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## The variation in methane emissions from sheep and cattle is not explained by the chemical composition of ryegrass

K.J. HAMMOND<sup>1</sup>, S. MUETZEL<sup>1</sup>, G.C. WAGHORN<sup>2</sup>, C.S. PINARES-PATINO<sup>1</sup>,  
J.L. BURKE<sup>3</sup> and S.O. HOSKIN<sup>1</sup>

<sup>1</sup>AgResearch Grasslands, Private Bag 11-008, Palmerston North 4442, New Zealand

<sup>2</sup>DairyNZ, Private Bag 3221, Hamilton 3240, New Zealand

<sup>3</sup>Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11-222, Palmerston North 4442, New Zealand

### ABSTRACT

A data base of over 3,000 ruminant methane emission records from individual animals used to compile New Zealand's greenhouse gas inventory, shows substantial variation in CH<sub>4</sub> yields (g/kg dry matter intake (DMI)) within and between forage types. Multiple regression analyses were undertaken using datasets from sheep and cattle fed fresh ryegrass to determine the extent to which variation in CH<sub>4</sub> yields could be explained by ryegrass chemical composition. Methane was measured using the SF<sub>6</sub> marker dilution technique with sheep and cattle, and also directly by respiration calorimetry from sheep. Data from sheep showed a similar average CH<sub>4</sub> yield from both techniques, but there was more variation in SF<sub>6</sub> data ( $23.4 \pm 5.73$ ) than from calorimetry ( $23.1 \pm 2.89$ ). Cattle CH<sub>4</sub> yields averaged  $19.1 \pm 3.70$  g/kg DMI. Multiple regression analysis of sheep data showed pasture chemical composition accounted for <2% of the variation determined by SF<sub>6</sub> and 20% of the variation from calorimetry. Only 13% of the variation in yield from cattle was explained by ryegrass composition, so the majority of variance (>80%) was not explained. We suggest the variation in CH<sub>4</sub> emissions is affected by digestive characteristics of individual animals, and interactions with forage physical and chemical components.

**Keywords:** methane; ruminants; pasture; chemical composition.

### INTRODUCTION

Methane (CH<sub>4</sub>) accounts for about 35% of New Zealand greenhouse gas emissions, with about 87% derived from ruminal fermentation (Ministry for the Environment, 2008). New Zealand is committed to mitigating greenhouse gas emissions in accordance with the Kyoto protocol and a ruminant CH<sub>4</sub> inventory has been created as part of this process. Inventory calculations are based on animal numbers, production, calculated feed intakes and methane yield per unit of dry matter intake (DMI) (g CH<sub>4</sub>/kg DMI). Measurements collected to build up the inventory for sheep and cattle in New Zealand have resulted in over 3,000 records of CH<sub>4</sub> emissions. Most values have been derived using the sulphur hexafluoride (SF<sub>6</sub>) tracer technique (Johnson *et al.*, 1994), but recently more precise measurements have been obtained for sheep by direct measurement using respiration calorimeters.

Methane yield from forage digestion averages about 20 g CH<sub>4</sub>/kg DMI, with the CH<sub>4</sub> derived from digestion of dry matter (DM), primarily in the rumen. The yield is lower when legumes are fed, compared to ryegrasses, even though more DM is digested from a legume diet, and there are significant variations in CH<sub>4</sub> yields when ryegrass is fed to sheep and cattle (McNaughton *et al.*, 2005; Waghorn & Woodward, 2006). The variation in

yield has been attributed to effects of intake, diet chemical composition, intrinsic animal factors such as the microflora, and more recently, the SF<sub>6</sub> technique itself (Pinares-Patino & Clark, 2008). Previous attempts to explain variation from unrelated trials on the basis of diet chemical composition accounted for up to 51% of the variation when ryegrass pastures were fed to sheep. No relationships could be established when legumes and herbs were fed to sheep or from pasture diets fed to cattle (Waghorn & Woodward, 2006).

