Effect of level of intake on methane production per kg of dry matter intake

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Stefan Muetzel

Table of Contents

1. Summary

Methane (CH_4) produced by ruminants is a major source of New Zealand's GHG emissions and obtaining accurate estimates of emissions on an annual basis is a critical component of our international reporting obligations. To estimate emissions New Zealand has adopted an IPCC Tier 2 approach which is based on estimating emissions from the average animal, based on feed intake and a CH_4 'yield factor'. This 'yield factor's currently based on a single value of methane produced per kg of feed dry matter intake (DMI). Literature indicates that methane yield (CH_4 g/kg DMI) decreases with increasing levels of feed intake. The effect is possibly driven by an increased rumen turnover and or changes in ruminal short chain fatty acid production. Literature data on methane emissions from animals fed fresh pastures however are scarce and equivocal partly because the more variable sulphur hexafluoride method has been used for most of the pasture based studies.
The study reported here was carried out to examine the effect of level of feed intake on methane yield in sheep fed a fresh forage diet covering the range of feed intake that will occur in practice on farms. Methane emissions of two groups of ewes (pregnant with a single lamb (Single) and non pregnant control group (Dry)) were measured over a period for 8 month over pregnancy, lactation and after weaning. During this 8 month period the Single ewes were fed from 1.2 to 3.6x maintenance energy requirements (MEr) while the control group was fed to 1.2 MEr over the whole experiment to account for possible effects of changes in diet composition on methane yield.

There was no difference in animal weights at the beginning of the experiment but at the end of the experiment the Single ewes had a significantly lower weight than the control animals. Dry matter intake in Single ewes varied from 0.7 to 3.3x MEr in Single ewes. The DMI of the control ewes also varied because of difficulties in an instant DM determination required when fresh forage is offered. Methane yield of the control animals was similar during the whole experiment. Methane yield differed between Single and Dry ewes in mid pregnancy, late pregnancy and mid lactation but during late lactation methane yields of the two groups were similar. Diet quality varied to a large extent over the seasons, but had no significant effect on methane yield. The overall relationship between DMI and methane yield was significant but relatively weak (adjusted $r^2 = 0.286$). However, a closer analysis of the data revealed that the data fall into two data sets; lactating and non lactating animals. Separation into these groups explained more than half of the variation in methane yield (adjusted $R^2 = 0.528$). The slope of the regression line between DMI and methane yield was identical for the two groups () but the lactating animals had a higher methane yield (5.01 g/kg DMI) compared to the non lactating animals Currently we cannot explain that result and more work to confirm and establish this observation is needed. Because the experimental design of this trial was to test the effect of feed intake on methane, we cannot exclude an interaction between level of intake and physiological status of the animal.

2. Introduction

In New Zealand, methane emission of ruminants make up to 35% of the total GHG produced. To estimate emissions New Zealand has adopted an IPCC Tier 2 approach which is based on estimating emissions from the average animal, based on feed intake and a CH_4 ‘yield factor’. This ‘yield factor’ is currently based on a single value of methane produced per kg of feed dry matter intake (DMI). Irrespective of ruminant species, diet quality or level of feed intake. This assumption however may not be correct and in a review on methane production of ruminants Blaxter and Clapperton (1965) showed that increasing the level of feed intake decreases methane yield (methane produced per kg DM eaten). This review however was based on trials carried out with conserved feeds and concentrates while in NZ ruminants are fed mainly on pastures. The data available on methane emissions from fresh pastures are equivocal. In a trial with wethers fed on 0.75 to 2.0x maintenance energy requirement (MEr) level Molano and Clark (2008) found no difference in methane yield using the sulphur hexafluoride method, while in a follow up experiment with sheep using the less variable open circuit calorimetry technique (Molano et al. 2008) it was demonstrated that methane yield decreases by an average of 6.6 g/kg DMI when DMI is increased by one level of MEr.

More research is required to establish the relationship between level of intake and methane yield over the range of feed levels observed on farm. Typically feeding levels range from 1 to 3x MEr depending on the physiological state of the animal. From the previous results in can be inferred that this relationship has to be established with the more accurate calorimetry method. In order to establish this relationship the trial reported here was carried out using pregnant ewes by fed to their energy requirements during pregnancy, lactation and after weaning. Lactating ewes were chosen because only they would consume up to 3x their maintenance energy requirements. Since the trial was going over a 8 month period, a group of control animals (dry ewes) was also measured which were fed the same amount of feed during the experiment to evaluate effects of diet quality on methane yield.

