from enteric fermentation and manure in total). In that case the increase of the milk yield of 970 kg FCM/cow.year had a stronger effect on specific

methane emission than the decrease of the service life of 6 months, methane emission per produced milk decreased by nearly 4%.

The dominating effect of the higher milk yield compared to the lower service life of the dairy cows was found by Rus et al. (2007) also for the cumulated energy demand per produced milk. But there was nearly no effect found for a milk yield higher than 8,000 kg FCM/cow.year.

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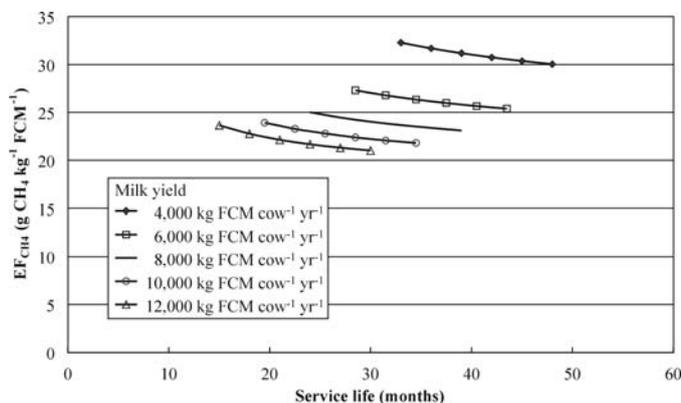


Figure 1. Specific methane emission per produced milk for different milk yields and service lives of the dairy cows.

A BASELINE PROJECTION OF METHANE EMISSIONS BY THE BRAZILIAN BEEF SECTOR: PRELIMINARY RESULTS

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INTRODUCTION

In the last 10 years, it is estimated that the Brazilian beef production has risen from about 5.8 to 9.0 million metric tons of carcass-equivalent (MMTCE) and exports increased from less than 0.3 to over 2.2 MMTCE. The continuous expansion of the Brazilian beef industry will face increasing restrictions of land availability and price, as the conversion of natural areas into pastures is going down rapidly and there is a projected expansion of sugarcane and other crops for biofuel production. Also greenhouse gases emissions maybe a problem, as enteric fermentation by bovines accounted for over 160 million metric tons of CO₂-equivalent (MMTCO₂e), i.e. around 11.6% of the anthropic sources of greenhouse gases in Brazil (Lima et al., 2002). In this context, a method to estimate Brazilian herd dynamics and a baseline projection of meat production, productivity and methane emissions is presented.

MATYERIAL & METHODS

In a discrete model the whole Brazilian herd was categorized into Cows, Calves (male and females up to 1 year old), Heifers1 (1 to 2 year old heifers), Heifers2 (2 to 3 year old heifers), Steers1 (1 to 2 year old steers), Steers2 (2 to 3 year old steers), Steers3 (3 to 4 year old steers) and Steers4 (steers older than 4 years). Population changes were calculated in annual time steps.

Changes in animal numbers in each category are defined by calving rates, mortality rates and slaughter coefficients. Initial numbers of animals in each category were based on the 1996 Brazilian agricultural census data (IBGE, 2007). Calving and mortality rates were adjusted using IBGE estimates for animal numbers and the slaughter between 1996 and 2006. In the projection, cow slaughter was fit to minimize the absolute deviation between beef supply and demand in the period of 2008 – 2030. Only the period from 2008 to 2025 were considered in the projections. External marked demand was assumed to increase 2% per year and internal market 1% per year in the period. The optimization problem is mathematically expressed as:

$$\text{minimize } \sum_{y=2008}^{2030} (D_y - S_y) \quad (1)$$

where D_y is the total beef demand (domestic + exports) and S_y is the total supply in the y^{th} year, calculated in Equation 2.

$$S_y = \sum_j G_{yj} * CW_j \quad (2)$$

where G_{yj} is the number of animals of the j^{th} category slaughtered in the y^{th} year; CW is the average carcass weight of the j^{th} category at slaughter. The current trend of improvement in production efficiency was considered by linearly change technical coefficients. Calving rates were increased from 55% to 68%; age at slaughter reduced from 36 to 28 month and calf mortality reduced from 7% to 4.5%. Methane emissions were calculated for each category based on the TIER 2 model, described in the IPCC Guidelines for National Greenhouse Gas Inventories (IPCC 1996).

RESULTS & DISCUSSION

Results presented in Table 1 suggest that the increasing demand will be met with no significant change in cow numbers and a small increase in the total number of animals. Despite the increase in animal numbers, the reduction in emissions per unit of product will result in almost no variation in methane emissions despite over 25% increase expected in production.

CONCLUSIONS

The expected raise in beef production in Brazil in the next 18 years will probably not contribute to the increase in methane emissions. The expected improvement in animal productivity and feed quality will allow meeting future production demand without major variation in the national herd population and methane emission.

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Table 1. Projected dynamics of bovine population, meat production and the methane emission in the Brazil in the period 2007-2025

Year	Cow number (million)	Animal number (million)	Slaughter (million)	Meat production (MMTCE)	Methane emission (MMTCH ₄)	CH ₄ /CWe
2007	64.3	208.0	43.0	8.83	9.56	1.08
2011	63.3	209.8	45.1	9.20	9.55	1.04
2015	63.0	214.0	48.0	9.73	9.65	0.99
2019	62.6	217.9	51.1	10.29	9.74	0.95
2023	62.1	221.4	54.1	10.81	9.80	0.91
2025	62.0	223.4	55.6	11.08	9.84	0.89
Variation	-3.6%	7.4%	29.3%	25.4%	2.9%	-18.0%

GROWTH AND METHANE PRODUCTION OF LAMBS WITH AND WITHOUT RUMEN PROTOZOA CONSUMING PASTURE AND ROUGHAGE DIETS

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SUMMARY

Ciliate protozoa are central organisms in the ecology of a normally faunated rumen, consuming bacteria and reducing the bacterial population (Williams and Coleman 1992) while also providing a physical and chemical substrate for methanogens in the rumen (Ushida *et al.* 1997). Consequently, sheep lacking protozoa typically have increased protein supply to the intestine and reduced methane production (Kreuzer 1986). This study was undertaken to verify the effects of the absence of rumen protozoa on growth and methane production of lambs.

Crossbred lambs were born to either faunated ewes or defaunated ewes and reared on pasture until weaning. After weaning, a subset of faunated lambs was chemically defaunated and the growth and methane production of naturally fauna-free (RF), defaunated (DF) and faunated (F) lambs were compared. Lambs were individually penned and fed a roughage diet *ad libitum* (9.2 MJ ME/kg DM, 12.3% CP) for 70 days. Female lambs ($n=8$) from each group were placed in open circuit respiration chambers for 2 x 21 h periods and their methane production determined.

Lambs born to defaunated ewes grew more quickly to weaning in the paddock than did lambs born to faunated ewes (284 v. 254 g/day singles; 225 v. 200 g/day twins). After weaning, no difference in voluntary feed intake, growth rate or daily methane production occurred due to the absence of protozoa (Table 1).

The data indicates that the absence of protozoa, either by chemical defaunation or by rearing animals in isolation, does not always affect voluntary feed intake or reduce daily methane emissions. The absence of protozoa did however lead to improved pre-weaning growth and increased wool growth by lambs, suggesting a greater protein supply to the intestine occurred in the absence of protozoa.

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Table 1. Intake, productivity and methane production of naturally fauna-free (RF), defaunated (DF) and faunated (F) crossbred lambs fed chaffed roughage *ad libitum*

Parameter	Protozoa treatment						Effect
	BF		DF		F		
	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.	
DM intake (g/day)	912	33	958	30	911	27	n.s.
Liveweight gain (g/day)	42	17	27	15	38	14	n.s.
Methane output (g/day)	28.4	1.42	26.7	1.14	24.9	1.19	n.s.
Wool growth (g/day)	8.1	0.19	7.9	0.22	7.2	0.19	$P<0.01$

TOWARDS A VACCINE AGAINST METHANOGENS

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SUMMARY

Almost half of New Zealand's emissions come from livestock. Methane is produced in ruminants predominantly by a group of archaeobacteria called methanogens. Within the forestomach of ruminants, methanogens produce methane from hydrogen and CO₂ produced by other micro-organisms. In addition to its environmental impact, the production of methane is recognised as a wasteful process which represents a loss of energy for ruminants. A variety of strategies have been considered to reduce emissions of methane from livestock. One possible avenue is the exploitation of the ruminant's immune response to curtail methanogen numbers and/or activity in the rumen. The concept is to induce a strong and sustained antibody salivary response that delivers a high yield of anti-methanogen antibodies to the rumen and either prevents the growth of methanogens in the rumen or inactivate the ability of these microbes to produce methane. Such an approach has been trialled using crude methanogens (Wright *et al.* 2004), but further studies have found this approach irreproducible (Clark *et al.* 2006), necessitating the need to identify defined antigens from methanogens to produce a more refined vaccine formulation.

Using a model methanogen, *Methanobrevibacter ruminantium*, we have initiated studies aimed at exploring the feasibility of generating an immune response in ruminants that can impair methanogen growth and/or methane production in the rumen. A number of fractions of *M. ruminantium* were prepared, and rabbits and lambs vaccinated with these fractions or whole cells. Basal levels (pre-vaccination) of antibodies against these antigens were minimal in rabbits and newborn lambs. Young unvaccinated lambs quickly developed a strong humoral response in the blood, but not saliva, against methanogens. Antisera from both vaccinated sheep and rabbits agglutinated methanogen cells *in vitro*. Analysis of the fractions by SDS-PAGE and Western blotting revealed the presence of a range of antigenic targets in the methanogens. These antigens are currently being identified by MALDI-TOF MS.

In summary, methanogens appear to be immunogenic for ruminants and animals develop a humoral response to methanogens, in keeping with the constant exposure of animals to these organisms. Antisera against fractions of methanogens impact on methanogens, based on an agglutination assay. Additional studies are currently aimed at optimizing the antibody levels in the saliva of immunized animals.

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USE OF CLONED DAIRY COWS TO DEFINE PHYSIOLOGICAL CONTRIBUTIONS TO RUMINAL METHANE PRODUCTION

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ABSTRACT

A trial was undertaken to determine the extent to which genetic makeup of cows can influence methane production. Ten non-lactating cows with permanent rumen fistulae (5 cloned and 5 unrelated cohorts) were fed ryegrass silage for six weeks, during which a range of physiological and microbiological measurements was undertaken. The trial comprised three phases; initially (Phase 1) the voluntary feed intake (VFI) was recorded, and the diet was fed at 95% of VFI thereafter (weeks 2-6). Baseline measurements (week 2) included dry matter intake (DMI), methane production, rumen pH, concentrations of ammonia and volatile fatty acids (VFA), liquid and solid digesta outflow and microbial growth. The same measurements were made in Phase 2 (weeks 3 and 4) to determine effects of switching rumen contents between clones and cohorts (day 15) on return to baseline values, and in Phase 3 (weeks 5 and 6), following ruminal administration of chloroform (1.3 mL/day) on days 29 and 30 to suppress methanogenesis. Average DMI (kg/day) was higher ($P < 0.05$) for the cloned cows than for the cohorts (10.2 v. 8.8), most likely related to larger mean body size (535 v. 440 kg). Expressed on the basis of DMI, however, mean values for methane production (g kg/DMI) were similar between clones (11.50) and cohorts (10.3). There were no differences between groups in rumen pH (6.76), total VFA concentration (74.5 mmol/L), molar proportions of acetate (A), propionate (P) or butyrate (0.72, 0.18, and 0.07, respectively), or passage rate of liquid (0.096/h) or solids (0.025/h) from the rumen. Exchanging rumen contents between clones and cohorts did not affect methane production in week 3 (13.0 g/kg DMI), but ammonia and VFA concentrations were lower than previously and A:P ratios increased ($P < 0.05$), which suggests a significant perturbation of fermentation. Chloroform reduced methane production in both groups by more than 100 g/day by the second day of treatment, after which production gradually returned to pre-treatment values. Chloroform also caused marked shifts in molar proportions of propionate (nearly doubled) and acetate (decreased) and reduced DM digestibility from about 71% to about 53%, but there were no differences between the clone and cohort groups. The two main observations from this trial included the very low levels of methane production, relative to typical values of 19-21g/kg DMI for cows fed forages, and some indications of a greater variance in methane production among cohorts than among clones. Relating methane (g/kg DMI; y) to liveweight (kg; x) yielded a negative relationship for the cohort cows ($y = -64x + 40.7$; $R^2 = 0.80$), but a weak positive relationship for the clones ($y = 0.72x - 24.1$; $R^2 = 0.33$). Average liveweight (LW) of cohort cows ranged from 391 to 495 kg, whereas the clones ranged from 517 to 561 kg. The variance in daily feed intake (kg DMI) was larger for cohorts (1.26) than for clones (0.06; $P = 0.02$), and when methane production was expressed on the basis of DMI as a proportion of metabolic liveweight ($\text{CH}_4/\text{DMI}/\text{LW}^{0.75}$; $\text{g}/\text{kg}/\text{kg}^{0.75}$), there was a significantly lower ($P = 0.07$) variance among the clones (0.00030) than among the cohorts (0.00201). These data suggest a reduced variance in methane production amongst the cloned cows relative to the unrelated cohorts, implying that the genetic makeup of animals may influence methane production.

Keywords: Cattle, genetics, methane production, rumen, ruminant

METHANE EMISSIONS FROM SHEEP USING PERMEATION TUBES MANUFACTURED IN AUSTRALIA AND NEW ZEALAND

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INTRODUCTION

The SF₆ tracer dilution technique is predominately used for estimating methane (CH₄) emissions from ruminant animals (Johnson *et al.* 1994). The basis of this technique is the same between countries but adjustments are made to the measuring equipment to deal with different trial conditions. The aim of this trial was to determine if differences in permeation tube design from Australia and New Zealand (NZ) would affect the estimation of CH₄ emissions when using the same animals under controlled feeding conditions.

MATERIALS & METHODS

Ten rumen fistulated wethers were used in a 5 x 5 cross-over trial. Animals were placed in individual crates to measure dry matter intake (DMI) from an *ad libitum* diet of ensiled lucerne. Methane emissions were measured twice from each animal, using alternatively an Australian (AusTubes) or New Zealand (NZTubes) permeation tube. Daily CH₄ emissions were collected over 4 consecutive days on 2 one week periods and analysed by gas chromatography (Johnson *et al.* 1994). Individual permeation tube flow rates were determined by sequential weighing over a 10 week period prior to insertion, with tubes inserted and removed from the rumen fistula by a permeable bag connected to string. All statistical data analyses were done using GenStat (version 9.0) with the main factors being week of measurement and tube type.

RESULTS

There were no effects of week of measurement or tube type on liveweight, DMI, CH₄ emission g/day and g/kg DMI (Table 1). The NZTubes had lower ($P < 0.05$) permeation flow rates than the AusTubes. There was a significant correlation for CH₄ emissions per day ($r = 0.82$, $P < 0.05$) but not per kg DMI ($r = 0.60$, $P = 0.29$) between the 2 types of permeation tubes when measurements were made in the same sheep.

DISCUSSION

The positive correlation determined between CH₄ emissions (g/day) and tube type suggests that animal rankings remained consistent for both tube measurements. Individual CH₄ emissions determined with NZTubes were lower in 70% of the animals which lead to mean CH₄ emissions (g/day) being 17% lower than the AusTubes. The main differences between AusTubes and NZTubes is in size, quantity of SF₆ (60 v. 30 mg) and mean flow rate (1.5 v. 1.2 mg/day; (Fig. 1).

Greater variation was found for individual CH₄ emissions using AusTubes (CV = 31.9) which was twice that of NZTubes (CV = 19.7; Fig 2). It is difficult to explain how the differences in tube design contributed to the variation, since other animal factors that were not tested may have also contributed to differences in CH₄ emissions. The large range in liveweight (48-95 kg) was spread evenly across both tube treatments so it was eliminated as a variable factor. This study did not determine any significant difference between the two types of permeation tubes but if the study was repeated with a greater number of animals, differences between AusTubes and NZTubes could become significant.

ACKNOWLEDGEMENT

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Table 1. Mean CH₄ emissions (± s.d.) dry matter intake (DMI), liveweight and SF₆ permeation rates

	New Zealand tube	Australian tube
Liveweight (kg)	75.2 ± 16.0	74.4 ± 16.0
DMI (kg/day)	1.18 ± 0.27	1.20 ± 0.19
CH ₄ (g/day)	17.8 ± 3.5	21.5 ± 6.9
Range CH ₄ (g/day)	11.5 – 22.4	12.6 – 34.1
CH ₄ /DMI (g/kg)	15.1 ± 2.7	17.8 ± 5.0
Range CH ₄ /DMI (g/kg)	12.5 – 19.9	13.4 – 30.0
Perm tube rate (mg/day)	1.21 ± 0.2	1.51 ± 0.3
Range - perm tube rate (mg/day)	1.000 – 1.808	1.211 – 2.205



Figure 1. Differences between a New Zealand (left) and Australian sheep permeation tube.

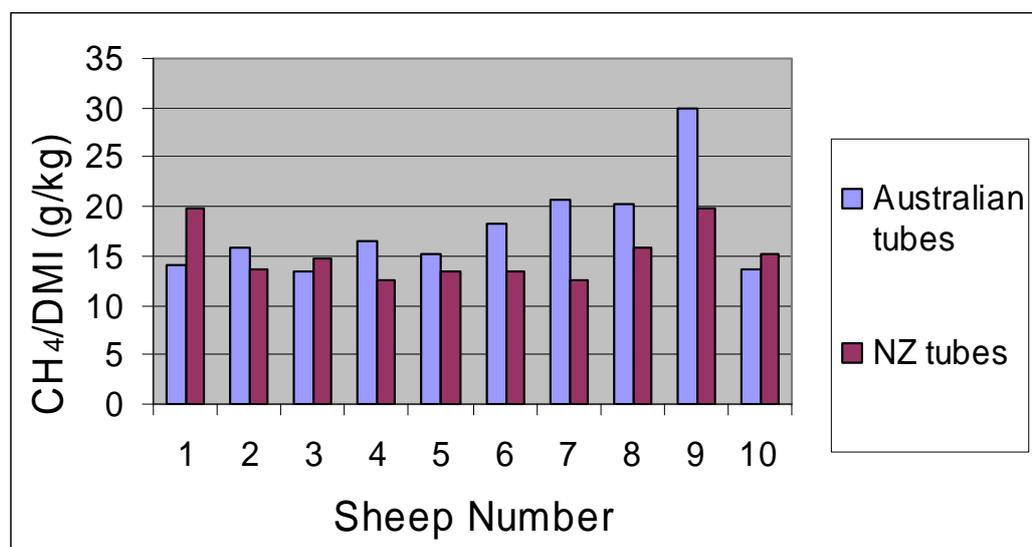


Figure 2. Individual methane emissions from 10 sheep using both an Australian and New Zealand permeation tube

AN UPDATED NEW ZEALAND ENTERIC METHANE INVENTORY CALCULATED USING ALGORITHMS DEVELOPED FROM AN ANALYSIS OF NEW ZEALAND EXPERIMENTS CONDUCTED BETWEEN 1997 AND 2005

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SUMMARY

Enteric methane (CH₄) emissions make up close to a third of all New Zealand's GHG emissions and to comply with guidelines published by the Inter-Governmental Panel on Climate Change (IPCC) a 'Tier 2' accounting approach has been adopted. This approach involves estimating the dry matter intake (DMI) of the 'average' New Zealand ruminant and converting this into an annual CH₄ emission using a constant value for the quantity of CH₄ emitted per unit of DMI (CH₄ yield). This is done separately for dairy cattle, beef cattle, sheep and deer. Currently data on CH₄ emissions per unit of DMI are derived from an analysis of experiments conducted in New Zealand between 1997 and 2002. This paper presents an updated analysis of experiments conducted in New Zealand between 1997 and 2005, limiting the analysis to pasture only diets so as to better represent the 'average' New Zealand animal.

The data set comprised 77 experiments; 6 beef cattle, 35 dairy cattle, 9 deer and 27 sheep. The total number of individual animal CH₄ emission values was 1933; 71 beef cattle, 1326 dairy cattle, 133 deer and 403 sheep. All CH₄ measurements were made using the SF₆ tracer technique and each individual animal emission value was the mean of 3-5 days measurement. These data were analysed using a between experiment model which worked on the mean value of each experiment. The four animal categories (beef cattle, dairy cattle, sheep and deer) were analysed separately. The variates used to model CH₄ emissions (g/day) were DMI, liveweight, age, and the feed descriptors dry matter digestibility (DMD), acid detergent fibre (ADF), neutral detergent fibre (NDF) and crude protein (CP).

The analysis revealed that a regression based simply on DMI can be used to predict CH₄ emissions from beef and dairy cattle and the addition of the other variates did not improve model fit. For sheep and deer the addition of animal age improved model fit (Table 1).

To compare with the current New Zealand methodology updated CH₄ yield values were also calculated from the 1997-2005 data sets. These values, along with the current values used to estimate New Zealand's enteric CH₄ emissions are shown in Table 2.

Two revised estimates of New Zealand's enteric CH₄ emissions for 1990 and 2005 were obtained from the analysis of the 1997-2005 data (Table 3). One used a regression equation approach (Table 1) while the other used the revised CH₄ yield values presented in Table 2. Using a regression equations approach to recalculate the New Zealand enteric CH₄ inventory for 1990 resulted in an upward revision in total emissions of 58.9 Gg CH₄ due to an increase in estimated emissions from dairy cattle and sheep. Revised total emissions for 2005 showed an increase of 17 Gg CH₄. The estimated increase in total emissions between 1990 and 2005 was reduced from 109 to 67 Gg CH₄, a decrease of 48Gg CH₄. Using updated yield factors resulted in estimated total emissions for 1990 and 2005 being reduced by 12.6 Gg CH₄ and 23.8 Gg respectively due to increased estimates for sheep and reduced estimates for cattle. The estimated increase in emissions between 1990 and 2005 were reduced from 109 to 90 Gg CH₄ a decrease of 19 Gg CH₄.