The bases for variation are important because they could offer opportunities for mitigation. The objective of this study was to relate CH<sub>4</sub> yields from individual animals, including those from unrelated experiments, to the chemical composition and intakes of the diets. All evaluations were based on data from sheep and cattle fed fresh ryegrass, with a range in quality and composition. Separate regression calculations evaluated chemical composition with CH<sub>4</sub> production (g CH<sub>4</sub>/d) and yield measured using either SF<sub>6</sub> marker dilution or respiration calorimetry with sheep and with SF<sub>6</sub> for cattle. The contribution of the SF<sub>6</sub> marker dilution technique to the variation in CH<sub>4</sub> production was indicated by comparing values with the calorimetry data from sheep.

**TABLE 1:** Ranges in pasture chemical component intakes and concentrations in sheep and cattle used to measure methane emissions by the SF<sub>6</sub> and calorimetry.

| Component                    | Sheep       |                 | Cattle          |
|------------------------------|-------------|-----------------|-----------------|
|                              | Calorimetry | SF <sub>6</sub> | SF <sub>6</sub> |
| Component intake (kg/d)      |             |                 |                 |
| Dry matter intake (DMI)      | 0.32 - 1.72 | 0.38 - 1.69     | 8.17 - 20.81    |
| DMI x maintenance            | 0.57 - 2.59 | 0.66 - 2.40     | 1.69 - 4.74     |
| Crude protein                | 0.05 - 0.25 | 0.05 - 0.42     | 1.77 - 4.58     |
| Lipid                        | 0.01 - 0.05 | 0.01 - 0.07     | 0.31 - 0.87     |
| Neutral detergent fibre      | 0.14 - 0.80 | 0.20 - 0.79     | 4.50 - 11.69    |
| Non-fibre carbohydrate       | 0.11 - 0.48 | 0.28 - 1.47     | 0.58 - 5.51     |
| Digestible organic matter    | 0.25 - 1.41 | 0.26 - 1.30     | 5.77 - 15.75    |
| Component composition (g/kg) |             |                 |                 |
| Crude protein                | 103 - 174   | 108 - 247       | 151 - 225       |
| Lipid                        | 16 - 35     | 24 - 50         | 33 - 42         |
| Neutral detergent fibre      | 431 - 626   | 397 - 609       | 396 - 569       |
| Non-fibre carbohydrate       | 175 - 398   | 123 - 225       | 71 - 305        |
| Digestible organic matter    | 487 - 821   | 636 - 800       | 694 - 808       |

## MATERIALS AND METHODS

The SF<sub>6</sub> database comprises 1,190 records from sheep and 1,880 from cattle with the records based on 2 to 4 days of measurements. This study was based on a selection of records including only animals fed fresh pastures. These were primarily ryegrass with no baleage, silage or legume dominant diets. Individual animal intakes were measured from indoor feeding trials. Values were excluded from animals fed pastures containing less than 10% crude protein (CP) or more than 68% neutral detergent fibre (NDF), because these were not representative of New Zealand pastures. Aberrant CH<sub>4</sub> values of <10 and >39 g CH<sub>4</sub>/kg DMI were also excluded. Application of these selection criteria to the SF<sub>6</sub> databases resulted in 196 records for sheep and 195 for cattle.

Calorimetry data were only available for sheep, with 472 records in the database. The application of the selection criteria resulted in 161 records from sheep.

Chemical components were measured by near infrared reflectance spectroscopy at FEEDTech, Palmerston North. Intakes were expressed as kg/d and also as multiples of maintenance energy requirements. Components were expressed as either intakes (kg/d) or proportions (g/100g DM) of lipid, CP, NDF, and NFC (non-fibre carbohydrate (1 - proportions of (ash + lipid + NDF + CP)).

