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Is rumen retention time implicated in sheep differences in methane emission?

C.S. PINARES-PATIÑO[1]*, S.H. EBRAHIMI[1], J.C. McEWAN[2], K.G. DODDS[2], H. CLARK[1] and D. LUO[1]

[1]AgResearch Grasslands, Private Bag 11-008, Palmerston North 4442, New Zealand
[2]AgResearch Invermay, Private Bag 50-034, Mosgiel 9053, New Zealand
*Corresponding author: cesar.pinares@agresearch.co.nz

ABSTRACT

Breeding ruminants for low methane (CH_4) emissions is an attractive mitigation approach for this greenhouse gas. During the last three years research has progressed at AgResearch to accurately identify individual sheep with contrasting low (Low) and high (High) methane yields. A study was conducted using these animal resources in order to explore the animal factors responsible for the observed differences between rankings. For this, the sheep were fed first on fresh pasture forage (pasture) and then on pellets containing lucerne hay and wheat grain (pellet). Methane emissions were measured in respiration chambers over two consecutive days. Feed dry matter digestibility (DMD) was estimated from a total faecal collection, whereas ruminal retention times of feed particles (PRRT) and solutes (SRRT) were estimated by single dosing of external markers. Methane yields from the High sheep were higher ($P > 0.05$) than from their Low counterparts both the pasture and pellet diets. High emission ranking sheep had higher ($P < 0.05$) DMD and SRRT than their Low counterparts, whereas PRRT was only numerically higher for the High sheep than for the Low sheep. Results of this study align with previous findings that retention time of digesta is implicated in animal differences in CH_4 emission.

Keywords: sheep; methane; emission ranking; retention time; diet.

INTRODUCTION

Globally, ruminant livestock are the single most important source of anthropogenic methane (CH_4) emissions and this source represents a major share of New Zealand's total greenhouse gas (GHG) emissions (Pinares-Patiño *et al*., 2009). Methane is a major GHG and as global demand for livestock products is rapidly increasing due to population growth and a shift in human food consumption patterns. As a consequence ruminant CH_4 emissions are predicted to continue to increase. Currently, there are few technologies to mitigate CH_4 emissions from ruminants. For example, mitigation technologies based on feed additives and supplements have shown some potential to reduce emissions, but these approaches may be uneconomical and impractical for grazing systems where animals are not handled on a daily basis. Besides, grazed forage constitutes the cheapest source of energy and nutrients for the animal, and use of additives and supplements could potentially undermine the product safety and price competitiveness of the industry. Development of cost effective CH_4 mitigation technologies suitable for pastoral farming systems is needed to ensure that these systems remain competitive in the global market.

Methane emission measurements carried out in New Zealand over the last 15 years have shown a common feature of large animal-to-animal variation in emissions, which persists after accounting for individual differences in feed dry matter intake (DMI). Thus, a CH_4 mitigation avenue by exploiting this natural variation to breed for low CH_4 emission seems an attractive option (Pinares-Patiño *et al*., 2011). However, the mechanisms responsible for animal variation in emissions remain mostly unknown. Enteric methanogenesis is carried out by archaea microbia housed in the forestomach of ruminants, and a relationship between rumen retention time of feed particles and the rate of CH_4 emission is a commonly accepted paradigm, which is based on the well established association between retention time and the extent of fibre degradation (Benchaar *et al*., 2001). However, studies directly addressing such a relationship are very scarce.

Retention time has been shown to be a repeatable trait (Ørskov *et al*., 1988) and also heritable (Smuts *et al*., 1995). It has been shown to be responsible for a large proportion of the between-sheep variation in CH_4 emission (Pinares-Patiño *et al*., 2003). This study was carried out in order to explore whether rumen retention time is implicated in differences in CH_4 emission between groups of sheep with known differences in CH_4 yield, measured as emissions per unit of DMI.

MATERIALS AND METHODS

This study was conducted at the Ulyatt-Reid Large Animal Facility of AgResearch Grasslands Research Centre, Palmerston North. The experimental protocols followed were approved by the Grasslands Animal Ethics Committee.