Table 1. Linear regression constant and slope values for the prediction of daily CH₄ emissions (g) obtained from a between experiment analysis of 6 beef cattle, 35 dairy cattle, 9 deer and 27 sheep experiments conducted in New Zealand between 1997 and 2005

Animal type	Regression model			Significance	R ²
	Constant	Parameter DMI	Parameter age		
Beef cattle	20.7	15.6		P<0.001	0.82
Dairy cattle	75	15.39		P<0.001	0.64
Sheep	5.4	11.77	3.21	P<0.001	0.80
Deer	-5.2	36.01	7.22	P<0.001	0.87

Table 2. Mean CH₄ yield (g kg/DMI) for beef cattle, dairy cattle, deer, sheep (>1 year old) and sheep (<1 year old) obtained from an analysis of 6 beef cattle, 35 dairy cattle, 9 deer and 27 sheep experiments conducted in New Zealand between 1997 and 2005 compared with current New Zealand values

	Beef cattle	Dairy cattle	Deer	Sheep	
				<1 year old	>1 year old
CH ₄ yield (1997-2005 data)	19.39	19.51	23.94	19.56	21.63
CH ₄ yield (current New Zealand values)	20.9	20.9	21.25	16.8	21.6

Table 3. Enteric CH₄ emissions from New Zealand ruminants 1990 and 2005 estimated using a regression equation approach (Table 1) and updated CH₄ yield factors (Table 2) compared current national inventory estimates

Animal type	Year	Current national inventory emissions	Re-calculated national inventory; regression equation approach (see Table 1)	Re-calculated national inventory; updated CH ₄ yield factors (see Table 2)
Beef cattle	1990	235.48	203.89	211.85
	2005	255.03	217.04	229.41
Dairy cattle	1990	237.72	273.17	214.49
	2005	405.24	443.44	365.70
Deer	1990	18.50	20.23	20.50
	2005	37.74	43.38	41.82
Sheep	1990	535.17	571.92	567.38
	2005	438.02	434.79	467.30
Total	1990	1026.87	1069.21	1014.22
	2005	1136.02	1138.65	1104.23

METHANOGENS DETECTED IN THE FOREGUTS OF THE TAMMAR WALLABY (*MACROPUS EUGENII*), THE RED KANGAROO (*MACROPUS RUFUS*) AND WESTERN GREY KANGAROO (*MACROPUS FULIGINOSUS*)

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INTRODUCTION

Macropods are native to the island continent of Australia and include many species of kangaroo and wallaby which have evolved in isolation to other herbivorous animals. The foregut microbial community of these animals coordinates the break down of plant biomass, but unlike ruminants and other herbivores, anaerobic fermentation of pasture in the macropod foregut produces relatively low methane emissions. To date, the limited data available suggests that macropods have a foregut flora that contains many species of micro-organisms not previously recognised. However, no published data exists about the presence or diversity of archaea in the macropod foregut. The diversity of methanogenic archaea in the foregut contents of four Tamar wallabies, two Western Grey kangaroos, and one Red kangaroo were investigated by using archaeal specific primers to create clone libraries. Preliminary results of these clone libraries suggest that archaeal species present in the foreguts of the above mentioned macropods are closely related to *Methanobrevibacter* spp. and to a group of uncultivated novel archaea that are distantly related to known methanogens. These novel archaea are believed to represent a new order of methanogens.

MATERIALS & METHODS

DNA was extracted from gut contents of one Red kangaroo, two Western Grey kangaroos, and five Tamar wallabies. PCR of archaeal 16S rRNA genes was performed on extracted DNA using primers Met86F and Met1340R (Wright and Pimm, 2003). 16S rRNA clone libraries were constructed using the TOPO-TA cloning kit (Invitrogen). From Red and Western Grey kangaroo foregut content libraries, clones with correctly sized PCR product inserts were sequenced. 16S rRNA gene sequences were identified using BLAST searches (Altschul *et al.* 1990). Correctly sized PCR products were isolated from clones of the Tamar wallaby foregut content library and initially identified using Restriction Fragment Length Polymorphism (RFLP) analysis (Wright and Pimm 2003).

RESULTS

Fifteen archaeal 16S rRNA gene sequences were obtained from the clone libraries of the Red and Western Grey kangaroos. Of the 15 16S rRNA gene sequences, only three were obtained from the Red kangaroo, these sequences were most closely related (98%) to *Methanobrevibacter gottschalkii* strain PG. The 12 sequences from the two Western Grey kangaroos were most similar to sequences from a recently discovered novel archaeal cluster. RFLP analysis of 16S rRNA gene sequences from the Tamar wallaby clone library also identified the presence of *Methanobrevibacter* spp. in these animals. However, many RFLP patterns from this clone library can not be identified against known *Hae*III restriction digest patterns, but most do resemble *Methanobrevibacter* spp. restriction patterns

DISCUSSION

To date no published data exists about the presence of archaea in the foregut of macropods. Archaea present in other animals with the capacity for foregut fermentation, like ruminants, appear to be predominately *Methanobrevibacter* spp. (Nicholson *et al.* 2007). Strains of *Methanobrevibacter* spp. were found in the clone library constructed from wallaby foregut contents. The three archaeal sequences identified from the Red kangaroo foregut contents were most similar (98%) to *M. gottschalkii*, a methanogen isolated from pig faecal material. Despite *Methanobrevibacter* spp. being present in the Red kangaroo and wallaby foregut contents, none were found in clone libraries from the Western Grey kangaroo foregut contents. The archaea present in the Western Grey kangaroo foregut belong to a novel cluster previously found in the rumens of sheep and are believed to be methanogens (Wright *et al.* 2006). These preliminary 16S rRNA gene clone libraries suggest most methanogens in the macropod foregut are similar to methanogens present in the rumens of cattle and sheep.

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EFFECT OF LEVEL OF DIETARY MALIC ACID SUPPLEMENTATION ON RUMINAL FERMENTATION AND METHANE EMISSIONS IN BEEF CATTLE

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INTRODUCTION

Methane (CH₄) emissions from enteric fermentation of ruminants account for over 53% of total emissions from Irish agriculture (Ireland National Inventory Report, 2006). One strategy to reduce methanogenesis is to promote metabolic pathways within the rumen which act as electron sinks for hydrogen (H₂). For example, organic acids such as malic acid (MA) act as propionate precursors in the rumen. Such acids are generally converted to propionate via the succinate propionate pathway and thus they can act as electron acceptors for H₂ and reduce the quantity available for CH₄ synthesis. Organic acids such as malic acid occur naturally in plants albeit at low levels. The aim of the current study was to examine the effect of level of dietary supplementation with MA on ruminal methanogenesis and fermentation in beef cattle.

MATERIALS & METHODS

Two latin square designed experiments were carried out. In the first, six beef heifers (452 ± 25 kg) were assigned to one of three dietary levels of malic acid (MA) over three periods in two replicates. In a second experiment, four rumen fistulated steers (895 ± 45 kg) were assigned to one of four dietary levels of MA over four periods. In both experiments animals were offered a 40:60 grass silage:concentrate diet. The concentrate portion of the diet was supplemented with one of three levels of MA (0%, 3.75%, or 7.5%) in experiment 1 and one of four levels of MA (0%, 2.5%, 5%, or 7.5%) in experiment 2. Each experimental period consisted of 28 days incorporating a 13-day acclimatisation period, with methane output and diet digestibility measured from days 14 to 18 in experiment 1 and rumen sampling taking place from days 16 to 18 in *experiment 2*. Dry matter intake (DMI) was recorded daily for both experiments. Methane emissions were measured using the SF₆ (sulphur hexafluoride) technique of Johnson et al. (1994) as described by Lovett *et al.* (2003). In experiment 2, rumen fluid was sampled at 2, 4, 6 and 8 h post feeding, and analysed for pH, VFA, ammonia and protozoa concentrations.

RESULTS & DISCUSSION

In experiment 1 incremental dietary inclusion of MA led to a linear reduction ($P < 0.05$) in DMI and also resulted in a linear reduction ($P < 0.05$) in total daily CH₄ emissions and CH₄ emissions per kg DMI (Table 1).

In experiment 2, supplementation with MA did not affect DMI ($P > 0.05$) or the digestibility of the ADF, NDF, total N, DMD or OMD fractions of the diet. There was no effect of treatment ($P > 0.05$) on ruminal concentrations of VFA. There was a tendency towards lower concentrations of ammonia with increasing dietary MA ($P = 0.05$). Ruminal pH was higher ($P < 0.05$) on 5 and 7.5% MA compared with either 0 or 2.5%. However there was no difference between any other treatment comparisons for pH ($P > 0.05$). Similarly, protozoal numbers were lower ($P < 0.05$) on 5 and 7.5% MA compared with either 0 or 2.5%.

In conclusion, dietary supplementation with MA reduced CH₄ emissions and may have been mediated through a reduction in the ruminal protozoal population. The lower DMI associated with MA supplementation in the younger animals used in experiment 1 may have been due to palatability issues and could have implications for performance and lifetime methane emissions.

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Table 1. Effect of dietary concentrate inclusion rate of malic acid on mean (\pm s.e.m.) feed intake and methane production (experiment 1)

DMI, dry matter intake; CH₄, methane; CDMI, concentrate dry matter intake.
Within rows, values followed by different letters are significantly different at $P=0.05$

Variable	Concentration of MA		
	0	3.75%	7%
CH ₄ (L/day)	344.50a (\pm 6.71)	322.78a (\pm 7.28)	289.77b (\pm 7.13)
CH ₄ /kg DMI (L/kg)	36.66a (\pm 0.76)	35.66ab (\pm 0.82)	33.28b (\pm 0.81)
CH ₄ /kg CDMI (L/kg)	60.57a (\pm 1.31)	59.14b (\pm 1.43)	55.60bc (\pm 1.40)
Silage DMI (kg)	3.70a (\pm 0.06)	3.60ab (\pm 0.06)	3.50b (\pm 0.06)
CDMI (kg)	5.69a (\pm 0.05)	5.47b (\pm 0.05)	5.23c (\pm 0.05)
Total DMI (kg)	9.39a (\pm 0.07)	9.06b (\pm 0.07)	8.72c (\pm 0.07)

IDENTIFICATION OF NOVEL POTENTIAL RUMEN ACETOGENS IN GNOTOBIOTICALLY-RAISED LAMBS

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INTRODUCTION

Reductive acetogenesis is an alternative sink for the disposal of excess hydrogen in the rumen and has been proposed as a mechanism to reduce ruminal methanogenesis (Joblin 1999). The bacteria responsible for this process are known as acetogens and are phylogenetically diverse (Lovell and Hui 1991). Acetogens dominate in the rumen of very young lambs before colonisation with methanogens (Morvan, Dore *et al.* 1994). We have therefore established gnotobiotically-raised lambs kept free from methanogens, to provide a unique environment where the natural rumen acetogen population can be maintained and further studied. The aim of the present study was to identify novel potential rumen acetogens using a gnotobiotic lamb model.

MATERIALS & METHODS

Two 15-hour old lambs were placed into isolators and gnotobiotically-raised on a diet of ultra-high temperature sterilised cows milk and later sterile alfalfa hay (Fonty, Joblin *et al.* 2007). Inoculants of cellulolytic bacteria (*Ruminococcus albus*, *R. flavefaciens*, *Fibrobacter succinogenes*) and methanogens (*Methanobrevibacter wolinii*) were provided after isolation at 8 and 150 days respectively. Two conventionally-raised lambs were also raised during this trial as controls. The inoculated cellulolytic bacteria and methanogens were quantified using real-time PCR (Denman and McSweeney 2006; Denman, Tomkins *et al.* 2007).

Rumen samples were taken from (i) gnotobiotically-raised lambs with cellulolytic bacteria before inoculation with methanogens, (ii) gnotobiotically-raised lambs with cellulolytic bacteria after inoculation with methanogens, (iii) conventionally-raised lambs, and analysed for microbial population differences using 16S rRNA gene clone libraries (Wright, Tajima *et al.* 2005) and denaturing gradient gel electrophoresis (DGGE) (Kocherginskaya, Cann *et al.* 2005). 16S rRNA gene sequences and DGGE bands unique to gnotobiotically-raised lambs before inoculation with methanogens were phylogenetically placed using ARB (Ludwig, Strunk *et al.* 2004).

RESULTS

Detection and quantification of cellulolytic bacteria, methanogens and acetogens

Cellulolytic bacteria were detected in gnotobiotically-raised lambs, with *R. albus* present at the highest levels (approximately 20% of total bacteria detected) and *R. flavefaciens* and *F. succinogenes* detected at levels 10^3 - 10^4 lower. Methanogens were detected in gnotobiotically-raised lambs after inoculation with *M. wolinii* and were present at levels 5.13×10^5 -fold lower than in conventionally-raised lambs. The methyl coenzyme-M reductase A (*mcrA*) gene was not detected in gnotobiotically-raised lambs before inoculation with *M. wolinii*. Acetogens were quantified by cultivation at between 10^7 and 10^8 cells/ml in the gnotobiotically-raised lambs void of methanogens (Dr E. Forano, INRA, pers. comm.)

Microbial diversity analysis

Comparison of the three 16 S rRNA clone libraries identified 19 clones unique to the methanogen-free, gnotobiotically-raised lambs. Of these, none showed >95% identity to any known acetogens and only 2 showed identity (>97%) to any presently cultured organism: *Bacteroides caccae* and *Clostridium ramosum*. Of the novel sequences, 5 were placed within *Bacteroidales* (grouping with *Bacteroides acidofaciens*, *B. distasonis*, *B. splanchnicus*), 1 within *Clostridiales* (grouping with *Clostridium orbiscindens*) and 4 within *Eubacteriales* (grouping with *Eubacterium rectale*). The remaining 8 were classified as *Firmicutes*, grouping near *Papillibacter* sp., *Ruminococcus* sp. or *Catonella* sp. Clones from two DGGE bands unique to the methanogen-free lambs had >98% identity to the 16S rRNA gene clones that grouped with *Eubacterium rectale* and those identified as *Firmicutes* grouping near *Ruminococcus* sp.

DISCUSSION

The bacteria and methanogens inoculated into the gnotobiotically-raised lambs to establish cellulolytic and methanogenic function appeared to colonise the rumen therefore these lambs were a suitable model for studying changes in rumen microbial populations involved in hydrogen utilisation.

It was hypothesised that investigating the rumen bacterial populations in gnotobiotically-raised lambs before and after inoculation with methanogens would reveal community changes that may lead to the identification of potential acetogens. Of the 19 sequences unique to the clone library from samples before inoculation with methanogens, 2 were assigned phylogeny and are not known to be acetogens. The remaining sequences were novel and phylogenetically diverse thus confirming that identification of acetogens is difficult based on phylogenetic information alone. However this information will provide a sound basis for linking phylogeny with function when DNA from these samples is investigated for functional genes involved in reductive acetogenesis.

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ESTIMATE OF METHANE EMISSIONS - ENTERIC FERMENTATION AND MANURE MANAGEMENT - FROM LIVESTOCK IN PERU

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INTRODUCTION

Peru's greenhouse gas emissions from anthropogenic sources were 720 megatonnes of carbon dioxide equivalent (Mt CO₂ eq.) with 48% of these emissions being attributed to livestock (CONAM. 2001). This was based on animal population of 1994 using the International Panel on Climate Change Tier-1 methodology which calculates CH₄ emissions for each animal category by multiplying the animal population by the average emissions factor associated with the specific animal category and for manure emissions the total population was divided according to their environmental conditions (IPCC 1997).

The objective of this study was to estimate enteric fermentation and manure emissions of methane (CH₄) during 2006 from animal population in Peru using the IPCC Tier-1 methodology and to identify regions and prevalent animal production systems of the country contributing more to those emissions.

MATERIAL & METHODS

Animal inventories and its distribution were obtained from Statistics Peru (INEI. 2007). A literature survey related to prevalent animal production systems was also conducted to complement that information. Methane emissions calculated were converted to CO₂ eq. by multiplying annual emissions by 23 (IPCC 2001).

RESULTS & DISCUSSION

Methane emissions from livestock in 2006 were 468.8 Gg (10.8 Mt CO₂ eq) and manure contributed with 13.7 Gg yr⁻¹ (0.3 Mt CO₂ eq). This is an increase of 24.7 and 22.3 % respectively comparing with data based on 1994 animal population from those sources. Among livestock, cattle are the major contributors (64.3%) to the total methane emissions in the country as indicated in Table 1.

In the case of cattle, the regions of Puno and Cajamarca represent 11.0 and 6.8% respectively of total methane emissions. These cattle are based mainly on low productivity systems on native pastures of the Andes. As referred by Leng (1993) 'Environment-Friendly' development of livestock production systems demands that the increased production be met by increased efficiency of production and not through increased animal numbers. This appears not to be the case in Peru as the defined increase in methane emissions is based on increase in number of cattle mainly for dairy production (Gomez *et al.* 2005) and its improvement is limited by low quality of range pastures based on not favorable environmental conditions and poor management (Florez and y Malpartida 1998).

Although estimates using the International Panel on Climatic Change Tier-1 methodology are limited by factors such as weight, age, gender, and feeding regime the data of this paper indicates the most prevalent system (dairy and beef) contributing to gases emissions by animals. There is a need to further measure methane emissions in practical production scenarios characteristic of those used in Peru with emphasis on cattle population. As well from a basic information side knowledge on methane emission area lacking for alpaca (*Lama paco*), llama (*Lama glama*) and cuy (*cavia porcellus*) which are important animal population in the Andes of Latin America.

CONCLUSION

Peru's greenhouse gas emissions from animal agriculture for 2006 were 10.8 Megatonnes of carbon dioxide equivalent. Methane from enteric fermentation and manure emissions were the main contributors and increased by 24.8 and 22.3% respectively comparing to data of 1994. Cattle (64.3%) are the main contributors to those total methane emissions followed by sheep (17.3%). It is important to fully characterize, on a regular basis, the cattle and sheep population, as well as the feeding/management strategies utilized to account for changes in management practices affecting green house gases emissions.

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Table 1. Estimation of methane from enteric fermentation and manure emissions

Animal population		Environmental structure population (%)			Enteric fermentation emission		Manure management emission		Total emission	
Species	(head/year)	Cool (-15°C)	Temperate (15-25°C)	Warm (+ 25°C)	Gg/year	(%)	Gg/year	(%)	Gg/year	(%)
Dairy cattle	693,651	20	70	10	54.6	12.0	0.6	4.6	55.2	11.8
Non-dairy cattle	4,900,349	65	25	10	240.1	52.8	6.1	44.7	246.2	52.5
Sheep	15,902,000	87	12	1	79.5	17.5	1.7	12.6	81.2	17.3
Horses	1,062,154	70	25	5	19.1	4.2	1.4	9.9	20.5	4.4
Alpaca	3,517,000	95	5	0	23.8	5.2	0.5	3.6	24.3	5.2
Mules or Asses	1,113,576	55	35	10	11.1	2.4	0.9	6.2	12.0	2.6
Goats	2,092,000	39	60	1	10.5	2.3	0.3	2.2	10.8	2.3
Llama	1,254,000	98	2	0	12.5	2.7	0.3	1.8	12.7	2.7
Others*	26,282,343	105	165	30	3.9	0.8	2.0	14.3	5.8	1.2
Total					455.1	100.0	13.7	100.0	468.8	100.0

METHANE EMISSIONS FROM FREE-RANGING CATTLE: COMPARISON OF TRACER AND INTEGRATED HORIZONTAL FLUX TECHNIQUES

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ABSTRACT

Accurate measurements of methane emission rates from livestock in their undisturbed natural environments are required to assess their impacts on radiative forcing (i.e. enhanced greenhouse effect) and the environment. Here we compare results from two non-intrusive techniques for the measurement of methane emissions from cattle. The cows were kept in an outdoor feeding strip that allowed them to follow natural behavioural patterns, but contained them within a well-defined space. In the first technique, nitrous oxide was released as a tracer at the upwind edge of the feeding strip, and the downwind concentrations of nitrous oxide and methane were measured simultaneously using Fourier transform infrared (FTIR) spectroscopy. Average methane emission per cow was calculated each half-hour on three separate days from the correlation between the two gases. The second technique was the well-established integrated horizontal flux (IHF) or 1-D mass-balance method, in which we used the measured vertical profiles of methane concentration and wind speed downwind of the cows to determine the total methane emission. Comparing the IHF results to the known release rate of nitrous oxide further allowed us to test the IHF technique independently. We found agreement within 10% for all comparisons on all days. The daily methane emission rate averaged over all tracer and IHF measurements was 342 g CH₄/head.day. This is within the range of previous measurements for mature lactating dairy cattle (200–430 g CH₄/head.day), but higher than expected for yearling cattle. The high CH₄ emissions are accompanied by high CO₂ emissions also determined from the FTIR measurements. The bias is most likely due to the measurements being made during and after supplementary feeding of the cattle.

Keywords: Methane, cattle, FTIR spectroscopy, greenhouse gases, tracer technique, integrated horizontal flux

SUMMARY

We have measured methane emissions from a small herd of free-grazing yearling dairy cows using two independent analysis techniques: a novel tracer technique and the more established integrated horizontal flux technique. The cattle were confined to a narrow feeding strip but free to graze normally, a tracer gas was released from the upwind boundary of the strip, and trace gas measurements were made in air sampled from a mast downwind of the strip. All trace gas measurements were made by FTIR spectroscopy. The results from both analysis methods agreed within 5–15% for individual profiles and 2–10% for daily means, with an average CH₄ emission over 3 days of 342 g/head.day. The level of emission was consistent with parallel measurements of CO₂ emissions measured with the same setup, but is higher than would be predicted using current IPCC Tier 1 guidelines. The discrepancy may be due in part to a high but unquantified bias of the order of 10% in both methods, in part to the restricted period during which measurements were taken (reflecting emissions following feeding rather than a true 24 h average), and in part be truly higher than IPCC guidelines infer for these cows. The tracer method introduced here has several advantages, including higher precision than the IHF method and the fact that no quantitative meteorological measurements are required. On the other hand, it requires simultaneous measurements of at least two trace gas species. The CO₂ emissions can be monitored in parallel with no extra experimental effort.

