

## **Rumen characteristics and total tract digestibility in low and high methane yield selection line sheep offered fresh good or poor quality pasture**

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### **Summary**

The objectives of the current study were to determine methane (CH<sub>4</sub>) emissions, rumen fermentation characteristics, rumen digestion kinetics and total tract digestibility in sheep from low and high CH<sub>4</sub> yield (g/kg dry matter intake; DMI) selection lines fed good or poor quality pasture (vegetative improved vs. very mature unimproved pasture). Methane emissions were determined over 48 h in open circuit respiration chambers, rumen samples were collected 16 h after feeding, and digestion kinetics and total tract digestibility were determined over six days in metabolism crates. Methane emissions and total tract digestibility were similar between low and high CH<sub>4</sub> yield selection line sheep fed pasture of two qualities. Phenotypic differences as previously found in selected extremes were observed including shorter rumen retention time of solubles and particles (P<0.05) and lower concentrations of total and individual volatile fatty acids (P<0.05). The lack of interactions between CH<sub>4</sub> yield selection line and pasture quality suggest that these phenotypic characteristics of the low CH<sub>4</sub> yield selection line sheep are expressed independent of diet quality.

*Keywords: enteric methane, rumen retention time, passage rate, fermentation, animal variation.*

### **Introduction**

Methane production (g/d) and yield (g/kg DMI) were found to be heritable and repeatable traits in sheep fed lucerne pellets (Pinares-Patiño et al., 2013a). An observation in the trial by Pinares-Patiño et al. (2011b) was that the magnitude of divergence between low and high CH<sub>4</sub> emission ranking sheep was greater on higher quality feed (with greater starch content). Further, when comparing sheep identified with phenotypic extremes of CH<sub>4</sub> yield, rumen retention time of particles and solubles were shorter (Pinares-Patiño et al., 2011a) and rumen size smaller (Elmes et al., 2014) in the low CH<sub>4</sub> yield sheep. It has not been determined, however, if these phenotypic differences are heritable and have been transferred to progeny of sheep bred for low or high CH<sub>4</sub> yield. The objectives of the current study were to determine CH<sub>4</sub> emissions and rumen characteristics in low and high CH<sub>4</sub> yield selection line sheep fed good or poor quality pasture.

## Material and methods

### *Animals and measurements*

The experiment was carried out at Grasslands Research Centre (AgResearch Ltd. Palmerston North, NZ) in March 2014 involving male sheep from the low ( $n=12$ ) and high ( $n=12$ ) CH<sub>4</sub> yield selection lines (Pinares-Patiño et al., 2013a). The animal experiment was reviewed and approved by the AgResearch Grasslands Animal Ethics Committee (Palmerston North, NZ; approval #13015). Animals were allocated to two sub-groups balanced for CH<sub>4</sub> emission ranking and each sub-group randomly assigned to a diet of fresh good or poor quality pasture (vegetative and mature) offered at approximately 2% of body weight. The sub-groups were first acclimatised in pens and then in metabolic crates followed by simultaneous measurements of digestion kinetics of particles and solubles [using pulse dose of chromium mordanted neutral detergent fibre (NDF) and Cobalt-EDTA, respectively, with kinetics calculated as described by Aharoni et al. (1999)] and total tract digestibility for six days as described in detail by Pinares-Patiño et al. (2011a), followed by gaseous emission measurements in respiration chambers as described by Jonker et al., (2017b). A rumen sample was collected via oral stomach tubing, after the sheep were removed from the chambers, which were analysed for volatile fatty acids as detailed by Tavendale et al. (2005). After the trial, animals were slaughtered and the reticulo-rumen weight recorded with and without contents as in Elmes et al. (2014).

### *Pasture and feeding*

The good quality pasture at a vegetative growth stage came from an irrigated plot sown with three ryegrass cultivars in strips, as described for Period 2 in Jonker et al. (2017b). Poor quality pasture came from unimproved pasture at a very mature stage of growth with lots of dead material. Forage from both plots were cut daily around 11 am and transported to the research facility and stored in a chiller till feeding. Good quality pasture contained 222 g crude protein (CP) and 526 g NDF, while poor quality pasture contained 92 g CP and 637 g NDF. Sheep were fed twice daily around 3.30 pm and 8.30 am the next morning.

