# METHANE EMISSIONS OF LOW AND HIGH METHANE YIELD SELECTION LINE SHEEP FED RYEGRASS-BASED PASTURE ACROSS MULTIPLE PERIODS

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# **SUMMARY**

There is a lack of information on actual CH<sub>4</sub> emissions from low and high CH<sub>4</sub> selection line sheep-fed pasture *ad libitum* across different periods of the year. The objective of the two experiments was to determine the CH<sub>4</sub> emissions in respiration chambers from low and high CH<sub>4</sub> yield selection line sheep while fed cut pasture *ad libitum* across multiple measurement periods. In both experiments, CH<sub>4</sub> yield was consistently less in low compared to high CH<sub>4</sub> yield selection line sheep being, respectively, 8.9, 8.4 and 9.7 % less in periods 1 to 3 in Exp. 1 and 7.6, 9.6, 7.7 and 8.9 % less in periods 1 to 4 in Exp. 2. These results indicate that the magnitude of CH<sub>4</sub> yield differences between selection lines is consistent across different periods of the year.

#### INTRODUCTION

Breeding for low CH<sub>4</sub> in sheep is a viable mitigation option (Pinares-Patiño *et al.* 2013) and breeding values (BV's) for low CH<sub>4</sub> emissions are now available for sheep farmers in New Zealand who genotype their sheep and whose sheep are phenotyped for CH<sub>4</sub> using portable accumulation chambers (Jonker *et al.* 2018; Rowe *et al.* 2020). However, the initial phenotyping for the research CH<sub>4</sub> BV's was performed while the sheep were fed lucerne pellets at a fixed feeding level of 2 × maintenance energy requirements (Pinares-Patiño *et al.* 2013), which is different from farm grazing conditions. The CH<sub>4</sub> emissions and CH<sub>4</sub> yield between the two CH<sub>4</sub> selection lines were also found to differ using the SF<sub>6</sub> tracer technique in the field while the sheep were gazing at ryegrass-based pasture (Jonker *et al.* 2017). However, the magnitude of difference between selection line sheep was not the same as on lucerne pellets and there were questions about the accuracy of the DMI estimates. Furthermore, the consistency of the CH<sub>4</sub> yield ranking across repeat periods or seasons with different pasture compositions was not determined. Therefore, there is a lack of robust information on actual 24 h CH<sub>4</sub> emissions from low and high CH<sub>4</sub> selection line sheep-fed pasture across different periods of the year when fed pasture under controlled conditions where both CH<sub>4</sub> emissions and DMI can be accurately determined.

The objective was to determine CH<sub>4</sub> emissions and DMI in respiration chambers from low and high CH<sub>4</sub> yield selection line sheep while fed *ad libitum* cut pasture across multiple measurement periods.

## MATERIALS AND METHODS

The two sheep studies were approved by the AgResearch Grasslands Animal Ethics Committee (Palmerston North, New Zealand; AE1364 and 14007).

Animal source. The sheep used are part of the 'Methane yield selection' flock sheep, being bred solely, since 2012, based on having a low or high CH<sub>4</sub> yield (Jonker *et al.* 2018). A cohort of 96 female yearling progeny born in spring 2014 were used in Exp. 1 and a cohort of 72 ram lambs, born in spring 2016 were used in Exp. 2 (same sheep as in Jonker *et al.* 2021). The sheep born in a birth year were raised as one flock at AgResearch Woodlands (Woodlands, New Zealand) and were transported to AgResearch Grasslands (Palmerston North, New Zealand) before the current two experiments in June 2015 and February 2017, respectively.

**Measurement periods.** The CH<sub>4</sub> measurements were performed in three periods between 7 October 2015 and 12 February 2016 in Exp. 1 and in four periods between 6 March to 13 December 2017 in Exp. 2. In both studies, the sheep were fed *ad libitum* cut ryegrass-based pasture.

Sheep were initially housed in group pens for at least 14 days on cut pasture before being moved into individual metabolic crates for two days followed by two days in 24 respiration chambers as described previously (Pinares-Patiño and Waghorn, 2018; Jonker *et al.* 2021). The sheep were grazing ryegrass-based pasture before and between measurement periods.

The pasture was cut daily at around 1100 h and stored in a chiller at 4°C till feeding at around 1530 h and 0830 h the next day. Representative samples from each daily pasture cut were dried at 105 °C for 24 h in triplicate for dry matter (DM) content determination. Refusals were collected once daily before the afternoon feeding, and an aliquot was dried at 65 °C for 48 h. The DMI was calculated as feed DM offered – feed DM refusals.