In a following paper, we extend the technique such that the tracer gas release is made from a canister mounted to each individual cow and measure downwind concentrations using open path FTIR spectroscopy to intercept the plume.

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EFFECT OF DIRECT-FED MICROBES ON ENTERIC METHANE EMISSION FROM SHEEP

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ABSTRACT

The present study deals with the effects of direct-fed microbes (BLCS Renscitur, Emeral Japan Co. Ltd, Shizuoka) on methane emission and rumen fermentation in sheep. The experiment was conducted using four rumen cannulated sheep and designed as a 4 x 4 Latin square with 22-day periods. Treatments were control (no additive), BLCS4g (4 g/day), BLCS8g (8 g/day), or monensin (20 mg/day). The basal diet consisted of 40% timothy hay, 40% alfalfa hay-cubes, and 20% concentrate (DM basis). The full automated head chambers were equipped to measure methane emissions from four animals. Rumen fluid was taken to analyze VFA and ammonia-N concentration. Adding BLCS to the diet tended to decrease total daily methane emissions ($P=0.7$). Total VFA concentration was increased by BLCS 4 and 8 g ($P=0.6$). Increasing in acetate/propionate ratio was observed by BLCS (4 and 8 g) addition. Numbers of protozoa were decreased in BLCS8g ($P=0.2$), which might be related to mitigation of methane emission.

INTRODUCTION

Methane emitted from the ruminant livestock contributes to global warming as one of greenhouse gases (GHG). Its greenhouse effect performed about 21 times of carbon dioxide.

Antibiotics such as monensin have been used widely to increase animal performance and decrease enteric methane emission. However, appearance of antibiotic-resistant bacteria restricts its convenient use. Moreover, the antibiotics excreted to manures without being absorbed have been scattered on the environment (Mwenya 2006). The alternative methods of antibiotics have been developed. For probiotics direct-fed microbes including lactic acid bacteria and *Saccharomyces cerevisiae* mitigate methane emission and reduced acetate: propionate ratio (Martin and Nisbet, 1992; Gamo *et al.* 2002; Lila *et al.* 2004).

The present study deals with the effect of direct-fed microbes (BLCS Renscitur, Emeral Japan Co. Ltd, Shizuoka) on methane emission and rumen fermentation in sheep.

MATERIALS & METHODS

The experiment was conducted using four rumen cannulated sheep and designed as a 4 x 4 Latin square consisted of BLCS4g (4 g/day), BLCS8g (8 g/day), monensin (20 mg/day), and control (no additive). All animals were fed a diet consisted of 40% timothy hay, 40% alfalfa hay-cubes, and 20% concentrate (DM basis) at a restricted level of 55 g of DM/kg BW^{0.75}. The fully-automated head chambers were equipped to measure methane emissions from four animals. Rumen fluid was taken to analyze VFA and ammonia-N concentration.

RESULTS & DISCUSSION

BLCS tended to decrease daily methane emission, and to increase VFA concentration and acetate: propionate ratio. However, monensin reduced the ratio along with the suppression of rumen methanogenesis. Numbers of protozoa tended to decline in BLCS8g.

In consequence, BLCS as a direct-fed microbial supplement seemed to mitigate rumen methane emission and reduction of number of protozoa. However, an increase in acetate: propionate ratio suggested an alternative mechanism for abatement of rumen methanogenesis different from antibiotic effect.

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Table 1. Effects of BLCS and monensin on rumen fermentation in sheep

Item	Control	BLCS4g	BLCS8g	monensin
pH	6.58	6.70	6.56	6.67
Protozoa, $\times 10^4$ /mL	26.3	26.3	22.9	7.9
Ammonia-N, mg/L	163.1	192.3	180.1	145.4
Volatile fatty acids, mM				
Total	78.6	88.1	91.1	74.7
Acetate	52.5	59.4	60.6	49.0
Propionate	14.2	15.4	15.6	14.3
Butyrate	9.5	10.3	12.1	8.6
Acetate:Propionate ratio	3.8	4.0	4.2	3.6
CH ₄ Production, L/kgDMI	44.3	43.1	41.5	36.7

ANTIBIOTICS IN CATTLE DIET AFFECT GREENHOUSE GAS EMISSIONS FROM MANURE COMPOSTING

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ABSTRACT

Antibiotics are used primarily in livestock production for the treatment or prevention of disease and, to a lesser extent, to improve feed efficiency. Concern over detrimental effects, including the potential risk of bacteria acquiring resistance to specific antibiotics, led to the 1999 European Union ban on most subtherapeutic use. As part of a larger study conducted to determine whether antibiotics in the diet would persist in manure from feedlot cattle, this study presents findings on the effects of dietary antibiotics on emissions of greenhouse gases (GHG) when the manure is composted.

Manure was collected at the end of two feeding trials in 2005 and 2006 in which feedlot cattle were assigned to one of five dietary groups: 1) Control: no antibiotics added; 2) AS700: chlortetracycline (Aureomycin[®]) and sulfamethazine, each at 350 mg/animal-day; 3) A11: chlortetracycline at 11 mg/kg feed; 4) T11: tylosin phosphate (Tylan[®]) at 11 mg/kg feed; and 5) A40: chlortetracycline at 350 mg/animal-day. Feeding trials ended 21 July 2005 and 20 July 2006. In 2005, manure was left in the feedlot pens for 76 days, then removed and composted for 126 days, beginning 4 October 2005. In 2006, manure was left in the pens for 33 days before composting (for 105 days, commencing 22 August 2006). Two open windrows were constructed for each treatment and treated as two replications. The rate of greenhouse gas surface emission was measured weekly for the first 8 weeks and biweekly for the rest of composting period using a vented static chamber technique. The total carbon content (TC) of manure used to construct the compost windrows was measured using a CNS analyzer (Carlo Erba, Milan, Italy).

In both years, surface emissions of CO₂ from the compost windrows were significantly higher ($P < 0.05$) with treatments A11 and AS700 than with the control. This indicates that treatments A11 and AS700 led to more rapid decomposition of organic matter (releasing CO₂) during manure composting. The CO₂ emission rates in 2005 were significantly lower than those in 2006 for all treatments, reflecting lower total carbon (TC) contents in manure from 2005 ($13.8 \pm 0.2\%$) than from 2006 ($24.5 \pm 0.2\%$). The combination of wet manure and cooler air temperatures at windrow construction time in 2005 delayed windrow heating and lowered CO₂ emissions. Surface emissions of CH₄ in 2005, which varied from 0.006 to 0.232 g C/m².day, were not significantly affected by the treatments. However, CH₄ emissions from the A11 and AS700 windrows were higher ($P < 0.05$) than from A40, but similar to control, in 2006. Except for treatments A11 (2.4%) and AS700 (1.7%) in 2005, CH₄-C emission accounted for <0.7% of total C emissions. Emissions of N₂O were affected by antibiotic treatments in both years. The rates of N₂O emission were highest with AS700 in 2005 and A11 in 2006, while A40 had the lowest ($P < 0.05$) N₂O emission in both years. Although the residual levels of antibiotics in the manure at the time of windrow construction are not yet known (analysis is currently in progress), it is possible that the use of antibiotics in cattle diets affected the microbial community in cattle manure which in turn may have affected the composting process and led to the observed differences in GHG emissions. Thus, the use of antibiotics in cattle diets may have an impact on GHG emissions when manure is composted. This should be taken into consideration when estimating agricultural GHG emissions from the livestock sector. While considerable research effort continues to be directed toward important issues surrounding bacterial resistance to antibiotics, the potential environmental impact of dietary antibiotics on GHG emissions during manure composting is also significant and warrants further study.

Keywords: Cattle feedlot manure, composting, greenhouse gas emission, veterinary antibiotics

VARIANCE IN PROTOZOA POPULATIONS IN FEEDLOT CATTLE AND GRAZING SHEEP

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SUMMARY

Rumen ciliate protozoa are often positively associated with enteric methane production by ruminants. A survey of the rumen protozoal population of grazing sheep ($n = 281$) was conducted to determine the between sheep variance in this trait. Correspondingly, two herds of cattle fed a cereal grain–ration based ($n = 87$ steers; $n = 89$ bulls) were assessed to determine between cattle variance in protozoal populations. In all cases, rumen fluid samples were collected by inserting a tube down the oesophagus of the animal, withdrawing rumen fluid using a mild suction, and preservation of the sample (4 mL) by mixing with 16 mL of formal saline.

Study 1. Merino rams ($n = 281$) were brought directly from the paddock (tall fescue + native species) and sampled. To maintain continuity of sampling time relative to time off feed, the study was completed over successive sampling days.

Studies 2 and 3. Angus steers (study 2; $n = 80$) and bulls (study 3; $n = 87$) consuming feedlot diets (80% barley grain, 11–12.2 MJ ME/kg ration DM) were adapted to diets for over 80 d before a rumen sample was collected.

Results indicate that the between-animal variation in the total population of ciliate protozoa in the rumen is far less in grazing sheep than in feedlot cattle, although the entodiniomorph protozoa dominate numerically in each system. Such large variation in feedlot cattle is in keeping with that found in cattle with low rumen pH (Franzolin and Dehority 1996). Since ciliate protozoa have been associated with enteric methane production, it is hypothesised that the lower variance in protozoa populations in grazing animals may in part explain the lower variance of prediction of daily methane production of ruminants fed low energy density diets compared to high energy density rations, as displayed in data reviewed by Pelchen and Peters (1998) and Johnston *et al.* 1991. Accounting for protozoal populations may allow further improvement in prediction of enteric methane production.

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Table 1. Description of the population of enteric protozoa in the rumen fluid of grazing sheep and feedlot cattle including the percentage of holotrichs (% holo) and entodiniomorph (% ento) protozoa present

Study	Protozoa ($\times 10^3$)/mL			CV (%)	% holo	% ento
	Mean	Max.	Min.			
1. Grazing sheep	738	1.578	247	32	8.1	91.9
2. Feedlot steers	254	3.229	0	182	17.2	82.8
3. Feedlot bulls	357	2.606	0	140	7.3	92.7

EFFECTS OF SLURRY INJECTOR DISK SHAPE ON REDUCTION OF SOIL-MACHINE RESISTANCE DURING OF ANIMAL EFFLUENT APPLICATION

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INTRODUCTION

Several systems for animal effluent application with slurry tankers and spreaders, or injectors, have been introduced in Hokkaido, Japan. However, to ensure that the intended amount of nutrients in slurry is applied to a given field, it is important to know the amounts of residual N, P and K in the soil beforehand. It is also important that suitable machinery is selected to ensure accurate slurry application.

Chambers *et al.* (2001) pointed out that the use of slurry injectors not only avoids odour diffusion, ammonia emission and the spreading of slurry spread on plant leaves, but also allows the intended amount of slurry to be spread. However, slurry injectors cause large soil tillage resistance. This means that the operation of slurry injectors requires more fossil fuel, though they provide an advantage for crops and lessen environmental pollution from chemical fertilizers. An important objective regarding slurry injectors is to reduce the fuel consumption by minimizing the cutting resistance of the injector disks. The reduction of soil-disk resistance and its impact on reducing the energy requirements of slurry spreader and injector implements could be achieved through experimentation conducted for this study.

METHODS

Figure 1 shows the circular disks of the slurry injector used in the experiments. A shallow injector for grassland is normally equipped with a solid disk (Fig. 1a) to open a slot at the soil surface. In order to reduce the friction between the disk side and soil, a newly designed perforated disk was made (Fig. 1b). A part of the disk surface was perforated to minimize the disk side-soil contact area. Both disks were 3 mm thick and 300 mm in diameter.

Experiments were conducted on an indoor soil bin containing loam soil. The composition of the loam was 48.0 % sand, 37.5% silt and 14.5% clay. Preparation of the soil for experiments was done by rotary tilling, compacting and leveling after adding adequate water for desired moisture content. The soil moisture content was about 14 % wet basis. The average cone index of the soil for a depth range of 0 to 10 cm was 374 kPa. The forward velocity of the tested device was adjusted to 0.17m/s. Cutting depths of the disk were set at 4, 6 and 8 cm. Perforated and solid disks of 300 mm diameter were used in the experiment to compare soil-disk and gauge wheel motion resistance.

RESULTS & DISCUSSION

Table 1 shows disk cutting resistance and the ratio of the resistance decrease of perforated disk to solid disk resistance at each cutting depth. The absolute value of cutting resistance of a disk increased with increasing cutting depth. The cutting resistances of the perforated disk were smaller than those of the solid disk at each cutting depth. The average decrease was 21%.

Table 2 shows the motion resistance and the ratio of the resistance increase of a gauge wheel with a perforated disk to the resistance with a solid disk at each cutting depth. The absolute values of motion resistance of a gauge wheel decreased with increasing cutting depth of the disk. The motion resistances of the gauge wheel of the perforated disk unit are larger than those of the solid disk at each cutting depth. The average increase in the ratio was 9.7%. This is caused by the increase of the reaction of the dynamic load to the gauge wheel. The interaction between the injector unit and soil is explained by the phenomena that the reduced vertical load to the wheel transferred to the disk (Kishimoto *et al.* 2002).

Table 3 shows total resistance and the ratio of the resistance decrease of the perforated disk unit to the total resistance of the solid disk unit at each cutting depth. The average decrease ratio was 4.5 %. From these results, total resistance (soil-disk resistance and the motion resistance of the gauge wheel) was considered to be less for the perforated disk unit (a cutting disk and a gauge wheel) than for the solid disk unit. The perforated disk unit produced less cutting resistance and more motion resistance than that of a solid disk unit. The absolute values of total resistance of both perforated and soil disk units increased with an increasing cutting depth of the disk. However, the total resistances of the perforated disk unit were smaller than those of the solid disk at each cutting depth. It was also found that disk perforation is effective in reducing total resistance.

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Table 1. Disk cutting resistance and the ratio of the decrease in resistance of the perforated disk compared to the solid disk resistance

Cutting depth (mm)	Solid disk (N)	Perforated disk (N)	Decrease (%)
40	91.4	66.1	27.7
60	200	162	19.1
80	282	233	17.5

Table 2. Gauge wheel motion resistance and the ratio of the resistance increase of a gauge wheel with a perforated disk to the resistance with a solid disk at each cutting depth

Cutting depth (mm)	Solid disk (N)	Perforated disk (N)	Increase ratio (%)
40	213	221	3.63
60	165	194	17.8
80	179	193	7.66

Table 3. Total resistance and ratio of the resistance decrease of perforated disk unit to the total resistance of solid disk unit

Cutting depth (mm)	Solid disk (N)	Perforated disk (N)	Decrease ratio (%)
40	304	287	5.77
60	365	356	2.45
80	461	425	5.31

Figure 1. Open slot disks used for experiments: (a) solid disk and (b) perforated disk.



EFFECT OF FEEDING CAUCASIAN CLOVER, WHITE CLOVER, RYEGRASS AND COMBINATIONS OF RYEGRASS AND CLOVERS ON THE METHANE EMISSIONS OF WETHER LAMBS

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INTRODUCTION

In vitro trials using rumen fluid from sheep have found that caucasian clover lowered methane (CH₄) production by 40-50% when compared with other legumes and grasses (Clark 2005). *In vivo* trials are needed to ascertain if this is the case when caucasian clover is consumed by animals either as a pure stand or with ryegrass, and to compare it with white clover.

MATERIALS & METHODS

The trial was conducted at the AgResearch Lincoln Research Centre using 38 wethers (5-6 months of age) randomised on liveweight into 5 groups on Day 0. The treatments were caucasian clover, white clover, a 70:30 mix of ryegrass : caucasian clover, a 70:30 mix of ryegrass : white clover ($n = 8$ for these groups) and ryegrass ($n = 6$). Pure swards of forage were cut each morning and fed at 1.5 times maintenance with half of each animal's allowance being fed at 0800 hours and the rest at 1500 hours. Wethers were penned in their groups except for the 2 periods (Days 9-17 and Days 37-43) when they were placed in metabolism cages for the measurement of CH₄ emissions, dry matter intake (DMI) and apparent digestibility. Emissions of CH₄ were measured by the sulphur hexafluoride technique. Digestibility of the diet, DMI, CH₄/day and CH₄/kg DMI for Periods 1 and 2 were analysed by analysis of variance. Liveweight was analysed by repeated measures analyses.

RESULTS

There were significant effects of the type of feed on DMI and digestibility in Periods 1 and 2 but no effects on liveweight (Table 1). The DMI was higher ($P < 0.05$) for wethers fed white clover than those fed caucasian clover in both periods. Digestibility of caucasian clover was higher ($P < 0.05$) than for the other forages except for the ryegrass : caucasian clover mixture in Period 1. There were no significant effects of the feed on CH₄/day although the trend in both periods was for higher CH₄ emissions from wethers fed caucasian clover. Wethers fed caucasian clover had higher ($P < 0.05$) CH₄/kg DMI than those fed white clover or the ryegrass : clover mixtures in Period 2, and a similar trend in Period 1.

DISCUSSION

Higher CH₄ emissions from wethers fed caucasian clover compared to white clover and to a lesser extent ryegrass contrasts with previous *in vitro* comparisons where CH₄ emissions were lower from caucasian clover (Clark 2005). There is no explanation for the differences in CH₄ emissions between the *in vivo* and *in vitro* analyses, especially given the high apparent digestibility of the caucasian clover.

Higher CH₄ emissions from sheep fed ryegrass compared to white clover has been reported previously for both *in vitro* (Clark 2005) and *in vivo* (Lee *et al.* 2004) comparisons. It is interesting to see that combining the ryegrass with as little as 30% white clover or caucasian clover can reduce CH₄ emissions of wethers by 17-24% compared with feeding ryegrass alone.

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Table 1. Mean liveweight, dry matter intake (DMI), digestibility of the feeds, CH₄/day and /kg DMI for the wethers fed caucasian clover (CC), white clover (WC), ryegrass (Ry), a 70:30 mix of ryegrass:caucasian clover (R:CC), and a 70:30 mix of ryegrass:white clover (R:WC) in Periods 1 and 2

	Period	CC	WC	Ry	R:CC	R:WC	SE	Signif.
Liveweight (kg)	1	37.5	38.0	37.4	37.6	37.8	1.50	n.s.
Liveweight (kg)	2	37.7	38.8	39.8	40.1	36.6	1.4	n.s.
DMI (kg/day)	1	0.89 ^b	0.98 ^a	0.79 ^c	0.87 ^{bc}	0.88 ^b	0.031	***
DMI (kg/day)	2	0.90 ^c	1.04 ^a	0.95 ^{bc}	0.99 ^{ab}	1.01 ^{ab}	0.024	***
Dig DM (%)	1	68.4 ^a	62.8 ^b	63.5 ^b	65.0 ^{ab}	62.2 ^b	1.33	**
Dig DM (%)	2	68.8 ^a	62.3 ^b	64.6 ^b	64.6 ^b	64.2 ^b	0.983	***
CH ₄ /day (g/day)	1	31.0	27.3	26.4	23.0	22.6	4.83	n.s.
CH ₄ /day (g/day)	2	39.5	28.4	34.2	28.1	30.2	4.10	n.s.
CH ₄ /DMI (g/kg DMI)	1	35.2	28.0	34.5	27.0	26.1	4.4	n.s.
CH ₄ /DMI (g/kg DMI)	2	45.5 ^a	27.1 ^b	35.5 ^{ab}	29.2 ^b	29.6 ^b	4.34	*

Means in row with different superscripts are significantly different ($P < 0.05$).

EFFECTS OF BLCS SUPPLEMENTS ON METHANE EMISSIONS FROM LACTATING DAIRY COWS

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INTRODUCTION

There is an urgent need for New Zealand farmers to have access to technologies that reduce agricultural green house gas emissions in a safe and cost effective manner. Bio Livestock Clean System (BLCS®; Japan Jinando Enterprises Inc) is a commercially available supplement, containing lactobacillus, bacillus natto and yeasts that is reputed to modify rumen activity resulting in reduced methane (CH₄) emissions *in vitro* and increased milk production *in vivo*. This paper presents the results of an experiment to test the ability of BLCS® to reduce CH₄ emission and enhance milk production in dairy cattle under New Zealand grazing conditions.

MATERIALS & METHODS

Thirty Friesian cows in early lactation were selected on the basis of similar milk production from the dairy herd at AgResearch Flock House. The cows were randomised into two groups ($n=15$) based on their milk production and composition, and grazed together on pasture at a high herbage allowance to ensure *ad lib* access. There was a 10-day adjustment period with no treatment at the end of which the cows were weighed and milk production and composition were measured and the values for each cow were used as covariates in subsequent analyses. All cows were then offered 400 g of triticale silage at the morning milking into which 10g of BLCS was mixed for the treated cows. The trial continued for 8 weeks with weekly measurements of liveweight, milk production and milk composition over the first 4 weeks, increasing to twice weekly over the second 4 weeks. Each cow received a permeation tube containing sulphur hexafluoride (SF₆) in the third week and CH₄ and SF₆ was collected in evacuated yokes over 24 hrs on each of 4 consecutive days at the beginning and end of the second 4 week period. The CH₄ and SF₆ collected was analysed by gas chromatography. The equations of Johnson *et al.* (1994) were used to calculate CH₄ emission over 24 hrs and the mean of the CH₄ emissions are presented in this paper. Intakes (DMI) were calculated using the equations from the Standing Committee on Agriculture (1990).