Statistical models were constructed to examine the extent to which DMI and chemical components explain variation in CH<sub>4</sub> production (g CH<sub>4</sub>/d) and CH<sub>4</sub> yield (g CH<sub>4</sub>/kg DMI). Models were determined separately for both the SF<sub>6</sub> and calorimetry datasets. All subsets of up to five variables (DMI, lipid, NDF, CP, NFC), were assessed by multiple regression for their ability to explain CH<sub>4</sub> production or yield. The 'all subsets regression' facility in GenStat software, version 10.2, (Payne *et al.*, 2008) was used to do this and the model which best explained the most variability identified.

## RESULTS

Mean ( $\pm$  standard deviation) for CH<sub>4</sub> yields (g CH<sub>4</sub>/kg DMI) from the SF<sub>6</sub> database for sheep averaged  $23.4 \pm 5.7$  compared with  $23.1 \pm 2.9$  from calorimetry, and the greater variances about the mean from SF<sub>6</sub> measurements for both production and yield are illustrated in Figure 1. Pastures fed in calorimeters included some with a lower digestibility and lower CP concentration than those used in the SF<sub>6</sub> measurements, but overall differences in composition between data sets were minor (Table 1).

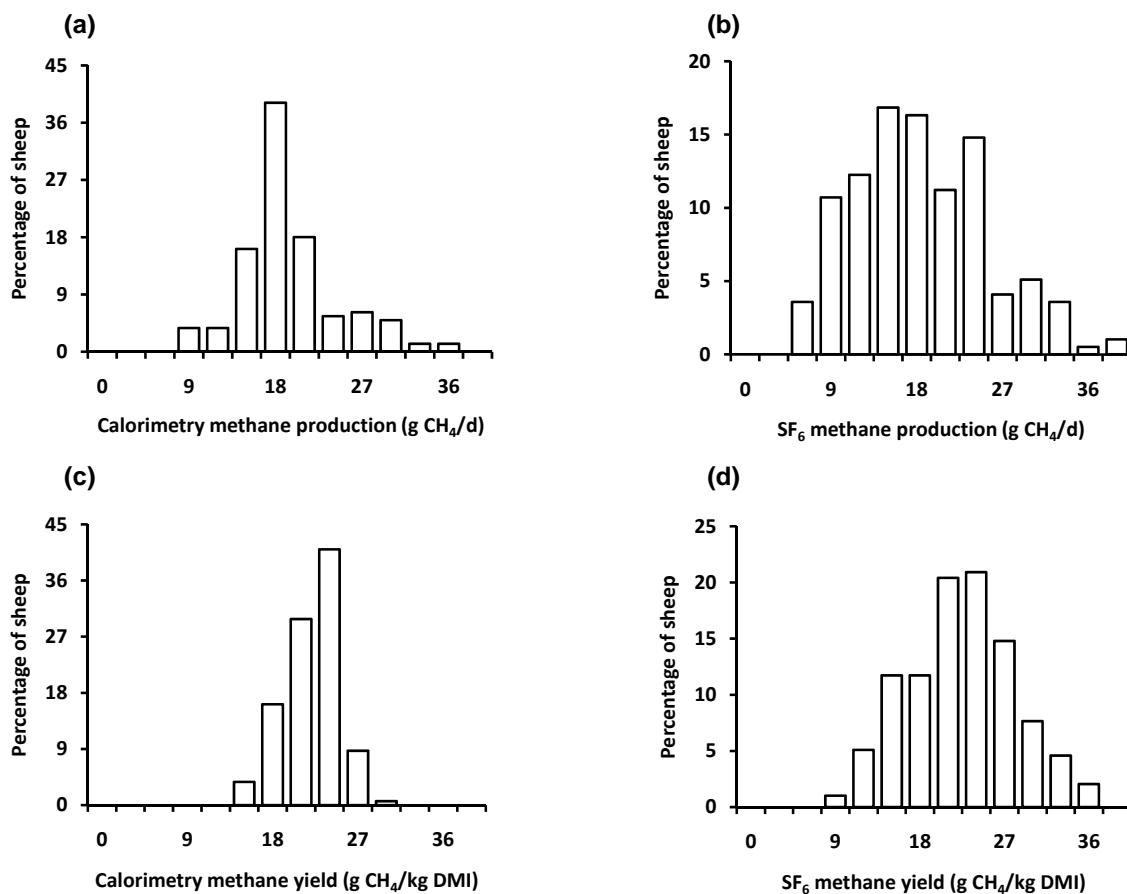
Intakes of pasture fed to sheep ranged from below maintenance to more than twice maintenance requirements and there were substantial ranges in pasture chemical composition (Table 1). For example, CP in pastures fed to sheep ranged from 108 to 247 g/kg DM, and NDF ranged from 397 to 609 g/kg DM with a 4 to 5 fold range in intakes of these constituents (Table 1). The range in intakes and composition of DM provided good opportunities to identify relationships with CH<sub>4</sub> production and yield from sheep.