3. Materials and Methods

3.1 Animals

3.1.1 Selection and management of pregnant and non-pregnant ewes

Sixty two-tooth Romney ewes at AgResearch Ballantrae were tagged and induced into oestrous by inserting CIDRs on 4 March 2008 and removing them on 14 March. The ewes were injected with Folligon at the time of CIDR removal and 6 rams introduced for 11 days. The ewes were pregnancy scanned on 9 May (Day 56 of gestation) and 14 pregnant ewes with single foetuses (Single ewes) and 15 dry ewes (Dry ewes) were transported to AgResearch Grasslands on 12 May. All the ewes were shorn on the 7 June and their pregnancy status confirmed by a second ultrasound scanning on the 11 June. The pregnant ewes lambed on 8 August (+/- 3 days) the lambs were weaned on 27 October. The ewes grazed on pasture at Grasslands Research Centre between methane measurements with the Dry and Single ewes grazing together up to lambing and separately after the Single ewes lambed. For the measurement of CH_4 emissions in the late lactating ewes the Dry and Lactating ewes grazed together for at least 24 hrs before entering the animal house for differential feeding. After weaning the two groups of ewes grazed together.

3.1.2 Measurement periods

The CH_4 emissions were measured on the 10-12 Single ewes on 6 occasions; three times over pregnancy, twice over lactation and once after weaning of the lambs (Table 1). The proposed levels of feed offered to the Single ewes are indicated in Table 1. The 10-11 Dry ewes had their methane emissions measured at the same 6 periods and were offered feed at 1.2 x MEr.

Before each CH_4 measurement period, the Single and Dry ewes were each randomly allocated to one of two pens in the Grassland's animal house for 3-7 days followed by 4 to 8 days in individual metabolism cages. The ewes were fed their respective diets twice a day over this period of adjustment before going into the calorimeters to have their CH_4 emissions measured. Over lactation, the Single ewes each had 2 metabolism cages side by side with the dividing partitions removed. This gave the ewe and lamb room to move about and to suck. The lambs were placed with the ewes from 0900 to 1600 hrs and removed over night.

Table 1 The dates and the days of pregnancy, lactation or days after weaning when the 6 periods of CH_4 measurements occurred.

Date	Period	Day of pregnancy /lactation/ weaning	Feed offered*
23 May	P1	Pregnancy Day 70 - 80	1.2x MEr
7 June		Shorn	
21 June	P2	Pregnancy Day P98 – 107	1.8x MEr
14 July	P3	Pregnancy Day P122 – 131	2.4x MEr
8 Aug		Lambing	
31 Aug	P4	Lactation Day 23 - 30	3.6x MEr
13 Oct	P5	Lactation Day 66 - 74	2.4x MEr
27 Oct		Weaning	
4 Dec	P6	Weaned Day 38 - 46	1.2x MEr

* Feed levels for the pregnant, lactating ewes or weaned ewes. The Dry ewes were offered 1.2 x energy maintenance.

Pasture for the ewes was cut in the mid-afternoon and half of the diet was fed at 1600 hr and the rest was stored in a chiller to be fed at 09:00 the next morning. The maintenance energy requirements of the ewes were calculated according to the Australian Feeding Standards (1990) according to the following equation:
$MEr = K\ S\ M\ (0.28\ W^{0.75}\ \exp(-0.03\ A)) / (0.02\ M/D + 0.5)$,
where K S and M are factors for species, sex and milk energy, W = liveweight, A = age and M/D is the energy density of the diet.

3.2 Measurement of methane emissions

In the first period of measurement there were 4 calorimeters available but in all later measurements there were 8 calorimeters. Similar numbers of Single and Dry ewes entered the calorimeters for 2 days at a time to have their methane emissions measured. Once the methane emissions had been measured over the 2 days the ewes were released into a paddock and a new group of Single and Dry ewes entered the calorimeters. When the Single ewes were lactating their lambs were placed in a pen in a sound proof room near the calorimeters and the lambs were allowed access to the ewes for 0.5 hrs twice a day before the ewes were being fed.

