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Within- and between-animal variance in methane emissions in non-lactating dairy cows

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Abstract. Several studies on methane (CH_4) emissions have focussed on selecting high and low CH_4-emitting animals. One challenge faced by this work is the lack of consistency, or repeatability, in animal rankings over time. Repeatability for individual animals over time needs to be high to reliably detect high and low CH_4-emitting animals. A possible explanation for the lack of repeatability is a relatively high within-animal variation in daily CH_4 emissions, meaning that animals could then change their ranking when compared at different points in time. An experiment was undertaken with four non-lactating dairy cattle to assess the within- and between-animal variation in CH_4 emissions over time when measured using the sulfur hexafluoride (SF_6) tracer technique. Two contrasting diets were fed to the cattle at maintenance energy levels: lucerne silage (diet 1) and a cereal + lucerne + straw mixed ration diet (diet 2). Daily CH_4 measurements were undertaken for 23 days on diet 1 and 30 days on diet 2.

There was a significant ($P < 0.001$) difference between diet 1 and diet 2 in daily CH_4 production, with mean (±s.e.) production of 124.3 (11.1) g CH_4/day from diet 1 and 169.8 (±11.0) g CH_4/day from diet 2. Lower CH_4 yield (g CH_4/kg dry matter intake) was recorded on diet 1 (22.8 ± 2.0) than diet 2 (32.0 ± 2.0). Cows differed significantly ($P < 0.05$) from one another in daily CH_4 yield (diet 1: cow 1 = 19.4 ± 0.6, cow 2 = 22.2 ± 0.8, cow 3 = 23.2 ± 0.7, cow 4 = 25.4 ± 0.6; diet 2: cow 1 = 26.0 ± 0.7, cow 2 = 36.4 ± 0.7, cow 3 = 29.3 ± 0.7, cow 4 = 36.6 ± 0.7). Variances for daily CH_4 yield were smaller for diet 1 (within animal = 6.91, between animals = 6.23) than for diet 2 (within animal = 10.09, between animals = 27.79). Estimates of repeatability (variation between animals/total variation) for daily CH_4 yield were 47 and 73% in diet 1 and 2, respectively. Coefficients of variation in average daily CH_4 emissions in this experiment ranged from 8 to 18% despite the fact that each animal received the same quantity and quality of feed each day. While further research is required, the high within-animal variability in CH_4 emissions measured using the SF_6 tracer technique may explain why there has been difficulty in obtaining consistent rankings in CH_4 yields when animals are measured on multiple occasions. The results also suggest that the SF_6 tracer technique may exaggerate apparent between animal differences in CH_4 emissions.

Introduction

Mitigation of ruminant methane (CH_4) has become an important area of research because the accumulation of CH_4 has been linked to global warming. One mitigation method that provides an inexpensive and long-term reduction is the use of natural variation to breed for animals with lower CH_4 yield [g CH_4/kg dry matter intake (DMI)]. Several studies have been undertaken to select high and low CH_4-emitting animals (Pinares-Patiño *et al*. 2003*a*, 2005; Goopy and Hegarty 2004). While high and low CH_4 emitters were identified, these animals did not maintain consistent rankings during subsequent measurement periods in any of the three studies. The lack of repeatability in rankings among animals may be due to natural variation in individual emissions, variation in the measurement technique or a combination of both.

Variation in CH_4 production both within- and between-animals has long been recognised from standardised calorimetric studies, with reported coefficients of variation (CV) of 7% within-animal and 7–8% between-animal (Blaxter and Clapperton 1965). More recently, Grainger *et al*. (2007) reported a CV of 4.3% within-animal and 17.8% between-animal when using open-circuit calorimetry. In grazing studies using the sulfur hexafluoride (SF_6) tracer technique, Lassey *et al*. (1997), Boadi and Wittenberg (2002) and McNaughton *et al*. (2005) obtained between-animal CV of 11.5, 15.5 and 25%, respectively. These studies indicate that substantial variation occurs both within- and between-animals in CH_4 production. Other researchers have described problems with poor repeatability over time, with animals changing their ranking within a group (Pinares-Patiño *et al*. 2003*a*, 2005; Goopy and Hegarty 2004). There is little quantification of the variation in within-animal CH_4 production in the published literature, apart from the early work of Blaxter and Clapperton (1965), and no proportioning to either within-

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or between-animal variance from studies that have used the SF_6 technique.

