Proc. Assoc. Advmt. Anim. Breed. Genet. 19:159-162

FACTORS AFFECTING VARIABILITY IN FEED INTAKE OF SHEEP WITH *AD LIBITUM* ACCESS TO FEED AND THE RELATIONSHIP WITH DAILY METHANE PRODUCTION

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SUMMARY

Feed intake accounts for a large proportion of between-animal variation in methane emissions. This study compared methane emissions in respiration chambers with *ad libitum* feed intake of 47 merino wethers on the day of, and the day before, measurement. All sheep were tested twice, first during the period from 1 to 18 November 2010, then during the period from 1 to 16 December 2010. Feed intake on the day before measurement (**FIP**) was significantly related to methane emissions ($P = 10^{-9}$). FIP increased with liveweight of the animals ($r = 0.39$, $P = 0.0001$), and was also subject to day-to-day variation ($P < 0.00002$). Feed intake in the respiration chamber was not significantly related to liveweight, nor feed intake on the previous day, and it was about 19% lower than feed intake on the previous day. It is concluded that feed intake during respiration chamber measurements differed from the animal's normal behaviour. Understanding and accounting for such changes in behaviour may help to increase the accuracy of predicting an animal's true methane emissions.

INTRODUCTION

Feed intake accounts for a large proportion of between-animal variation in methane emissions. Some researchers therefore analyse and report methane emissions per kg of feed intake, which is known as 'methane yield' (Lassey 2007; Pinares-Patiño *et al.* 2011).

However, when animals have *ad libitum* access to feed, methane emissions over a 23-hour period in a respiration chamber are expected to depend not just on the amount of feed consumed in the respiration chamber, but also the amount already fermenting in the rumen, which represents a proportion of the feed consumed before the start of the measurement period. This paper explores the repeatability and day-to-day variability in feed consumption, including the effect of confinement in the respiration chamber, and the relationship between feed intake and methane emissions.

MATERIALS AND METHODS

Merino wethers (n = 47; 2 years old) had daily methane production (**DMP**) measured twice, 4 weeks apart, the first replicate being measured from 1 to 18 November 2010 and the second from 1 to 16 December 2010. DMP was measured over 23 hours using open circuit respiration chambers. A total of 4 respiration chambers were available, so the 47 wethers were tested, four at a time, over an 18-day period in November and then again over a 16-day period in December.

Sheep had *ad libitum* access to a mixed ration (90% chaffed oaten hay and 10% cracked lupins) for 10 weeks before the first methane measurement, then throughout the two measurement periods and the time in-between. The sheep also had *ad libitum* access to food and water in the respiration chambers, with 20% more food offered than the previous day's intake. Feed intake (**FI**) was determined for each animal by weighing refusals. The CSIRO Animal Ethics Committee approved the use of animals and the experimental procedures.

Methane measurements. The construction, operation and calculation of DMP over 23 hours in respiration chambers are described in detail by Klein and Wright (2006).

Statistical analyses. As well as calculating methane emissions per kg of feed intake, REML methodology (Robinson 1987) was used to fit mixed linear models using ASREML-R software (Butler *et al.* 2009) to determine the factors affecting the variability of feed intake on the day of, and the day before, respiration chamber measurements, by fitting the models:
Y = intercept + Lwt + rep + week + day + Lwt.rep + Lwt.week + Lwt.day + animal + chamber + error, where the dependent variate, Y, was either feed intake in the chamber (**FIC**), or the previous day (**FIP**), Lwt = live weight of the animal, rep = replicate, and week and day are the week and day of measurement. All terms except Lwt and the intercept were fitted as random. Terms explaining little or no variation were then dropped to obtain the final models:
FIP = intercept + Lwt + day + animal + error
FIC = intercept + week + animal + error
DMP was also analysed by fitting exploratory fixed linear models, followed by a REML analysis including terms for FIC, FIP, rep, week, day and their interactions, with terms accounting for little or no variation dropped, to obtain a final model:
DMP = intercept + rep.FIC + FIP + Lwt (fixed effects) + **chamber** + **animal + error** (random).

