Contributed paper

IMPACT OF BREEDING FOR DIVERGENT METHANE YIELD ON MILK COMPOSITION IN BREEDING EWES

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SUMMARY

Previous research into breeding sheep based on methane yield has shown that low emitting animals appear to have neutral or superior economic and environmental value compared to high emitting animals. However, the impact of breeding for methane yield on milk composition has not been studied in depth. We investigated differences in detailed fatty acid (FA) profiles and rumen volatile fatty acids (VFA) associated with methane selection line across two lactation years in lactating ewes from a sheep flock selected for divergent methane yield. Changes in FA profiles due to selection line were observed, with increased polyunsaturated fatty acids levels in the milk and VFAs associated with less hydrogen formation in rumen samples from lower methane emitting animals. There was evidence that these differences were partly driven by changes in the rumen microbial profile. These results have important implications in screening for, and processing milk from, low methane emitting animals in industry.

INTRODUCTION

Methane is a greenhouse gas associated with climate change and approximately 84% of methane emissions in New Zealand are produced from grazing livestock (MFE 2020). Reducing methane emissions from livestock is therefore of environmental and economic importance and is achievable by breeding for animals that emit less methane. Ruminant animals primarily produce methane as a by-product of the complex microbial fermentation process in their rumen that breaks down feed to VFAs, which are absorbed through the gut wall and are a major source of energy for the animal (Matthews *et al.* 2019). The mammary gland also uses these VFA in the de novo synthesis of milk fatty acids (FA) (Negussie *et al.* 2017). Changes in a herd's methane emission levels via breeding is therefore likely to be associated with changes in FA composition.

Over the past decade, a sheep flock has been selected for divergent methane yield, with low-methane sheep emitting 10-12% less methane than the high-methane animals (Rowe *et al.* 2019). Using lactating ewes from this flock, we analysed milk FA profiles and rumen fluid VFA to investigate changes associated with methane selection line, and whether these changes relate to changes in the rumen microbial profile.

MATERIALS AND METHODS

Animals. This study selected 60 out of 100 ewes from the high methane line (HML) and 60 out of 100 ewes from the low methane line (LML) in a divergent methane yield sheep flock (Rowe *et al.* 2019) that were lambing from September each spring. This selection was made in two lactation years (2018 and 2019), with 25 HML and 23 LML ewes retained in the flock and selected in the study in both years leaving 192 unique ewes. The average difference in methane breeding values for these ewes between the two lines was 1.98 g CH4 per kg dry matter intake (DMI) for 2018 and 2.21 g CH4 per kg DMI for 2019 (average methane value was 16 ± 1.45 g CH4 per kg DMI).

Traits. Milk and rumen samples were collected after lambing in October 2018 and 2019 at two

time-points two weeks apart. These time-points were approximately 2 and 4 weeks post lambing if the ewe lambed late (last week of September or October) and approximately 4 and 6 weeks post-lambing if the ewe lambed early (early September). An 8-mL sample of milk was processed and methyl esters of the fatty acids measured using gas chromatography as described by Agnew *et al.* (2019). Rumen fluid were collected via oral stomach tubing and was divided into three 2-mL samples that were processed using the method described by Jonker *et al.* (2019) to obtain volatile fatty acid (VFA) profiles, and into a 30 mL sample for DNA extraction and sequencing to generate a rumen microbial profile as described by Hess *et al.* (2020).

Statistical Analysis. Univariate linear mixed models (LMMs) were fitted for each trait using ASREML v4.1 (Gilmour *et al.* 2015). Model equations were:

$$\log_{10}(y) = \mu + cdat*bg + age + nll + lwt + line + p_e \quad (1)$$

$$\log_{10}(y) = \mu + cdat*bg + age + nll + lwt + M \quad (2)$$

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 (2, 3, 4+), nll is the number of live lambs (1, 2, 3+), lwt is the ewe's liveweight (kg) at sampling, line is the methane line (low or high), p_e is the permanent environment random effect, and M is the reference-based microbial relationship matrix as described by Hess *et al.* (2020). Most ewes were 2 (47%) or 3 (33%) years old and had 1 (31%) or 2 (58%) lambs. The trait values were log transformed to improve variance homogeneity. 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

Milk FAs. Results from fitting univariate LMMs on the milk FAs are given in Table 1. FA percentages for each individual polyunsaturated fatty acids (PUFA) (e.g., C18:2 n6, CLA) and the total PUFA value were significantly greater in the LML compared to the HML for both years, with differences ranging between 4.3% to 13.5%. The total saturated fatty acids (SFA) value was significantly smaller in the LML for both years, with a difference around -1.1% to -1.3%, although changes in individual SFA (e.g., C12:0, C17:0) were not consistent across years. There was little evidence of changes in the monounsaturated fatty acids (MUFA). The repeatabilities were moderate across the FA, but greater for the PUFA and the total SFA value across both years.

