



Methane emissions from sheep fed different intakes of high quality pasture

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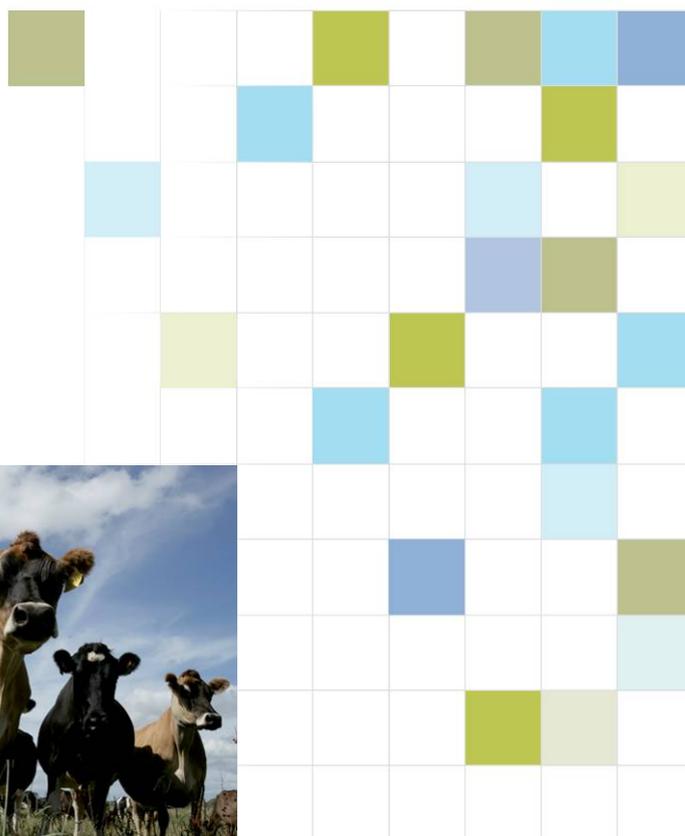
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1. Summary

There are suggestions in the literature that the methane (CH₄) emission per kg dry matter intake (DMI) decreases as the intake increases above maintenance energy requirements. This trial aimed to determine if this relationship exists in sheep fed fresh perennial ryegrass based pasture. Methane emissions were measured in calorimeters on 24 lambs randomised into 4 groups that were offered feed at 0.8, 1.2, 1.6 and 2 times maintenance energy requirements. There was a significant linear increase in CH₄ emissions per day with increasing DMI ($R^2 = 93\%$; $P < 0.001$), but a significant linear decrease in CH₄ emissions per kg DMI with increasing DMI ($R^2 = 68\%$; $P < 0.001$). CH₄ emissions per kg DMI also significantly, linearly decreased when they were related to the level of feeding above maintenance (actual intake expressed as a proportion of maintenance intake; $R^2 = 69\%$; $P < 0.001$). Methane emissions of the 10 ewe and 14 wether lambs used did not differ. These results suggest that assuming CH₄ emissions per kg DMI are constant, as is currently done in the New Zealand inventory and as recommended by the IPCC, may not be appropriate when estimating the quantity of CH₄ produced by pasture fed ruminants. However, more work on animals of different ages and consuming different quantities and perhaps qualities of feeds is needed before an alternative approach to that used currently in the New Zealand national inventory can be recommended.

2. Introduction

The method for estimating agricultural CH₄ emissions for Kyoto Protocol inventory purposes in NZ is currently based on a constant value of CH₄ emitted per kg of dry matter intake (DMI) produced by the average ruminant animal. Emissions are therefore assumed to be independent of quantity or quality of feed consumed, and the physiological state of the animal. This assumption may not be correct. In an extensive review of 48 trials Blaxter and Clapperton (1965) found that CH₄ production per unit of feed intake decreased as feed intakes above maintenance energy level increased. However, Molano and Clark (2008) found no effects of level of feeding (0.75 to 2 x maintenance) on CH₄ emissions per kg DMI from wethers in a trial at Agresearch Grasslands. A key difference between the New Zealand work and that of Blaxter and Clapperton (1965) is that the New Zealand work looked at forage-only diets whereas the United Kingdom work included results from ruminants fed mixed concentrate and forage diets. Another important difference is that in the Blaxter and Clapperton (1965) study, CH₄ emissions were measured using calorimetry whereas the study of Molano and Clark (2008) estimated emissions using the sulphur hexafluoride (SF₆) technique. Recent studies by Vlaming (2008) have suggested that results obtained using the SF₆ technique