Between July and September 2008 measurements of CH_4 emissions were conducted on 105 ewe lambs belonging to the Central Progeny Test programme (CPT) of Beef and Lamb New Zealand, while housed in respiration chambers and fed on a standard diet. Then, 10 extreme low (Low) and 10 extreme high (High) CH_4 yield individuals were selected and their rankings confirmed on subsequent repeated measurements, results of which have been published elsewhere (Pinares-Patiño *et al.*, 2011). In January 2010 (Period 1) and March 2010 (Period 2), two trials were conducted with the Low and High CH_4 emission sheep, while fed first on perennial ryegrass (*Lolium perenne*) pasture forage (pasture) (Period 1) and then on a mixed forage:concentrate (40% lucerne hay:60% wheat grain, fresh basis) pelleted diet (pellet) (Period 2). The crude protein and neutral detergent fibre (NDF) contents of the pasture and pellet diets were 130 and 638, and 203 and 280 g/kg dry matter (DM), respectively.

The sheep were brought indoors from grazing and acclimatised to the forage and pelleted diets over 10 and 21 days, respectively, while group-fed in pens. Pasture forage was fed *ad libitum*, allowing for 15% refusal, whereas feeding of the pelleted diet was set at 2.2 times the maintenance metabolisable energy requirement (CSIRO, 2007). Daily feed allowances were delivered in equal portions twice a day at 08:30 h and 16:00 h. Following acclimatisation in pens, the sheep were housed individually in metabolic crates for feed digestibility and digesta kinetics measurements over a seven-day period. Then, they were moved to respiration chambers for measurements of CH_4 emissions over two consecutive days. Due to the availability of eight respiration chambers, the sheep were allocated to three working groups of eight, eight and four animals, and groups staggered on time. Each working group contained equal number of Low and High CH_4 emitters.

Daily DMI was calculated from the amounts of feed offered and refused and their corresponding DM contents. Feed DM coefficient of digestibility (DMD) was estimated from the total collection of faecal output and DMI. Rumen retention times of feed particles (PRRT) and solutes (SRRT) were estimated using Cr-mordanted grass NDF and Co-EDTA, respectively as external markers (Pinares-Patiño *et al.*, 2007). For this, the sheep received a single oral dosing of the markers with samples of faeces collected at pre-defined intervals of time after dosing, then a multi-compartmental digestive tract model (Pinares-Patiño *et al.*, 2007) was fitted to the faecal excretions of Cr and Co to calculate PRRT and SRRT.

Effects of CH_4 ranking group, hereafter referred to as Low and High; diet, referred to as Pasture forage and Pellet, and their interaction on CH_4 yield, DMD, PRRT and SRRT were analysed using a mixed effects model by GenStat (Payne *et al.*, 2009). In this study, effects of diet and period were confounded. Consequently, given that effects of these factors cannot be separated, assumption was made that all effects were due to diet rather than period. Two animals belonging to the High emission ranking group could not acclimatise to the pellet diet, so they were removed from the trial. Data are presented as mean ± the standard error of mean.

RESULTS

As expected, CH_4 yield was higher ($P < 0.001$) for the High CH_4 emission ranking sheep than for their Low emission counterparts (17.7 ± 0.68 vs. 14.0 ± 0.62 g/kg DMI), and emission from the Pasture diet was much higher ($P < 0.001$) than from the Pellet diet (23.2 ± 0.62 vs. 8.6 ± 0.68 g/kg DMI). No ranking × diet interaction effect on CH_4 yield was observed ($P > 0.05$), but on the Pellet diet the difference in CH_4 yield between High and Low emission ranking sheep was much higher than on

TABLE 1: Mean ± standard error of mean of effect of methane (CH_4) emission ranking of sheep as either Low or High, when fed either a Pasture or Pellet diet on CH_4 yield, feed dry matter coefficient of digestibility and rumen retention times of digesta particulate and solute phases. Bolding of P values indicates significance ($P < 0.05$).

Measurement	Low ranking		High ranking		P value		
	Pasture	Pellet	Pasture	Pellet	Ranking	Diet	Ranking x Diet
Methane yield (g/kg dry matter intake)	21.7 ± 0.8	6.4 ± 0.9	24.6 ± 0.9	10.8 ± 1.0	**<0.001**	**<0.001**	0.41
Dry matter digestibility (%)	59.5 ± 1.9	58.1 ± 2.0	62.9 ± 2.1	63.4 ± 2.2	**0.05**	0.81	0.64
Particulate matter rumen retention time (hours)	27.0 ± 1.8	36.4 ± 1.7	32.9 ± 1.8	37.3 ± 2.1	0.17	**0.001**	0.19
Solute rumen retention time (hours)	13.1 ± 0.7	14.1 ± 0.7	16.0 ± 0.7	15.0 ± 0.8	**0.04**	0.92	0.07

the Pasture diet (69 vs. 13%) (Table 1). Feed DMD was higher (P = 0.05) for the High emission ranking sheep than the Low emission group (63.2 ± 1.5 vs. 58.8 ± 1.4%). On the other hand, effects of diet and ranking × diet interaction on DMD were not significant (P >0.05) (Table 1).