RESULTS

There were no significant differences between control and BLCS cows for the liveweights at the start and end of the experiment but liveweight gain was higher ($P<0.05$) for BLCS cows (Table 1). Even over the second month of the experiment the liveweight gain of BLCS cows were 62% higher than for control cows. Milk yield was 3.3% higher with no effects on milk composition, and CH₄ emissions were 3.4% lower for BLCS than for control cows, although none of these differences were significant. Estimated DMI were slightly higher for BLCS cows but this was also not significant. BLCS cows had 10% lower ($P<0.05$) CH₄/kg DMI than for control cows.

DISCUSSION

The results from this trial suggest that BLCS has some positive impact on productivity and CH₄ emissions but that these effects are relatively small. During the trial not all treated cows ate their BLCS supplemented forage on all days and so the average quantity of BLCS consumed per day was less than 10g. Since this is the first time that BLCS has been tested with a fresh grass diet we cannot assess whether the quantity of BLCS offered each day was the most appropriate daily allowance for pasture-fed animals.

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Table 1. Mean values for liveweight, liveweight change, milk volume and characteristics, DMI and CH₄/day and /kg DMI for the control and BLCS supplemented cows

	Control	BLCS	s.e.d.	Significance
Start liveweight (kg)	450.3	442.3	13.15	n.s.
End liveweight (kg)	454.6	458.8	12.55	n.s.
Liveweight gain (kg/day)	0.194	0.377	0.075	*
Milk volume (kg)	23.0	23.8	0.52	n.s.
Fat (%)	4.40	4.35	0.121	n.s.
Protein (%)	3.67	3.70	0.048	n.s.
Estimated DMI (kg DM/day)	15.6	16.7	0.82	n.s.
CH ₄ (g/day)	295.8	285.8	9.87	n.s.
CH ₄ /kg DMI (g/kg DMI)	19.0	17.1	0.81	*

EFFECTS OF TANNIFEROUS PLANTS ON *IN VITRO* ENTERIC METHANE AND OTHER RUMEN FERMENTATION PRODUCTS

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ABSTRACT

Tanniferous plants have demonstrated abilities to reduce enteric methane by ruminants, however, the mechanism of action is not completely understood. The aim of this study was to investigate the influence of tanniferous plants on methane mitigation. The *in vitro* Hohenheim Gas Test technique was applied to evaluate the four tannin-rich plants *Stylobium aterrimum* (STA), *Stylobium deeringianum* (STD), *Leucaena leucocephala* (LEU), *Mimosa caesalpiniaefolia* Benth (MIC) and *Cynodon x cynodon* (CYN) as control, containing 20, 64, 56, 105 and 0.2 g condensed tannin (CT)/kg dry matter (DM), respectively. The production of total gas (GP), methane (CH₄), ammonia, short chain fatty acids (SCFA), microbial mass (MM) and true substrate degradability (TSD) were measured at two main timepoints: $t_{1/2}$ (time of half maximal gas production) and 24h. Methane production at $t_{1/2}$ was reduced ($P<0.05$) with addition of legumes by 17% and when related to TSD this reduction reached on average 50% with LEU and STA and 25% with MIC and STD. LEU at $t_{1/2}$ resulted also in the lowest GP (129 mL/gTSD), which was accompanied by the highest TSD (727 g/kg). In contrast, CYN and MIC presented the highest GP value (211 and 182 mL/gTSD). Additionally, high MM/SCFA ratios in $t_{1/2}$ were found in LEU (15) and STA (14) and followed by STD (6), MIC (6) and CYN (5). The higher MM in LEU and STA suggested higher ATP production; however, the different proportion of the SCFA demonstrated different routes of ATP acquisition. In general, tanniferous plants were able to reduce enteric methane with different fermentation products proportions, which suggested that only LEU and STA could contribute to increase animal production with higher efficiency.

NITROUS OXIDE EMISSIONS FROM ‘PROTOTYPE FARMS FOR DAIRY FARMING’S FUTURE’

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ABSTRACT

This study is examining nitrous oxide (N₂O) emissions from “Prototype farms for dairy farming’s future”. The experimental programme for the Prototype farms is being run as farmlets (e.g. Control farmlet and “Tight N” farmlet). The control farmlet consists of industry average inputs and current typical management practices; while the “tight N” farmlet consists of use of both a restricted grazing regime and a nitrification inhibitor. We are measuring N₂O on these dairy farmlets during several typical grazing patterns/seasons. During late spring/early summer 2006 and autumn 2007, N₂O emission rates were similar between the two farmlets, except during November 2006 when the rates were higher from a “tight N” paddock than those from a control paddock. Preliminary results from winter 2007 show emission rates to be higher than in other seasons and that rates are lower in the “tight N” farmlet than in the control farmlet. We will continue to measure N₂O emissions until winter 2009.

INTRODUCTION

Nitrous oxide is formed in soils during the microbiological processes of nitrification and denitrification, and these processes are affected by soil and climatic factors. N₂O emissions from grazed pastures in New Zealand are highest in winter and spring when soils are wet (Luo *et al.* 2007). There are a number of possible management options to reduce N₂O emissions from grazed pastures, including using a restricting grazing regime during wet conditions (Luo *et al.* 2007) and using a nitrification inhibitor (Di *et al.* 2007).

A farmlet trial is being conducted by Dexcel to develop “Prototype farms for dairy farming’s future”. A key goal of this study is to bring together the best available technologies to increase farm productivity and environmental efficiency. We are using this trial to determine the potential reduction in N₂O emissions obtained by integrating both a winter restricted grazing strategy and a nitrification inhibitor on dairy farms.

MATERIALS & METHODS

The experimental farmlets are at Scott Farm near Hamilton, New Zealand and represent highly productive dairy pastures. We are using two farmlets: Control farmlet – with a stocking rate of 3.0 cows/ha, and managed under a rotational grazing regime; “Tight N” farmlet –also with a stocking rate of 3.0 cows/ha. The grazing regime on this farmlet is similar to that on the control farmlet, except during the non-lactating period between late autumn and early spring when cows graze for 6 hours per day on pasture with the remaining 18 hours spent in an animal shelter. A nitrification inhibitor (Eco-N) is also applied onto the “tight N” farmlets on three occasions after grazing events in winter and early spring.

Measurements are being made during three contrasting seasons of the year: (i) spring/early summer with a short-medium grazing rotation period, (ii) late summer/early autumn with a short grazing rotation period, and (iii) winter with a long grazing rotation period. A chamber technique is being used to measure N₂O emissions (Luo *et al.* 2007).

RESULTS & DISCUSSION

Data from measurements during late spring/early summer 2006 and autumn 2007 indicated that N₂O emission rates were similar between the “tight N” farmlet and the control farmlet in both of the seasons, except for November 2006 when the rates were higher from a “tight N” paddock than those from a control paddock. The magnitude of overall N₂O emission rates during the early autumn season of 2007 was lower than those during the late spring/early summer season of 2006. Preliminary results from winter 2007 show emission rates to be higher than in other seasons and that rates are lower in the “tight” N farmlet than in the control farmlet. These should be viewed as preliminary data from three sampling seasons only, and further measurements are being conducted to determine any differences between the two farms or seasons.

ACKNOWLEDGEMENT

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QUANTITATIVE EVALUATION OF GREENHOUSE GAS EMISSIONS BY LAND APPLICATION OF DAIRY COW SLURRY

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SUMMARY

Based on the Kyoto Protocol of 1997, the Government of Japan is obligated to reduce the amount of Japan's greenhouse gas emissions by 6%, making 1990 into a base year. However, the amount of greenhouse gas emissions in Japan in the 2005 fiscal year was 1,364 million t (CO₂ equivalent), about 8.1% more than that in the base year. Thus we see that Japan must take more effective measures to reduce its greenhouse gas emissions. Greenhouse gas emissions from the agricultural sector are calculated in five categories, with emissions from the three categories of enteric fermentation, manure management, and rice cultivation assessed as the main sources. The total methane and nitrous oxide emissions from Japanese agricultural sources in 2004 were estimated to be 50.2 million t (CO₂ equivalent), and the emissions from manure management are also very large (7.3 million t; 14.5% of total emissions from agricultural sources).

Since various kinds of processing systems are used in livestock excrement management, precise inventory data corresponding to each processing system are required. However, emissions from dairy cow excrement slurry spread on pastures have not yet been investigated. Since spreading dairy cow excrement slurry on pastures is the main processing system used in Hokkaido, which contains about 52.6% of Japan's total dairy cow population, a precise estimate of greenhouse gas emissions is important.

In this study, we constructed a chamber system in order to evaluate greenhouse gas emissions from a pasture when dairy cow excrement slurry is spread. This system can quantitatively estimate environmental impact gases, including not only greenhouse gases but also odorous substances. The chamber system is made from vinyl chloride with a capacity of 0.1 m³ (1 x 0.5 x 0.2) and has a vent of 100 mm in diameter equipped with a small fan. In order to quantitatively evaluate gas emissions, we controlled the airflow of the surface of the pasture through use of the fan. Gas emissions can be estimated quantitatively by calculating gas concentration (mg/m³) x the ventilation rate (m³/h) of an exhaust port. The ventilation rate of the chamber was 123 times/h. When conducting measurements in an actual pasture, an iron frame was driven into the pasture and the chamber was placed on the frame. The application rate was 4t/10a. After the slurry had been applied, air samples were immediately taken from the exhaust port, and ammonia, methane, and nitrous oxide concentrations were measured by using an infrared photoacoustic detector (INNOVA, Multi Gas Monitor type 1412) continuously for a week. The odour concentration was measured by Japanese standard odour analysis using six human panels.

The gas emissions of a shallow injection of dairy cow excrement slurry were compared with those of surface-applied slurry, using our chamber system. The application went three times (Runs 1-3) during August from July. The average temperature of the ambient air of Runs 1-3 was 17.8 ± 0.8, 19.7 ± 0.7, 22.9 ± 0.9°C, respectively. The NH₄⁺ concentration of the slurry was 284.8 ± 0.4, 181.6 ± 20.8, 201.7 ± 2.0 (mg/L), respectively.

A sharp peak of ammonia volatilization just after slurry application was recognized in all repeated examinations (3.2, 7.8, 8.6 mg/m³) of the surface applications. Approximately 68 to 84% of the volatilization of ammonia, which is a potential greenhouse gas, occurred on the day of application in both systems. Shallow injection reduced the volatilization of ammonia to about 66 to 87% of that in the case of the surface application. This value is the same as that reported by Misselbrook *et al.* (2002). Shallow injection also reduced odour concentration to about 42% of that in the case of the surface application.

Methane and the nitrous oxide emissions were small, and the reduction obtained by the shallow injection method was not appreciable during the experimental period. Because Sherlock (2002) reported that nitrous oxide emissions increased two weeks after slurry application, measurement over a long term may be needed.

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METHANE EMISSIONS BY NELLORE BEEF CATTLE CONSUMING *BRACHIARIA BRIZANTHA* WITH DIFFERENT STAGES OF MATURATION

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INTRODUCTION

The methane produced from ruminal fermentation, constitutes an important greenhouse effect gas and a loss in the potential energy production that has been studied per decades (HOWDEN; REYENGA, 1999). In the ruminants, the quantification of rumination gases production has been used as indicative of the micro-organism activity in the ruminal environment, that the methane eructation of the animals represents a loss of energy of approximately 2 to 14% of the food crude energy and has implications in the global warming (JOHNSON; JOHNSON, 1995; DEMARCHI *et al.* 2003). The objective of this study was to evaluate methane (CH₄) emission rate by sulfur hexafluoride (SF₆) tracer technique (Primavesi *et al.* 2004) in bovines of Nellore breed fed with *Brachiaria brizantha* hay in different maturation stages.

MATERIALS & METHODS

The three treatments were: I- Hay of *Brachiaria brizantha* with 15 days of maturation, II- Hay of *Brachiaria brizantha* with 45 days of maturation and III- Hay of *Brachiaria brizantha* with 90 days of maturation. Six bovines of Nellore breed, males, castrated, rumen-cannulated, with 402 ± 51.62 kg of initial average weight were used in a duplicated 3 x 3 latin square design. The trial lasted 60 days. The adaptation period to the diets lasted 7 days. In the eighth day, faeces collections (5 days) and feed sampling were done. In 13th day the methane collections were initiated using the sulfur hexafluoride (SF₆) tracer technique. The 20th day was used for ruminal fluid sampling at 0, 2, 4, 6, 8, and 10 hours after 1st meal for determination of pH, amoniacal-N, and volatile fatty acids in ruminal fluid.

RESULTS

Methane data are presented in Table 1. The cutting ages did not affect the total concentration substantially or the molar ratio of the VFA, as well as pH, even so the ruminal concentration of NH₃-N decreased as the cutting ages increased. For the digestion data, only the digestibilidade of the CP increased and the data of the NFC decreased as the cutting ages increased. The methane production for animal/day was not affected by the different cutting ages. The animals fed with the hay produced with 45 days of age had presented reduction of DM intake, what it resulted in bigger methane production for unit of DM intake.

CONCLUSION

The quality of the hay did not affect the methane emission for animal, but it affects the consumption of dry matter, what results in differences in the methane emission for unit of dry matter or ingested nutrients.

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Table 1. Means, coefficient of variation (CV) and statistical probabilities for methane emissions

	Treatments			CV (%)	Probabilities	
	15	45	90		Linear	Deviation
CH ₄ (g/day)	132.65	138.32	133.93	25.14	0.9658	0.6767
CH ₄ (g/h)	5.53	5.76	5.58	25.13	0.9655	0.6778
CH ₄ (g/kg body weight)	0.33	0.34	0.33	20.96	0.9166	0.6395
CH ₄ (g/kg ^{0.75})	1.48	1.54	1.51	21.72	0.8853	0.7043
CH ₄ (g/ kg dry matter)	17.38	23.41	20.02	22.07	0.3812	0.0299
CH ₄ (g/kg organic matter)	22.32	32.03	26.39	24.14	0.3614	0.0175
CH ₄ (g/kg digestible OM)	36.86	56.40	46.18	30.29	0.3348	0.0340
CH ₄ (% gross energy)	6.18	9.02	7.42	24.77	0.3249	0.0168
CH ₄ (% digestible energy)	11.04	16.89	13.83	30.30	0.3344	0.0340

GREENHOUSE GAS EMISSIONS PRODUCED BY A POULTRY MANURE TREATMENT FACILITY USING FORCED AERATION COMPOSTING

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INTRODUCTION

About 88 million tons of waste is excreted by livestock annually in Japan. According to recent data estimates, about 4,726 Gg CO₂ eq of N₂O and 2,540 Gg CO₂ eq of CH₄ might be emitted from all livestock manure management processes (Ministry of the Environment, Japan 2006). These amounts account for about 0.6% of Japan's annual greenhouse gas (GHG) emissions. In Asian countries, the compost process is widely used for the treatment of livestock waste. Enclosed and forced aeration-type composting facilities involving a deodorization system are widely used in Japan for poultry manure treatment. Not only the compost, but the emission factor of each treatment system should be evaluated in consideration of each country's treatment procedure and general conditions, because these factors might vary widely. It is important that each country has a technique for measuring GHG emissions, not only for inventory data but for the development of greenhouse gas regulations and technologies. In the present report, the amount of harmful gas emissions produced by the process of composting poultry manure with forced aeration and continuous mixing were determined using an actual processing facility on a working farm.

MATERIALS & METHODS

Enclosed and forced aeration-type composting facilities equipped with a deodorization system are widely used in Japan for treating poultry manure. Amounts of harmful gas emissions from the process of composting poultry manure were determined using an actual processing facility (COMPO S-18ET, CYUBU ECOTEC CO. LTD.) on a working farm. Under a fill-and-draw-type operation, about 20 t of raw poultry manure was introduced into a cylinder-type reactor and processed under continuous mechanical agitation and aeration forced from the bottom of the reactor. Exhaust gas from the reactor was diluted with fresh air, and was introduced into the deodorization facility by means of an external blower. During several days of manure treatment periods, CH₄, N₂O, and NH₃ concentrations of the diluted exhaust gas from the composting facility and ambient air (inlet air) were measured using an infrared photoacoustic detector (IPD, multi gas monitor type 1312, INNOVA, Copenhagen DK) at 30-min intervals. Airflow rate analyses using pressure sensors were also performed at the same intervals.

RESULTS & DISCUSSION

The temperature of the poultry manure in the reactor rose to around 60 - 70°C within a few hours and remained at that temperature range for 3 - 4 days. During this composting process, the total amount of nitrogen in the raw materials was reduced only by 3.6% and 10.3%, as demonstrated by a comparison before and after this processing. As a result, final products with high nitrogen content (4.75% and 5.38%) were produced.

According to the results, 1.7 to 3.4% of the initial nitrogen in the raw manure was lost as NH₃ and 0.01 to 0.07% was lost as N₂O during the composting process. CH₄ emissions could be kept lower with forced aeration. Only 0.23 - 0.59 g of CH₄ were generated from 1 kg of the initial organic materials. These emission factors were similar to the CH₄ and N₂O emission factors of a piled-type composting system reported previously (Osada *et al.* 2004). However, about 19 - 40% of the initial nitrogen was lost as NH₃ during piled-type composting. In the present study, under continuous mechanical agitation and forced aeration, most of the raw poultry manure in the reactor was kept oxidative and over 60°C from the beginning of treatment. These treatment conditions increased manure uric acid and total nitrogen retention by reducing NH₃ volatilization. Forced aeration-type composting should be recognized to reduce NH₃ emissions because NH₃ might be a potential greenhouse gas.

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ESSENTIAL OILS AND THE DIVERSITY OF RUMEN METHANOGENS

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ABSTRACT

The objective of this study was to evaluate the impact of selected essential oils with known antimicrobial properties on archaeal communities in the rumen, using an ovine model. Forty weaned Canadian Arcott ewes fed a barley-based diet were allotted to one of four dietary treatment groups for 13 weeks (10 ewes per treatment). The treatments were: cinnamaldehyde, garlic oil or juniper berry oil (included at 0.02 g/kg DM) and a control, containing no additive. Ruminal content samples (approx. 50 mL) were collected from each ewe at slaughter and pooled by treatment. DNA was extracted from the pooled samples (approx. 400 µL) and analyzed for methanogenic archaea using quantitative-PCR, denaturing gradient gel electrophoresis (DGGE), cloning, and sequencing. Our results suggest that the total copy number of archaeal 16S rRNA was not significantly affected by the treatments. Phylogenetic analysis indicated that cinnamaldehyde, garlic, and juniper berry oils increased the diversity of methanogenic archaea related to *Methanosphaera stadtmanae*, *Methanobrevibacter smithii*, and some uncultured groups in the ovine rumen. Rumen methanogens share non-random symbiotic and commensal relationships with rumen protozoa and essential oils have been shown to alter protozoal populations. Therefore the changes in the diversity of the methanogenic archaea observed with the essential oil supplementation may have resulted from the changes in their associated protozoal species. Supplementation of ruminant diets with essential oils may alter the diversity of rumen methanogens, although the methanogenic capacity of the rumen may not be affected.

Additional keywords: essential oil, methanogen, ovine rumen.

EFFECT OF SF₆ TRACER PERMEATION RATE UPON THE CALCULATED RUMINAL METHANE PRODUCTION RATES USING RUMEN HEAD SPACE GAS COMPOSITION

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INTRODUCTION

The sulphur hexafluoride (SF₆) tracer technique for estimating methane (CH₄) emissions from ruminants relies on the release of a known quantity of SF₆ gas from a pre-calibrated permeation tube inserted into the reticulo-rumen of the test animal and the CH₄/SF₆ ratio of concentrations (above the background air) on representative breath samples collected from each participating animal (Johnson *et al.* 1994). However, the growing evidence (Vlaming *et al.* 2007; Pinares-Patiño *et al.* 2007) for a positive relationship between the pre-calibrated permeation rate (PR) of SF₆ and the calculated CH₄ emission, suggests that the molar proportion of SF₆ in the breath sample relative to that of CH₄ decreases as the PR of SF₆ increases. Since gas-mixing processes within the rumen headspace as well as during expulsion through the throat and the mouth are highly turbulent, it would not be expected that such mixing to discriminate between CH₄ and the 9-fold heavier SF₆ molecules. Alternatively, the fate of the SF₆ gas from release in the rumen to disappearance from it (e.g. SF₆ outflow to the lower digestive tract) might be implicated in the systematic lower abundance of SF₆ (relative to that of CH₄) in the breath sample. This study explored the above hypothesis by calculating CH₄ emissions based on ruminally-collected gases.

MATERIALS & METHODS

Six adult non-lactating Holstein cows fitted with permanent ruminal cannulas equipped with stoppers allowing collection of rumen head space gas samples without having to open the cannula (Jouany and Senaud 1979) were used. The experiment lasted 39 days, which included 21-day acclimatisation, followed by two periods (P1 and P2) of gas measurements over days 23–25 and 37–39, respectively. Cows were randomly subdivided into two groups (A and B) of 3 animals each and randomly assigned to permeation tubes deployment with low PR (LPR, 10.05 ± 1.8 µL/h) or high PR (HPR, 20.05 ± 3.6 µL/h) of SF₆ in a crossover design over days 17–25 and 31–39. Between days 25 to 31 tubes were maintained in the laboratory at 39°C. The cows were kept in individual stalls and fed maize silage at 80% their *ad libitum* intake, delivered in two equal meals at 0800 and 1600 hours. Rumen gases (50 mL) were collected immediately before the morning feeding and then hourly over 8 h. Concentrations of CH₄ and SF₆ in rumen gas head space (i.e. their molar mixing ratios) were determined by gas chromatography after dilution (1:150, vol:vol) with N₂ gas.

RESULTS & DISCUSSION

There was no treatment effect (P=0.80) upon mean concentrations of CH₄ (305 and 291 ppm for HPR and LPR, respectively). Despite the two fold difference in PR between LPR and HPR permeation tubes, treatment effects on SF₆ concentrations only approached statistical significance (P=0.09) (381 and 212 ppt for HPR and LPR, respectively). The CH₄/SF₆ ratio of molar concentrations significantly differed (P=0.001) between the treatments (0.651 and 1.197 for HPR and LPR, respectively). When the CH₄/SF₆ ratio of concentrations in the rumen head space and pre-calibrated PR of SF₆ were used to calculate CH₄ production rates, the HPR treatment yielded consistently higher hourly CH₄ production rates than the LPR tubes (Figure 1). The mean CH₄ production calculated for the HPR tubes were 8.5% higher than that for the LPR tubes (12.9 v. 11.9 L/h), although this difference was not significant (P=0.34).

