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# Persistence of differences between sheep in methane emission under generous grazing conditions

# C. S. PINARES-PATIÑO<sup>1,3</sup>, M. J. ULYATT<sup>1</sup>\*, K. R. LASSEY<sup>2</sup>, T. N. BARRY<sup>3</sup> AND C. W. HOLMES<sup>3</sup>

<sup>1</sup> AgResearch Limited, Grasslands Research Centre, Tennent Drive, Private Bag 11008, Palmerston North, New Zealand

<sup>2</sup> National Institute of Water and Atmospheric Research Ltd., P.O. Box 14-901, Kilbirnie, Wellington, New Zealand

<sup>3</sup> Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand

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# SUMMARY

Four low and four high methane (CH<sub>4</sub>) emitters were selected from a flock of 20 Romney sheep on the basis of CH<sub>4</sub> production rates per unit of intake, measured at grazing using the sulphur hexafluoride (SF<sub>6</sub>) tracer technique. Methane emissions from these sheep were monitored at grazing for four periods (P): October, November, January and February 1999/2000. All measurements were carried out on perennial ryegrass/white clover pasture under generous herbage allowance, and the sheep were maintained on similar pastures during non-measurement periods. The tracer technique was used for all the CH<sub>4</sub> measurements and feed DM intake was calculated from total faecal collection and estimated DM digestibility. Data for liveweight (LW), gross energy intake (GEI) and CH<sub>4</sub> emission were analysed using split-plot analysis of variance. In addition, a between-period rank order correlation analysis was carried out for CH<sub>4</sub> emission data.

Low CH<sub>4</sub> emitters were heavier (P < 0.05) than the high emitters in all the periods, but they did not differ (P < 0.05) in their gross energy intakes (GEI: MJ/kg LW<sup>0.75</sup>). Low and high CH<sub>4</sub> emitters consistently maintained their initial rankings in CH<sub>4</sub> yield (% GEI) throughout the subsequent periods and the correlation analysis of rank order for CH<sub>4</sub> yield showed strong between-period correlation coefficients, although this was weaker in the last period. It is suggested that feeding conditions that maximize feed intake (e.g. generous allowance of good quality pasture under grazing) favour the expression and persistence of between-sheep differences in CH<sub>4</sub> yield.

# INTRODUCTION

Rumen methanogenesis results in the loss of up to 12% of gross energy intake (GEI) (Johnson *et al.* 1993). Methane (CH<sub>4</sub>), a potent greenhouse gas, is estimated to contribute about 24% of anthropogenic global warming, second only to carbon dioxide (CO<sub>2</sub>) (Houghton 1997), and its atmospheric concentration has increased over recent years at the rate of about 0.9% per year (Crutzen 1995). New Zealand's pastoral farming contributes about 88% of the national CH<sub>4</sub> emission (UNFCCC 1999), which on a per capita basis is 10 times greater than the global average

(Ministry for the Environment 1997), a consequence of large ruminant livestock and small human populations.

Compared with the other sources of CH<sub>4</sub> emission, ruminant CH<sub>4</sub> can be manipulated relatively simply (Leng 1993). With appropriate policies, current and potential future technologies and management practices could reduce CH<sub>4</sub> emissions per unit of animal product by 25–75% (Gibbs *et al.* 1989; Leng 1993; Mosier *et al.* 1998). However, with the exception of improved feeding management, the current technologies to control CH<sub>4</sub> emission from ruminants are seen with pessimism (Johnson *et al.* 1996; van Nevel & Demeyer 1996). In addition, for ruminant production systems based on forages, the necessary improvement in feeding management might not only

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<sup>\*</sup> To whom all correspondence should be addressed. Email: mwu@paradise.net.nz

be unaffordable, but it may undermine the traditional role of ruminants, which is to utilize low-cost fibrous feed resources. Therefore, the development of cost-effective strategies to mitigate ruminant  $CH_4$ , without causing a negative impact on ruminant production, continues to be a major challenge for ruminant nutritionists and microbiologists (McAllister *et al.* 1996).