**Statistical analysis.** The DMI and  $CH_4$  emissions were analysed separately for each measurement period in each experiment using a standard least squares model (SAS 2012) with a repeated measures model using ASREML (Gilmour *et al.* 2009), fitting animal as a random effect and selection line, sampling group (24 in a chamber measurement group) and day as fixed effects, and live weight (LW) as a covariate for the three  $CH_4$  emission parameters. The covariate was not used to analyse DMI to enable the expression of *ad libitum* DMI. Significance was declared at P < 0.05.

#### RESULTS AND DISCUSSION

The DMI was on average 6.4% greater (P>0.05) across the three periods in Exp. 1 in low compared to high CH<sub>4</sub> yield selection line sheep, while the DMI was similar between selection lines in Exp. 2 (Tables 1 and 2).

Table 1 Dry matter intake (DMI) and methane (CH<sub>4</sub>) emissions in low and high CH<sub>4</sub> yield selection sheep fed ad libitum cut pasture across three periods in Experiment 1

Item	DMI	CH <sub>4</sub>	CH <sub>4</sub>	CH <sub>4</sub> /CO <sub>2</sub>
	(kg/day)	(g/day)	(g/kg DMI)	(mol/mol)
Period 1, Oct/Nov 15				
High line	$1.44\pm0.030$	$34.4\pm0.48$	$23.6 \pm 0.29$	$0.070 \pm 0.0005$
Low line	$1.55 \pm 0.029$	$32.2 \pm 0.46$	$21.7 \pm 0.28$	$0.067 \pm 0.0005$
Difference (%)	-6.6	6.8	8.9	5.3
High – Low	$-0.10 \pm 0.04$	$2.19 \pm 0.68$	$1.93 \pm 0.42$	$0.004 \pm 0.001$
P-value	0.018	0.002	< 0.0001	< 0.0001
Period 2, Nov/Dec 15				
High line	$1.56 \pm 0.030$	$35.1 \pm 0.47$	$22.3\pm0.32$	$0.069 \pm 0.0006$
Low line	$1.66 \pm 0.029$	$32.9 \pm 0.45$	$20.6 \pm 0.31$	$0.065 \pm 0.0006$
Difference (%)	-5.8	6.7	8.4	5.4
High – Low	$-0.10 \pm 0.040$	$2.19 \pm 0.67$	$1.73 \pm 0.46$	$0.0035 \pm 0.0009$
P-value	0.0244	0.002	0.0003	0.0001
Period 3, Jan/Feb 16				
High line	$1.84\pm0.031$	$38.4 \pm 0.46$	$20.7 \pm 0.24$	$0.068 \pm 0.0005$
Low line	$1.97 \pm 0.030$	$36.2 \pm 0.44$	$18.9\pm0.23$	$0.063 \pm 0.0005$
Difference (%)	-6.7	6.1	9.7	7.2
High - Low	$-0.13 \pm 0.04$	$2.22\pm0.65$	$1.83\pm0.34$	$0.005 \pm 0.001$
<i>P</i> -value	0.003	0.001	< 0.0001	<0.0001

This DMI followed the same trend as LW, which was on average 5.3% greater across the three periods in Exp. 1 in low compared to high CH<sub>4</sub> yield selection line sheep, while LW was similar

between selection lines across the four periods in Exp. 2. It is generally expected that a sheep with a larger LW would be able to consume more feed dry matter.

In both experiments, CH<sub>4</sub> yield was consistently less in low compared to high CH<sub>4</sub> yield selection line sheep being, respectively, 8.9, 8.4 and 9.7 % less in periods 1 to 3 in Exp. 1 and 7.6, 9.6, 7.7 and 8.9 % less in periods 1 to 4 in Exp. 2. The repeatability for CH<sub>4</sub> yield was 0.43 in Exp. 1 and 0.32 in Exp. 2 (Jonker *et al.* 2021). To note, when interpreting the results of the two experiments, the difference between CH<sub>4</sub> selection lines was likely bigger in Exp. 1 than in Exp. 2 because the 12 extreme low and 12 extreme high CH<sub>4</sub> rams in Exp. 2 were not part of this study as they were retained in the main flock for the breeding season.