An assumption behind studies that are seeking to identify high- and low-emitting animals is that CH_4 yield is constant for a given feed type and that a measurement at one point in time is an accurate reflection of overall CH_4 yield potential. The focus of this study is to assess the within- and between-animal variation in daily CH_4 yield so as to test the hypothesis that large within-animal variation in CH_4 yield is a factor in the lack of repeatability found in studies that have tried to identify consistently high and low CH_4-emitting animals.

Materials and methods

Experimental design

Two contrasting diets were fed to four Friesian × Jersey dairy cows: a forage-based diet (diet 1) and a cereal + lucerne + straw ration diet (diet 2). CH_4 emissions from each cow were measured daily using the SF_6 tracer technique. Intake was restricted to energy maintenance levels for indoor cattle calculated using the equation, metabolisable energy_m (MJ/day) = 8.3 + 0.091 × liveweight (ADAS 1984). The experiment ran for 74 days, with the first 9 days for adaptation to diet 1, 23 days of measurement for diet 1, 12 days adaptation to diet 2, and 30 days of measurement on diet 2.

Animals and feeding

Cows had an average weight (± s.d.) of 417 ± 24 kg at the beginning of the experiment. Housing was in individual stalls in a well ventilated barn, with exercise periods twice daily on an outdoor sawdust pad for 30–60 min. Cows had *ad libitum* access to fresh drinking water throughout the experiment, with feeding twice daily at 0800 and 1600 hours.

Diet 1 consisted of ensiled lucerne (*Medicago sativa*), which contained small, but unknown, quantities of molasses (ChaffHage, The Great Hage Co., Reporoa, NZ). The silage came as three separate batches, so individual cows were fed from single batches within dietary periods. Transition to diet 2 was carried out progressively in 3-day periods, with cows receiving 30, 50, 80 then 100% of the concentrate pellet as a proportion of that fed during diet 2. Diet 2 included a concentrate-based pellet, barley straw, and the same lucerne silage as diet 1. On a dry matter (DM) basis, the diet comprised 60% pellet, 17% straw and 23% lucerne silage. Samples were collected for DM determination (oven drying for 48 h at 60°C) and chemical composition analysis by wet chemistry. The cows ate all the feed offered each day, so no refusals were collected.

CH_4 measurements

Administration of SF_6 permeation tubes to the animals was undertaken on day 7 of the experiment, with CH_4 measurements beginning on day 9. Release rates of SF_6 (mg SF_6/day) for each cow were: cow 1 = 3.677, cow 2 = 3.975, cow 3 = 3.547, cow 4 = 4.228. The permeation tubes were selected to minimise differences in absolute SF_6 permeation rate. This follows from the study by Vlaming *et al*. (2007), which showed that the absolute release rate of SF_6 from permeation tubes can influence estimates of CH_4 emission when using the SF_6 tracer technique; high permeation tube release rates result in higher CH_4 yield. Using the relationship between permeation tube release rate and calculated CH_4 yield suggested by Vlaming *et al*. (2007), the difference in estimated CH_4 yield between the highest (4.228) and lowest (3.547) release rate used in this experiment would be a maximum of 0.5 g CH_4/kg DMI.

CH_4 emissions were measured daily from day 9 until the completion of the experiment on day 74 using the SF_6 tracer technique (Lassey *et al*. 1997; Ulyatt *et al*. 1999). Briefly, gas samples were collected via a tube ~5 cm dorsal to the nostrils, held in place by a halter. An in-line capillary tube restricted airflow to ~1 mL/min, delivered via a QuickConnect valve to a pre-evacuated PVC canister (yoke) mounted on the animal's back. Yokes were approximately half-filled over the 24-h collection period and were fitted or exchanged immediately before feeding at ~0800 hours, daily. Two background air samples were collected from inside from the barn each day. Samples were measured with flame ionisation and electron capture detectors for CH_4 and SF_6, respectively, using a gas chromatograph (Hewlett Packard 5890 Series II or Simadzu 2010).

The release rate of the SF_6 tracer gas and the ratio of SF_6 to CH_4 in the breath were used to calculate the CH_4 emissions of each animal (Q_{CH_4}):

$$Q_{CH_4} = Q_{SF_6} \times (CH_4)/(SF_6)$$

where (CH_4) and (SF_6) denote the concentrations in the yokes after background corrections, and Q_{SF_6} is the release rate of SF_6 from the permeation tube(s). As the experiment ran for almost 11 weeks, the method of Lassey *et al*. (2001) was used to obtain a corrected weekly SF_6 release rate for each permeation tube. The only correction resulting from this procedure was an increase in the release rate of the tube inserted into cow 4 (4.228–4.229 mg SF_6/day) from week 5 onwards.