Rumen retention time of feed particles (PRRT) did not differ (P = 0.17) with sheep CH_4 emission ranking, but it was numerically longer in the High emission ranking sheep than in the Low group (35.1 ± 1.4 vs. 31.7 ± 1.3 hours). The PRRT of the Pasture diet was shorter (P <0.01) than that the Pellet diet (29.9 ± 1.3 vs. 36.9 ± 1.4 hours). In turn, SRRT was longer (P <0.05) for the High emission sheep than for their Low emission counterparts (15.5 ± 0.6 vs. 13.6 ± 0.6 hours), and no effect (P >0.05) of diet on SRRT was observed. The ranking × diet interaction effects on PRRT and SRRT were not significant (P >0.05) (Table 1).

DISCUSSION

Using the same experimental animals, results of this study confirmed previously reported findings (Pinares-Patiño *et al.*, 2011) that sheep selected for contrasting CH_4 yields maintain their emission rankings across time and diets, and that difference in emissions between the Low and High CH_4 emission ranking sheep is higher on concentrate-containing pelleted diets than on pasture forage.

Results of the present study align with findings from an earlier study at AgResearch Grasslands (Pinares-Patiño *et al.*, 2003) that CH_4 yield is positively associated with both feed digestibility and PRRT. Structural carbohydrates are fermented at slower rates than non-structural carbohydrates, such as starch and sugars, and yield more CH_4 per unit of substrate fermented (Holter & Young, 1992; Moe & Tyrrell, 1979). Thus, longer PRRT increases the rate of CH_4 emission, most probably by increasing the extent of digestion of structural carbohydrates (Pinares-Patiño *et al.*, 2011). The fact that in this study PRRT was only numerically different between the High and Low CH_4 emission sheep reflects the higher variability of this variable compared to CH_4 yield and DMD.

Results of the present study support findings from previous studies (Okine et al., 1989) that CH4 emission is associated with SRRT. The solute fraction of digesta is responsible for the flow of small feed particles out of the rumen, and shorter rumen retention times of the solute phase are associated with shorter residence time of protozoa in the rumen (Michalowski et al., 1986) and potentially, also methanogens as there is a symbiotic association between these two groups of microorganisms (Ushida et al., 1997).

A low CH_4 yield from sheep fed the concentrate-containing diet was expected, and it probably was associated with decreased ruminal pH and higher rates of ruminal fermentation favouring a shift of fermentation from acetate to propionate (Martin *et al.*, 2010). In addition, the physical form of the ground pelleted diet may have favoured a rapid passage of particles throughout the digestive tract (Hironaka *et al.*, 1996), contributing to a reduction in CH_4 yield. However, the longer PRRT for the Pellet than the Pasture diet observed in this study was unexpected, but similar findings have been observed previously by other researchers (Faichney, 1983). In this respect, Bernard *et al.* (2000) concluded that the effect of grinding and pelleting of forages on retention time remains conflicting, whereas Faichney *et al.* (2004) suggested that grinding of forages may slow microbial colonisation on account of the destruction of the fibre matrix. Attempting to draw any valid conclusion on effects of physical form of diet on PRRT is not easy in the case of the present study given that the forages belonged to distinct families.

This study, based on small sample size, indicates that differences in CH_4 yield per unit of feed intake between sheep with known low and high CH_4 emission rankings, was due to rumen retention time of digesta, with high CH_4 emission sheep having higher CH_4 yields due to longer retention times of digesta in rumen and therefore greater extent of digestion of structural carbohydrates. Although the above conclusion needs to be confirmed with a larger sample size, it implies that the possibility of exploiting animal-to-animal variation for mitigating CH_4 emissions should be considered in relation to its association with net feed efficiency.

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REFERENCES

Benchaar, C.; Pomar, C.; Chiquette, J. 2001: Evaluation of dietary strategies to reduce methane production in ruminants: a modelling approach. *Canadian Journal of Animal Science* **81**: 563-574.