are inherently more variable than those using calorimetry and this may make it difficult to detect more subtle relationships such as those between the level of intake and the quantity of CH₄ emitted per unit of intake when using this technique. This research repeats the work of Molano and Clark (2008) in that it looks at the relationship between CH₄ emissions and intake on a forage only diet but, importantly, measures CH₄ emissions directly using calorimeters rather than relying on the indirect SF₆ tracer technique.

3. Aim

- To determine the CH₄ emissions per kg DMI from groups of lambs fed intakes of 0.8, 1.2, 1.6 and 2 times maintenance.
- Compare the CH₄ emission from ewe and wether lambs.

4. Methods

4.1 Animals

Fifteen Romney wether- and 15 ewe-lambs, 8-9 month of age, were weighed on 19 May 2008 and after stratification on liveweights the lambs were allocated to 4 groups, which were penned separately in the animal house at Grasslands. The four groups were fed with freshly cut ryegrass at 0.8, 1.2, 1.6 or 2 times maintenance (M) (i.e. treatment groups 0.8M, 1.2M, 1.6M and 2M). Two ewe lambs that had not settled into the pen feeding schedule were removed from the trial after 4 days and adjustments were made to the groups so that there were 7 lambs per group (i.e. 6 lambs plus one spare lamb).

The lambs remained in their pens for 10 days, then on the eleventh day all the lambs were weighed and two lambs from each treatment were put into individual metabolism cages while continuing to be fed their respective diets. After 4 days in the metabolism cages, one lamb from each treatment group was put into one of the four calorimeters to measure CH₄ emissions (Replicate 1). The lambs continued to be fed their respective diets over the two days they were in the calorimeters. The refusals for each lamb were accumulated over the two days and collected at the end of the two day period.

Once the CH₄ emissions had been measured over the two days the lambs were released into a paddock and a new replicate entered the calorimeters. As each replicate of four lambs was removed from the metabolism cages to go into the calorimeters another replicate of four lambs, one from each treatment, was placed in the metabolism cages.

This was repeated 6 times over 12 days for the 24 lambs in the trial. One spare lamb in each treatment did not enter the calorimeters.

Lambs were weighed immediately before they entered the pens, two days after they entered the pens, the day they entered the metabolism cages, and after they were released from the calorimeters.

4.2 Pasture samples

The pasture was a perennial ryegrass dominated pasture with no sown clover in the sward. Samples of the freshly cut pasture were collected the day the lambs entered the pens, the day they entered the metabolism cages and the day before and each day the lambs were in the calorimeters. Rapid estimates of the dry matter (DM) content of the pasture were made by micro-wave drying a weighed sub-sample for 20 minutes and used to indicate the weight of fresh feed to prepare for the lambs. A second weighed sub-sample of about 50g was dried at 105 C° for 16 hr to determine true DM and a third sample was sent for analyses of nutrient content by Infrared Reflectance Spectrophotometry (NIRS; Corson et al. 1999). The weighed refusals collected over the two days each lamb was in a calorimeter were also dried at 105 C° for 16 hr to determine DM and sent for analyses of nutritional composition by NIRS.

Pasture for the lambs was cut in the mid-afternoon and half of the diet was fed at 16.00 hr and the rest was stored in a chiller to be fed at 09 00 hr the next morning. The maintenance requirements of the lambs were calculated from the Australian Feeding Standards (1990) and a metabolisable energy (ME) content of 11.8 MJ ME / kg DM. Lambs in each group were fed a pasture allowance calculated from the average liveweight of the group using the liveweight on the day the lambs entered the pens.