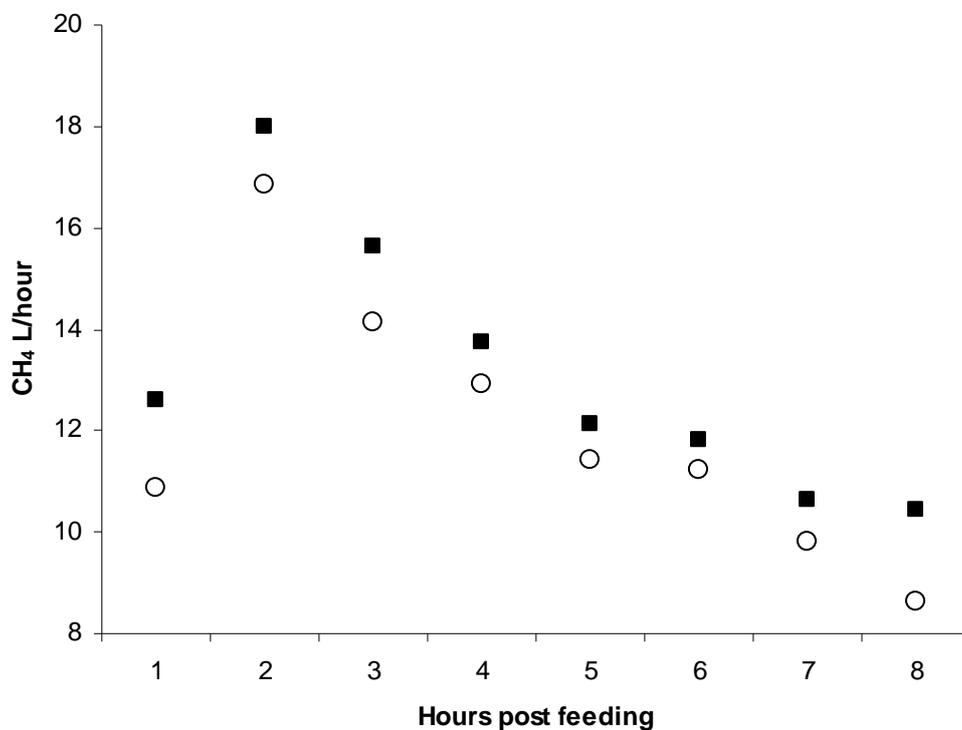
CONCLUSION

The results suggest that there is a portion of the SF₆ released in the rumen which is not accounted in the rumen head space (the pool available for eructation) and although probably small, this unaccounted portion likely increases with increasing PR of SF₆.

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Figure 1. Calculated hourly rates of CH₄ production in the rumen of cows deployed with low (○) or high (■) PR of SF₆. The rumen gases were collected unobtrusively throughout rumen cannulae. Each data point represents mid-points of consecutive sample collections.



PLANT SECONDARY METABOLITES PRESENT IN *TERMINALIA CHEBULA* AND *ALLIUM SATIVUM* REDUCE METHANE EMISSION IN SHEEP

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ABSTRACT

Sixteen adult male sheep divided in four groups were used to study the effect of *Terminalia chebula* (HAR), *Allium sativum* (GAR) and the mixture of two (MIX) as feed additives on *in vivo* nutrient digestibility and methane emission and compared to the control (CON) animals (without any feed additive). All the animals were fed on wheat straw and concentrate mixture in the ratio of 1:1. The intake of feed was similar ($P > 0.1$) in all the treatments. The organic matter (OM) digestibility increased ($P = 0.10$) by 12.0%, 10.2% and 6% in HAR, GAR and MIX, respectively in comparison to CON. Methane production expressed as L/kg digestible DM intake was lower ($P = 0.09$) in HAR and MIX than in CON. *T. chebula* appears to have a potential to be used as feed additive to improve nutrient digestibility and reduce enteric methane emission in ruminants.

INTRODUCTION

The use of plant secondary metabolites for reducing enteric methanogenesis appears to be a better alternative to chemical feed additives that sometimes may affect the animals adversely. In a screening experiment using *in vitro* gas production test, *Terminalia chebula* (harad) and *Allium sativum* (garlic) reduced methane production significantly (Patra *et al.* 2006). In the present experiment it is proposed to study the effect of these plants additives on *in vivo* nutrient utilization and methane production in sheep.

MATERIALS & METHODS

Sixteen adult male sheep (30.0 ± 1.69 kg BW; 22-months-old) were divided into 4 groups in a randomized block design on the basis of their body weight. Wheat straw (OM, 91.1%; CP, 3.1% and NDF, 78.2%) and concentrate mixture (OM, 92.1%; CP, 21.4% and NDF, 39.9%) were fed to all sheep in the ratio of 1:1. *Terminalia chebula* (HAR), *Allium sativum* (GAR) and the mixture of two in equal proportion (MIX) were supplemented at the rate of 10 g/kg feed in three experimental groups and the animals of control group (CON) were fed none. After 35 days of feeding a metabolism trial was conducted and respiration calorimetric studies were done in an open circuit respiration chamber suitable for sheep for 2 consecutive days for each of the animals.

RESULTS & DISCUSSION

The intake of DM, concentrate mixture and wheat straw was similar ($P > 0.10$) in all the treatments. DM digestibility was greater ($P = 0.06$) in HAR and GAR than in CON or MIX treatments (Table 1). The organic matter (OM) digestibility increased ($P = 0.10$) by 12.0%, 10.2% and 6.0% in HAR, GAR and MIX, respectively, compared with CON, which might be due to increased ($P = 0.02$) digestibility of neutral detergent fibre.

Methane production (L/d) by sheep was similar ($P > 0.10$) among the treatments, but when expressed as L/kg digestible DM intake, it was lower ($P = 0.09$) in HAR and MIX than in CON treatment. Methane energy losses expressed as per cent of gross energy intake were not affected ($P > 0.10$) by any of the treatments, but when expressed as per cent of digestible energy intake, the losses decreased ($P = 0.08$) in HAR and MIX compared with CON and GAR treatments. The reduction of methanogenesis by *T. chebula* could be due to the presence of high concentration of phenolic compounds.

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Table 1. Effects of feed additives on intake, digestibility of nutrients and methane emission by sheep
 Within rows, means followed by different letters are significantly different ($P < 0.10$)

	CON	HAR	GAR	MIX	± s.e.	<i>P</i> -value
Total DM (g/day)	587	630	784	642	48.3	0.53
Total DM (g/kgW ^{0.75})	47.2	47.7	58.2	46.5	3.12	0.53
Digestibility (g/kg)						
Dry matter	523b	593a	580a	553ab	10.6	0.06
Organic matter	549	615	605	582	10.3	0.10
Neutral detergent fibre	375b	480a	474a	449a	14.4	0.02
Methane production						
(L/day)	15.6	15.6	17.2	13.7	0.88	0.54
(L/kg digestible DM intake)	52.1a	39.6b	45.9ab	39.8b	2.09	0.09
Methane energy loss						
Percent of GE intake	5.82	5.12	5.70	4.82	0.22	0.41
Percent of DE intake	10.91a	8.72b	10.07a	8.20b	0.44	0.08

METHANE PRODUCTION FROM SHEEP GRAZING EITHER WILLOW FODDER BLOCKS OR DRYLAND PASTURE

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ABSTRACT

A 79-day rotational grazing experiment was conducted over the late summer/autumn period of 2007 at Massey University's Riverside dryland farm, in the East of the Southern North Island of New Zealand, to compare methane (CH₄) emissions from hoggets grazing willow (*Salix* spp.) fodder blocks or dryland perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture. Relative to sheep grazing dryland pasture, those grazing fodder blocks containing a mixture of pasture and small trees had reduced ($P = 0.06$) CH₄ emissions (25.4 ± 1.96 versus 31.0 ± 2.02 g/day) after 5 weeks, when adjusted to equal live weight (LW). A likely reason for CH₄ mitigation is the presence of condensed tannins (CT) and other phenolic compounds in willow.

INTRODUCTION

The livestock sector accounts for 9 and 37 % of worldwide anthropogenic carbon dioxide and methane (CH₄) emissions, respectively (Steinfeld *et al.* 2006; FAO). One of the possibilities for reducing rumen CH₄ emissions is the use of alternative temperate forages containing condensed tannins (CT) and other plant secondary compounds (Ramírez-Restrepo and Barry 2005). The objectives of this study were to measure the effects of grazing willow (*Salix* spp.) fodder blocks, which contain CT and phenolic glycosides versus perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture, upon CH₄ production and animal performance in young sheep.

MATERIALS & METHODS

Experimental design

The protocol of the study was approved by the Massey University Animal Ethics Committee. Eighty Suffolk x Romney ewe hoggets were balanced for live weight (LW) and randomly allocated to pasture ($n = 40$) or willow fodder blocks ($n = 40$; 6000 stems/ha). There were two ($n = 20$) replicates of each treatment. Half of each replicate group was dosed with Sulphur hexafluoride capsules at day 22; all animals received oral anthelmintic on days 0 and 28.

Liveweight (LW) was measured fortnightly using electronic scales (Tru-test, Auckland, New Zealand). Methane emissions were measured by collecting expired gases in yokes over a five-day collection period during weeks 5 and 11, followed by gas chromatography analysis (Ulyatt *et al.* 1999). Values of carcass weight (CW) and carcass fatness (GR; Ramírez-Restrepo *et al.* 2005) were also recorded for all hoggets at slaughter in a commercial abattoir.

Grazing management

Hoggets were rotationally grazed in fortnightly breaks at a feed allowance of 4.5 kg/DM per hogget.day, using front and back electric fences. Sheep had free access to water from moveable water troughs at all times. For hoggets grazing willow fodder blocks, DM allowance used refers to the combined total of trees and herbage growing underneath the woody plants.

Forages

Pre-grazing and post-grazing herbage mass measurements for each replicate fortnightly break in perennial ryegrass/white clover pasture and the tree/pasture system were assessed by cutting six random quadrats (0.180 m²) to ground level and four trees to stump level, respectively. Pasture samples were washed and the woody samples were cut into small pieces (i.e. 2 cm). Samples were dried overnight (16 h) at 80°C in a forced-air oven (Contherm: Thermotec 200; Petone, New Zealand). Botanical composition in terms of green and dead matter was estimated from dissecting pooled samples of pasture before grazing.

RESULTS

Pre and post-grazing pasture masses for the summer/autumn growth season did not to exceed 3700 and 2800 kg DM/ha, respectively (Table 1).

Pre-grazing herbage mass/ha was similar for control pasture and fodder block pasture, whilst post-grazing herbage mass and dead matter were higher ($P<0.05$) for the pasture component in the silvopastoral system than in the control pasture sward.

Although LW was similar at day 29 for hoggets grazing both control pasture and willow fodder blocks (Table 2), total LW gain and CW were lower ($P<0.001$) in hoggets grazing the fodder blocks. Grazing on willow fodder blocks decreased CH₄ emissions (corrected to equal LW) over week 5 ($P = 0.06$) relative to hoggets that grazed pasture, but not during week 11 (Table 2).

DISCUSSION

From the pre and post-grazing masses measured in the fodder blocks it can be calculated that trees comprised approximately 17.3 % of the diet of the grazing sheep. Compared to grazing control pasture, grazing willow fodder blocks was successful in reducing CH₄ emissions after 5 weeks, but not after 11 weeks. This difference may be attributed to the sheep eating some willow each day during week 5, but consuming all the willow leaf (containing CT) in week 11 before CH₄ measurements commenced. Future experiments are planned involving polyethylene glycol administration, to define if CT is responsible for the reduced CH₄ production found in week 5. Plant CT reduce rumen methanogenesis through reducing hydrogen formation and by inhibiting methanogens (Scalbert 1993; Tavendale *et al.* 2005).

Willow supplementation during mating can be used to increase the reproductive performance of ewes grazing dryland pastures (McWilliam *et al.* 2005; Pitta *et al.* 2005). The present study suggests that this practice may also reduce CH₄ production, but this aspect requires further research.

ACKNOWLEDGEMENT

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Table 1. Least square mean values for (least square means \pm s.e.) pre-grazing and post-grazing herbage mass (t DM/ha), green and dead matter content from dryland perennial ryegrass–white clover (*Lolium perenne*–*Trifolium repens*) pasture and willow (*Salix* spp.) fodder blocks grazed over the summer–autumn season of 2007

Number of fortnightly breaks (*n*)
 Within rows, means with different letters are significantly different (a b; $P < 0.05$)

	Perennial pasture		Willow fodder blocks			
			Pasture		Trees	
	Pre-grazing	Post-grazing	Pre-grazing	Post-grazing	Pre-grazing	Post-grazing
	<i>Whole experiment – secondary + third growth</i>					
<i>n</i>	6	6	6	6	6	6
Herbage mass	3363.9 (\pm 286.93)	2342.5a (\pm 156.07)	3697.6 (\pm 254.73)	2821.0b (\pm 134.08)	902.1 (\pm 254.73)	719.3 (\pm 134.08)
Green DM	2375.1 (\pm 171.30)	n.d.	2079.7 (\pm 171.30)	n.d.	902.1 (\pm 254.73)	n.d.
Dead matter	988.8a (\pm 185.01)	n.d.	1637.6b (\pm 177.76)	n.d.	0	n.d.

n.d., not determined.

Table 2. Effect of grazing hoggets on perennial ryegrass (*Lolium perenne*) –white clover (*Trifolium repens*) pasture or willow (*Salix* spp.) fodder blocks on animal productivity and methane (CH₄) production (g/day) in a dryland farming system in the Wairarapa, on the East Coast of North Island, New Zealand

Values are least square mean \pm s.e.

Within rows, means with different letters are significantly different (a b; $P < 0.001$), (c d; $P = 0.06$)

	Pasture	Willow fodder blocks
<i>n</i>	40	40
Initial liveweight (kg)	32.8 \pm 0.29	32.8 \pm 0.41
Liveweight at day 29 (kg)	35.3 \pm 0.45	35.0 \pm 0.47
Final liveweight (kg)	40.9 \pm 0.51a	38.0 \pm 0.46b
Total liveweight change (g/day)	102.7 \pm 6.94a	65.3 \pm 6.35b
CH ₄ emissions (g/day; week 5) ^A	31.0 \pm 2.01c	25.4 \pm 1.96d
CH ₄ emissions (g/day; week 11) ^A	22.0 \pm 0.94	23.3 \pm 0.91
Carcass weight (kg) ^B	18.3 \pm 0.36a	16.1 \pm 0.36b
Carcass fatness (GR, mm) ^C	11.0 \pm 0.84	9.2 \pm 0.83

^AAdjusted to equal liveweight.

^BAdjusted to equal initial liveweight.

^CAdjusted to equal carcass weight.

STUDIES ON DESIGNING BIOLOGICAL DESULFURIZATION SYSTEM OF BIOGAS

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ABSTRACT

Using the biogas obtained from livestock manure is effective to reduce greenhouse gas emission. However, in order to use it safely and effectively, it is necessary to remove hydrogen sulphide from it. The objective of this study is to determine the factors of designing economical and efficient biological desulfurization system using methanogenic digested slurry. The highest effect was obtained by the tube mesh carrier filling system. It was suggested that desulfurization efficiency involved the contact with H₂S and bacterium.

INTRODUCTION

Using the biogas obtained from livestock manure is effective to reduce greenhouse gas emission. However, the biogas contains corrosive gas, hydrogen sulphide (H₂S) and it causes negative effects such as corrosion of systems and influence on a human body. Although the biological desulfurization is considered as a method with an easy maintenance and low cost, enough data on the stability of the effects and technical reliability have not been reported.

The objective of this study is to determine the factors of designing economical and efficient biological desulfurization system using methanogenic digested slurry.

MATERIALS & METHODS

Biogas was supplied into the biological desulfurization system having various patterns (Fig. 1.), and the factors of design were investigated. The systems examined were a babbling system, a tube mesh carrier (carrier type: polypropylene meshed tube, outside diameter 50 mm, length 50mm, 3mm mesh, 3mm wire) filling system, a spray system, and the compound system that combined three systems above-mentioned.

RESULTS & DISCUSSION

The highest effect was obtained by the tube mesh carrier filling system (Fig. 2.). The compound system showed earlier rise in desulfurization efficiency than spray system. Therefore, it is effective to be filled with the mesh carriers. Because the compound system required high cost, it is thought the cube mesh carrier filling system is more desirable.

These results suggested that the rate-determining step of desulfurization was not the dissolution of H₂S to the digested slurry but biochemistry reaction of H₂S and bacteria or maintenance of bacterial activity by the effective removal of the oxidate.

Further studies are needed to examine the optimal interval of spraying the digested slurry. Moreover, since carriers are easy to blockade with spraying digested slurry contained solid content and by depositing sulfur, it is needed to select the carriers of high percentage of void that sacrificed bacteria maintenance capability and contact interval to some extent.

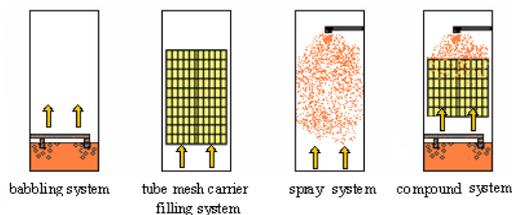


Fig. 1. Patterns of desulfurization system

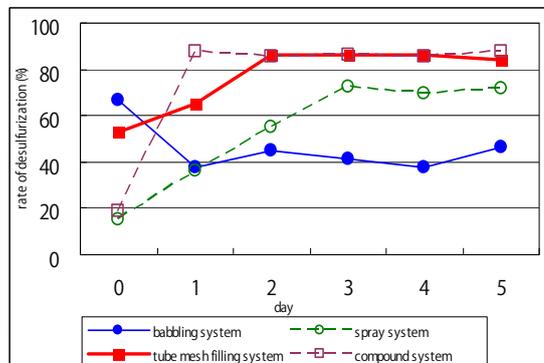


Fig. 2. Comparison of rate of desulfurization

EFFECT OF AGE ON METHANE EMISSIONS OF RED DEER STAGS FROM WEANING UNTIL ONE YEAR OF AGE GRAZING PERENNIAL RYEGRASS-BASED PASTURE

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SUMMARY

Immature ruminants appear to produce less methane per unit of dry matter intake (DMI) than adult animals. Reduced methane yields (g CH₄/kg DMI) of up to 17-20% have been reported in sheep and cattle less than one year of age compared with mature animals. This has implications for New Zealand's National Greenhouse Gas Emissions Inventory (NZGHGEI), if values for adult animals are used across all livestock classes.

As at June 2005, up to 38% of the New Zealand deer herd was less than one year of age. If immature deer produce less methane than adult deer, then total methane emissions in the New Zealand GHG inventory for deer may be over-estimated. Therefore, the aim of this study was to test the hypothesis that methane emissions from young deer grazing permanent perennial-ryegrass-based pasture (pasture) increase with age. Methane emissions of 20 grazing red deer stags were measured four times post-weaning, at 4.5, 6.5, 9 and 11.5 months of age, using the SF₆ technique, as described by Pinares-Patiño *et al.* (2003) Methane equipment was modified to fit young deer.

During the experiment, deer rotationally grazed on a permanent pasture containing ryegrass (*Lolium perenne* cv. Nui) (approximately 87% of the DM) and white clover (*Trifolium repens* cv. Huia) (approximately 2% of the DM) and were offered pasture *ad libitum*. Pasture to estimate the metabolisable energy content of feed on offer was collected by hand plucking. Dry matter intake of deer was estimated using the energy requirements for maintenance (0.57 MJ ME/liveweight kg 0.75/day), adjusted for season, and growth rate (37 MJ ME/kg liveweight gain) according to the methods of Fennessy *et al.* (1981). Analyses of data were carried out using SAS (Statistical Analysis System, version 9.1; SAS Institute Inc., Cary, NC, USA).

Methane production (g/day), as shown in Table 2, was found to increase with age ($P < 0.0001$); 4.5 months < 6.5 = 9.5 < 11.5 months of age. Similarly, methane yield (g/kg DM) was also found to increase with deer age ($P < 0.0001$); 4.5 months < 6.5 = 9 = 11.5 months of age. There was also a trend for methane per kg live weight to decrease with age ($P < 0.078$); methane per kilogram of live weight was lower at 9 and 11.5 months of age than at 4.5 and 6.5 months of age.

As methane production and methane yield from immature red deer increase with age, and appear to be less than those previously recorded in adult deer, this implies that there may be an overestimation of methane from deer in the NZGHGEI if a separate value for deer less than one year of age is not adopted. However, as this study did not simultaneously measure methane from adult and immature animals consuming the same diet at the same time, the absolute effect of age could not be determined from this study. A further confounding factor, in this and other studies showing apparent effects of age on methane emissions from young ruminants, is that in the grazing or pasture-fed situation the chemical composition of the ingested diet is not constant over time. The measurements made in this study do not indicate the mechanism by which methane production and yield is reduced in adolescent animals and this needs further research.

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Table 1. Dry matter intake (DMI) and methane emissions (CH₄), production (CH₄, g/day) and yield (CH₄ per kilogram DMI) of weaned red deer stags at 4.5 (March), 6.5 (May), 9 (August) and 11.5 (October) months of age

Values are means \pm s.e.

Within rows, mean values followed by different letters are significantly different.