Table 2 Dry matter intake (DMI) and methane (CH<sub>4</sub>) emissions in low and high CH<sub>4</sub> yield selection sheep fed ad libitum cut pasture across four periods in Experiment 2

T4	DMI	CII	CII	CII /CO
Item	DMI	CH <sub>4</sub>	CH <sub>4</sub>	CH <sub>4</sub> /CO <sub>2</sub>
	(kg/day)	(g/day)	(g/kg DMI)	(mol/mol)
Period 1, March/April 17				
High line	$1.23 \pm 0.019$	$23.4 \pm 0.27$	$19.1 \pm 0.26$	$0.065 \pm 0.0006$
Low line	$1.25 \pm 0.018$	$22.0 \pm 0.26$	$17.8 \pm 0.25$	$0.060 \pm 0.0006$
Difference (%)	-1.3	6.4	7.6	8.5
High – Low	$-0.02 \pm 0.03$	$1.40\pm0.37$	$1.36\pm0.36$	$0.005 \pm 0.001$
P-value	0.523	0.0004	0.0003	< 0.0001
Period 2, June/July 17				
High line	$1.29 \pm 0.019$	$26.2 \pm 0.44$	$20.3 \pm 0.41$	$0.064 \pm 0.0008$
Low line	$1.34 \pm 0.019$	$24.6 \pm 0.45$	$18.5 \pm 0.41$	$0.061 \pm 0.0008$
Difference (%)	-3.7	6.2	9.6	5.7
High – Low	$-0.05 \pm 0.02$	$1.52\pm0.58$	$1.79 \pm 0.54$	$0.004 \pm 0.001$
P-value	0.044	0.012	0.0016	0.0009
Period 3, Sep 17				
High line	$1.53 \pm 0.022$	$36.3 \pm 0.52$	$23.7 \pm 0.26$	$0.070 \pm 0.0006$
Low line	$1.54 \pm 0.022$	$33.6 \pm 0.52$	$22.0 \pm 0.26$	$0.065 \pm 0.0006$
Difference (%)	-0.7	7.9	7.7	7.8
High – Low	$-0.01 \pm 0.03$	$2.66 \pm 0.74$	$1.69 \pm 0.36$	$0.005 \pm 0.001$
P-value	0.744	0.0006	< 0.0001	< 0.0001
Period 4, Nov/Dec 17				
High line	$1.87 \pm 0.028$	$39.9 \pm 0.48$	$21.5 \pm 0.31$	$0.078 \pm 0.0006$
Low line	$1.86\pm0.029$	$36.6 \pm 0.50$	$19.7 \pm 0.31$	$0.071 \pm 0.0006$
Difference (%)	-0.6	8.9	8.9	10.0
High - Low	$0.01 \pm 0.04$	$3.27 \pm 0.69$	$1.75 \pm 0.44$	$0.007 \pm 0.0009$
P-value	0.782	< 0.0001	0.0002	< 0.0001

The findings of the current two experiments were similar to the findings of Pinares-Patiño *et al.* (2011ab), who observed 11 and 12 % differences in CH<sub>4</sub> yield between sheep selected to have extremely low or high CH<sub>4</sub> yield measured approximately 8 months apart while fed cut pasture. The mean research BV's for CH<sub>4</sub> yield for the low and high CH<sub>4</sub> yield selection line sheep (on lucerne pellets) were, on average 8.4 and 11.9 % different in 2016 and 2018, respectively (Rowe *et al.* 2019), again being similar differences between selection lines to that observed in the current study. In absolute terms, the g of CH<sub>4</sub> yield difference between low and high CH<sub>4</sub> selection line sheep was 1.7-1.9 g in Exp. 1 and 1.4-1.8 g in Exp. 2, which was also similar to the difference in CH<sub>4</sub> yield selection BV's of 1.4 and 2.0 g in 2016 and 2018, respectively. This was even though the average CH<sub>4</sub> yield was lower at 16.0 g/kg DMI during phenotyping for BV's on lucerne pellets (Rowe *et al.* 2019) than the average CH<sub>4</sub> yield of 20.8 g/kg DMI on fresh pasture in the current two studies. The

CH<sub>4</sub> yield in the current two experiments was in a normal CH<sub>4</sub> yield range for sheep fed fresh pasture only (Swainson *et al.* 2018).

#### **CONCLUSION**

The results of the current study indicate that the magnitude of CH<sub>4</sub> yield differences (as % and g difference) between low and high CH<sub>4</sub> yield selection lines sheep were consistent across different periods when fed *ad libitum* cut pasture in both experiments. Therefore, the CH<sub>4</sub> yield BV's generated for NZ sheep can be used to adjust CH<sub>4</sub> emission predictions in farm carbon calculators across all seasons.

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