Rumen VFAs. Results from fitting univariate LMMs on the rumen fluid VFA are given in Table 2. Percentages of caproic and propionic acid were on average significantly greater in the LML than in the HML in both lactation years, while changes in the other VFA were inconsistent across years. The two VFA ratios were consistently smaller in the LML compared to the HML and significant at the 5% threshold, indicating that the percentage of acetic and butyric relative to propionic and valeric was smaller in the LML. This is consistent with stoichiometric principles, as formation of acetic and butyric acids is connected with hydrogen formation (utilised by methanogens to form methane) while propionic and valeric acids are associated with less hydrogen formation (Janssen 2010). The repeatabilities were between 0.23 and 0.39 for all VFA, except for caproic acid which had very low repeatability. Similar results in terms of ruminal VFA composition and repeatabilities were found in growing methane selection line sheep fed pasture as in this trial (Jonker *et al.* 2020).

Microbiability: Estimates of microbiability for milk FA and rumen VFA are given in Table 3. The microbiability for all the milk PUFA and all the rumen VFA ranged between 0.21 to 0.54 and was greater than 2 standard errors from zero across both years. This was not the case for the milk SFA and MUFA. These results suggest that differences between the selection lines in rumen VFA and milk PUFA are, at least partially, driven by changes in the rumen microbial profile.

CONCLUSIONS

This study shows that breeding for methane impacts milk FA and rumen fluid VFA profiles and

suggests that changes in these profiles are partially driven by changes in the rumen microbial profile. These results suggest there is potential for milk FA and rumen VFA to be used as a proxy measure for methane, but the results also have implications on milk processing, as changes in FA profiles affects the quality and type of products produced from the milk.

Table 1. Fatty acid (FA) composition of milk samples from low and high selection lines

FA (%)	2018			2019		
	Mean ± s.e.	% diff‡	Repeatability	Mean ± s.e.	% diff‡	Repeatability
Total SFA[1]	43.4 ± 2.36	**-1.3%**†	0.53 ± 0.08	44.8 ± 2.25	**-1.1%**†	0.44 ± 0.09
C12:0	4.35 ± 1.07	1.5%	0.36 ± 0.09	4.33 ± 1.20	-2.9%	0.19 ± 0.11
C14:0	7.83 ± 1.21	-0.5%	0.34 ± 0.10	8.06 ± 1.40	-2.1%	0.26 ± 0.10
C15:0	0.84 ± 0.10	0.7%	0.41 ± 0.09	0.93 ± 0.10	**2.1%**†	0.12 ± 0.11
C16:0	17.6 ± 1.67	**-1.6%**†	0.51 ± 0.08	18.1 ± 1.79	-1.2%	0.39 ± 0.09
C17:0	0.65 ± 0.18	-1.9%	0.31 ± 0.10	0.66 ± 0.12	-0.6%	0.28 ± 0.10
C18:0	12.1 ± 2.30	-2.8%	0.26 ± 0.10	12.6 ± 2.70	0.4%	0.28 ± 0.10
C20:0	0.12 ± 0.02	-2.8%	0.14 ± 0.10	0.12 ± 0.03	-1.3%	0.30 ± 0.10
Total MUFA[2]	18.3 ± 3.75	-1.6%	0.28 ± 0.12	19.1 ± 3.51	-1.0%	0.11 ± 0.11
C14:1	0.04 ± 0.04	-0.7%	0.20 ± 0.23	0.07 ± 0.03	-4.7%	0.51 ± 0.08
C16:1	0.48 ± 0.11	-1.0%	0.26 ± 0.10	0.49 ± 0.13	**-5.3%**†	0.45 ± 0.09
C17:1	0.24 ± 0.08	-1.4%	0.32 ± 0.10	0.23 ± 0.05	**-4.4%**†	0.33 ± 0.09
C18:1 c9	17.3 ± 3.65	-2.2%	0.21 ± 0.10	18.0 ± 3.47	-0.8%	0.11 ± 0.11
C18:1 c11	6.94 ± 1.63	4.5%	0.70 ± 0.07	6.94 ± 1.13	1.0%	0.34 ± 0.09
Total PUFA[3]	3.85 ± 0.66	**5.4%***	0.57 ± 0.07	3.92 ± 0.54	**7.2%***	0.53 ± 0.08
C18:2 n6	0.63 ± 0.14	**4.8%***	0.38 ± 0.09	0.57 ± 0.13	**9.4%***	0.44 ± 0.09
C18:3 n3	0.98 ± 0.24	**7.1%***	0.62 ± 0.06	0.97 ± 0.22	**13.5%***	0.48 ± 0.08
CLA	2.24 ± 0.61	**4.5%**†	0.61 ± 0.07	2.38 ± 0.42	**4.3%**†	0.56 ± 0.07