4.3 Statistical analyses

All the animal traits were analysed by analyses of variance (GenStat, 2005) with the main factor being feed intake. The gender of the lambs, the calorimeter chamber used to measure the CH₄ emissions, and the replication (i.e. the order the 6 groups of four lambs entered the calorimeters) were included separately with treatments as main factors but since they were not significant they were removed from the model. Relationships between actual DMI and CH₄ emissions per day and per kg DMI were determined using regressions analysis. In addition a relationship between energy requirements above maintenance and CH₄ emissions kg DMI was determined using regressions analysis

5. Results

5.1 Pasture quality

The pasture cut each day to be fed to the lambs was a perennial ryegrass sward with no clover and few weeds. Organic matter digestibility (OMD) was high while the crude protein (CP) content was lower than is often found in New Zealand pastures presumably because of the lack of clover in the sward (Table 1). The proportions of neutral detergent fibre (NDF) and the acid detergent fibre (ADF) were also high, which again was likely to be associated with of the lack of clover in the sward.

Table 1: Mean (\pm SD) nutrient composition measured by NIRS, or oven drying at 105 C° in the case of DM, on samples collected daily from the pasture fed to the lambs over the period the CH₄ emissions were being measured in the calorimeter. Samples were collected for 13 days including the 12 days lambs were in the calorimeters and from the day before lambs entered the calorimeter.

	Mean feed quality
DM	17.4 \pm 1.43
Ash	9.5 \pm 0.61
NDF	44.7 \pm 1.82
ADF	26.4 \pm 1.08
Lignin	2.7 \pm 0.48
CP	15.5 \pm 1.97
Lipid	3.1 \pm 0.48
SSS	15.9 \pm 1.61
OMD	80.1 \pm 2.69
ME	11.8 \pm 0.36

DM = dry matter; NDF = neutral detergent fibre; ADF = acid detergent fibre; CP = crude protein; SSS = soluble sugars and starch; OMD = organic matter digestibility; ME = metabolisable energy. All the values are in percentages except ME, which is in MJ / kg DM.

5.2 Liveweights and DMI

The average liveweight (\pm SD) of the 24 lambs that were used in the measurement of CH₄ emissions was 38.0 \pm 1.45kg when they were weighed off grass before entering the pens

at Grasslands. There were no differences in liveweight between the treatment groups at this date (Day = 0), but by Day 2 the 0.8M lambs had lower liveweights ($P < 0.05$) than the other groups (34.0, 36.3, 35.4, and 35.4 ± 0.75 (sed) kg for 0.8M, 1.2M, 1.6M and 2M lambs, respectively). When the first group of lambs entered the metabolism cages on Day 11 the liveweight differences had increased ($P < 0.001$) with the 0.8M lambs having lower liveweights ($P < 0.05$) than the 1.2M lambs (33.0 vs 37.0 ± 0.80 kg), which in turn had had lower liveweights than the 2M lambs (38.7 ± 0.8 kg). The 1.6M lambs had intermediate liveweights between the 1.2M and the 2M lambs. These relative differences in liveweight between treatments were probably attributed to differences in gut fill and were maintained until the lambs were removed from the calorimeters. There were no significant differences in the liveweights between ewe and wether lambs at any time in the trial.

Table 2: Mean (\pm SED) pasture dry matter offered (kg DM/day) to the four treatment groups, daily refusals (g/day), DMI (kg DMI/day), and CH₄ emissions per day (g/day) and per kg DMI (g/kg DMI) for the treatment groups 0.8M, 1.2M, 1.6M and 2M fed at the indicated levels of maintenance (M).