### *Statistical analysis*

Data were analysed using a standard least squares model (SAS, 2012) fitting the main effects of CH<sub>4</sub> yield progeny (high, low) and feed quality (good, poor) and their interaction. Interactions were not significant and are therefore not shown. Linear regression between traits were determined by plotting the traits (y against x) and fitting a straight line. Significance was declared at  $P < 0.05$ , unless otherwise stated.

## Results and discussion

Methane yield was similar for sheep fed poor and good quality pasture ( $P = 0.702$ ) (Table 1), which is consistent with findings in a meta-analysis of data from sheep fed a wide range of pasture qualities (Muetzel and Clark, 2015).

Rumen particle and liquid retention time were shorter for the low compared with the high CH<sub>4</sub> selection line sheep ( $P < 0.05$ ), which was consistent with previous findings in sheep with the lowest and highest CH<sub>4</sub> yields (Pinares-Patiño et al., 2011a; Pinares-Patiño et al., 2003). Total tract particle and liquid retention time were similar between both CH<sub>4</sub> yield selection lines and all digestive kinetic parameters were similar for sheep fed poor or good quality pasture. Overall, there was a moderate positive relationship between CH<sub>4</sub> yield and rumen retention time of solubles and particles ( $R^2 = 0.39$  and  $0.30$ , respectively) (Figure 1), which was consistent with previous findings in sheep fed forages (Hammond et al., 2014;

Pinares-Patiño et al., 2003). The differences in rumen kinetics did not, however, affect total tract digestibility of either DM or NDF, while good quality pasture had a much greater digestibility of DM and NDF compared with poor quality pasture. Rumen weights, full and empty, were similar for low and high CH<sub>4</sub> yield selection line sheep, which was different to previous findings (Elmes et al., 2014), although numerically the trend was similar.

Total VFA concentration and concentrations (*mM*) of acetate, butyrate, propionate and valerate were greater ( $P < 0.05$ ) for the high than the low CH<sub>4</sub> yield line sheep, while VFA proportions (% of total VFA) and VFA ratios remained similar for both CH<sub>4</sub> yield selection lines (data not shown). These trends were consistent with previous findings of strong genetic and moderate phenotypic correlations (positive) for total VFA concentration and acetate and butyrate concentrations (*mM*) with CH<sub>4</sub> yield (g/kg DMI) in sheep fed lucerne pellets (Pinares-Patiño et al., 2013b).

Dry matter intake and CH<sub>4</sub> production (g/d) and yield (g/kg DMI and % of GEI) were similar between animals of the two CH<sub>4</sub> yield lines. The lack of variation in CH<sub>4</sub> yield was, however, most likely due to the large difference in diet quality and variation in DMI. For half siblings of the sheep in the current study born in the same year, CH<sub>4</sub> yield was approximately 6% lower for the low vs. high CH<sub>4</sub> yield selection lines sheep (Jonker et al., unpublished), which suggests that the progeny used in the current study are genetically divergent for CH<sub>4</sub> emissions. Thus, the genetic potential of the progeny were not expressed or were diminished by the variation in the current experiment. Conversely, this differs from recent experiments where low CH<sub>4</sub> yield selection line progeny fed lucerne pellets also had lower CH<sub>4</sub> yield when grazing pasture (Jonker et al., 2017a) or when fed cut pasture (Jonker et al., 2017c).

## Conclusion

Methane emissions were not statistically different between low and high CH<sub>4</sub> yield selection line sheep fed pasture of two qualities, however, phenotypic differences as previously found in selected extremes were observed including shorter rumen retention time of solubles and particles and lower concentrations of VFAs. The lack of significant interactions between CH<sub>4</sub> yield selection line and pasture quality suggest that these phenotypic characteristics of the low CH<sub>4</sub> yield selection line sheep are expressed independent of diet quality.