RESULTS

Table 1. Means, variances, CVs and correlations (cor) with DMP for methane emissions (DMP), feed intake in the respiration chamber (FIC), on the previous day (FIP), and Lwt, by replicate

	DMP (g)		FIC (kg)		FIP (kg)		Lwt (kg)	
Replicate	1	2	1	2	1	2	1	2
Mean	17.9	17.6	1.53	1.43	1.83	1.83	64.2	65.8
Variance	14.2	10.3	0.10	0.16	0.10	0.10	33.0	30.1
CV(%)	21%	18%	21%	28%	17%	17%	9%	8%
Cor with DMP			0.75	0.72	0.60	0.45	0.22	0.32

Table 1 shows means, variances and CV(%) for DMP, FIC, FIP, Lwt, plus correlations with DMP in each replicate. The correlation between DMP in the first and second replicates was 0.58. DMP was strongly related to feed intake both in the respiration chamber and on the previous day. Cumulative R-squared values from the exploratory fixed liner models were 17% (chamber), 69% (chamber + rep.FI), 83% (chamber + rep.FI + FIP) and 84% (chamber + rep.FI + FIP + Lwt). The fitted relationships (coefficients ± SE) from the REML analysis were:
DMP, rep 1 = 17.75 + (6.8±0.62)*(FIC – 1.5) + (3.82±0.51)*(FIP – 1.8) + (0.09±0.03)*(Lwt - 65)
DMP, rep 2 = 17.75 + (5.6±0.50)*(FIC – 1.5) + (3.82±0.51)*(FIP – 1.8) + (0.09±0.03)*(Lwt - 65)
The regression coefficient for feed intake on the previous day (3.82 ± 0.51) was highly significant ($P = 10^{-9}$). For replicate 1, eating an extra kg of feed on the day before measurement increased DMP by 56% (i.e. 3.82/6.8) of the increase from eating an extra kg of feed in the respiration chamber. For replicate 2, eating an extra kg feed on the day before measurement increased DMP by 68% (i.e. 3.82/5.6) of the increase from eating an extra kg in the respiration chamber.

Feed intake in the respiration chamber was substantially lower than on the previous day suggesting that confinement in the respiration chamber discouraged normal eating behaviour. Fig 1a shows the day-to-day variability of feed intake on the day before and during respiration chamber measurements, illustrating that the day-to-day variation present the day before measurement was largely absent for feed intake in the respiration chamber.

Analysis of emissions per kg feed intake. The simple analysis of methane emissions per kg of feed eaten in the respiration chamber showed a strong negative relationship with feed intake (r = -0.63, Fig 1b), implying that, when animals have *ad libitum* access to feed, use of this measure will tend to favour the animals that eat the most. However, that calculating total feed intake over 2 days: FIT = FIC+FIP resulted in a lower correlation of -0.19 between FIT and CH4/kgFI.