Treatments	Feed offered (kg DM/day)	Refusals (g/day)	DMI (kg DM/day)	DMI as a proportion of maintenance intake	CH ₄ (g/day)	CH ₄ (g/kg DMI)
0.8M	0.355	0.3	0.355	0.75	8.95	25.25
1.2M	0.559	4.9	0.554	1.13	13.20	23.82
1.6M	0.714	11.8	0.702	1.47	16.22	23.10
2.0M	0.904	32.6	0.871	1.80	18.05	20.77
SED	0.017	9.00	0.21		0.45	0.768
Probability	< 0.001	0.009	< 0.001		< 0.001	< 0.001
LSD (5%)	0.0334	18.77	0.0446		0.940	1.603

The four treatment groups were deliberately offered different levels of feed and both the feed offered in kg DM per day and the DMI in kg DM intake per day were significantly different between the treatments (Table 2). The daily refusals of feed tended to increase ($P < 0.1$) with increasing amounts of feed offered, but the only significant differences in daily refusals ($P < 0.05$) were between the 2M lambs and the lambs receiving lower amounts of feed offered. There were no effects of gender or order in which the groups of four lambs entered the calorimeter (Replicates) on any of these traits. Since not all of the

feed offered was eaten, the 'achieved' treatments, expressed in terms of actual intake as a proportion of maintenance intake, differed from the 'imposed' treatments which were based on feed offered as a proportion of maintenance intake. In all cases the 'achieved' treatments were lower (Table 2) than the 'imposed' treatments (range 0.75M – 1.8M). However, actual intake on the highest treatment (nominal 2M) was still more than double the intake on the lowest treatment (nominal 0.8M).

5.3 CH₄ emissions

Emissions of CH₄ per day increased (P < 0.001) with increasing intakes and all treatment groups were significantly (P < 0.05) different from each other (Table 2). In contrast, CH₄ emissions per kg DMI decreased (P < 0.001) with increasing intakes offered to the treatment groups. The 0.8M lambs had significantly (P < 0.05) higher CH₄ emissions per kg DMI than the other treatment groups, whereas the 2M lambs had significantly (P < 0.05) lower CH₄ emissions per kg DMI than the groups consuming less feed.

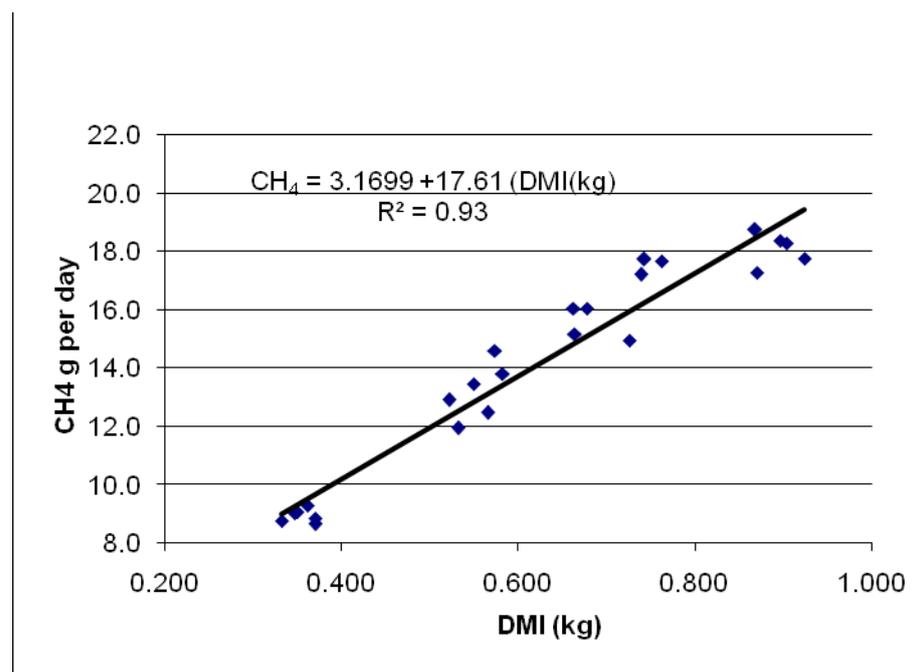
There was a significant (P < 0.001) positive relationship between CH₄ emissions per day and DMI which accounted for 93.4% of the variation in CH₄ emissions per day (Figure 1). The regression equation (1) was:

$$\text{CH}_4 \text{ emissions (g/day)} = 3.16 + 17.61 (\text{DMI (kg)}); R^2 = 93.4\%$$

$$\pm 0.63 \quad \pm 0.97$$

$$P < 0.001 \quad P < 0.001$$

Figure 1. The linear relationship between methane emissions (g/day) and DMI in growing lambs fed high quality ryegrass based pasture.



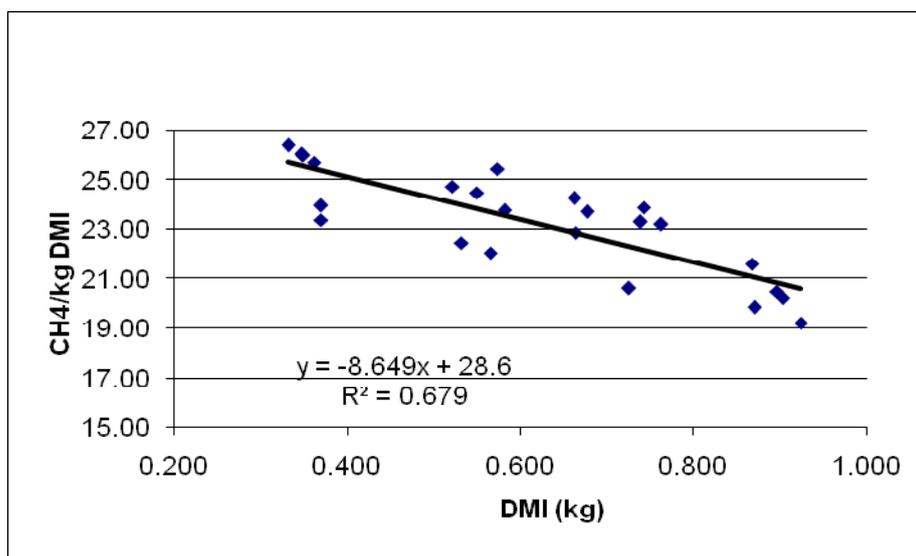
The relationship between CH₄ emissions per kg DMI and DMI was negative (P < 0.001) and accounted for 66.5% of the variation in CH₄ emissions per kg DMI (Figure 2). The regression equation (2) was:

$$\text{CH}_4 \text{ emissions g/kg DMI} = 28.60 - 8.65 (\text{DMI}); R^2 = 67.9\%$$

$$\pm 0.82 \quad \pm 1.27$$

$$P < 0.001 \quad P < 0.001$$

Figure 2. The linear relationship between methane emissions (g/kg DMI) and DMI (kg) in growing lambs fed high quality ryegrass based pasture.



There was no significant difference in the CH₄ emissions per day or per kg DMI between ewe and wether lambs (14.0 vs 14.2 ± 0.36 CH₄ g/ day and 22.9 vs 23.5 ± 1.21 CH₄ g/ kg DMI for ewes and wethers, respectively).