[1]SFA = saturated fatty acids (Total = C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0)
[2]MUFA = monounsaturated fatty acids (Total = C14:1 + C16:1 + C17:1 + C18:1 c9 + C18:1 c11)
[3]PUFA = polyunsaturated fatty acids (Total = CLA + C18:2 n6 + C18:3 n3)
†Significant at 5% threshold, *Significant at 0.1% threshold, ‡Difference (Low – High)

Table 2. Volatile fatty acids (VFA) in rumen fluid samples from low and high selection lines

VFA (%)	2018			2019		
	Mean ± s.e.	% diff‡	Repeatability	Mean ± s.e.	% diff‡	Repeatability
Acetic	65.9 ± 2.73	-0.4%	0.22 ± 0.10	66.1 ± 2.77	**-1.0%***	0.23 ± 0.10
Butyric	10.2 ± 1.41	0.2%	0.25 ± 0.10	9.94 ± 1.35	**2.5%**†	0.31 ± 0.10
Caproic	0.30 ± 0.12	**6.8%**†	0.00 ± 0.00	0.31 ± 0.12	**12.7%***	0.01 ± 0.11
Isobutyric	1.33 ± 0.37	-2.0%	0.36 ± 0.09	1.19 ± 0.32	0.6%	0.30 ± 0.10
Isovaleric	1.49 ± 0.52	-2.8%	0.39 ± 0.09	1.29 ± 0.43	0.2%	0.29 ± 0.10
Propionic	19.5 ± 1.28	**1.3%**†	0.37 ± 0.09	19.9 ± 1.31	**1.5%**†	0.35 ± 0.10
Valeric	1.24 ± 0.28	-0.1%	0.11 ± 0.11	1.21 ± 0.32	**4.5%**†	0.25 ± 0.11
A/P[1]	3.40 ± 0.33	**-11.6%**†	0.31 ± 0.10	3.34 ± 0.33	**-17.2%***	0.26 ± 0.10
(A+B)/(P+V)[2]	3.69 ± 0.32	**-11.4%**†	0.35 ± 0.09	3.62 ± 0.33	**-16.6%***	0.30 ± 0.10

[1]A/P = Acetic/Propionic
[2](A + B)/(P + V) = (Acetic + Butyric)/(Propionic + Valeric)
†Significant at 5% threshold, *Significant at 0.1% threshold, ‡ Difference (Low – High)

Table 3. Microbiability estimates (% ± s.e) for milk fatty acids and rumen volatile fatty acids

FA (%)	2018	2019	FA (%)	2018	2019
Total SFA	0.18 ± 0.07	0.13 ± 0.07	Total PUFA	0.33 ± 0.09	0.34 ± 0.09
C12:0	0.11 ± 0.07	0.21 ± 0.09	C18:2 n6	0.21 ± 0.09	0.40 ± 0.08
C14:0	0.04 ± 0.05	0.13 ± 0.08	C18:3 n3	0.28 ± 0.09	0.46 ± 0.09
C15:0	0.01 ± 0.04	0.05 ± 0.06	CLA	0.26 ± 0.08	0.27 ± 0.09
C16:0	0.16 ± 0.07	0.00 ± 0.00	VFA (%)	2018	2019
C17:0	0.07 ± 0.07	0.27 ± 0.09	Acetic	0.38 ± 0.09	0.44 ± 0.09
C18:0	0.07 ± 0.06	0.17 ± 0.09	Butyric	0.48 ± 0.08	0.36 ± 0.09
C20:0	0.07 ± 0.06	0.17 ± 0.08	Caproic	0.32 ± 0.08	0.33 ± 0.09
Total MUFA	0.08 ± 0.08	0.22 ± 0.09	Isobutyric	0.54 ± 0.09	0.32 ± 0.09
C14:1	0.00 ± 0.00	0.11 ± 0.08	Isovaleric	0.52 ± 0.08	0.29 ± 0.09
C16:1	0.01 ± 0.05	0.00 ± 0.00	Propionic	0.28 ± 0.09	0.46 ± 0.09
C17:1	0.15 ± 0.09	0.16 ± 0.09	Valeric	0.41 ± 0.08	0.42 ± 0.09
C18:1 c9	0.16 ± 0.09	0.21 ± 0.09	A/P	0.31 ± 0.09	0.48 ± 0.09
C18:1 c11	0.41 ± 0.10	0.09 ± 0.07	(A+B)/(P+V)	0.30 ± 0.09	0.47 ± 0.09

Abbreviations for FA, VFA, SFA, MUFA, PUFA, A/P and (A+B)/(P+V) are as in Tables 1 and 2.

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