Data from cattle measurements were all based on the SF<sub>6</sub> technique. Fresh pastures contained 396 to 569 g/kg NDF and 151 to 225 g/kg CP. They were predominantly ryegrass-based pastures (Table 1). CH<sub>4</sub> production ranged from 110 to 491 g CH<sub>4</sub>/d and it averaged  $294 \pm 76$  g CH<sub>4</sub>/d while CH<sub>4</sub> yield averaged  $19.1 \pm 3.7$  g CH<sub>4</sub>/kg DMI (Figure 2).

### CH<sub>4</sub> production and plant composition

When CH<sub>4</sub> production from sheep was measured by SF<sub>6</sub>, the single component best able to explain the variation was DMI, which explained 51% of the variation. The combinations of component variables best able to explain the variation were CP intake and NDF intake which also explained 51% of the variation (Table 2). The

**FIGURE 1:** Histogram showing the spread of data for (a) Measuring CH<sub>4</sub> production using calorimetry; (b) Estimating sheep CH<sub>4</sub> production using the SF<sub>6</sub> technique; (c) Measuring sheep CH<sub>4</sub> yield using calorimetry; (d) Estimating sheep CH<sub>4</sub> yield using the SF<sub>6</sub> technique.



addition of other components into the model made little improvement to the prediction. When CH<sub>4</sub> production was measured in the calorimeters the single component best able to account for the variance was DMI which explained 81% of the variation. The combination of component variables best able to explain the variation were intakes of lipid, NDF and NFC which explained 80% of the variation. Additional variables had minor effects on prediction of CH<sub>4</sub> production.

The range of CH<sub>4</sub> production in cattle was

driven by intake associated with lactation. The single component best able to account for variance in cattle CH<sub>4</sub> production was DMI which explained 52% of the variation. Combined intakes of lipid, CP and NDF were able to explain 51% of the variation (Table 2).

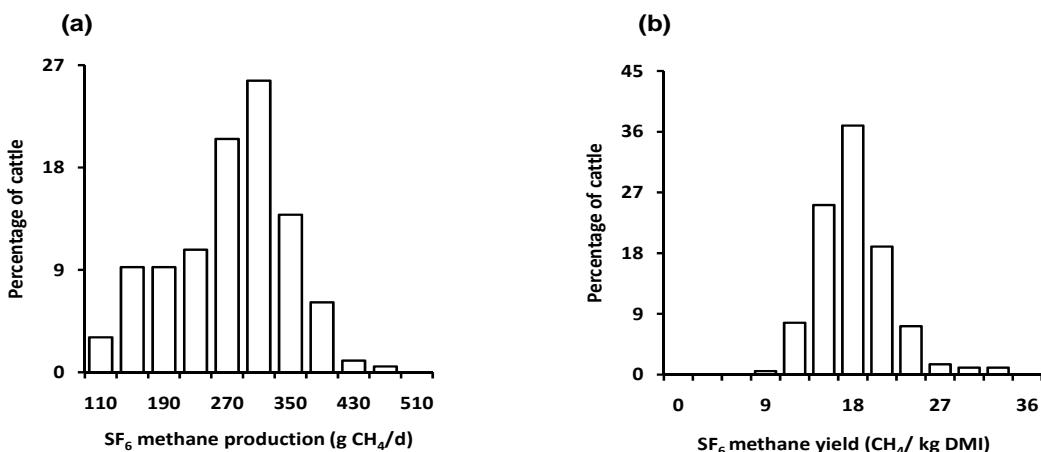
#### CH<sub>4</sub> yield and plant composition