Ewes in the calorimeters continued to be fed their respective feed allowances twice a day at 16:00 and 09:00. Refusals were accumulated over the 2 days the ewes were in the calorimeters but for the Single ewes fed at 2 to 3 times maintenance, when there were often large amounts of refusals, the refusals were collected at each feeding and pooled for the 2 days. The refusals were weighed immediately after they were collected then dried at 65 ℃ for 48 h. Ewes were weighed immediately before they entered the pens from pasture, after 3 days on their diets and after they were released from the calorimeters. The first of the weightings was used for calculation of MEr.

3.3 Pasture samples

Samples of the perennial ryegrass were collected the day the ewes entered the pens, the day they entered the metabolism cages and the day before and on each day the ewes were in the calorimeters. Rapid estimates of the dry matter (DM) content of the pasture were made by drying a weighed sub-sample for 20 min in a micro-wave oven. This was used to indicate the weight of fresh feed to prepare for the ewes. A second weighed sub-sample was dried at 105 C°for 16 hr to determine true DM and a third sample was sent for analyses of nutrient content by Near Infrared Reflectance Spectrophotometry (NIRS; Corson et al. 1999). The refusals collected while the ewes were in the calorimeters were weighed and dried at 105 C°for 16 hr to determine DM and sent for analyses of nutritional composition by NIRS.

3.4 Statistical analyses

All data were analysed by analyses of variance (GenStat V10.2, 2009) with Dry vs Single ewes being the main factor. The numbers of ewes in each group was not constant over the 6 periods of methane measurements because of the failure of the one or more calorimeter on several occasions and because ewes stopped eating in the metabolism cages and were replaced with spare ewes. There were spare ewes in each group which had been carried through under the same treatment regime in each period and in the latter periods the methane emissions on these spare ewes were measured.

Regression analyses were also conducted to investigate the relationships between feed composition, DMI and methane emissions.

4. Results

4.1 Diets and Animals

Composition of the diets varied considerably during the experiments with quality decreasing markedly in periods 5 and 6 (Table 2).

Table 2 Nutrient composition, digestibility of organic matter (DOM) and metabolic energy content (ME) of the grass pasture fed over the 6 experimental periods

Date	Period	Ash	Protein	Lipids	Sugars	NDF	DOM	ME
		[g/kg]						[MJ/kg]
May.08	1	92.5	143.8	30.7	150.0	460.4	778.6	11.6
Jun.08	2	101.6	144.9	31.4	131.6	481.4	767.1	11.3
Jul.08	3	93.9	152.2	25.4	118.0	523.1	726.9	10.8
Sep.08	4	81.8	145.5	30.6	155.7	466.0	811.5	12.0
Oct.08	5	66.6	93.6	25.8	186.2	477.5	808.5	12.0
Dec.08	6	65.2	65.6	26.1	145.2	523.3	693.2	10.4
sed		0.98	2.36	0.32	3.87	3.11	2.98	0.04
P value		>.001	>.001	>.001	>.001	>.001	>.001	>.001

sed, standard error of the difference

On average the feed quality was not universally high , but reflected pasture quality over a year where conditions were very dry during periods 5 and 6. The protein contents were especially low, but are a reflection of a pure grass pasture going into the reproductive state in early summer due to a lack of rain. The design of the trial included a control group fed on the same intake level during the whole experiment in order to account for effects of changing pasture quality on methane emissions during in each period.

On average the control animals were 1.5 kg heavier than the Single ewes. This difference however was due a significant increase in weight of the Control animals during the last two periods of the experiment while the weight of the Single ewes remained similar (Table 3). Only in P6 the difference between Single and Dry ewes became significant. The apparent weight loss observed from P1 to P2 in both groups is due to the shearing of the animals between these periods.