Between-sheep variation in  $CH_4$  emission has long been recognized from measurements in respiration chambers (Blaxter & Clapperton 1965) and *in vitro* (Demeyer & van Nevel 1975), and recently confirmed under grazing conditions (Lassey *et al.* 1997; Ulyatt *et al.* 1999). The latter authors reported that about 85% of the variation in daily  $CH_4$  production (g/day) from sheep grazing temperate pastures was due to variation between animals. If such between-animal variability is persistent in the long term, and the animal trait(s) that account for such variation is (are) inherited, breeding of animals for low  $CH_4$  emission might be viable (Gibbs *et al.* 1989).

The present study was planned to test the hypothesis that sheep grazed on pasture will maintain existing differences between animals in methane emitted per unit of feed intake in the medium term.

## MATERIALS AND METHODS

#### Experimental design and animals

In early October 1999, 20 Romney wethers, approximately 14 months old, grazing on perennial ryegrass (Lolium perenne)/white clover (Trifolium repens) pasture at AgResearch Grasslands, Palmerston North, New Zealand, were selected on the basis of uniform liveweight (~45 kg) from a flock of 200. Methane production rates (g/kg dry matter intake, DMI) from each of these sheep were measured by the sulphur hexafluoride (SF<sub>6</sub>) tracer technique (Johnson *et al.* 1994) for 3 consecutive days. Based on these measurements, eight sheep with contrasting CH<sub>4</sub> production rates (four low and four high, P = 0.001) were selected and defined as the first measurement period  $(P_1)$ . Three subsequent  $CH_4$  measurement periods, using the tracer technique, were carried out on the selected sheep in late November 1999 (P2), early January 2000  $(P_3)$  and late February 2000  $(P_4)$ . Because one sheep ranked as a low emitter developed chronic lameness at the end of  $P_1$  and had to be excluded, the final sheep numbers were three low and four high emitters.

In each period sheep were acclimatized for 5 days to the experimental and management conditions before a 5-day measurement phase began. CH<sub>4</sub> production and herbage intake were measured over days 1-4 and samples of rumen contents for volatile fatty acid (VFA) analysis were taken on day 5. Acclimatization was set at only 5 days because the pasture was not changed during or between measurement periods. Sheep liveweights (LW) were measured at the beginning and end of each period. Weather conditions (maximum and minimum air temperatures and relative humidity) were recorded daily.

# Pasture and grazing management

Two 0.25-ha paddocks of perennial ryegrass/white clover pasture were used in this study. Both paddocks were rotationally grazed. One of the paddocks was exclusively used during each of the 10-day measurement periods, whereas sheep grazed the other paddock between measurement periods. Herbage in both paddocks was maintained in a vegetative stage by grazing with sheep and when required by irrigation.

During the measurement periods a fresh area of pasture was offered each day and controlled by electric fences to give about 12% of sheep LW as total herbage dry matter (DM) offered, in order to maximize intake (Hodgson 1990).

Fresh drinking water was available *ad libitum* during all the experimental periods.

#### Sample collection and laboratory analyses

Daily CH<sub>4</sub> production (g/day) was measured over days 1-4 by the SF<sub>6</sub> tracer technique, following the procedures described by Lassey *et al.* (1997). Three successful CH<sub>4</sub> sampling days were required from each animal.

The DMI (kg/day) of individual sheep was estimated from the pasture's DM digestibility (DMD), determined by the near-infrared reflectance spectrometry technique (NIRS), in conjunction with the twice-daily collection of total faeces output using a harness and canvas collection bag. Faeces were collected for 3 days and delayed 1 day relative to breath collection to allow for transit time in the gastrointestinal tract. Faeces from each animal were weighed, pooled within each day and sampled (10%) for DM determination (100  $^{\circ}$ C, 48 h).

Samples of herbage for DMD determination were collected each morning before grazing by hand plucking at the height to which the sheep had grazed on the previous day, in order to imitate any selective grazing of sward components. Herbage samples were dried (60 °C, 72 h), ground to pass through a 1-mm sieve, pooled for each period of CH4 measurement and analysed by NIRS for chemical composition and apparent DMD. The NIRS was calibrated against a wet chemistry database for chemical composition estimates and against samples from previous in vivo digestibility measurements for DMD (Corson et al. 1999). Gross energy (GE, MJ/kg DM) content in herbage was determined by an adiabatic bomb calorimeter (Gallenkamp Autobomb; Loughborough, Leics., UK).