Bernard, L.; Chaise, J.P.; Baumont, R.; Poncet, C. 2000: The effect of physical form of orchargrass hay on the passage of particulate matter through the rumen of sheep. *Journal of Animal Science* **78**: 1338-1353.

CSIRO. 2007: Nutrient requirements of domesticated ruminants. CSIRO Publishing, Collingwood, Victoria, Australia. 296 pp.

Faichney, G.J. 1983: The effect of physical form of lucerne hay on the passage of markers through the rumen of sheep. *Proceedings of the Nutrition Society of Australia* **8**: 186.

Faichney, G.F.; Teleki, E.; Brown, G.H. 2004: Effect of physical form of a lucerne hay on digestion and rate of passage in sheep. *Australian Journal of Agricultural Research* **55**: 1253-1262.

Hironaka, R.; Mathison, G.W.; Kerrigan, B.K.; Vlach, I. 1996: The effect of pelleting of alfalfa hay on methane production and digestibility by steers. *Science of the Total Environment* **180**: 221-227.

Holter, J.B.; Young, A.J., 1992: Methane production in dry and lactating Holstein cows. *Journal of Dairy Science* **75**: 2165-2175.

Martin, C.; Morgavi, D.P.; Doreau, M. 2010: Methane mitigation in ruminants: from microbe to the farm scale. *Animal* **4**: 351-365.

Michalowski, T.; Harmeyer, J.; Breves, G. 1986: The passage of protozoa from the reticulo-rumen through the omasum of sheep. *British Journal of Nutrition* **56**: 625-634.

Moe, P.W.; Tyrrell, H.F. 1979: Methane production in dairy cows. *Journal of Dairy Science* **62**: 1583-1586.

Okine, E.K.; Mathison, G.W.; Hardin, R.T. 1989: Effects of changes in frequency of reticular contractions on fluid and particulate passage rates in cattle. *Journal of Animal Science* **67**: 3388-3396.

Ørskov, E.R.; Ojwang, I.; Reid, G.W. 1988: A study on consistency of differences between cows in rumen outflow rate of fibrous particles and other substrates and consequences for digestibility and intake of roughages. *Animal Production* **47**: 45-51.

Payne, R.W.; Murray, D.A.; Harding, S.A.; Baird, D.B.; Soutar, D.M. 2009: GenStat for Windows, 12th Edition. Introduction. VSN International Ltd., Hemel Hempstead, Hertfordshire, UK.

Pinares-Patiño, C.S.; McEwan, J.C.; Dodds, K.G.; Cárdenas, E.A.; Hegarty, R.S.; Koolaard, J.P.; Clark, H. 2011: Repeatability of methane emissions from sheep. *Animal Feed Science and Technology* doi:10.1016/j.anifeedsci.2011.04.068.

Pinares-Patiño, C.S.; Ulyatt, M.J.; Lassey, K.R.; Barry, T.N.; Holmes, C.W. 2003: Rumen function and digestion parameters associated with differences between sheep in methane emissions when fed chaffed lucerne hay. *Journal of Agricultural Science* (Cambridge) **140**: 205–214.

Pinares-Patiño, C.S.; Waghorn, G.C.; Hegarty, R.S.; Hoskin, S.O. 2009: Effects of intensification of pastoral farming on greenhouse gas emissions in New Zealand. *New Zealand Veterinary Journal* **57**: 252-261.

Pinares-Patiño, C.S.; Waghorn, G.C.; Machmüller, A.; Vlaming, B.; Molano, G.; Cavanagh, A.; Clark, H. 2007: Methane emissions and digestive physiology of non-lactating dairy cows fed pasture forage. *Canadian Journal of Animal Science* **87**: 601-613.

Smuts, M.; Meissner, H.H.; Cronje, P.B. 1995: Retention time of digesta in the rumen: its repeatability and relationship with wool production of Merino rams. *Journal of Animal Science* **73**: 206-210.

Ushida, K.; Tokura, M.; Takenaka, A.; Itabashi, H. 1997: Ciliate protozoa and ruminal methanogenesis. *In*: Rumen microbes and digestive physiology in ruminants. Onodera, R.; Itabashi, H.; Ushida, K.; Yano, H.; Sasaki, Y. eds. Japan Scientific Society Press, Tokyo, Japan and S. Karger AG, Basel, Switzerland. p. 209-220.