Table 3 Animal weights [kg] during the experiment

Period	Dry	Single	sed	P value
P1 (May)	47.5	46.5	1.32	0.451
P2 (Jun)	42.7	43.0	1.34	0.863
P3 (Jul)	42.6	44.6	1.51	0.204
P4 (Sep)	46.3	45.7	1.32	0.632
P5 (Oct)	48.4	45.4	1.57	0.066
P6 (Dec)	51.4	45.1	2.22	0.011
Average	46.5	45.0	0.66	0.027
sed	1.75	1.56		

P value	>.001	0.370		
Group*Period				0.009

4.2 Dry matter intake

Between periods DMI varied significantly for both the Dry and Single ewes (Table 4). The differences in DMI observed for the Dry ewes however are due to differences in the feed offered because of the inaccuracy the microwave method used to determine dry matter content of the fresh cut forage. Refusals for this group were below 3% of the DM offered during the whole experiment. The differences in the DMI of the Single ewes were driven by the differences in the amount of feed offered in different periods. The dry matter intakes achieved in the Single ewes however were not as high as expected because the animals refused up to 40% of the feed offer in P3 during late pregnancy. Refusals during lactation were also high where the animals left 29.6 and 28.3% of the diet offered in P4 and P5 respectively. The refusals of the Single ewes in P1, P2 and P6 as well as the refusals of the Dry ewes during the whole experiment were less than 5% of the feed offered.

Table 4 Dry matter intake (DMI) [kg/d] and as a multiple of maintenance energy requirements [MEr] of Dry and Single ewes

	DMI [kg/d]				**DMI [MEr]**			
Period	Dry	Single	sed	P value	Dry	Single	sed	P value
P1 (May)	0.74	0.75	0.023	0.607	1.3	1.4	0.04	0.312
P2 (Jun)	0.71	1.07	0.021	>.001	1.3	2.0	0.04	>.001
P3 (Jul)	0.86	1.20	0.032	>.001	1.5	2.0	0.05	>.001
P4 (Sep)	0.76	1.47	0.081	>.001	1.5	2.8	0.15	>.001
P5 (Oct)	0.67	1.19	0.055	>.001	1.2	2.3	0.09	>.001
P6 (Dec)	0.75	0.75	0.025	0.954	1.1	1.2	0.06	0.069
Average	0.7	1.1	0.019	>.001	1.3	1.9	0.03	>.001
sed	0.018	0.065			0.04	0.12		
P value	>.001	>.001			>.001	>.001		>.001
Group*Period				>.001				>.001

4.3 Effects on methane emissions

When Single and Dry ewes were fed the same amount of feed, their methane production (g CH_4/d) and yield was similar (Table 5). Higher feed intake in the Single ewes during periods P2 to P5 led to higher methane production but to a lower methane yield except for P5 where methane yields were similar between the two groups. On average the Single ewes had a 33.5% higher methane production but their methane yield was around 5.8% lower than the Dry ewes. However in both cases there were strong interactions between period of measurement and the two groups indicating an effect of time and/or diet quality which differed significantly over the course of the experiment.

Table 5 Methane production [g/d] and methane yield [g/kg DMI] of Single and Dry ewes when fed ryegrass based pastures

	CH_4 [g/d]				CH_4 [g/kg DMI]			
Period	Control	Single	sed	P value	Dry	Single	sed	P value
P1 (May)	18.7	19.1	0.54	0.424	25.3	25.5	0.68	0.760
P2 (Jun)	16.6	22.7	0.77	>.001	23.6	21.3	0.90	0.021
P3 (Jul)	20.7	25.1	0.71	>.001	24.0	20.2	0.67	>.001
P4 (Sep)	18.6	32.1	1.34	>.001	24.5	22.3	1.04	0.021
P5 (Oct)	16.9	28.9	0.89	>.001	25.3	24.4	0.84	0.274
P6 (Dec)	18.4	18.9	0.60	0.416	24.7	25.4	0.82	0.421
Average	18.3	24.5	0.35	>.001	24.6	23.2	0.34	>.001
sed	0.518	1.12			0.73	0.974		
P value	>.001	>.001			0.117	>.001		
Group*Period				>.001				0.002

The methane yield of the control animals was similar during the whole experiment despite considerable variation in diet quality. . Variation in methane yield from these animals was mainly explained by variations in DMI and less than 2% of the variation could be explained by changes in plant chemical composition. However the organic matter digestibility (OMD) accounted for 8.5% of the variation in methane yield when expressed on an organic matter basis. In addition when methane emissions were expressed relative to OMD intake (g/kg DOMI) significant differences are observed for the control group in P6 when diet quality was very poor (Table 6).