At the start of  $P_1$  and  $P_4$  two 0.15-m<sup>2</sup> quadrats of herbage were cut to ground level using hand-clippers. Samples were pooled, washed, dried (60 °C, 72 h) and used for determination of herbage mass and botanical composition (% DM; by manual separation).

At the end of the last day of breath collection in each measurement period rumen contents (15–20 ml) were sampled from each sheep by stomach tube within 1 h of removal from grazing (08.00 h). Samples were squeezed through one layer of cheese cloth, processed (acidified, deproteinized and centrifuged) following the procedures described by Domingue *et al.* (1991) and then analysed for VFA as described by Hoskin *et al.* (1995). Because in some cases the rumen content samples were contaminated with saliva, only the ratios of molar proportions of acetic acid to propionic acid (A/P) were calculated. It was assumed that A/P did not vary with the sampling position (Bryant 1964).

#### Data calculation and statistical analyses

Daily GE intake (GEI) was calculated from the estimated DMI and GE content of forages and expressed as MJ/kg LW<sup>0.75</sup>. In this study, the term 'CH<sub>4</sub> production' is the absolute daily production (g/day), whereas the proportion of the daily GEI (% GEI) lost as CH<sub>4</sub> is the CH<sub>4</sub> yield. The term CH<sub>4</sub> emission refers both to CH<sub>4</sub> production and CH<sub>4</sub> yield, as well as to CH<sub>4</sub> production rate per unit of intake.

The persistence of sheep rankings (low or high) for CH<sub>4</sub> emission was assessed from: (1) a split-plot analysis of variance and (2) a between-periods rank order correlation analysis. In the split-plot analysis of variance (Gill 1986), the daily CH<sub>4</sub> production and CH<sub>4</sub> yield, were analysed using the GLM procedure of SAS (SAS 1987). Effects of CH<sub>4</sub> emission subgroups (S, low or high emitters) were tested using the animal (A) within emission subgroups (A(S)) component as the error term; whereas the effects of periods (P) and the interaction  $S \times P$  were tested using  $P \times A(S)$  as the error term. The PDIFF option in SAS (SAS 1987) was used to test the differences between least squared means. If the  $S \times P$  interaction from the analysis of variance of CH<sub>4</sub> production (or yield) data was statistically significant, it implied that sheep subgroups (S) were not persistent in  $CH_4$  production (or yield) even when the S main effects were significant. The influence of LW or LW<sup>0.75</sup> on CH<sub>4</sub> production (and yield) was assessed by including it as a covariate in the model of analysis of variance.

For the correlation analysis, the mean  $CH_4$  production and  $CH_4$  yield values for individual sheep (irrespective of  $CH_4$  emission subgroups) were ranked within each period using the rank procedure of SAS (SAS 1987).

Split-plot analyses of variance, with similar sources of variation to those for analysis of  $CH_4$  emission,

 Table 1. Chemical composition (g/kg DM) and
 apparent DM digestibility (DMD) of sheep diets during

 the experimental periods (P)

		Feed of	composi	tion* (g	kg DM)	)	
	СР	SC	NDF	ADF	Lipid	Ash	DMD
$\begin{array}{c} P_1 \\ P_2 \\ P_3 \\ P_4 \end{array}$	242 221 225 293	118 104 113 87	365 413 375 397	206 236 216 210	47 42 47 47	108 108 107 110	0.831 0.783 0.803 0.833

\* Abbreviations: DM, dry matter; CP, crude protein; SC, soluble carbohydrates; NDF, neutral detergent fibre; ADF, acid detergent fibre.

Table 2. Mean ( $\pm$  s.p.) daily maximum and minimum temperatures (°C) and relative humidities (%) during the experimental periods (P)

	Maximum	Minimum	Relative
	temperature	temperature	humidity
	(°C)	(°C)	(%)
$\begin{array}{c} P_1 \\ P_2 \\ P_3 \\ P_4 \end{array}$	$\begin{array}{c} 19 \cdot 1 \pm 2 \cdot 26 \\ 17 \cdot 6 \pm 2 \cdot 96 \\ 20 \cdot 3 \pm 1 \cdot 90 \\ 22 \cdot 7 \pm 1 \cdot 72 \end{array}$	$ \begin{array}{r} 11.2 \pm 1.75 \\ 9.1 \pm 3.75 \\ 11.0 \pm 1.65 \\ 12.0 \pm 3.08 \end{array} $	$95.2 \pm 1.40 \\ 79.8 \pm 10.8 \\ 82.2 \pm 9.05 \\ 80.5 \pm 5.95$

were also carried out for data concerning LW, GEI and A/P ratio.