Both sets of sheep data suggest variation in CH<sub>4</sub> production was due mainly to feed intake and imply little association with intake of specific

**TABLE 2.** Dietary component intakes and diet component concentration best able to explain variation in either CH<sub>4</sub> production or CH<sub>4</sub> yield for sheep using calorimetry and SF<sub>6</sub> techniques and cattle using the SF<sub>6</sub> technique. NDF = Neutral detergent fibre, NFC = Non-fibre carbohydrates, CP = Crude protein.

| Technique   | Component variables best able to account for variation in the model | Variation explained (%) |
|---|---|-------------------------|
| CH <sub>4</sub> production (g CH <sub>4</sub> /d) | Component intake (kg/d)   |                         |
| Sheep Calorimetry                                 | Lipid + NDF + NFC   | 80                      |
| Sheep SF <sub>6</sub>                             | NDF + CP <sup>1</sup>   | 51                      |
| Cattle SF <sub>6</sub>                            | Lipid + CP + NDF  | 51                      |
| CH <sub>4</sub> yield (g CH <sub>4</sub> /kg DMI) | Component concentration (g/kg)                                      |                         |
| Sheep Calorimetry                                 | Lipid+ CP + NDF + NFC   | 20                      |
| Sheep SF <sub>6</sub>                             | No significant effects  | <2                      |
| Cattle SF <sub>6</sub>                            | CP + NFC  | 13                      |

**FIGURE 2:** Histogram showing the spread of data for (a) Estimating cattle CH<sub>4</sub> production using SF<sub>6</sub>; (b) Estimating cattle CH<sub>4</sub> yield using the SF<sub>6</sub> technique.



components such as lipid, NDF, CP and NFC, despite the range in pasture chemical composition (Table 1). This was highlighted by multiple regression analysis of the concentration of the component on CH<sub>4</sub> yield (Table 2). With the SF<sub>6</sub> data from sheep, regression of multiple component concentrations explained less than 2% of the variation in CH<sub>4</sub> yield. However, when the calorimetry database was evaluated, 20% of the variation ( $P < 0.001$ ) in the CH<sub>4</sub> yield of sheep was explained by a combination of component concentrations of lipid, NDF, CP and NFC (Table 2). Additional components had little effect on the accuracy of prediction.

The cattle data showed the chemical composition of pasture explained only 13% of the variation in CH<sub>4</sub> yield (Table 2).

## DISCUSSION

The principal finding from this study was that the chemical composition of fresh ryegrass pasture was only able to explain up to 20% of the variation in CH<sub>4</sub> yield. Thus at least 80% of variation was independent of NDF, CP, NFC and lipids in the ryegrass. Methane production was best explained by DMI. Inclusion of chemical components did not account for more of the variation. These results appear contrary to the findings of Waghorn & Woodward (2006). Although these workers were able to explain 51% of the variation in CH<sub>4</sub> yield from sheep fed pasture on the basis of plant chemical components, they were unable to account for the variations in CH<sub>4</sub> yield when legumes and herbs were fed to sheep, and when cows were fed fresh pasture.