Table 6 Methane yield [g/kg DOMI] of control and treatment animals

Period	Dry	Single	sed
P1 (May)	32.4	32.7	0.68
P2 (Jun)	30.7	27.7	0.90
P3 (Jul)	33.0	28.8	0.67
P4 (Sep)	30.2	27.4	1.04
P5 (Oct)	31.3	30.4	0.84
P6 (Dec)	35.6	36.6	0.82
Average	32.2	30.6	
sed	0.94	1.24	
P value	>.001	>.001	

4.3.1 Relationship between DMI and methane production

Regression analysis revealed that 86.3% of the variation in methane production is explained by DMI [kg/d] where methane production increases by 17.85 g for every kg of additional DMI (Figure 1). A more detailed multiple regression analysis of the data revealed that significantly more of the variation (adjusted R^2 = 91.0%) in methane production is explained when data are separated into lactating and non lactating animals (Figure 1). Both, slope and intercept are significantly different for the between the two regression lines. The slope of the lactating animals is much less steep while the intercept nearly doubled compared to the non lactating animals.

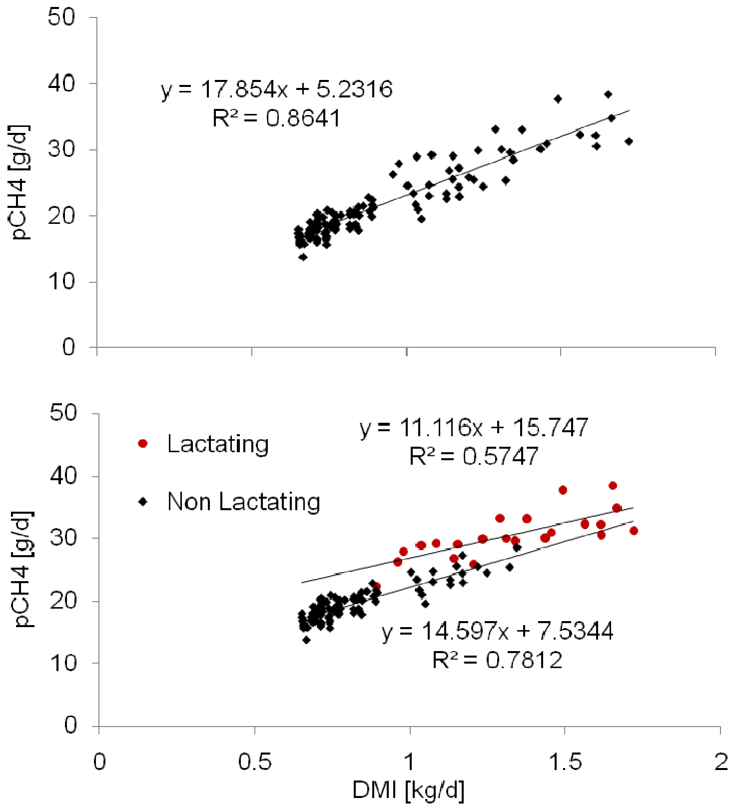

Figure 1 Relationship between DMI and methane production and methane yield of Dry and Single ewes and when grouped into lactating and non lactating animals

No other parameter improved the prediction of methane yield to that extent, but the experimental period also influenced the overall relationship since 89.3% of the variance im methane production could be explained when experimental period was used as a grouping factor (data not shown). This indicates either an influence of the changing diet quality, or an interaction between feeding level and lactation.

4.3.2 Relationship between DMI [MEr] and methane yield

The fact that the intercept of the relationship between methane production and DMI is different indicates that the amount of methane produced per kg of DMI (methane yield) varies between the two groups. The relationship between methane yield and DMI as a multiple of maintenance energy requirements [MEr] is shown in **Error! Reference source not found.**. The overall relationship (yCH_4 = -2.36 DMI [MEr] + 27.8) between methane yield and DMI [MEr] was rather poor and only 28.6% of the variation in methane yield was explained by DMI [MEr].

Analysing the data in groups (lactating and non lactating) significantly improved the overall variance explained to 52.6% (**Error! Reference source not found.**). The resulting dataset is best explained by two parallel lines for lactating and non lactating animals where methane yield is decreased by 5.14 g/kg DMI for when intake is increase by one multiple of MEr. The intercept is 5.01 g/kg DMI higher in lactating animals than in non lactating animals, an observation that is not easily explained on a biological base.