## RESULTS

#### Diet and weather conditions

Herbage masses at the start of  $P_1$  and  $P_4$  were 3050 and 2300 kg DM/ha, respectively. At the start of  $P_1$ , perennial ryegrass and white clover constituted respectively 83 and 13% of the herbage DM on offer, whereas the corresponding values for  $P_4$  were 71 and 21%, respectively.

The diet judged to be selected by the sheep (handplucked samples) was of good quality as seen from its high digestibility and crude protein content (Table 1). Statistical analysis of trends in feed composition was not possible because only one bulked sample was chemically analysed for each period. The diet in  $P_2$ appeared to be higher in NDF and ADF and lower in digestibility than other periods, while  $P_4$  had a relatively high crude protein and soluble carbohydrate content. Lipid and ash were similar in all periods.

Differences in weather conditions between consecutive periods were small (Table 2). However, the weather tended to be slightly warmer and drier as the experiment progressed.

		Low	CH <sub>4</sub> en	nitters			High	CH <sub>4</sub> er	nitters		St	andard err	ors
	$P_1$	$P_2$	$P_3$	$P_4$	Mean	$P_1$	$P_2$	$P_3$	$P_4$	Mean	S	Р	$\mathbf{S}\times\mathbf{P}$
LW (kg/head)	45.5	49·7	54.7	55.7	51.4	44·0	46.6	50.7	50.2	47·9	2·13 <sup>N.S.</sup>	0.31***	0.46**
GEI (MJ/kg LW <sup>0.75</sup> )	2.39	1.95	2.29	2.36	2.25	2.21	1.83	2.15	2.39	2.14	0·110 <sup>N.S.</sup>	0.058***	0.088 <sup>N.S</sup>
CH <sub>4</sub> emission													
g/day	23.0	32.8	32.0	27.3	28.8	37.3	37.3	31.3	36.4	35.5	2·44 <sup>N.s.</sup>	2·19 <sup>№·s·</sup>	3·30 <sup>N.s.</sup>
% GEI	3.04	4.98	3.85	3.13	3.75	5.41	6.42	4.22	4.56	5.15	0.261**	0.322**	0·487 <sup>N.5</sup>
A/P	3.20	2.67	2.90	3.63	3.10	3.53	2.90	3.15	3.50	3.27	0·106 <sup>N.S.</sup>	0.070***	0.107 <sup>N.s</sup>

Table 3. Mean liveweight (LW), daily gross energy intake (GEI), methane (CH<sub>4</sub>) production (g/day) and yield (% GEI) and acetate/propionate (A/P) ratio for sheep subgroups (S: low or high CH<sub>4</sub> emitters) during the experimental periods (P). Statistical non-significance (N.S. = P > 0.05) or significance (\*\*P < 0.01 and \*\*\*P < 0.001) of S, P and S × P effects are indicated as superscripts of their respective standard errors

Degrees of freedom for S, P and  $S \times P$  are respectively 5, 15 and 15.

Table 4. Between-periods rank order correlation coefficients for methane (CH<sub>4</sub>) production (g/day) and yield (% GEI)

	Cl	H <sub>4</sub> (g/da	ıy)	CH4 (% GEI)			
	P <sub>1</sub>	$P_2$	$P_3$	P <sub>1</sub>	$P_2$	$P_3$	
$P_2$	0.89			0.86			
$P_3$	-0.14	0.04		0.54	0.72		
$P_4$	0.82	0.64	-0.61	0.71	0.68	0.00	

## Methane emission and other animal parameters

There were no significant effects of LW or LW<sup>0.75</sup> (covariate) on CH<sub>4</sub> production (g/day) or CH<sub>4</sub> yield (% GEI) (P > 0.05), however there was a significant S × P interaction (P < 0.01) between methane emission subgroup and period for LW (kg) (Table 3). In P<sub>1</sub>, low emitters were 1.51 kg heavier (P = 0.03) than the high emitters and this difference became larger as the experiment progressed (3.10, 3.97 and 5.58 kg at P<sub>2</sub>, P<sub>3</sub> and P<sub>4</sub>, respectively; P < 0.001). Liveweight of both low and high emitters increased (P < 0.001) from P<sub>1</sub> to P<sub>3</sub>, but was similar (P > 0.05) between P<sub>3</sub> and P<sub>4</sub> (Table 3).