Age (months)	4.5		6.5		9		11.5	
DMI (kg) ^A	1.66a	\pm 0.030	1.93b	\pm 0.030	1.85b	\pm 0.031	2.27c	\pm 0.031
CH ₄ (g/day)	24.6a	\pm 1.11	32.8b	\pm 1.63	32.3b	\pm 1.79	40.1c	\pm 1.68
CH ₄ /kg DMI ^A	14.9a	\pm 0.57	17.0b	\pm 0.72	17.4b	\pm 0.85	17.7b	\pm 0.75
CH ₄ /kg LW	0.51	\pm 0.022	0.57	\pm 0.026	0.48	\pm 0.024	0.48	\pm 0.023

^ADMI based on calculations of energy requirements for maintenance and growth.

THE EFFECT OF, COCONUT OIL AND MONENSIN ON METHANE EMISSIONS FROM SHEEP FED EITHER FRESH PERENNIAL RYEGRASS PASTURE OR CHICORY

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SUMMARY

New Zealand's pastoral livestock production systems place severe constraints on the type of measures available to farmers for the abatement of enteric methane emissions. Research has shown that some legumes and herbs can reduce methane emissions in sheep, deer and cattle. Similarly, research has also shown that mitigation agents, such as oils (e.g. coconut oil), and monensin supplements can reduce methane emissions per unit of intake. However, much of this work has been based on grain and/or conserved forage as the main dietary constituents and it is not known if the same effects can be achieved with fresh forage diets. The aim of this study was to investigate and compare the effect of different potential methane mitigation technologies, singly or in combination, on methane emissions from sheep fed fresh forage diets.

The experimental design was a two-factorial design with two measurements two weeks apart, with animals receiving the experimental diet and mitigation agent(s) for 10 days prior to the first methane measurement. There were four main treatment groups; (1) no agent ($n = 8$), (2) monensin ($n = 8$), (3) coconut oil ($n = 12$) and (4) coconut oil + monensin ($n = 12$) and within each main treatment group animals were further split into sub-groups and fed either perennial ryegrass (*Lolium perenne*)-based pasture or chicory (*Cichorium intybus*) cut fresh daily and fed 1.5 times estimated energy requirements for maintenance on a per animal basis. Animals receiving either mitigation agent were dosed twice daily. Monensin was dosed via a drench gun at a rate of 15 mg/day and coconut oil was dosed at a rate of 3% dry matter intake (DMI)/day on an individual animal basis. The coconut oil was administered by a 20 mL syringe. Methane production was measured using the sulphur hexafluoride (SF₆) technique when sheep were housed individually in metabolism cages.

Preliminary results found that sheep fed chicory (17.0 g CH₄/day and 24.3 g CH₄/kg DMI) produced 37% and 22% less methane per day and per kilogram of DMI, respectively than sheep fed pasture (26.9 CH₄/day and 31.0 g CH₄/kg DMI) ($P < 0.001$). Monensin (20.2 g CH₄/day and 25.7 g CH₄/kg DMI) and monensin + coconut oil (18.1 g CH₄/day and 22.1 g CH₄/kg DMI) reduced methane production per day and per kg DMI by up to 33% ($P < 0.01$) compared with the control (25.1 g CH₄/day and 33.2 g CH₄/kg DMI) and coconut oil (24.5 g CH₄/day and 29.8 g CH₄/kg DMI) groups. There were no significant interactions between the types of forage fed and administered mitigation agents ($P > 0.7$).

This research shows that significant reductions in both methane production and yield can be achieved with fresh forage feeding. Based on these results, monensin appears to be able to reduce methane emissions from animals fed fresh pasture or chicory, although results from other New Zealand experiments using monensin are less encouraging. Supplementing pasture based diets with coconut oil alone does not appear to be an effective mitigation approach, although there was some benefit from supplementing with coconut oil and monensin. Further research is required to firstly determine the effectiveness of other forage herbs and legumes in reducing ruminant methane emissions, the mechanisms of action of alternative forages such as chicory, and whether inclusion of alternative forages in pasture mixes or as a proportion of the total diet will impact on methane emissions. Secondly, research is needed to determine the effectiveness of methane mitigation agents in the long-term grazing situation.

COMPARATIVE METHANE PRODUCTION AND YIELDS FROM ADULT CATTLE, RED DEER AND SHEEP

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SUMMARY

Methane emissions from ruminant livestock in New Zealand (NZ) represent 31.8% of New Zealand's total greenhouse gas emissions and accurate inventories of methane production for all farmed livestock classes are required by the Kyoto Protocol. Currently, methane yields used to calculate total ruminant enteric methane inventories for adult (greater than one year of age) sheep (20.9 g CH₄/kg DMI), dairy cattle (21.6 g CH₄/kg DMI) and deer (21.5 g CH₄/kg DMI) appear to be similar. However, the value used for deer is an average of adult sheep and dairy cattle, gained from actual methane measurements in those species. This is despite possible differences in digestive physiology and apparent digestibility. These findings are also confounded by methane measurements of each species being taken at different times with different diets. The aim of this study was to directly compare the methane yields of cattle, sheep and red deer when animals were fed the same diet of ensiled lucerne (*Medicago sativa*). Methane measurements were taken at the same time from animals housed under similar conditions and to compare differences in seasonality.

An experiment to measure methane production and yields from non-lactating dairy cows (cattle) ($n = 11$), sheep ($n = 11$) and red deer (deer) ($n = 11$) was conducted at AgResearch Grasslands (sheep and cattle) and Massey University (deer), Palmerston North, NZ, in summer (January 2007) and winter (June 2007). Each measurement period was conducted over two weeks, of which the first ten days consisted of an adaptation period to the diet and housing, followed by four days of methane and DMI measurements. All animals were individually housed in metabolism cages to allow for individual DMI to be measured directly. Animals were fed ensiled lucerne chaff 'Chaffhage' to ensure consistency of diet between species and across seasons, at a rate of 1.2 times estimated energy requirements for maintenance of each species. Calculations used for energy requirements were based on the Australian feeding standard for ruminants. Methane measurements were made using the SF₆ technique and collection equipment used was modified to suit each species.

The preliminary results of this study are presented in Table 1. Dry matter intake per kg of metabolic live weight was greater for cattle (0.060 kg DMI/kg LW^{0.75}) compared with deer (0.046 kg DMI/kg LW^{0.75}) and sheep (0.048 kg DMI/kg LW^{0.75}) ($P < 0.0001$). Methane production of cattle (140.4 g CH₄/day), was greater compared to deer (31.5 g CH₄/day) and sheep (18.3 g CH₄/day) ($P < 0.0001$), and deer produced more methane than sheep ($P < 0.005$). Methane yields also differed with species, cattle (20.6 g CH₄/kg DMI) > sheep (18.4 g CH₄/kg DMI) > deer (16.5 g CH₄/kg DMI) ($P < 0.0003$). An interaction of species by season of measurement for both methane production and yield was found, which appeared to be due to the increase in methane production and yield of cattle from summer to winter ($P < 0.0002$). There was found to be no effect of season on methane yields of deer or sheep.

The preliminary findings of this experiment show that there is a difference between ruminant species in methane emissions when measured at the same time on the same diet. This could mean that the current inventory value for deer, which is an average of sheep and cattle, overestimates total methane yields. Differences in the digestive processes and physiology or microbial populations between ruminants may be responsible for the differences in methane yields. Measurements of apparent digestibility, rumen fermentation, digesta flow and microbial populations have also been taken and may help to explain the results presented here.

Table 1. Dry matter intake (DMI) (kg/day and kg/kg LW^{0.75}) and methane (CH₄) emissions, production (CH₄ grams per day) and yield (CH₄ per kilogram DMI) of non-lactating dairy cows (cattle), sheep and red deer

Within rows, means followed by different letters are significantly different at $P = 0.05$.

* $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$.

	Cattle		Deer		Sheep		s.e.m.	Significance		
	Summer	Winter	Summer	Winter	Summer	Winter		Species	Season	Spp. x S
DMI (kg)	7.0	6.8	2.0	1.9	1.0	1.0	0.05	***	*	n.s.
DMI/kg LW ^{0.75}	0.060	0.061	0.046	0.046	0.045	0.045	0.0012	***	n.s.	n.s.
CH ₄ (g/day)	126.3a	154.4b	34.7c	28.3cd	18.2d	18.3d	4.82	***	n.s.	**
CH ₄ g/kg DMI	18.1a	23.0b	17.1c	15.8c	18.6d	18.2d	0.95	***	n.s.	*

GREENHOUSE GASES AND AGRICULTURE IN CAMBODIA – INITIAL PERSPECTIVE

(REVIEW PAPER OF CAMBODIA’S INITIAL NATIONAL COMMUNICATION)

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INTRODUCTION

Cambodia has lost much valuable data and human resources from decades of war and genocide, but with assistance from the United Nations Development Programme (UNDP)/the Global Environment Facility (GEF), Cambodia has been able to initiate action in response to the United Nations Framework Convention on Climate Change (UNFCCC). This review takes a look at specific data compiled to date and future directions.

GREENHOUSE GAS PROJECTIONS

Greenhouse gas (GHG) projection under the Cambodia Climate Change Enabling Activity Project (CCEAP), which is the only GHG projection study in Cambodia so far, was conducted for four sectors: energy, agriculture, waste and Land Use Change and Forestry (LUCF). Projection of GHG emissions from the industry sector was not performed since the industrial development plan for the period of up to the year 2020 concerns mainly light industries such as garments, textiles and food processing which do not emit GHGs. A number of assumptions were made based on historical trends in these sectors in the last 10 years. Results from this projection analysis of greenhouse gas emissions and removals by sectors (baseline scenario) indicated that in the year 2000 Cambodia was already a net emitter of GHGs. The net emissions were approximately 6244 Gg of CO₂ equivalents. In 2020, the net emissions would increase to approximately 43 848 Gg of CO₂ equivalents. Among the sectors, LUCF would be the main source of GHG emissions (63%), followed by agriculture (27.5%). Energy would only contribute to approximately 9.0% of the total national emissions.

GREENHOUSE GAS EMISSIONS FROM AGRICULTURE

Greenhouse gas emissions from the agriculture sector include CH₄, NO_x, N₂O and CO. The emissions are produced by several subsectors such as livestock, rice fields, agricultural soils and burning of agricultural residues and grassland. Each subsector emits different forms and magnitudes of GHGs. In Cambodia, livestock and rice fields are the major source of CH₄ (78% of total CO₂ equivalent emissions), while agricultural soils are the main source of N₂O (21% of total CO₂ equivalent emissions).

Methane emissions from domestic livestock in Cambodia mostly come from enteric fermentation with small amounts from manure management. The 1994 inventory has indicated that domestic livestock emitted 184.8 Gg of CH₄ equivalents to 48.1% of the total CO₂ equivalent emissions from the sector. Economic and population growth leads to an increase in the consumption of meat and eggs, for example an increase of 6.5% in 1998, and hence emissions of methane from livestock are likely to increase.

The results of the projection showed that GHG emissions from agriculture would increase quite significantly. In 2020, methane emissions will be about three times the 1994 emissions while nitrous oxide will more than double. The rate of increase in methane emissions for livestock would be slightly higher than that from rice paddy. In total, GHG emissions from agriculture in 2000, 2010 and 2020 would be approximately 12,030; 17,789; and 26,821 Gg of CO₂ equivalents respectively.

GHG MITIGATION OPTIONS FOR AGRICULTURE

Mitigation options evaluated for the agriculture sector only covered rice paddies. Mitigation options for the livestock sub-sector were not assessed due to insufficient data, even though livestock is the biggest contributor of CH₄ emissions in the agriculture sector. It is also important to note that in Cambodia, animal husbandry is still considered as a family application as there is neither household dairy farming nor investment in this field. The government plan, however, provides for the vigorous development of the livestock industry to meet national consumption.

Results of the analysis showed that reduction of emissions, which would result from the mitigation options, ranged from 71 to 304 kg CH₄ per ha per season (Tables 1 and 2). Using mitigation potential provided by studies conducted in Indonesia (Pawitan *et al.* 1999), it was found that all options evaluated in this study gave positive benefits with a range of 10 to 71 US\$/ha. In terms of methane reduction, the incremental benefit ranged between 116 and 774 US\$/Mg CH₄. Based on profitability, yield, mitigation potential, applicability and acceptability of the options, it was

found that options with low barrier are dry season intermittent and organic matter management, options with medium barrier are direct seeding and wet season zero tillage, while that with high barrier is dry season zero tillage.

As land is allocated for implementing these options, on-going monitoring will help determine the amount of area required to achieve satisfactory emission reductions. Also, more research needs to be done in the livestock subsector to be able to assess appropriate mitigation options.

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Table 1. Projection of GHG emissions and removals by sectors (Gg)

Source/Sink	1994 ^B Gg (%)	2000 Gg (%)	2010 Gg (%)	2020 Gg (%)
Emissions				
Energy	1,881 (2.8)	2,622 (3.6)	4,780 (5.9)	8,761 (9.0)
Industry	50 (0.1)			
Agriculture	10,560 (15.5)	12,030 (16.4)	17,789 (22.1)	26,821 (27.5)
Waste	273 (0.4)	331 (0.4)	425 (0.5)	523 (0.5)
LUCF ^A	55,216 (81.2)	58,379 (79.6)	57,627 (71.5)	61,512 (63.0)
Total emissions	67,980 (100.0)	73,362 (100.0)	80,621 (100.0)	97,617 (100.0)
Removal by				
LUCF	-73,122	-67,118	-61,090	-53,769
Net emissions	-5,142	6,244	19,531	43,848

^ALUCF (Land Use Change and Forestry).

^B1994 inventory (IPCC methodology).

Table 2. Projection of GHG emissions from agriculture sector (Gg)

Activity	GHGs	1994	2000	2010	2020
Domestic livestock	CH ₄	185	195	323	545
	N ₂ O	4	4	7	13
Rice cultivation	CH ₄	150	198	254	303
Grassland burning	CH ₄	2	2	2	2
	N ₂ O	0	0	0	0
Agriculture residue burning	CH ₄	2	4	4	5
	N ₂ O	0	0	0	0
Agricultural soils	N ₂ O	7	7	10	16
Total CH₄		339	399	584	854
Total N₂O		11	12	18	29
Total CO₂ equivalents		10,560	12,030	17,789	26,821

GROWING PIGS AT A HIGH AMBIENT TEMPERATURE ARE DEFICIENT IN LYSINE

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INTRODUCTION

Low protein and amino acid balanced diet reduces the nitrogen excretion from pigs without hindering the growth performances. Supplementation of crystalline amino acids to diets is a useful technique to improve the amino acids balance. Improved amino acid balance reduces the nitrogen excretion from pigs and hence decreases N₂O emission. Here, the experiment was conducted to evaluate the amino acids state of growing pigs at a high ambient temperature.

MATERIALS & METHODS

Twelve castrated male pigs weighing 45 kg were individually housed in metabolism cages maintained in temperature- and humidity-controlled rooms. They were assigned to two experimental groups. One was control temperature of a constant ambient temperature of 23 ± 0.5°C and the other was 28 ± 0.5°C. Relative humidity of each room was 60 ± 10%. Experimental period was for three weeks and pigs had free access to diet and water during experiment. Faeces and urine were collected during the final three days of experiment and we measured nitrogen contents of these samples. Pigs were slaughtered at the final day of experiment and blood was collected into heparinized test tube. Liver was removed, weighed and stored at -80°C until analysis. Plasma-free amino acids concentrations and liver enzyme activity of lysine-oxoglutarate reductase (LOR), which was a rate limiting enzyme of lysine degradation, were determined.

RESULTS & DISCUSSION

Feed intake and daily weight gain were lower in the 28°C group than in the 23°C group ($P < 0.05$), but feed efficiency was not different between the 23°C and the 28°C groups. Nitrogen excretion (g/d) in urine was lower in the 28°C group than in the 23°C group ($P < 0.05$). This response may be explained by the lower nitrogen intake in the 28°C groups. Plasma lysine concentration in the 28°C group was lower than in the 23°C group ($P < 0.01$). Similar responses were observed in tryptophan, arginine, alanine and tyrosine concentrations. Contrarily, plasma threonine and serine concentrations in the 28°C group were higher than in the 23°C group ($P < 0.01$). The changes of plasma free amino acids such as lower lysine and higher threonine concentrations were typical responses in lysine deficiency. The liver LOR activity in the 28°C group was lower than in the 23°C group ($P < 0.01$). It is known that the reduction of this enzyme activity occurs when lysine is deficient.

On the basis of plasma lysine and threonine concentrations and LOR activity, we infer that the growing pigs at a high ambient temperature are deficient in lysine, suggesting that high amount of supplementation of lysine to diets at a high ambient temperature is effective to reduce nitrogen excretion from pigs, leading to decrease N₂O emission.

PURIFICATION OF NITROGEN AND PHOSPHORUS RICH WASTEWATER RELATED TO MILKING BY SIMPLE PROCESSING METHOD IN THE GLASSLAND DAIRY FARMS

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ABSTRACT

The final goal of this study is to develop the purification system by combing the series of three settling tanks and the artificial marsh. The artificial marsh was composed of *Phragmites australis* (Cav.) Trin. When the precondition is satisfied the purifying effect was demonstrated to achieve the quality standard of wastewater in the targeted parameters related to total nitrogen and phosphorus except BOD. The pollution of wastewater (contamination by waste milk), however, varied considerably depending on the farming condition. Therefore the combination of settling tanks and artificial marsh may be used to improve the purifying effect.

INTRODUCTION

Wastewater from milking systems in dairy farming has been a serious environmental pollutant in Hokkaido, Japan. We gathered information by field survey and questionnaire on the routs and amount of wastewater discharged from individual dairy farm in the grassland dairy farming area in Hamanaka, Eastern Hokkaido. It was thought that wastewater was able to be purified by the simple processing method such as the settling tanks according to the feature of the waste water. The present study deals with the development of simple system for purifying wastewater from dairy farms in grassland area by combining a series of three settling tanks and a artificial marsh composed of *Phragmites australis* (Cav.)Trin.

MATERIALS & METHODS

The experimental installation for waste water purification combining series of three settling tanks and artificial marsh was facilitated at a farm in Hamanaka (Fig. 1). Three cylindrical tanks with a capacity of 1.6 m³ were set in a row and wastewater overflows in order. For establishing artificial marsh, *Phragmites australis* (Cav.)Trin growing naturally in Hamanaka was planted by simple and low-cost procedure using Culm Laying Method(Taizo and Fuyuki 2005). BOD, COD, T-N and T-P of the waste water were monitored from October, 2006 through July 2007.

RESULTS & DISCUSSION

The improvement of purification by series of three settling tanks was confirmed from the results of measurements in 2006 in comparison with those in 2007. However, the pollution of wastewater (contamination by waste milk and livestock excreta) varied considerably depending on the farming condition. It was revealed that when livestock excreta and waste milk are not introduced into the wastewater the purification can be made only by the series of three settling tanks. On the other hand, it was demonstrated that artificial marsh absorbed large amounts of T-N and T-P from the results obtained in analyses of soil and root. Thus, a simple purifying system of wastewater combining series of three settling tanks and artificial marsh is an effective procedure to purify dairy wastewater.

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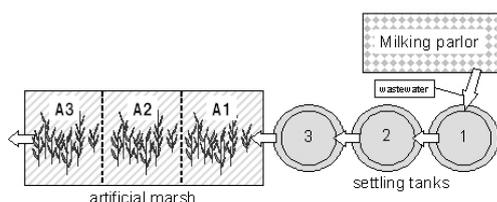


Fig.1. Purification system by combing the series of settling tanks and the artificial marsh in A farm

CHARACTERIZATION AND FUNCTION OF WATER-SOLUBLE HUMIC SUBSTANCES IN DIGESTED DAIRY SLURRY

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INTRODUCTION

Many studies have been carried out to evaluate the plant-nutritional effects of using digested dairy slurry as a liquid fertilizer, mostly focusing on the dynamics of nitrogen (e.g. Matsunaka *et al.* 2006); however, the properties and function of organic matter, especially dissolved (water-soluble) organic matter, in the digested dairy slurry remain unclear. In our previous study, we noted that there were considerable differences in quantity and quality of water-soluble humic substances (WSHS) between digested dairy slurry and raw slurry (Tani *et al.* 2006). Humic substances exhibit stimulatory effects on plant cell growth and development. This function seems to be carried out more readily by low-molecular-size humic fractions (Nardi *et al.* 2002). The objectives of this study were to characterize the amount and structure of WSHS in digested dairy slurry and to evaluate chemical and biological effects of these substances on plant growth.

MATERIALS & METHODS

Two types of digested dairy slurry were used in the present study, one collected from a thermophilic biogas plant (with an averaged process temperature of $55 \pm 1^\circ\text{C}$) at the Obihiro University of Agriculture and Veterinary Medicine in Obihiro, Hokkaido (designated as T-DS), and the other from a methophilic biogas plant (with an averaged process temperature of $36 \pm 1^\circ\text{C}$) at a private farm in Shihoro, Hokkaido (designated as M-DS). Original raw dairy slurry (not fermented) was also collected from each plant, and these slurries are designated as T-RS and M-RS, respectively. The slurry samples were mixed with distilled water and shaken for 16 h. The water-soluble fraction of the slurry suspension was separated by centrifugation and ultrafiltration (0.2 μm). Water-soluble humic acid (HA) and fulvic acid (FA) were fractionated by acidification and using PVP resin, respectively. These WSHS were characterized by carbon and nitrogen analyses, UV-VIS spectroscopy, and FT-IR spectroscopy. The biological function of the WSHS was evaluated by using water cress (*Lepidium sativum*) to assess auxin-like activity (Muscolo *et al.* 1999) and komatsuna (*Brassica rapa* var. *peruviridis*) to examine the inhibitive effect on its root growth.

RESULTS & DISCUSSION

The carbon and nitrogen contents of water-soluble HA in the digested slurry were higher than those in the raw slurry, especially in the case of the carbon content of HA in T-DS (1.24 mg/g), which was three times as high as that in T-RS (0.39 mg/g). Humification indexes (relative darkness of HA, designated as *RF* values) of T-DS, T-RS, M-DS, and M-RS were 25, 14, 30, and 16, respectively, indicating that the humification of HA progressed after the digestion, irrespective of the processing temperature. The FT-IR spectra of the FA in the digested slurry showed more distinct bands of carboxylic group (-COOH) than did the raw slurry.