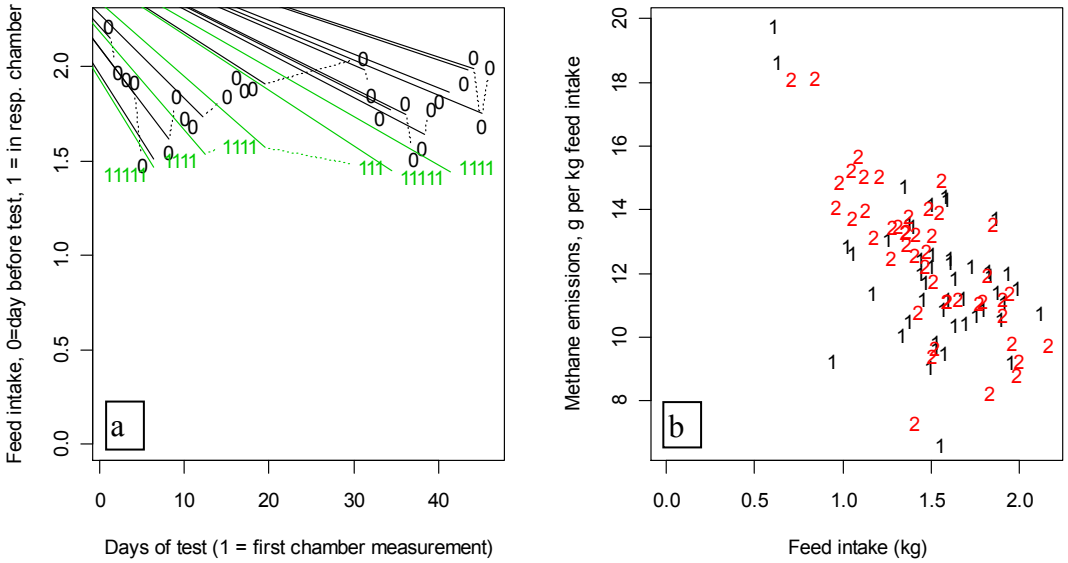

Figure 1. (a) Variation in feed intake on the day before respiration chamber measurement (0), and in the respiration chamber (1); (b) negative relationship (r = -0.63) between feed eaten in the respiration chamber and 'methane yield', i.e. methane emissions per kg of feed intake in the chamber, by replicate (1 or 2).

DISCUSSION

In this experiment, a large proportion (84%) of the variation in DMP was explained by feed intake, both in the respiration chamber and on the previous day, plus liveweight and respiration chamber effects. Understanding the variation in these factors will make it easier to predict methane emissions in different situations and also help improve tests to select animals for low methane emissions relative to their level of production. For example, when insufficient resources are available to test animals more than once, some repeat tests are necessary to avoid confounding animal, day and respiration chamber effects.

In beef cattle, low residual feed intake (RFI) cows had lower CH_4 emissions per kg liveweight of cows and their calves (if present) when grazing high quality, but not low quality, pasture (Jones *et al.* 2011). When molecular microbial profiling techniques were used to investigate rumen microbial composition, diet was found to significantly alter all microbial communities. Moreover, significantly different archaeal and methanogenic communities for high and low RFI cows were found only when the cattle were fed high quality pasture (Torok *et al.* 2011).

Similar results have also been reported for sheep selected for high and low methane emissions. The difference between the high and low groups was only 13% when the animals were fed a grass diet, compared to 36% when fed a pelleted diet (Pinares-Patiño *et al.* 2011). Such results suggest that tests to select low methane emitting animals may have higher accuracy when animals have *ad libitum* access to high quality feed.

Research shows that the digestibility and quantity of feed consumed affects the total amount of methane produced by livestock, and that improving livestock growth rates will reduce methane emission per unit of product (called emissions intensity, Hegarty et al, 2010). This suggests that emissions measurements are needed for animals grazing high quality pasture, which can perhaps be mimicked by providing animals with *ad libitum* access to feed. In addition, methane reduction strategies will need to take account not just of the relationships between methane emissions and feeding and management strategies, but also how these strategies are expected to interact with genotypes selected for low methane emissions or RFI.

A new development is the use of portable chambers to measure methane emissions of grazing animals for 1 hour directly off pasture. Measurements from portable chambers have moderately high correlations (0.56 to 0.66) with DMP measured over the previous day in respiration chambers (Bickell *et al.* 2011). Measurements under field conditions have moderate repeatability (r = 0.47, before and 0.32 after adjusting for liveweight, Robinson *et al*. 2010). This suggests that portable chambers provide similar information to the Open Path Fourier Transform Infrared Spectrophotometer used by Jones *et al*. (2011) to obtain methane emissions of grazing beef cattle, except that individual animal information is also available, so that low-emitting animals can be selected. As in respiration chambers, when feed intake before entering that portable chambers has been measured, it is highly correlated (r = 0.82) with predictions calculated from the animal's feed intake and liveweight (Robinson personal communication). Understanding the relationships between methane emissions, feed intake and liveweight will therefore be critical to successful methane reduction strategies.

CONCLUSIONS

Methane emissions measured for 23 hours in respiration chambers are related to feed intake in the respiration chamber and on the previous day. Feed intake when animals are confined in respiration chambers differed from the animal's normal behaviour, showing very low day-to-day variation compared to feed intake on the previous day. Understanding and accounting for such changes in behaviour may help to increase the accuracy of predicting an animal's true methane emissions.

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