GEI (MJ/kg LW<sup>0.75</sup>) did not differ between CH<sub>4</sub> emission subgroups (S), but was significantly different between periods (P < 0.001). GEIs (MJ/kg LW<sup>0.75</sup>) were similar during P<sub>1</sub> (2.30), P<sub>3</sub> (2.22) and P<sub>4</sub> (2.38) and higher than values during P<sub>2</sub> (1.89; P < 0.001).

Over the four periods the low emitters did not produce significantly less total CH<sub>4</sub> (g/day) than the high emitters (28·9 v. 35·5; P=0.09), however the CH<sub>4</sub> yields (% GEI) of the low emitters were significantly lower than those of the high subgroup (3·75 v. 5·15; P<0.01) (Table 3). There were also significant

period effects on CH<sub>4</sub> yield (P < 0.01), but not upon CH<sub>4</sub> production (g/day). Methane yields (% GEI) at P<sub>1</sub> (4.23), P<sub>3</sub> (4.03) and P<sub>4</sub> (3.84) were similar, but lower (P < 0.01) than P<sub>2</sub> (5.70).

Low and high emitters did not differ in A/P ratio (Table 3), however there were significant effects of period (P). A/P ratio in rumen fluid differed throughout all the periods of this study, being 3.36, 2.78, 3.03 and 3.57 in P<sub>1</sub>, P<sub>2</sub>, P<sub>3</sub> and P<sub>4</sub>, respectively (P < 0.001).

# Persistence of between-sheep differences in $CH_4$ emission

The fact that there was no  $S \times P$  interaction (P > 0.05) for CH<sub>4</sub> yield (% GEI) and that this parameter was significantly influenced by S (Table 3), indicates that there was a consistent difference between low and high CH<sub>4</sub> emitters throughout the 5-month study. This observation was corroborated by the generally high (P < 0.05) rank order correlation coefficients for CH<sub>4</sub> yield between the periods (Table 4), except that for P<sub>3</sub> and P<sub>4</sub>, which was null. The persistence of the difference between the high and low emission subgroups in CH<sub>4</sub> yield can be seen in Fig. 1: the high subgroup was higher than the low subgroup throughout, significantly so in P<sub>1</sub>, P<sub>2</sub> and P<sub>4</sub>.

CH<sub>4</sub> production (g/day) did not show the same pattern since differences between low and high CH<sub>4</sub> emitters were not significantly different (P = 0.09) despite the lack of S × P interaction (P > 0.05) (Table 3). In addition, for CH<sub>4</sub> production, no clear pattern was observed in the rank order coefficients of correlation between consecutive periods (Table 4).

#### DISCUSSION

The objective of the work was to test whether the observed large differences in  $CH_4$  yield (% GEI)

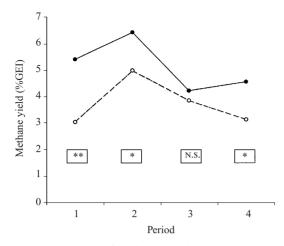


Fig. 1. The pattern of methane emission (% gross energy intake; % GEI) in sheep selected as high  $(\bullet - \bullet)$  or low  $(\bigcirc - \bigcirc)$  emitters across the four experimental periods. The symbols in the boxes indicate the significance of differences between means within periods: \*\*P < 0.01; \*P < 0.05; N.S., not significant.

between animals reported by Blaxter & Clapperton (1965), Demeyer & van Nevel (1975), Lassey et al. (1997) and Ulyatt et al. (1999) persist in time under grazing conditions. The significant effect of S (Table 3, Fig. 1), the lack of effect of  $S \times P$  upon  $CH_4$  yield (Table 3), and strong between-period correlation coefficients for CH<sub>4</sub> yield (Table 4) indicate that sheep in the present study persisted in their rankings for CH<sub>4</sub> emission (low or high). This finding contrasts with our preliminary observations (Pinares-Patiño 2000), when there was a lack of persistence when sheep selected as low or high CH4 emitters (g/kg DMI) under grazing, were brought indoors, fed dried feeds at restricted levels (1.2 maintenance), and had their feeding conditions changed between periods of measurement.