Laboratory analyses

Feed samples were dried and ground before analysis. Analysis procedures were predominantly those of the Association of Official Analytical Chemists (AOAC 2005). Nitrogen was measured using a Carlo Erba NA1500 nitrogen analyser (Carlo Erba Strumentazione, Milan, Italy). Analyses for *in vitro* DM digestibility were undertaken using neutral detergent solubilisation, followed by cellulytic enzyme break-down. Digestibility values were validated with coefficients derived from animal experiments fed maintenance level diets of similar feed types (Corson *et al*. 1999). Gross energy of the feedstuffs was measured using a Leco Automatic bomb calorimeter AC-350 (Leco Corporation, St Joseph, MI, USA) at the Massey University Nutrition Laboratory (Palmerston North, New Zealand).

Statistical analyses

Both daily CH_4 values and CH_4 yield were analysed with a repeated-measures analysis using the MIXED procedure of SAS (SAS 2002). The model included the fixed effect of diet, day and the diet × day interaction, and the random effect of animal. Estimates of variances within- and between-animals were obtained across dietary periods and for each dietary period. Repeatability of CH_4 emission was calculated as the proportion of variance between animals with respect to the total variance,

Table 1. Nutrient composition (g/100 g) of the three batches of lucerne silage, barley straw and concentrate pellet offered to cows during the experiment

DMD, dry matter digestibility; ADF, acid detergent fibre; NDF, neutral detergent fibre; ESS, ethanol soluble sugar; GE, gross energy

	Silage 223	Silage 173	Silage 093	Barley straw	Pellet
DMD	65.49	66.48	68.80	51.59	87.44
Ash	9.56	9.34	9.94	5.47	5.91
ADF	42.38	42.77	41.28	52.49	8.18
NDF	50.86	48.79	47.26	83.42	24.87
Lipid	1.69	1.62	1.33	1.54	3.24
Nitrogen	3.69	3.60	3.53	0.77	2.43
ESS	1.53	1.72	1.77	1.44	4.83
Starch	0.00	0.00	0.00	0.00	43.93
GE (MJ/g)	19.56	19.44	19.11	18.14	18.36

which was estimated as the sum of the within- and between-animal variances. A Fisher test ($P = 0.05$) was used to test whether repeatability measures differed significantly between dietary periods.

Results

Feed intake

The nutrient analyses of the three lucerne silages, barley straw and concentrate pellet fed are shown in Table 1. Small differences between animals in intake occurred due to differences in DM content of the batches of silage. The DM intake of the cows was between 5.3 and 6 kg DM/cow.day for diet 1 and between 5.2 to 5.4 kg DM/cow.day for diet 2.

CH_4 production

Absolute daily CH_4 production from cows differed ($P < 0.001$) between diets, with mean (± s.e.) production of 124.3 (± 11.1) g CH_4/day from diet 1 and 169.8 (± 11.0) g CH_4/day from diet 2. Lower CH_4 yields (g CH_4/kg DMI) were recorded on diet 1 (22.8 ± 2.0) than diet 2 (32.0 ± 2.0). Cows differed significantly ($P < 0.05$) from one another in CH_4 yield (Table 2).

CV in daily CH_4 yield (g CH_4/kg DMI) from individual cows in this experiment ranged from 8 to 18% despite each animal receiving the same quantity and quality of feed each day. Variances for daily CH_4 yield were smaller for diet 1 (within animal = 6.91, between animals = 6.23) than for diet 2 (within animal = 10.09, between animals = 27.79); daily absolute CH_4 emissions followed a similar pattern (Table 3). Estimates of repeatability (variation between animals/total variation) for daily CH_4 yield were 47 and 73% in diet 1 and 2, respectively. While repeatability was almost 60% for daily CH_4 production and 55% for daily CH_4 yield when using combined data from both diets.