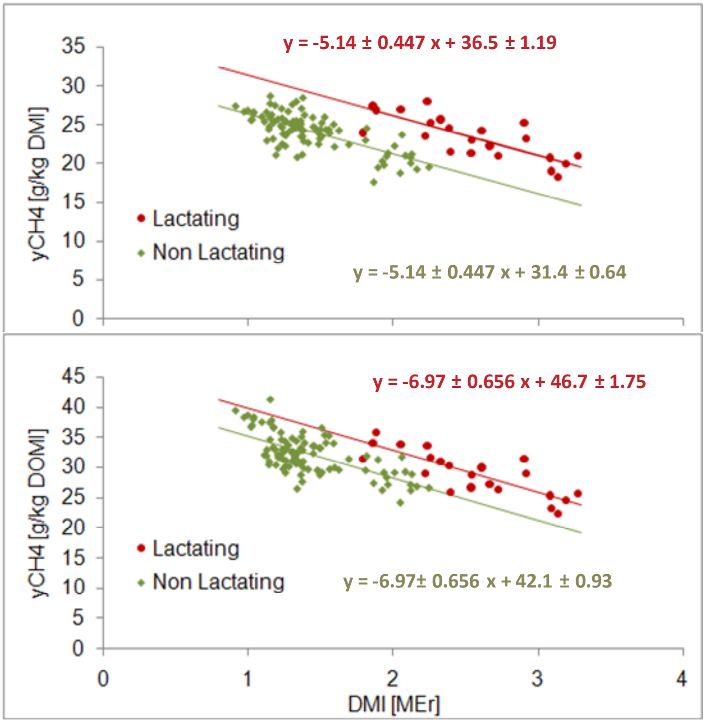

Figure 2 Relationship between DMI as a multiple of maintenance requirement and methane yield expressed relative to DMI and digestible organic matter intake (DOMI)

4.3.3 Interaction with diet quality

From the analysis of methane production data we speculated that diet quality may influence methane production and consequently methane yield. In order to account for such an interaction methane yield (domyCH_4) was calculated relative to the digestible organic matter intake as g/kg DOMI the rather than DMI. The overall regression of domyCH_4 and DMI [MEr] was domyCH_4 = -4.40 (± 0.441) DMI [MEr] + 38.7 (± 0.76) with 45.1% of the variation in domyCH_4 explained by intake. This is a significant improvement compared to the overall relationship of methane yield (28.6%). Grouping the data into lactating and non lactating animals however increased the adjusted R^2 to 54.2 (**Error! Reference source not found.**). Again analysis suggests a common slope and two different intercepts. The difference between the intercepts (4.63 g/kg DOMI) is only slightly lower than that observed for the comparison with methane yield.

5. Discussion

This study is part of a series of trials looking into the effect of the level of feed intake on methane emissions. In order to achieve high intakes above maintenance ewes were used in this experiment and they were followed throughout their reproduction cycle from pregnancy during lactation till the lambs were weaned. In order to make the work relevant to New Zealand farming fresh forage diets were used meaning that, as occurs in practice, changes in level of feed intake, physiological status and diet quality occur independently and there are multiple factors to consider when interpreting the drivers of CH_4 emissions.

5.1 Effect of diet composition

Since the animals were fed with fresh pasture (ryegrass dominated without clover or weeds) the diet composition changed during the experiment and therefore the methane emissions from a control group of dry ewes were measured simultaneously with the pregnant and lactating ewes in each period. The methane production (g/day) of the control animals (Dry ewes) was significantly different between the periods because of some differences in the quantity of feed offered. However, despite these differences, and the large variability in chemical composition of the pastures, the methane yield (g CH4/kg DMI)of the Dry ewes was similar in all the experimental periods. Differences in diet composition (content of ash, crude protein, lipids, NDF and sugars) explained less than 2% of the variation in methane yield. This is in contrast to observation by Waghorn and Woodward (2006) who found that over 50% of the variation in methane yield can be explained by nutrient composition. However, this comparison was made with a variety of fresh legumes and herbs while our data are based on grass only. A recent analysis of CH_4 emissions from New Zealand ruminants fed pasture only diets by Hammond et al 2009 has shown that diet composition is a poor predictor of methane yield when applied to one feed type (grass dominant pasture) only. Organic matter digestibility of the diet was estimated by NIR and explained 8% of the variability in methane yield. When methane yield was calculated on the base of digestible organic matter intake (domyCH_4) significant differences in the Dry ewes were observed (Table 6) and also the overall relationship with DMI [MEr] was improved. This indicates that diet composition has an influence on methane yield but this is not reflected in the parameters with which nutritional quality of a feed is assessed.