The high repeatability of  $CH_4$  yield between periods observed in the present study, suggests that feeding conditions which maximize voluntary feed intake (e.g. generous allowance of good quality pasture), favour the expression and persistence of betweensheep differences in  $CH_4$  yield. It must be noted, however, that these differences weakened as the experiment progressed (Table 3).

Sheep selected as low or high CH<sub>4</sub> emitters did not differ (P > 0.05) in their daily GEI (MJ/kg LW<sup>0.75</sup>) within any period (Table 3). Nevertheless, while the initial difference in LW between them became larger with the progress of the experimental periods (Table 3), the differences in CH<sub>4</sub> yield were maintained. This suggests that in comparison with the high emitters, the low emitters retained a greater proportion of their daily GEI as body energy.  $CH_4$  yield is negatively correlated with rumen particulate outflow rate (particulate FOR) (Blaxter & Clapperton 1965; Demeyer & van Nevel 1975; Okine *et al.* 1989; Pinares-Patiño *et al.* 2003), and on forage-based diets, lower A/P ratios and CH<sub>4</sub> yields are expected from the ingestion of diets with higher contents of legumes and soluble carbohydrates (Demeyer & van Nevel 1975; Beever 1993; Benchaar *et al.* 2001). In the present study, the A/P tended to be lower in the low emitters compared with the high emitters. However, whether differences in particulate FOR or diet selection (or both) were responsible for the consistent differences in CH<sub>4</sub> yield between low and high CH<sub>4</sub> emitters, is unknown.

Despite the fact that the perennial ryegrass/white clover-dominant pasture used for this experiment covered the period from spring (early October;  $P_1$ ) to mid summer (late February;  $P_4$ ), there were no dramatic changes in pasture chemical composition (Table 2). DM digestibility was high throughout the measurement periods. The small decrease in DM digestibility, together with increased fibre in P2 (late November), probably reflected an increase in grass stem due to flowering (Ulyatt 1980). It is probable that the relatively high CH<sub>4</sub> yields (% GEI) and low GEIs (MJ/kg LW<sup>0.75</sup>) in both the high and low emitters in P2 (Table 3, Fig. 1) were due to this decrease in pasture quality. This is in agreement with the observations by Blaxter & Clapperton (1965), who concluded that above maintenance levels of feeding, CH4 yield is inversely related to feeding level and feed apparent digestibility.

The mean daily CH<sub>4</sub> yield (% GEI) observed in the present study (4.5) was similar to that (4.6) reported by Lassey *et al.* (1997) for grazing sheep. Both these experiments used young wether sheep and the SF<sub>6</sub> tracer technique for CH<sub>4</sub> measurement. It has been observed (Ulyatt *et al.* 2002), also in grazing conditions, that mature sheep have a methane yield around 6.0% GEI. These latter values are similar to those commonly found in respiration calorimeters measurements for mature sheep fed on grass hay (e.g. 6.5; Blaxter & Wainman 1964). Thus, it can be suggested that young sheep have a lower CH<sub>4</sub> yield compared with mature sheep, perhaps because they select a more nutritious component of the pasture, or perhaps because their rumens are not fully developed.

The results of the work showed that the differences between sheep in CH<sub>4</sub> yield found by Blaxter & Clapperton (1965), Demeyer & van Nevel (1975), Lassey *et al.* (1997) and Ulyatt *et al.* (1999) can persist for up to 6 months. This result needs confirmation and testing over a longer time span, because if it is true and is under genetic control, it raises the possibility of selecting for sheep of reduced methane emission. If these differences can be shown to persist there is a need to determine how the individual animal controls an activity that is the prerogative of the methanogenic microorganisms.

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