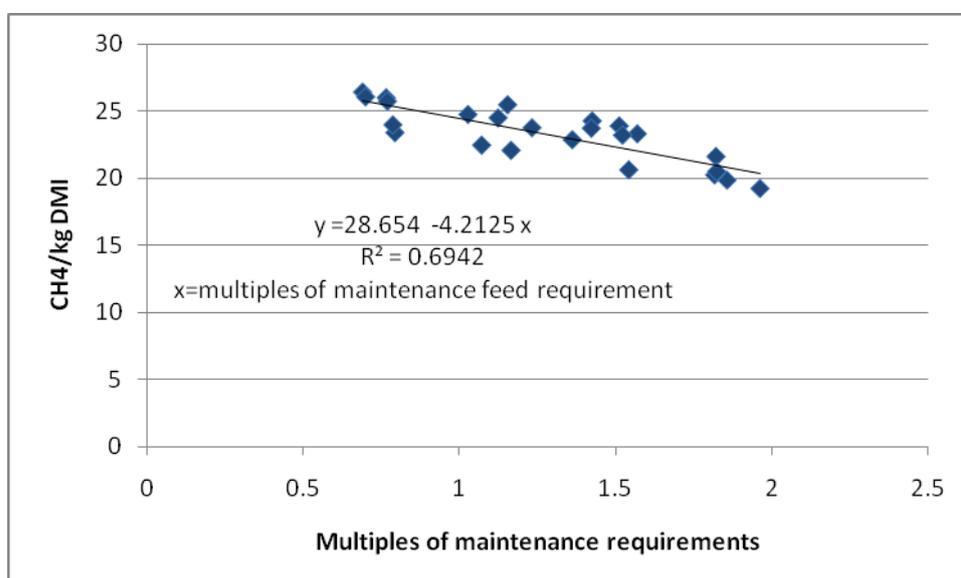
The relationship between CH₄ emissions per kg DMI and intake expressed as a proportion of maintenance intake was negative and accounted for 69% of the variance in CH₄ emissions per kg DMI (Figure 3). The regression equation (3) was:

$$\text{CH}_4 \text{ emissions g/kg DMI} = 28.65 - 4.21 (\text{relative intake}); R^2 = 69.4\%$$

$$\pm 0.80 \quad \pm 0.60$$

$$P < 0.001 \quad P < 0.001$$

Figure 3. The linear relationship between methane emissions (g/kg DMI) and DMI expressed as multiples of maintenance intake in growing lambs fed high quality ryegrass based pasture.



6. Discussion

The results clearly indicate that when young sheep are fed high quality pasture CH₄ emissions per day can be almost exclusively explained by DMI. In addition, over the range of feeding levels used in this trial of approximately 0.7 to 1.8 times maintenance CH₄ emissions per kg DMI (calculated by simple division of CH₄ emissions per day by the DMI) linearly decrease with increasing intakes. Methane emissions per kg DMI were approximately 22% higher at the lower levels of feed intake than at the higher levels. These results disagree with the results of Molano and Clark (2008), who found no effects of level of feeding on CH₄ emissions per kg DMI when CH₄ was estimated using the sulphur hexafluoride technique to measure CH₄ emissions. This may simply be a reflection of the fact that the calorimeters used to measure CH₄ in this experiment have a coefficient of variation that is less than half of that found when estimating CH₄ using the SF₆ tracer technique (Stefan Muetzel, personal communication).

The excellent relationship between DMI and CH₄ emissions per day suggests using this relationship would lead to a more accurate estimation of CH₄ emissions from New Zealand sheep. However, this relationship has been derived from a single group of young animals and more work needs to be done before the results can be generalised to all sheep on all diets. A priority would be assessing the relationship between intake and CH₄ emissions per day in adult sheep whose intakes will be considerably higher than those

used in this experiment and who, in past experiments, have consistently had higher emissions per unit intake than younger animals. At present in the national CH₄ inventory, emissions from young sheep (<1 year of age) are estimated using a constant value of 16.8 g CH₄/ kg DMI. In 2007, the estimated forage intake of a lamb between birth and slaughter was 137kg, giving an estimated CH₄ emission of 2.3kg. per lamb. Using the relationship $CH_4 \text{ (g/day)} = 3.16 + 17.63 \cdot \text{kg DMI/day}$, estimated emissions would increase to 2.95 kg/lamb.