5.2 Effects on methane production

This experiment confirmed that DMI is the main driver of methane emissions and that there is a negative relationship between methane yield and DMI as suggested by Blaxter and Clapperton (1965). DMI [kg/d] explained 86.3% of the variation in methane production. However this relationship seems to be influenced by the physiological status of the animal (lactation) as the prediction increased to 91.0% when data were grouped into lactating and non lactating animals. When the data (lambs) from Molano et al. (2008) are included into the analysis 88.2% of the variation of methane production is explained by DMI [kg/d] and 93.0% are explained when data are separated into lactating, non lactating and lambs (Figure 3). The slope of the non lactating animals and the lambs are similar but different form the lactating animals.

This indicates that the relationship is fairly similar for young and old animals, a result that was also found by Knight et al (2008) and the main difference is due to lactation. When the lambs are included into the non lactating group to simplify the model 91.0% of the variation in methane production can be explained by DMI.

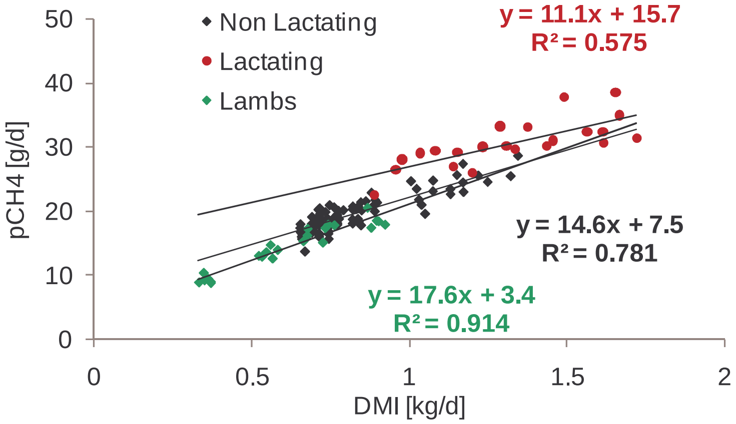

Figure 3 Relationship between methane production and DMI including an experiment with lambs by Molano et al. (2008)

5.3 Effects on methane yield

The overall relationship between methane yield and DMI [MEr] was relatively poor with only 28.6% of the variation explained compared to the lamb study (Molano et al. 2008) where 60.7% of the variation in methane yield was explained by DMI [MEr]. The response (slope) was also nearly twice as high in the lambs than in the present study suggesting that there is a difference between young and adult sheep in their response to feeding level. However the separation into lactating and non lactating animals revealed that no difference in response (slope) was observed for lactating, non lactating ewes and the lambs but the intercepts of the three lines were all significantly different. There seems to be a small difference between young and old non lactating animals but again the biggest effect was observed between the lactating and non lactating animals. The common slope of the lines suggest that the basic driver behind the effect is similar for the three groups and currently we speculate that this effect is driven by an increase in rumen turnover as DMI increases, leading to a lower methane production because of a reduced fibre digestibility.