The concentration of iron, copper, and zinc in the water-soluble fractions of the T-DS and M-DS was much higher than that of the T-RS and M-RS, respectively, suggesting that high chelating abilities of the WSHS in the digested slurry could contribute to the complexation and dissolution of these essential metals for plant growth. The auxin-like activity of the WSHS in the dairy slurry was not clear, probably due to the complex and diversified nature of WSHS. The IAA-like activity increased with increase in the HA concentration of the T-DS and revealed that the HA at a concentration of 1 mg C/L showed the same hormone-like activity as 1.2 mg IAA/L (Fig. 1). The inhibitive effects of water-soluble HA in the T-RS and M-RS on root growth of komatsuna increased as their concentration increased; however, no inhibition was observed in the digested slurry (Table 1). Thus, the WSHS in the digested dairy slurry seems to positively influence chemical and biological function; however, the still unknown nature of WSHS prevents us from drawing more conclusive results concerning the effects on plant growth of WSHS in digested slurry.

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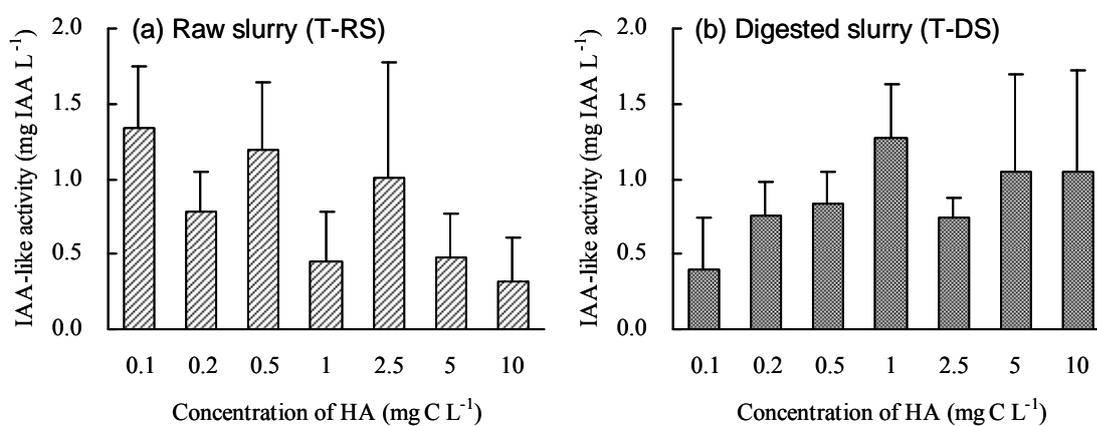
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Table 1. Inhibitive effects of water-soluble HA in the raw and digested dairy slurry on root growth of komatsuna (root growth index)

Concentration of HA (mg C L ⁻¹)	Root growth index ^A			
	M-RS	M-DS	T-RS	T-DS
0.1	86 ± 5.6	95 ± 6.4	108 ± 7.7	101 ± 6.1
0.2	91 ± 13.2	103 ± 17.4	93 ± 9.2	98 ± 6.8
0.5	90 ± 4.4	110 ± 6.5	88 ± 11.5	109 ± 15.0
1	82 ± 10.0	123 ± 11.1	94 ± 6.4	128 ± 14.5
2.5	64 ± 8.2	104 ± 17.9	88 ± 9.3	116 ± 11.4
5	32 ± 10.0	119 ± 19.3	28 ± 2.4	101 ± 10.8
10	10 ± 0.4	114 ± 4.4	15 ± 2.5	108 ± 13.1

^A Percentage of root length to that of control (without water-soluble humic acid).

Figure 1. IAA-like activity of water-soluble humic acids at different concentration (0.1 - 10 mg C/L) in the raw and digested dairy slurry from the thermophilic bio-gas plant (T-RS and T-DS, respectively).



METHANE AND HYDROGEN CONCENTRATIONS IN THE BREATH OF SHEEP

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SUMMARY

Methane (CH₄) and nitrous oxide (N₂O) are the most important agricultural greenhouse gases. In New Zealand emissions of enteric CH₄ from grazing livestock are the most important source of GHG from agriculture. This 'ruminant' CH₄ is derived mainly from hydrogen (H₂) produced during anaerobic fermentation of dietary carbohydrates in the rumen. There are few reports on the changing levels of H₂ in the rumen during digestion, and little is known about levels of H₂ or the relationships between CH₄ and H₂ in exhaled breath of ruminants.

A gas-measuring monitor (mBA-5000, Mitleben R&D Associates, Osaka, Japan), which quantifies CH₄, H₂ and carbon dioxide (CO₂) concentrations at 10-s intervals via a semiconductor gas sensor, was used to monitor gas levels in the breath of sheep fed on lucerne silage or a mixed lucerne silage: concentrate diet. Four 2-year-old castrated sheep were used in two periods (P1 and P2). The sheep had SF₆ permeation tubes deployed in their rumen and they were part of a series of CH₄ emission measurements using the SF₆ tracer technique. In P1 the sheep were fed on the silage diet and after 3 days adaptation, the mixed diet was fed in P2. The feeding level was selected to provide 1.2 times maintenance energy requirements and daily feed was delivered in two equal portions. A transparent acrylic head box with front and top openings and fitted in front of a metabolic crate was used to collect breath samples. A canvas sleeve attached to the front opening was secured to the neck of the sheep using a drawstring allowing the sheep to move freely. Air from the top opening moved freely around the neck of the animal. Concentrations of breath CH₄, H₂ and CO₂ were monitored (using the mBA-5000) continuously over a 3-h period following feeding. Breath samples over this period were also collected into evacuated yokes and analysed for SF₆ and CH₄ concentrations by gas chromatography.

The mBA-5000 measurements showed that the mean H₂ concentrations in the breath of sheep fed on the forage and mixed diet were 2.1 ± 1.5 and 14.8 ± 8.6 ppm, respectively, and differed significantly. The mean H₂ concentration in all samples over the 3 h post-feeding period was 8.4 ppm and amounted to around 2.1% of the mean CH₄ concentration measured by the monitor. In contrast, there was no significant difference between CH₄ emissions from silage and mixed-diet fed sheep (1.99 v. 2.52 g CH₄/3 h), when CH₄ was determined by the SF₆ technique. For forage-fed sheep, the mean H₂ concentration (using the mBA-5000) showed a positive correlation ($r^2=0.966$) with CH₄ emission derived from the SF₆ tracer technique, whereas for mixed-diet fed animals no correlation was found. On both diets, CH₄ production was detected immediately after feeding, whereas H₂ production was first detected at 638 ± 153 s following the end of feeding.

These findings show that there can be periods of high H₂ emission from the rumen when H₂ formation occurs at a faster rate than methanogenesis. Our results suggest that this occurs when there is a change of diet and/or when diets contain concentrates. Further work is needed to determine whether H₂ is emitted from the rumen after a longer period of acclimatization following diet change.

AN OPEN AIR TRACER METHOD FOR MEASURING CH₄ EMISSIONS FROM CATTLE

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SUMMARY

Methane is the dominant source of greenhouse gas emissions from agriculture, and its principal source is anaerobic fermentation in ruminants. Accurate, non-intrusive measurements on animals kept as closely as possible to their normal living routines are required to quantify methane emissions and assess potential mitigation techniques. In this paper we describe a novel tracer technique in which methane emissions are measured downwind of a small herd of free-ranging cattle by open path Fourier Transform Infrared (FTIR) Spectroscopy. Each cow in the herd carries a small canister of nitrous oxide on a halter; the nitrous oxide is used as a tracer which is released continuously at a measured rate of around 10 g/h from each canister. The emission rate of methane from the cattle can be determined from the ratio of CH₄:N₂O concentrations and the known N₂O release rate. Open path FTIR spectroscopy is capable of quantifying both methane and nitrous oxide simultaneously in the plume downwind. Under favourable conditions, the CO₂ emission rate from the cattle can also be determined to provide a CH₄: CO₂ ratio.

The paper will describe trials of this method at the DPI Ellinbank Research Centre in Victoria in December 2005. Sixteen lactating dairy cows were monitored continuously (except for milking times) in an open, fenced grazing enclosure over a period of 4 days. Methane emissions were also measured simultaneously using the SF₆ tracer technique, with excellent agreement between the two methods.

SURVIVAL OF *COLI-AEROGENES* AND *ENTEROCOCCUS* DURING ANAEROBIC DIGESTION OF DAIRY MANURE IN FULL SCALE BIOGAS PLANTS

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ABSTRACT

The paper deals with the hygienic advantages as sanitation to treat dairy manure in full scale biogas plants. The slurry samples were collected from two thermophilic biogas plants (55°C) and two mesophilic biogas plants (38°C) in Hokkaido Japan. The detectable number of *Coli-aerogenes* group and *Enterococcus* in the slurries after anaerobic digestion couldn't be found in both thermophilic biogas plants. In both mesophilic biogas plants, however, the viable numbers of *Coli-aerogenes* group and *Enterococcus* were detected in the slurries even after anaerobic digestion. The mean decimation reduction time (T_{90}) values of *Coli-aerogenes* group and *Enterococcus* in the slurries during mesophilic digestion were 13.3 days and 16.7 days, respectively.

INTRODUCTION

Anaerobic digestion in biogas plants (BGPs) is an alternative way to handle animal manure and reduce greenhouse gas emission. The digested manure may be used as a fertilizer on agricultural land. Animal manure is known to contain pathogenic bacteria that may be a health risk for both human and animals. The digested manure must be proven hygienically safe in order to be recycled (Sahlstrom, 2003). However, there is no regulation concerning the hygienic standard of the BGPs residue in Japan. The growing interest in BGPs in Japan makes it important to consider biosecurity aspects of recycling digested residues. Umetsu *et al.* (2002) studied the survival of coliform bacteria during mesophilic anaerobic digestion of dairy manure slurry. Minato *et al.* (2003) also reported the monitoring of *Coli-aerogenes* group and *Enterococcus* in practical mesophilic BGPs. But there is no data on hygienic requirements in full-scale thermophilic BGPs in Hokkaido Japan. In thermophilic process, the temperature were known to be beneficial for the conversion rate, but were originally feared to be more difficult to be control. Umetsu *et al.* (2005) and Aoki *et al.* (2005) demonstrated the practicality of thermophilic temperature of BGPs in the cold regions. Thus, the thermophilic process has pervasive by this domination. The objective of the present paper is to clarify the survival of *Coli-aerogenes* group and *Enterococcus* in full-scale mesophilic and thermophilic BGPs in Hokkaido Japan.

MATERIALS & METHODS

Indicator organisms

Bacteria used as the most common indicator for public health monitoring is faecal *E. coli*. *Enterococci* can validate the hygienic treatment of biowaste in BGPs (Larsen *et al.* 1994). In this study, indicator organisms were detected *Coli-aerogenes* group and *Enterococcus*. *Coli-aerogenes* group and *Enterococcus* were isolated from the slurry samples on Desoxycholate *E. coli* selective agar plates incubated at 35°C for 20 h, M- *Enterococcus* selective agar plates were incubated at 35°C for 48 h.

Sampling

The slurry samples were collected from reception pit, digester and storage tank in two thermophilic (55°C) and two mesophilic BGPs (38°C) in 5 August 2005. Specification of these BGPs and operating conditions of this study were presented in Table 1. Ambient temperature of the day was around 30°C.

Analytical methods

Total solids (TS) were determined by drying in a fan-assisted oven at 105°C for 24 h. Volatile solids (VS) were determined by combusting the oven dried material at 550°C for 4 h. The pH values of the slurry samples were measured with pH meter (TOA Inc, Tokyo, Japan). Total volatile acids (TVA) determined with a Shimadzu HPLC (LC-10A), using a Shim-pack SGR-102H. The concentration of lactic, formamide, acetic, propionic and butyric acids were measured. The detail of the analytical procedure for TVA was described in a previous paper (Kimura *et al.* 1994).

RESULTS & DISCUSSION

Anaerobic digestion can be performed either mesophilic at 30-38°C or thermophilic at 50-55°C. The advantages for thermophilic temperatures include increased reaction rates, and therefore less capital costs as results of smaller digester size; increased efficiency of organic matter destroyed. Disadvantages of thermophilic system include higher energy requirements to heat influent substrate and maintain digester temperature. The survival patterns of *Coli-aerogenes* group and *Enterococcus* in the thermophilic (A, B) and mesophilic BGPs (C, D) were shown in Figs 1 and 2. The number of *Coli-aerogenes* group and *Enterococcus* in the slurries after Anaerobic digestion couldn't be found in both thermophilic BGPs. In both mesophilic BGPs, the viable numbers of *Coli-aerogenes* group and *Enterococcus* in the slurries were detected, though the number declined to low level after Anaerobic digestion. The decay rate of viable bacteria has been reported to depend on temperature, retention time, pH, volatile acids, bacterial species and available nutrients (Farrah and Bitton, 1983; Kearney *et al.*, 1993). The temperature is the most important factor concerning survival of pathogenic bacteria during Anaerobic digestion (Dumontet *et al.* 1999). Bacterial inactivation due to temperature is related to time (Olsen and Larsen 1987).

The time required for a 90% reduction of viable counts of a population of microorganisms or a decrease by one logarithmic unit (\log_{10}) is called the decimation reduction time (T_{90}) (Schlundt, 1984). The T_{90} value indicates the differences of inactivation of bacterial pathogens in anaerobic digestion. T_{90} for many bacteria can be counted in hours in thermophilic digestion and in days in mesophilic digestion (Gibbs *et al.* 1995). In the present investigation, *Coli-aerogenes* group and *Enterococcus* in the digested slurries of thermophilic BGPs (A, B) were not detected. The mean T_{90} values of both *Coli-aerogenes* and *Enterococcus* in mesophilic BGPs (C, D) were 13.3 days and 16.7 days, respectively. These findings agreed with the experimental results obtained by Olsen and Larsen (1987). *Salmonella* and *M. paratuberculosis* are inactivated within 24 h in thermophilic AD compared to weeks and even months in mesophilic anaerobic digestion (Plym-Forshell, 1995; Olsen *et al.* 1985).

Figure 3 shows the changes in TS and VS in the slurries of the thermophilic (A, B) and mesophilic (C, D) biogas plants. The TS contents of the slurries taken from the reception pits ranged from 3.97 to 11.95% and the VS contents remained relatively constant averaging 83.0% of the TS. The slurries taken from the A plant had higher water content because of the dilution by milking parlor wastewater. On the contrary, the TS content of the slurries taken from the C plant, over 12 %, was much higher because of no dilution. Figure 4 shows the changes in TVA in the slurries of the thermophilic (A, B) and mesophilic (C, D) biogas plants. The main volatile acids present in the slurries in the reception pits were acetic acid, propionic acid and butyric acid. The TVA content of the slurries taken from the reception pits were similar to those of TS or VS contents as shown in Fig. 3. During AD, the concentrations of TVA decreased sharply with the decomposition performed each biogas plants. Fig. 5 shows the changes in pH value of the slurries for each BGPs. The pH value is one of the important clues for understanding methane bacteria activity. In general animal manure has sufficient alkalinity to maintain the pH in an anaerobic digester so methane production from animal manure is a relative stable process. However, as a result of overloading, excessive concentration of volatile acids and ammonia nitrogen can develop, and this has been considered to hinder methane production because of the drastic changes in pH values. But in this observation, no special differences in pH were found between each of the biogas plants. From the data of composition of the digested slurries, the authors have concluded that each biogas plant was running favourably.

CONCLUSIONS

In Japan, many farmers spread raw or untreated manures straight to land. However, it is important that appropriate management practices are implemented to minimize the risks of pathogen transfer to the food chain from the management of animal manures. The present investigation was undertaken to study the hygienic and sanitation in full scale biogas plants treating dairy manure. This paper reported the survival of *Coli-aerogenes* group and *Enterococcus* in full-scale mesophilic and thermophilic BGPs in Hokkaido Japan. *Coli-aerogenes* group and *Enterococcus* in thermophilic BGPs were not detected. In both mesophilic BGPs, the viable numbers of *Coli-aerogenes* group and *Enterococcus* in the slurries through the number declined to low level after anaerobic digestion. In this study, digester high temperature would have a suppressing effect on the population of *Coli-aerogenes* group and *Enterococcus* in the slurries during anaerobic digestion. Finding of this study suggest that BGPs offers benefit towards net reduction of pathogenic bacterial numbers in dairy manure slurry. However, pasteurization is the best way to ensure non-pathogenic condition of mesophilic anaerobic digestion.

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Table 1. Specification and operating conditions of the biogas plants of this study

Parameter	Units	Plant A	Plant B	Plant C	Plant D
Digester temperature	(°C)	55	55	38	38
Digester volume	(m ³)	60	540	424	671
Amount of feeding per day	(m ³ /day)	4	45	16	18
Hydraulic Retention Time (HRT)	(day)	15	12	26.5	37.3

Table 2. The mean T_{90} values of *Coli-aerogenes* and *Enterococcus* for each biogas plants

Plant	<i>N</i>	T_{90} for <i>Coli-aerogenes</i>	T_{90} for <i>Enterococcus</i>
A	Reception pit	6	
	Digester	5	
	Storage tank	5	—
B	Reception pit	6	
	Digester	6	
	Storage tank	6	—
C	Reception pit	5	
	Digester	6	
	Storage tank	5	14.5
D	Reception pit	6	
	Digester	6	
	Storage tank	6	12.1

N, number of samples.

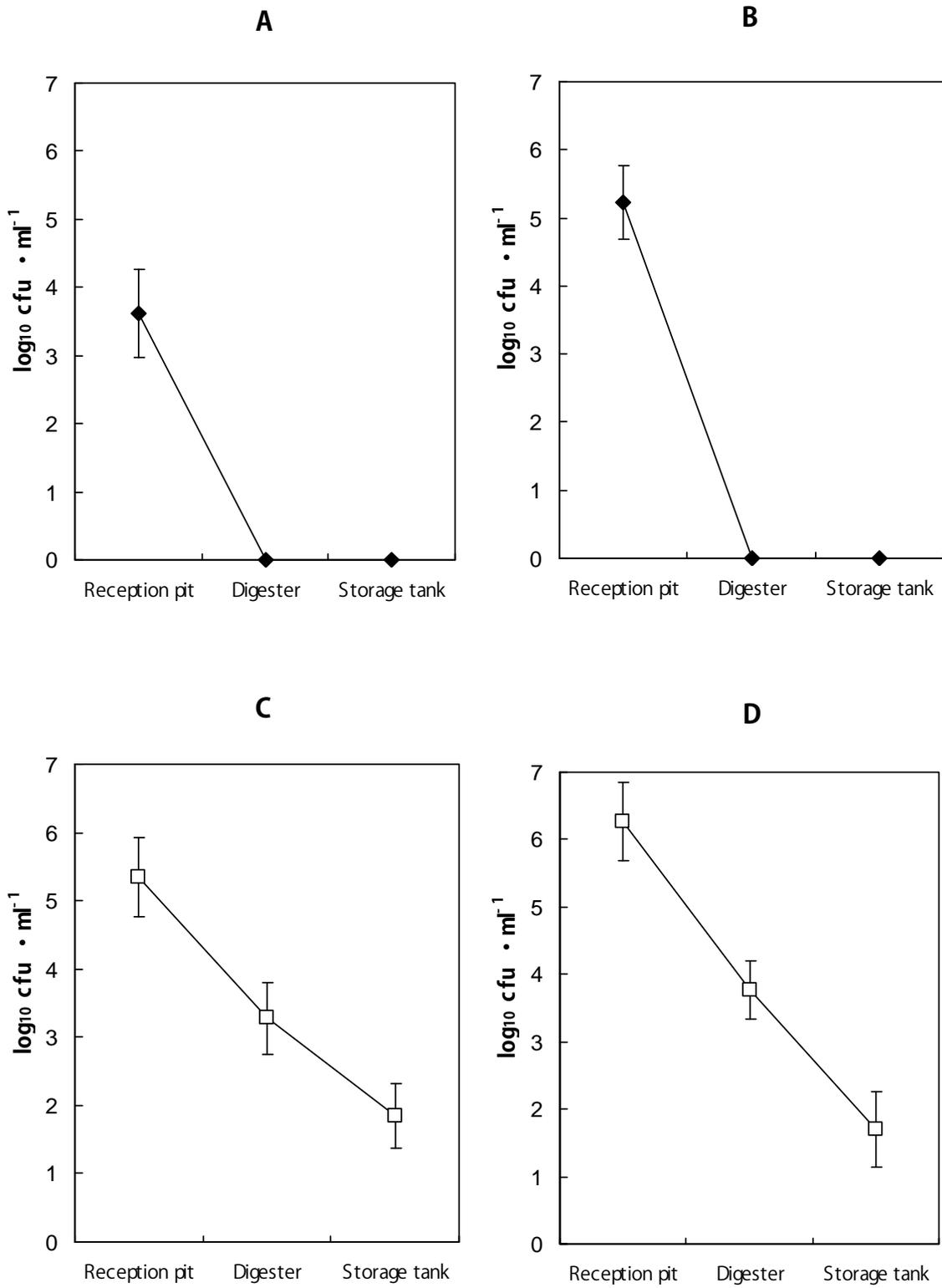


Figure 1. Arithmetic mean viable counts of *Coli-aerogenes* group in the thermophilic (A ,B) and mesophilic (C, D) biogas plants