The lower variation in CH<sub>4</sub> yield measured by calorimetry compared to SF<sub>6</sub> marker dilution in sheep has considerable implications for mitigation. Mean values were not affected by technique, but

direct measurement by calorimetry are expected to be more accurate than the SF<sub>6</sub> marker dilution. Weaknesses have been demonstrated in the SF<sub>6</sub> technique (Vlaming *et al.*, 2007; Pinares-Patino *et al.*, 2008), whereas, calorimetry is a direct measure of CH<sub>4</sub> emissions including that in the flatus. The lower variation from calorimetry suggests fewer opportunities for mitigation by selecting for “low CH<sub>4</sub> emitting” individuals than was previously envisaged.

### Variation between measurement techniques

The SF<sub>6</sub> technique is based on dilution of the SF<sub>6</sub> marker gas which is released at a known rate from permeation tubes in the rumen. This method has enabled CH<sub>4</sub> to be measured from grazing animals and large numbers of animals simultaneously. There is however growing evidence that CH<sub>4</sub> emission estimates by the SF<sub>6</sub> technique are influenced by the SF<sub>6</sub> gas permeation rate from the tubes (McNaughton *et al.*, 2005; Vlaming *et al.*, 2007; Pinares-Patino *et al.*, 2008; Pinares-Patino & Clark, 2008). Another contributor to variance associated with SF<sub>6</sub> measurements is an inconsistent equilibration of the SF<sub>6</sub> gas with rumen gases, for example, if it is entrapped and later released from particulate matter in the rumen.

Calorimetry allows CH<sub>4</sub> to be measured directly and includes emissions from flatus, as well as eructation. Animal behaviour is however constrained relative to grazing (Johnson *et al.*, 1994; Johnson & Johnson, 1995; Boadi & Wittenberg, 2002). Calorimetry was able to account for 81% of variance in CH<sub>4</sub> production associated with DMI compared to only 51% from SF<sub>6</sub>.

The variation around the mean for CH<sub>4</sub> emissions from cattle are also likely to be overestimates. Calorimeters have been constructed at the AgResearch Grasslands campus for direct cattle measurements.

## Diet composition and CH<sub>4</sub> yield

The calorimetry data showed a minor association between fresh pasture chemical composition and CH<sub>4</sub> yield, requiring alternative explanations of the variation. Expectations of a direct association between CH<sub>4</sub> yield and diet composition are based on the physiology of digestion which affects different proportions of volatile fatty acids (VFA) as well as hydrogen (H<sub>2</sub>), which is a precursor of CH<sub>4</sub>, carbon dioxide (CO<sub>2</sub>) and microbial biomass. Theoretical calculations show a low H<sub>2</sub> yield when propionate is produced during digestion and higher yields of H<sub>2</sub> when fibrous components are digested to produce acetate and butyrate. Thus a lower CH<sub>4</sub> yield might be expected from immature pasture with a high NFC and low NDF content, and a higher CH<sub>4</sub> yield from more mature, fibrous pasture (Beever, 1993). More extreme diets containing 70 to 90% grain do result in very low CH<sub>4</sub> emissions from cattle and high proportions of propionate (Johnson & Johnson, 1995), but these diets and their digestion are atypical in New Zealand.

More recent analyses of VFA production by Bannink and Tamminga (2005) have shown very poor relationships between predicted and actual propionate production in cattle, so the theoretical stoichiometric relationships between substrate digestion and CH<sub>4</sub> production (Beever, 1993) seem to have little application *in vivo*. Put simply, the microflora degrades feeds in order to survive, and theoretical optima do not apply *in vivo*. Bannink and Tamminga (2005) suggest a higher CH<sub>4</sub> yield from NFC than cellulose and lower values from CP. Their calculations complement suggestions that methyl groups contribute to CH<sub>4</sub> production and H<sub>2</sub> may be utilised through other routes, including nitrogen disposal as ammonia (Waghorn et al., 2006). We postulate that most of the variation in CH<sub>4</sub> yield is due to interactions between structural and chemical forage characteristics, combined with the effect of chewing on digesta presented to the rumen microflora, and digestive physiology involving rumen pH regulation, and digesta turnover. All of these characteristics will differ between individuals in a group (Pinares-Patino *et al.*, 2003; 2007).

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