## Acknowledgements

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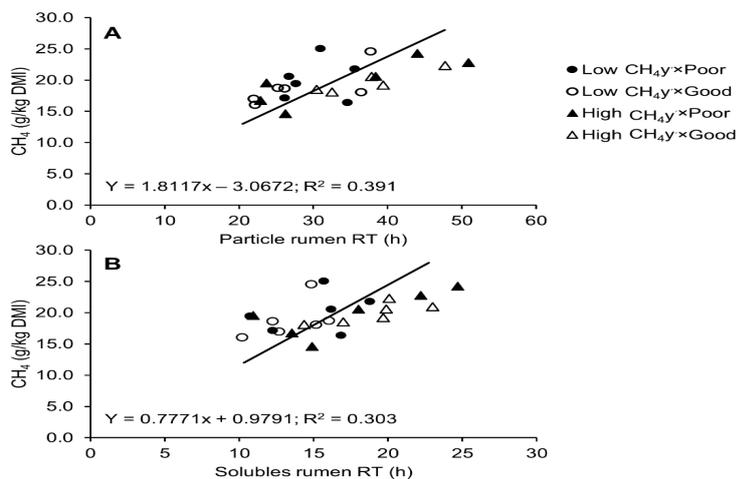
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**Table 1 Average methane emissions and rumen and total tract characteristics of progeny from the low and high methane yield selection lines fed poor or good quality pasture.**

Parameter <sup>2</sup>	Progeny			Pasture quality			SEM <sup>1</sup>
	Low	High	<i>P</i> value	Poor	Good	<i>P</i> value	
DMI (kg/d)	0.83	0.87	0.564	0.76	0.94	0.006	0.04
CH <sub>4</sub> (g/d)	15.8	17.0	0.166	14.8	18.0	0.001	0.6
CH <sub>4</sub> (g/kg DMI)	19.4	19.9	0.762	19.9	19.4	0.702	0.9
Particle kinetics							
Rumen RT (h)	29.3	39.3	0.048	32.3	36.3	0.412	3.3
Total tract RT (h)	45.4	53.6	0.132	47.1	51.9	0.367	3.7
Liquid kinetics							
Rumen RT (h)	14.3	18.2	0.019	16.2	16.3	0.980	1.1
Total tract RT (h)	27.8	29.4	0.367	27.9	29.3	0.428	1.2
Total tract digestibility							
DMD (% of DM)	61.9	63.8	0.215	53.2	72.6	<0.0001	2.1
NDFD (% of NDF)	63.7	66.1	0.137	55.1	74.7	<0.0001	1.1
Rumen weights							
Rumen full (kg)	7.08	7.42	0.405	6.80	7.70	0.040	0.40
Rumen empty (kg)	0.78	0.83	0.170	0.76	0.86	0.009	0.03
Rumen content (kg)	6.29	6.59	0.456	6.05	6.84	0.056	0.39
Rumen fermentation							
Total VFA ( <i>mM</i> )	56.7	63.1	0.032	57.3	62.5	0.076	1.9
Acetate (A; <i>mM</i> )	39.4	43.7	0.048	39.8	43.2	0.113	1.4
Propionate (P; <i>mM</i> )	9.3	10.6	0.035	9.8	10.1	0.644	0.4
Butyrate (B; <i>mM</i> )	4.7	5.3	0.045	5.1	4.9	0.571	0.2
Valerate (V; <i>mM</i> )	0.38	0.45	0.024	0.34	0.49	0.0003	0.02
AB/PV	4.6	4.5	0.499	4.5	4.6	0.567	0.1

<sup>1</sup>SEM, standard error of the mean for the main effects, interactions were not significant.

<sup>2</sup>DMI, dry matter intake; RT, retention time; DMD, dry matter digestibility; NDFD, neutral detergent fibre digestibility; VFA, volatile fatty acids.



**Figure 1 Regressions of methane yield (CH<sub>4</sub>y; g/kg dry matter intake; DMI) and rumen retention time (RT) of particles (A) and solubles (B) in progeny of the low or high CH<sub>4</sub> yield selection lines fed good or poor quality pasture.**