Differences in milk composition associated with enteric methane emissions

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Abstract

Milk samples from sheep were analysed for fat, protein, lactose, somatic cell counts and detailed fatty acid composition. The sheep were from two lines each of 100 adult ewes, grazed together but differing by an average of 10% in enteric methane yield (g CH_4 /kg dry matter intake). Milk samples were taken at 2, 4 and 6 weeks post lambing. Rumen fluid was also sampled for volatile fatty acid profiles and sequencing of the rumen microflora. There were significant differences in the rumen microbiome, rumen volatile fatty acids and milk fatty acid composition between the two lines. This suggests that the ruminant hosts have been co-selected for divergent fermentation profiles affecting milk composition. The next step is to explore whether milk composition profile in ruminants is a potential predictor of enteric methane status. These results have important implications for the selection of low methane emitting ruminants and subsequent effects on product composition.

Keywords: methane, milk, rumen, microbiome.

Introduction

Around 1/3 of New Zealand's greenhouse gases are emitted as enteric methane, a by-product of ruminant digestion (Mfe, 2020). Heritable individual variation in enteric emissions has been shown in ruminant livestock populations (Pinares-Patino *et al.* 2013, Jonker *et al.*, 2018), and selection for lowered methane emissions has been shown to be an effective mitigation tool in sheep (Pinares-Patino *et al.*, 2013). As enteric methane is produced during the fermentation of ingested feed to energy sources for the animal, this selection against methane production has been associated with different microbial populations in the gut and differing amounts of volatile fatty acids in rumen outflow (Jonker *et al.*, 2019, 2020). Sheep selection lines exist that have been bred divergently for 3 generations creating two divergent lines that differ in methane emissions by approximately 11% (Rowe *et al.*, 2019). Because these sheep selection lines that are divergent for methane emitted per kg DMI also differ in fermentation energy sources to the animal, and in rumen microbial composition, we hypothesised that synthesis of milk fatty acids and therefore milk composition may also vary.

Methods

Ewes To create the selection lines, the 100 lowest emitting and the 100 highest emitting ewes and the 10 lowest and 10 highest emitting sires were selected from an initial population of 1000. Lines were closed and selected over 3 generations for high and low enteric methane emissions (CH_4) per kg of dry matter eaten (DMI) to attain an average of 11% difference. Ewes from both lines were grazed together on a mixed ryegrass-clover pasture.

Milk At 3 time-points post lambing, at 2-weekly intervals starting 2 weeks after the first ewe lambed, all ewes were taken off pasture at 8am. Lambs were removed and after one hour a milk sample was collected from each ewe. The foremilk was discarded and a mixed sample of ~25ml was collected from both teats. A 5-ml sample of milk was processed, and the fatty acids measured as methyl esters, using gas chromatography as described by Agnew *et al*. (2019). The remaining 20ml was sent to the LIC testing laboratory (Christchurch, NZ) for standard herd test profiling of fat, protein, lactose and somatic cell count.

Rumen Fluid. Within 30 min of milking, a 30-ml rumen fluid sample was collected via stomach intubation. Short chain fatty acid analysis was carried out using a 2-ml subsample, as described by Attwood *et al*. (1998). The remainder was snap-frozen and freeze dried prior to DNA extraction and microbial sequencing as described by Hess *et al*. 2020.

Methane All selection line ewes were measured for methane emissions through portable accumulation chambers (Jonker *et al*., 2018) at 4 and 6 weeks post lambing. Breeding values for each ewe were estimated to ensure that differences were retained in early lactation.

Analysis Data were analysed using univariate linear mixed models. Models (1) and (2) were fitted for each trait using ASREML v4.1 (Gilmour *et al*. 2015).

$$y = \mu + cdat*bg + age + nll + lwt + line + pe \quad (1)$$

$$y = \mu + cdat*bg + age + nll + lwt + M \quad (2)$$

where

y is the trait of interest, cdat is the collection date of the sample, bg indicates if the ewe lambed late or early, age is the ewe's age (years) at sampling, nll is the number of live lambs, lwt is the ewe's live weight (kg) at sampling, line is the methane line (low or high), pe is the permanent environment random effect, and M is the reference-based microbial relationship matrix computed as described by Hess *et al*. (2020). Model (1) was fitted to investigate the effect of selection line on each trait while Model (2) was fitted to estimate the microbiability (proportion of variance explained by the rumen microbial profile).

Results and discussion

Average milk constituents for the two selection lines from a standard milk test are given in table 1. No significant differences were found between the high and low methane selection line sheep for total fat, total protein or lactose. Significant differences were reported for somatic cell count and for methane breeding value. There were no obvious reasons why the low line sheep should have higher somatic cell counts. Udders were in good condition with no signs of infection. Sheep had been grazed together since immediately post mating. The low line sheep have been previously shown to have greater parasite resistance (Rowe *et al*., 2019). This may suggest potential differences in immune status.

In contrast to the non-significant differences in total fat percentage, there were clear differences in individual milk fatty acids analysis between the selection lines (Table 2).