The relationship between CH₄/kg DMI and DMI is not as strong as the relationship between CH₄ per day and DMI, but it is a significant relationship and does imply that the use of a single constant value, such as the one used by New Zealand or that recommended by the IPCC, may be inappropriate. Since this relationship has been obtained from a single group of young sheep extreme caution needs to be exercised in generalising this relationship to other classes of sheep. However, it can be used to generate an estimate of emissions from young sheep which can be compared with those in the current national inventory. As already stated, the 2007 estimate of emissions from a growing lamb are 2.3kg and these rise to 3.2kg using the relationship between CH₄/kg DMI and DMI found in this experiment. A particular feature of this relationship is that it predicts very low emissions from animals with high absolute intakes. Data from other New Zealand experiments would not support this (Clark et al 2008).

This experiment was designed to see if it is possible to derive a relationship between CH₄/kg DMI and intake expressed as a proportion of maintenance intake. The advantage of using a relationship of this kind is that it may have more universal applicability than a relationship with absolute intake; if CH₄ emissions per unit of feed digested are related to the rate of passage of digesta the relationship will be a relative one since the rate of passage will be related both to the size of the main digestive organ, the rumen, and the quantity of feed eaten. The use of multiples of intake above maintenance is simply a proxy for a more complex relationship between intake and passage rate and CH₄ emissions. In this experiment we did find a significant relationship between CH₄/kg DMI and intake expressed as a proportion of maintenance and, in common the results of Blaxter and Clapperton (1965), emissions per kg DMI fall as the intake relative to maintenance intake rises. Re-calculating 2007 emissions from growing lambs using the relationship between CH₄/kg DMI and the proportion of intake above maintenance found in this experiment gives a value of 2.2kg compared with the national inventory value of 2.3kg.

Based on the results from this trial it appears that the use of a constant value for the quantity of CH₄ emitted per unit of feed eaten may not be appropriate. However, given that it is possible for a given set of data to yield several relationships, it is not a simple matter to decide which is the most appropriate relationship to use when attempting to predict emissions from the general population. The relationship found here between CH₄ emissions per day and DMI is the strongest relationship and explains most of the variation between animals and treatments in CH₄ emissions. However, since it has previously been found that young sheep emit less CH₄/kg DMI than older sheep, it cannot be assumed that this relationship will apply to older animals. There is also a good relationship between CH₄/kg DMI and intake, but again, how applicable this relationship is to older animals whose intake is well beyond the range tested here is questionable. The relationship between CH₄/kg DMI and intake expressed as a proportion of CH₄ should in theory be more applicable to all animals since it is based on a physiological attribute. However, it still may not address the issue of young versus old animals and it suffers from the problem of defining exactly what maintenance is.

In this trial, maintenance energy demands were simply assessed as the amount of energy needed for a housed animal to maintain its weight and grow 6g wool per day (AFS 1991); this is known as the basal energy requirement. This basal energy requirement was then converted into a maintenance feed requirement based on the estimated metabolisable energy of the diet consumed. In the Australian Feeding Standards, the maintenance energy of a grazing animal that is gaining weight comprises the basal energy requirement plus the energy required to harvest food plus a proportion (0.1) of the energy needed for liveweight gain. These additional components of the maintenance energy needs mean that the calculated maintenance energy requirements of grazing animals putting on weight are much higher than the calculated maintenance energy requirements of an indoor animal. When re-calculating the emissions from the average New Zealand lamb we have used the basal energy requirements, meaning that dry matter intake as a proportion of maintenance intake is approximately 3. If we had used the calculated maintenance requirements of a grazing and growing animal the value would have been closer to 2. The rationale for using multiples of maintenance intake is that it is a proxy for a relationship between intake, rate of passage and CH₄ emissions and we therefore feel that the most appropriate relationship is between basal maintenance intake and actual intake since this is stable for any animal of a given weight.

The experiment described and discussed in this report is one of a pair of experiments looking at the relationship between intake and CH₄ emissions. The companion trial is looking at this relationship in older animals consuming quantities of feed that in some

circumstances are almost double those used in this experiment. It is possible that once these all trials are completed all the data can be combined to produce a robust relationship that can be used to more accurately predict CH₄ emissions in all classes of sheep.

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