Table 2. Mean (± s.e.) daily methane (CH_4) yield (g CH_4/kg dry matter intake) of four cows fed either lucerne silage (diet 1) or a cereal + lucerne + straw ration (diet 2)

	Cow 1	Cow 2	Cow 3	Cow 4
Diet 1	19.4 ± 0.6	22.2 ± 0.8	23.2 ± 0.7	25.4 ± 0.6
Diet 2	26.0 ± 0.7	36.4 ± 0.7	29.3 ± 0.7	36.6 ± 0.7

Discussion

The results presented here confirm the work of Blaxter and Clapperton (1965) and Grainger et al. (2007), which showed considerable within- and between-animal variance in CH_4 production from animals receiving the same diet. However, the within-animal values in our study (7–10%) obtained using the SF_6 technique, are higher than those found in studies using calorimetry (4–7%). This may help explain why animals selected as high or low emitters have been found to change their ranking when measured at different points in time (Pinares-Patiño et al. 2003a, 2005; Goopy and Hegarty 2004). In addition to demonstrating that within-animal variance in daily CH_4 yield is higher from SF_6 than other published results obtained using calorimetry, our study suggests that between-animal variance in CH_4 yield can differ markedly between diets. This would again create difficulties when trying to identify consistently high- or low-emitting animals.

It is not clear why the CH_4 yield increased for diet 2, which had higher levels of starch and reduced fibre levels, but may be due to the straw portion. Straw is known to break down slowly, and may have reduced rumen outflow rates, thus increasing residence time in the rumen and CH_4 production (Okine et al. 1989). Reduced rumen outflow rate is linked to rumen volume (Pinares-Patiño et al. 2003b), greater acetate and, therefore, CH_4 production per unit of feed eaten (McAllister et al. 1996).

Estimated repeatability of CH_4 yield for cows was higher on diet 2, which had the higher total variance. This can be explained by the within-animal variance being only slightly higher for diet 2, while the between-animal variance was much greater. As the CH_4 yield was also much higher on diet 2, the repeatability for animals could also be higher.

Results from studies using the SF_6 technique are usually expressed as the mean of a 4-day measurement period. Using this standard protocol, the mean (± s.d.) of the daily CH_4 yield for each animal on each diet are presented in Table 4. Using these data and some simplified assumptions it is possible to provide approximate guidelines for selecting individual animals that differ significantly in their CH_4 yield. Using the 95% confidence level and the t-statistic for a large sample ($n > 30$) two means will differ significantly from each other if they are ~2 standard deviations apart. In this study, individual animal variances

Table 3. Within- and between-animal variance for both absolute daily methane (CH_4) production and daily CH_4 yield for four cows for the combined data for diet 1 and diet 2 separately

	Combined data	Diet 1	Diet 2
Absolute CH_4 production (g CH_4/day)			
Within-animal	464.85	211.85	770.67
Between-animal	320.80	217.56	281.69
Total	785.65	429.41	1052.36
Repeatability	0.59	0.49	0.73
Daily CH_4 yield (g CH_4/kg dry matter intake)			
Within-animal	14.83	6.23	27.79
Between-animal	12.17	6.91	10.09
Total	27.00	13.14	37.88
Repeatability	0.55	0.47	0.73

Table 4. Mean (± s.d.) of the 4-day mean methane (CH_4) yields (g CH_4/kg dry matter intake) of four cows fed either lucerne silage (diet 1) or a cereal + lucerne + straw ration (diet 2)

	Cow 1	Cow 2	Cow 3	Cow 4
Diet 1	19.3 ± 0.6	22.5 ± 3.0	23.4 ± 3.0	25.4 ± 0.9
Diet 2	26.2 ± 2.0	36.0 ± 2.4	29.3 ± 2.1	36.6 ± 2.1

were not constant making it difficult to compare mean values. However, assuming the largest standard deviation (s.d. = 3.0 g CH_4/kg DMI), it is possible to estimate a conservative value for the differences between mean CH_4 yield required to be confident that these differences are statistically significant differences between animals (~6 g CH_4/kg DMI).

However, this value only applies to animals measured indoors and fed a constant quality and quantity diet and in a situation where permeation tube release rates can be equalised for every animal (Vlaming *et al*. 2007). If animals are measured under less controlled circumstances, i.e. in grazing animals where the quality of the diet isn't strictly controlled for each animal, and intakes are estimated rather than measured, within-animal variation in CH_4 yield is likely to be greater than that found in this study. Differences between animals in mean daily CH_4 yield will, therefore, need to be larger than the 6 g CH_4/kg DMI suggested by this study for there to be a high probability that they are significantly different.

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