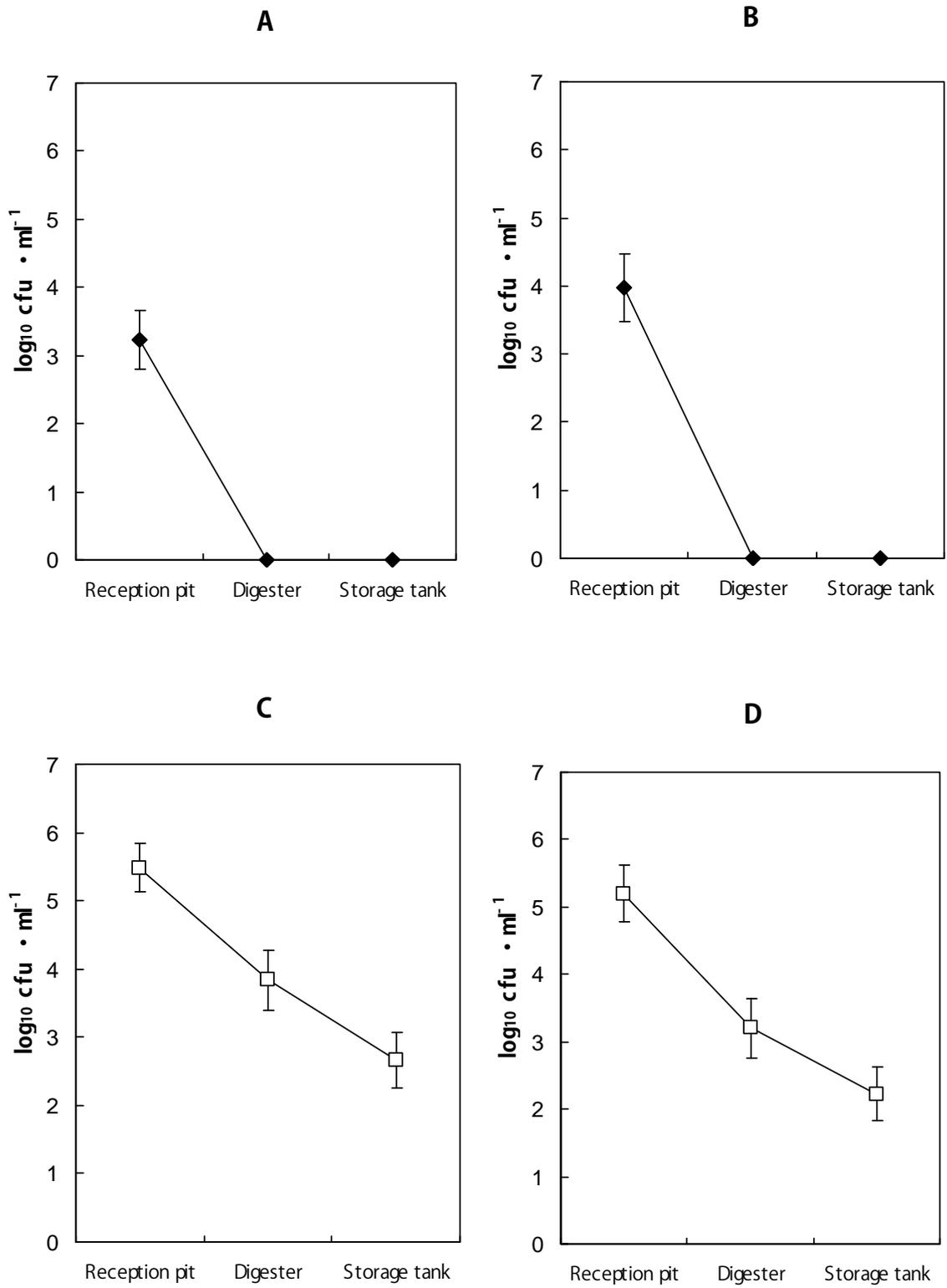


Figure 2. Arithmetic mean viable counts of *Enterococcus* in the thermophilic (A ,B) and mesophilic (C, D) biogas plants

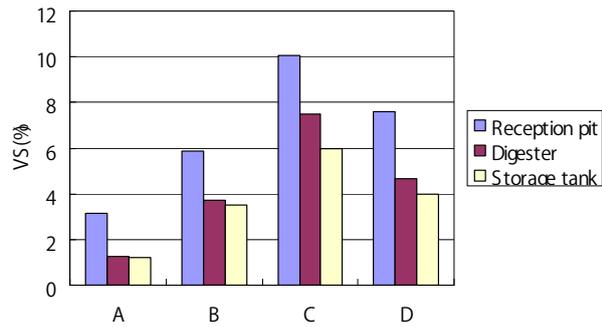
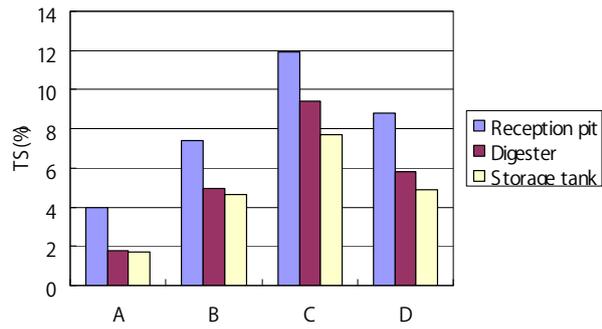


Figure 3. Changes in total solids (TS) and volatile solids (VS) in the slurries of the thermophilic (A, B) and mesophilic (C, D) biogas plants

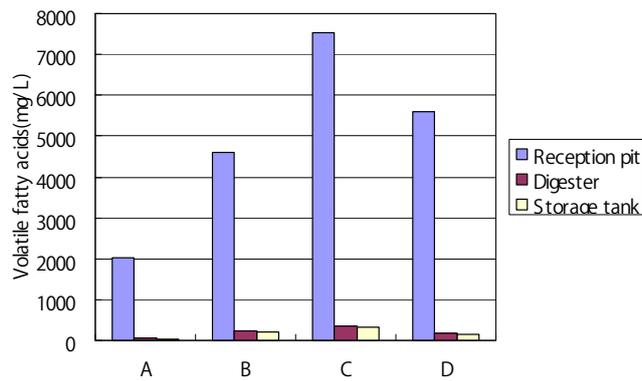


Figure 4. Changes in total volatile acids (TVA) in the slurries of the thermophilic (A, B) and mesophilic (C, D) biogas plants

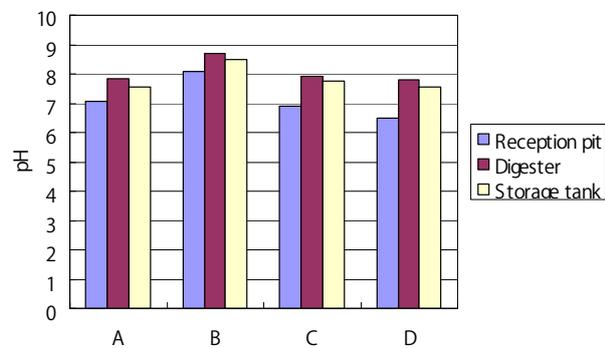


Figure 5. Changes in pH value of the slurries in the thermophilic (A, B) and mesophilic (C, D) biogas plants.

EFFECT OF VENTILATION RATE IN HEADSPACE OF DIGESTED SLURRY STORAGE TANK ON GREENHOUSE GAS EMISSIONS

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ABSTRACT

The objective of this study is to examine the relationship between degree of air tightness of digested slurry storage and greenhouse gas emission from the surface. Headspace of model digested slurry storage tank was ventilated at fixed ventilation rates. And quantities of gas emissions were measured. The results suggest that the quantities of greenhouse gas emissions when ventilation rate is about 80 ac/h are similar to that of the digested slurry storage tank without cover.

INTRODUCTION

It is thought that greenhouse gas emission from surface of digested slurry storage tank may be controlled by some cover. The objective of this study is to examine the relationship between degree of air tightness of digested slurry storage tank and greenhouse gas emission from the surface.

MATERIALS & METHODS

A 1/23-scale model of prototypical digested slurry storage tank was used. A ventilation system was installed so that ventilation rate at the headspace of the model tank can be controlled. Levels of ventilation rates were set in four levels: 0.25, 1.7, 11.7 and 80 ac/h. Densities of ammonia, dinitrogen oxide, carbon dioxide and methane gases were measured with Infrared Photoacoustic Detector (INNOVA) and a multi-point sampler at input and output ducts. Measuring was continued more than 24 h after temperature of the slurry reached to the room temperature.

Quantities of gas emissions (GE, mg/h) were calculated by the following equation:

$$GE = (D_o - D_i) \times V$$

where D_o and D_i are densities of output and input gases (mg/m^3) and V is volume of ventilation (m^3/h).

RESULTS & DISCUSSION

Gas emission from the surface of digested slurry was low when ventilation rate at headspace of the tank was low, that is, when the air tightness of the tank was high. Carbon dioxide gas emission at 80 ac/h was lower than that at 11.7 ac/h probably because of the dry scum formed on the surface of the slurry. As a generated tendency gas emission seems saturate when ventilation rate was 80 ac/h. Therefore it is thought that the state of digested slurry storage tank at 80 ac/h was close to the situation that the tank has no cover.

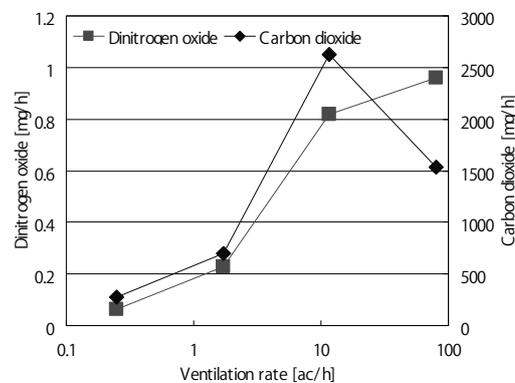
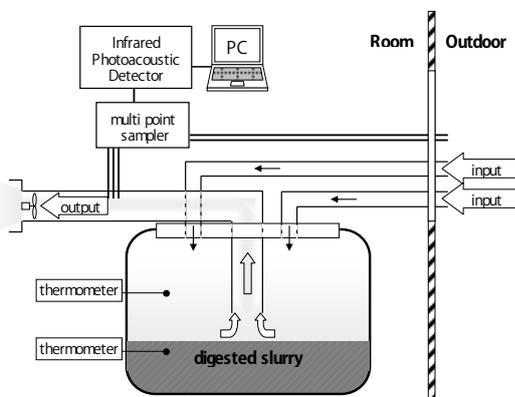


Figure 1. Model of digested slurry storage tank.

Figure 2. Quantities of gas emissions and ventilation rate.

NITROUS OXIDE EMISSION FROM A MAGNETIC ACTIVATED SLUDGE (MAS) PROCESS TO TREAT THE DAIRY MILKING PARLOUR WASTEWATER

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ABSTRACT

Nitrous oxide (N₂O) can be emitted as a by-product of the process of nitrogen removal from agricultural wastewater. Magnetic activated sludge (MAS) process was introduced to an activated sludge process to improve the solid-liquid separation characteristics of the sludge. To reduce N₂O emission from MAS treating dairy milking parlour wastewater, N₂O emissions were compared between the continuous aeration process and the intermittent aeration process using a 4 L aeration tank contained a magnetic separation apparatus. The N₂O gas concentrations during MAS dairy milking parlour wastewater treatment were obtained to estimate total emission to profile its release.

INTRODUCTION

N₂O is a potent greenhouse gas produced from animal production systems. N₂O emission from animal wastes is about 6% of total N₂O emission (IPCC 1995). Milking parlour system produces a large volume of wastewater. In which the disposal poses a significant problem. MAS process uses a magnetic separation to improve the solid-liquid separation characteristics of the activated sludge. This study was carried out to estimate N₂O emission comparing the continuous aeration process and the intermittent aeration process of MAS system and to evaluate the efficiency of treatments in removing organics and nitrogen from dairy milking parlour wastewater.

MATYERIALS & METHODS

Two units of 4 L aeration reactors containing a magnetic separation apparatus, which composed of a magnetic drum and a scrapper (Fig. 1), were prepared for dairy milking parlour wastewater treatment. Activated sludge was magnetized by supplementing with ferromagnetic powder (Fe₃O₄), which exhibits ferromagnetic properties and deposited on the surface of magnetic drum due to magnetic force, separated from the treated water and returned to the aeration tank by means of a scraper (Sakai *et al.* 1994). The ratio of magnetite/MLVSS (mixed liquid suspension solid) was 1:1. The wastewater was taken from K dairy farm in Akan chio, Kusiro, Japan. The reactor I was used for continuous aeration process. The reactor II was used for intermittent aeration process. The aeration rate was 2 L/min. In the anoxic phase of reactor II, the mixed liquor is stirred to maintain the particles in suspension. The wastewater was continuously added into both reactors, at a flow rate of 1.0 L/day. In both reactors, the hydraulic retention time (HRT) was 4 days. In the reactor II, intermittent aeration was carried out under aeration/non-aeration ratios of 30/60 min in a cycle. The gases samples were collected from each head space of both reactors and N₂O concentrations were measured at 10-min intervals during one treatment cycle.

RESULTS & DISCUSSION

Table 1 shows the characteristics of Influent wastewater and Effluent of both treatments. The COD removal efficiencies are 89.5% and 90.0% at the continuous aeration process and intermittent aeration process, respectively. Fig. 2 shows the changes in N₂O concentration in exhaust gases of both processes in a typical cycle. The concentrations of N₂O in both reactors were higher than that of the background air. In the intermittent aeration process of 30min aeration/60min non-aeration cycles in reactor II, the concentrations of N₂O in aeration phase and non-aeration phases were higher than that of continuous aeration process in reactor I. Furthermore, in the reactor II the N₂O concentrations in non-aeration phase after 30min were higher than that of aeration phase. Our next step is to determine the quantity of N₂O emission from MAS process to treat the dairy milking parlour wastewater.

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Table 1. Characteristics of influent wastewater and effluent of both treatments

	Influent	Effluent I	Effluent II
COD (mg/L)	2133.7	223.4	213.1
NH ₄ ⁺ -N (mg/L)	63.9	0.87	0.51
NO ₃ ⁻ -N (mg/L)	0.6	59.7	53.1
TS (mg/L)	1696.7	992	963
pH	6.79	7.24	7.22

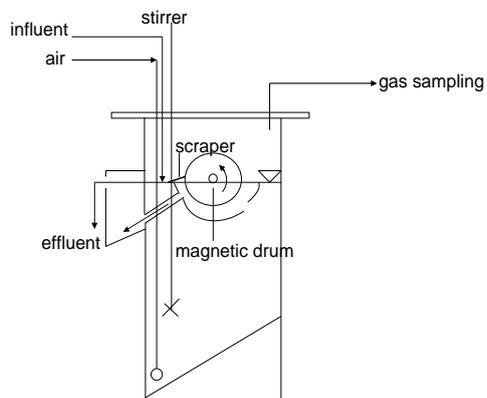


Fig. 1 A schematic diagram for the laboratory-scale MAS reactor

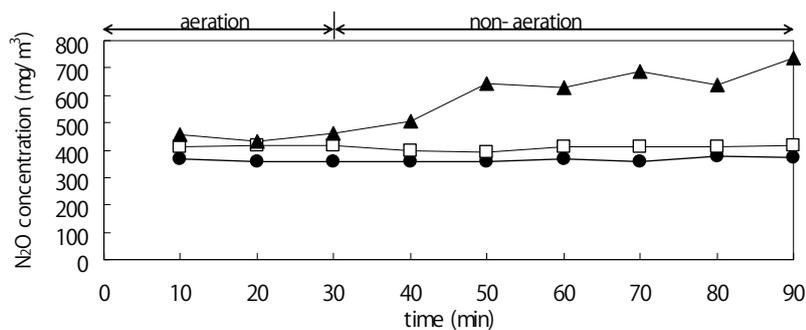


Fig. 2 Changes of N₂O concentrations during the continuous aeration process (reactor I) and the intermittent aeration process (reactor II) and background air: □, continuous aeration process (reactor I); ▲, intermittent aeration process (reactor II); ●, background air

EFFECT OF FORAGE/CONCENTRATE RATIO AND ETHYL LINOLENATE LEVEL ON METHANE EMISSION AND FERMENTATION PARAMETERS OF HUZHOU SHEEP

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INTRODUCTION

Methane produced by enteric fermentation within ruminants means not only a severe loss of feed energy for the animal, but also causes, as an important greenhouse gas, ecological problems (Johnson and Johnson 1995). Therefore, developing feeding strategies with methane-suppressing impact is desirable. Long-chain unsaturated fatty acids are known to have a high potential in suppressing ruminal methanogenesis (Moss *et al.* 2000). In the previous study, we investigated the effects of type and level of octadeca-carbon fatty acids on *in vitro* rumen fermentation and methane emission and found that linolenic acid had the most efficient methane-suppressing effect. In this experiment, as part of a wider project, the effect of ethyl linolenate (LNE) was studied on methane emission from finishing sheep given diets differing in the forage to concentrate ratio (F:C) using simple box chamber.

MATERIALS & METHODS

The experimental design was a 4×4 Latin square including four dietary treatments. Eight male Huzhou sheep were paired with fistulated and not fistulated each at the beginning of the experiment and the pairing of animals was consistent throughout the experiment. Four pairs of sheep were fed a forage-based diet without (F; F:C=70:30, dry matter basis) or with LNE (FL; F:C=70:25, 5% LNE); a concentrate-based diet without (C; F:C=30:70) or with LNE (CL; F:C=25:70, 5% LNE), respectively. The diets were given in equal portions twice a day at 8:00 and 16:00. Methane emission and fermentation parameters such as ammonia-N, volatile fatty acids and microbial protein mass were determined using the methods described by Hu *et al.* (2005).

RESULTS & DISCUSSION

Addition of LNE in different F/C ratio diets had the same diurnal pattern of methane emission (Fig. 1). Methane emission was rapidly increased to peak two or three hours after the feeding, and then decreased slowly until the next feeding.

Addition of LNE decreased methane emission by 17.3 and 33.8% in forage-based and concentrate-based diet, respectively (Table 1). Ruminal pH was increased by inclusion of LNE. Diet type had no effect on total volatile fatty acid, but LNE supplementation decreased total volatile fatty acid significantly ($P<0.05$). Inclusion of LNE decreased molar proportion of acetate and butyrate and increased propionate proportion in concentrate-based diet, while had little effect on the fermentation pattern in forage-based diet. Ammonia-N concentration and microbial protein mass were decreased significantly ($P<0.05$) by addition of LNE. Forage-based diet had lower ammonia-N concentration than concentrate-based diet. However, diet type had no effect on microbial protein mass. It is concluded that addition of LNE can inhibit methane emission in both forage- and concentrate-based diets significantly, which is beneficial for economy and environment.

ACKNOWLEDGEMENTS

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Table 1. Methane emission and ruminal parameters for sheep fed different forage/concentrate ratio diet with or without ethyl linolenate

Within rows, means followed by different letters are significantly different at $P = 0.05$

Diet	Forage-based		Concentrate-based		s.e.m.	<i>P</i> -values		
	0	50	0	50		D	S	D×S
Ethyl linolenate (g/kg)								
Methane (L/day)	28.9a	23.9b	26.6a	17.6c	0.8	<.01	<.01	0.02
Ruminal pH	7.14ab	7.33a	6.90b	7.13ab	0.09	0.02	0.03	0.84
Volatile fatty acids (mmol/L)	68.6a	54.6ab	62.2ab	49.7b	4.1	0.26	0.02	0.88
Molar proportions (%)								
Acetate	77.4a	76.9a	71.7b	69.3c	0.7	<0.01	0.14	0.30
Propionate	13.7b	14.8 b	16.6b	22.6a	1.3	<0.02	0.03	0.11
Butyrate	9.0ab	8.3b	11.7a	8.1b	0.8	0.15	0.02	0.10
A/P ratio	5.74a	5.35ab	4.38b	3.16c	0.30	<0.01	0.08	0.34
NH ₃ -N (mg/dL)	12.3c	10.7d	20.7a	16.8b	0.4	<0.01	<0.01	0.15
Microbial protein (mg/mL)	1.95a	1.55b	1.95a	1.69ab	0.11	0.25	0.01	0.76

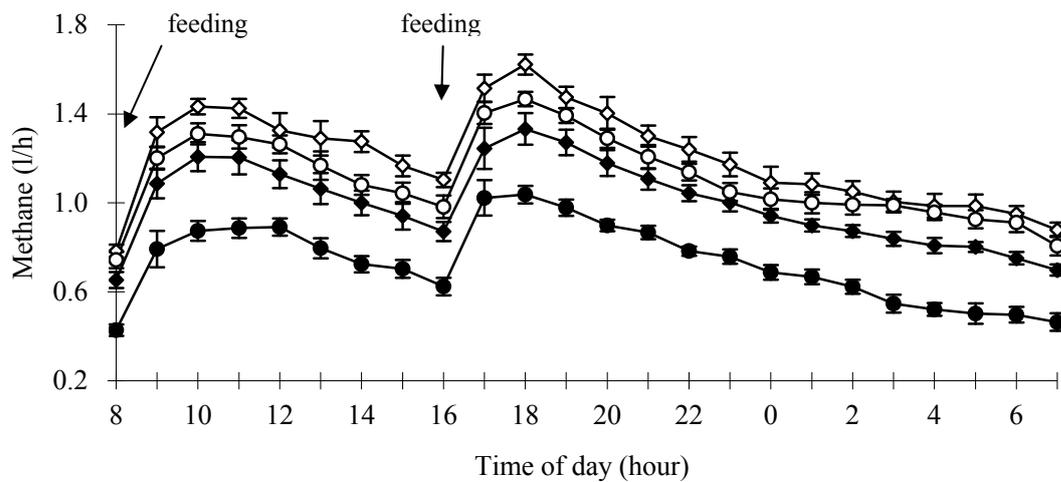


Figure 1. Diurnal pattern of methane emission from sheep fed a forage-based diet without (\diamond) or with ethyl linolenate (\blacklozenge ; 5g/kg DM); concentrate-based diet without (\circ) or with ethyl linolenate (\bullet ; 5g/kg DM), respectively. Vertical bars indicate standard error of means.