In particular, medium chain fatty acids were lower in the low methane emitting sheep, and polyunsaturated fatty acids were higher. There was also a significant difference between the lines in iso C14 and anteiso C15 and C14:1 (Table 2). The relationship between rumen fermentation, bio-hydrogenation and the presence of odd and branched chain fatty acids in milk was reviewed by Vlaeminck (2006). These iso and anteiso acids in the milk might be related to the ruminal iso volatile fatty acids (Table 3). Regardless of whether this is the case and they are from de novo synthesis in the rumen or derived from propionate in the mammary gland, they warrant further investigation as potentially important predictors in milk.

Table 3 reports differences in the volatile fatty acids measured in rumen fluid of the lines. In particular, there were differences in acetic acid and in the ratio of acetic to propionic acids. Propionate dominated fermentations are often associated with lower methane emissions due to lower hydrogen production. Similar results were reported by Jonker *et al.* (2020). Table 4 shows the variance explained by the rumen microbes present, which was estimated using the microbial relationship matrix M in model 2. There is a clear link between the microbial community composition obtained by sequencing and described by Hess *et al.*(2020), and the fatty acids found in the milk and the volatile fatty acids in the rumen. Next steps are to measure methane emissions from animals identified with divergent profiles and to explore the impact that changes in fatty acid composition of milk may have on neonatal nutrition and product processing.

Table 1. Average milk constituents of high and low selection line sheep.

	Low	High	P-value
Fat (%)	5.07	5.00	0.910
Protein (%)	5.04	5.14	0.169
Lactose (%)	5.79	5.78	0.451
Total solids (%)	16.28	16.28	0.797
Somatic cell count ,000	260	182	<0.001*
Methane yield g/kg DMIbreeding value	–1.22	+1.14	<0.001*

*Significant at 5% threshold.

Table 2. Fatty acid composition of milk from high and low selection line sheep.

Fatty Acid (%)	Low – High	% Diff from high	P-value
iso C14	0.009	8.2	<0.001*
C14:0	–0.199	–2.6	0.218
iso C15	0.008	3.2	0.077
anteiso C15 +C14:1[1]	0.022	4.0	0.021*
C16:0	–0.455	–2.6	0.024*
C18:1 t9	0.012	4.8	0.036*
C18:1 t11	0.613	8.7	0.001*
C18:1 c11	0.032	9.7	0.018*
C18:2 n6	0.082	13.0	<0.001*
C18:3 n3	0.176	17.3	<0.001*
C20:0	–0.006	–5.0	0.008*
CLA	0.255	10.9	0.001*
SFA[2]	–1.220	–2.8	<0.001*
PUFA[3]	0.512	12.9	<0.001*

[1]Antesiso C15 and C14:1 could not be separated in the spectral analysis and are reported together.
[2]SFA = saturated fatty acids = C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0.
[3]PUFA = polyunsaturated fatty acids = CLA + C18:2 n6 + C18:3 n3.
*Significant at 5% threshold

Table 3. Volatile fatty acid (VFA) profile of rumen fluid.

Rumen VFAs	Low – High	% Diff from high	P-value
Concentrations			
Acetic (mM)	–4.423	–7.9	0.009*
Butyric (mM)	–0.555	–7.0	0.060
Caproic (mM)	0.013	6.1	0.243
Isobutyric (mM)	–0.106	–9.4	0.012*
Isovaleric (mM)	–0.124	–10.2	0.030*
Propionic (mM)	–0.837	–5.2	0.122
Valeric (mM)	–0.062	–6.5	0.113
Total(mM)	–4.423	–7.9	0.009*
Proportions[1]			
Acetic		–0.4	0.346
Butyric		–0.8	0.073
Caproic		4.6	0.013*
Isobutyric		–1.5	0.282
Isovaleric		–2.1	0.261
Propionic		1.1	0.003*
Valeric		–0.3	0.733
Ratios			
Acetic/Propionic	–0.102	–2.9	0.012*
(A + B)/(P + V)[2]	–0.104	–2.7	0.009*

[1]Proportions were log transformed to satisfy assumptions of homogeneous variance when fitting the models.
[2](A + B)/(P + V) = (Acetic + Butyric)/(Propionic + Valeric).
*Significant at 5% threshold

Table 4. Proportion of variance of milk fatty acids (FA) and volatile fatty acids (VFA) explained by the rumen microbial profile.

Milk FAs (%)	Variation explained	Rumen VFAs (%)	Variation explained
iso C14	0.13 ± 0.05 *	Proportions	
C14:0	0.07 ± 0.04	Acetic	0.30 ± 0.07 *
iso C15	0.10 ± 0.04 *	Butyric	0.34 ± 0.06 *
anteiso C15 + C14:1	0.10 ± 0.05 *	Caproic	0.23 ± 0.06 *
C16:0	0.11 ± 0.04 *	Propionic	0.28 ± 0.07 *
C18:1 t9	0.09 ± 0.05 *	Valeric	0.34 ± 0.07 *
C18:1 t11	0.28 ± 0.06 *	Ratios	
C18:1 c9	0.16 ± 0.05 *	Acetic/Propionic	0.26 ± 0.06 *
C18:1 c11	0.13 ± 0.05 *	(A + B)/(P + V)	0.26 ± 0.06 *
C18:2 n6	0.17 ± 0.06 *		
C18:3 n3	0.26 ± 0.07 *		
C20:0	0.14 ± 0.05 *		
CLA	0.25 ± 0.06 *		
SFA	0.16 ± 0.05 *		
PUFA	0.31 ± 0.07 *		

*Greater than 2 standard deviations above zero.

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