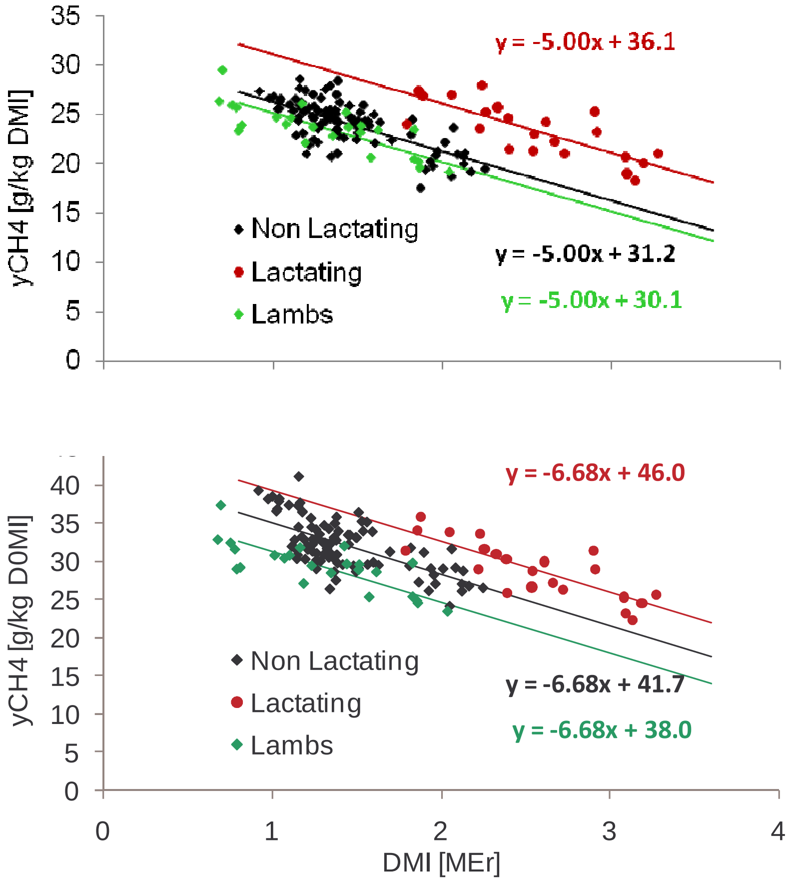

Figure 4 Relationship between methane yield and DMI including an experiment with lambs by Molano et al. (2008)

This is the first study where an effect of lactation on methane emissions in sheep has been shown and our results are in contrast to reported data in cattle where no differences in methane yield were found before and during lactation (Muenger and Kreuzer, 2007). These results are not simple to interpret since only an increase in ruminal digestibility during lactation could explain such a difference. However increasing digestibility with high intakes is not easily achieved in the rumen since plant material has to clear the rumen at the same rate than feed eaten. In order to increase ruminal digestibility either an increased rumen volume or an increased microbial activity is required. Another complication in the interpretation of the data is that the experiment was not actually designed for testing the effect of the physiological status of the animal and at present we cannot exclude an interaction between level of feed intake, feed quality and physiological status.

5.4 Interaction with diet quality

Although chemical composition of the feed could not explain any variation of methane yield the estimation of the digestibility of the organic matter explained more than 8% of the variation. This is an indication that the chemical description of the diet does not reflect very well the complex pathways and interaction when feed is fermented in the rumen. When methane yield was calculated at basis of DOMI the overall relationship with DMI was significantly improved (r^2 = 45%) compared to methane yield on the basis of DMI (r^2 = 28.6%). Grouping for non lactating and lactating animals and combining the data with the lamb experiment of Molano et al. (2008) shows that the difference between lactating and non lactating animals became smaller (4.63 g/kg DOMI vs. 5.14 g/kg DMI) but also the difference between young and old animals increased (3.65 g/kg DOMI vs. 1.12 g/kg DMI). This is indicates an interaction between feeding level and diet quality and needs to be addressed in experiments already planned.

6. Conclusion

DMI is the single most important driver for methane production and explains 86.2% of the overall variation. However, physiological status (Lactation) seems to affect this relationship and more than 90% of the variation of methane production is accounted for when taken into account.
The basic driver behind that relationship however seems to be independent of physiological status as the response of methane yield over DMI as a multiple of MEr was similar for lactating and non lactating animals. Currently we have no biological explanation for the higher methane yields in lactating animals but since the design of the study did not include physiological status as a variable no final conclusions can be drawn from our data.

Diet quality as determined by chemical analysis seems to have a minor affect on methane yield and methane production but this might be mainly due to the fact that simple chemical analysis of the diet is a poor descriptor for the complex breakdown of plant material in the rumen. An indicator would be the differences observed when methane production is expressed relative to the intake of digestible organic matter intake as opposed to dry matter intake.

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