Kelly, S., Bilton, T., Agnew, M., Booker, F., Shortall, A., Peers-Adams, J., Bain, W., Hampton, I., McRae, K., Dodds, K., Johnson, P., McEwan, J., Rowe, S. (2026) Genetic variation for methane emissions and meat fatty acid composition implicates rumen microbiota in New Zealand sheep, *Proceedings 13th World Congress on Genetics Applied to Livestock Production*
23511: https://doi.org/10.31274/wcgalp.23511 ©2026 Kelly, et al. CC BY-NC-ND

Genetic variation for methane emissions and meat fatty acid composition implicates rumen microbiota in New Zealand sheep

S.Y. Kelly[1], T.P. Bilton[1*], M. Agnew[2], F. Booker[1], A. Shortall[1], J. Peers-Adams[1], W. Bain[1], I. Hampton[1], K.M. McRae[1], K.G. Dodds[1], P.L. Johnson[1], J.C. McEwan[1], and S.J. Rowe[1]

*[1]Bioeconomy Science Institute, Mosgiel, New Zealand; [2]Bioeconomy Science Institute, Palmerston North, New Zealand:*Corresponding author: timothy.bilton@agresearch.co.nz*

Introduction

Anthropogenic greenhouse gas emissions have been identified as a factor driving climate change. Mitigation of emissions of carbon dioxide (CO_2) and methane (CH_4) is crucial to reducing global impacts of climate change and meeting New Zealand's international obligations, including a commitment to reduce net greenhouse gas emissions by 50% below 2005 levels by 2030 under the Paris Agreement (2020) targets (United Nations, 2015). As the agricultural sector contributes a significant proportion of New Zealand's economy and atmospheric emissions, impacts of livestock producing CH_4 must be addressed. Breeding has been demonstrated to reduce CH_4 emissions in ruminants while continuing to make genetic gains on production traits (Booker *et al.*, 2024; Hickey *et al.,* 2022). In principle, it should be feasible to implement breeding at-scale to reduce CH_4 emissions.

Accurately measuring CH_4 emissions at an individual animal level is necessary for breeding but is costly and laborious. The fermentation process in the rumen produces CH_4 emissions but is also biologically linked to fatty acid production in the animal. Rumen microbiome composition and host tissue fatty acid profiling are therefore both being explored as proxy traits for CH_4 emissions. Fatty acids are synthesised from 2-carbon chains with the majority of odd- and branched-chain fatty acids (OBCFAs) in the human diet originating from rumen bacteria (Vlaeminck *et al.*, 2006; Toral *et al*., 2020). Fatty acid composition is an established measure of quality for sheep milk and meat products (Carta *et al.*, 2008; Junkuszew *et al.*, 2020) and it is important to balance selection for low CH_4 animals and meat quality (Johnson *et al.*, 2021; Rose *et al.*, 2023). Fatty acid composition in sheep milk has also been linked to genetics of CH_4 emissions (Bilton *et al*., 2025; Carta *et al*., 2008). We aim to develop effective tools for balanced genetic gains of conventional and low-emissions traits sheep. To achieve this, the genetic relationship between meat fatty acids and CH_4 measured using portable accumulation chambers (PAC) was investigated.

Materials & Methods

This work has been approved by the AgResearch Animal Ethics committee (approval numbers 15293, 15294, and 2357). All animals in this study were managed in a pastoral grazing system at the Invermay or Woodlands research farms (near Dunedin and Invercargill, New Zealand respectively). Samples were collected between February 2023 and October 2025 from 686 ram lambs in 3 research flocks of composite breeds (predominant breeds include Coopworth, Romney, and Texel, among others) born between 2022 and 2024. The research flocks have different breeding strategies with single-trait selection or selection based on multiple trait indices so that CH_4 emissions vary widely (McLean *et al.*, 2006; Jonker *et al.*, 2018; Booker *et al.*, 2024).

All animals had a single CH_4 measures collected at around 6 months using PAC (Jonker *et al.*, 2018). PAC measures are moderately repeatability with moderate-to-high genetic correlation

with methane measured in respiration chambers (Jonker *et al.*, 2018). These animals have been genotyped using SNP chips and production traits were collected as per the SIL Best Practice Guide (Beef and Lamb Genetics 2016). Genotyping was performed using the AgResearch Sheep Genomic 60K SNP chip. A genomic relationship matrix was computed using the software KGD (Dodds *et al.*, 2015) from a filtered set of ~40,000 SNPs for 2253 animals that included all the animals sampled and animals from previous generations from the 3 flocks.
Loin meat fatty acid composition was evaluated from ram lamb carcasses sampled post-slaughter at approximately 6-12 months of age. A single freeze dried sample of around 2cm of loin meat from each ram lamb were processed at the AgResearch Grasslands facility by gas chromatography of triglyceride esters using a previously published protocol (Agnew *et al.*, 2019; Johnson *et al.*, 2021). Fatty acids were reported percentages of total fat content computed from mg per gram of wet loin muscle weight.

Univariate models. Each fatty acid (FA_n) was calculated as a proportion of wet weight. Heritabilities were estimated with ASReml v4.2ni (Gilmour *et al.*, 2015) using variances computed from the following single trait model:

$$y_{ipqrst}^{FA_n} = \mu + cg_p + bdev_q + brr_r + aod_s + cwgt_t + a_i + e_{ipqrst} \quad (1)$$

where μ is the overall mean, with fixed effects: cg_p is a contemporary group (defined as flock and birth year), brr_r is the birth rearing rank and aod_s is the age of dam fitted as factors, and $bdev_q$ is the birth date deviation relative to the average birth date within each contemporary group and $cwgt_t$ is carcass weight fitted as a covariate. The genetic component is the random effect $a_i \sim N(0,\sigma_a^2\mathbf{G})$ and the residual term is $e_{ipqrst} \sim N(0,\sigma_e^2\mathbf{I})$, where **G** denotes the genomic relationship matrix and **I** denotes the identity matrix. Live weight at 6 months of age (LW6) was evaluated with the same model excluding carcass weight. PAC measurements for CH_4 (scaled grams per day) were evaluated in a single trait model with live weight at PAC ($paclwt_u$) fitted as a covariate:

$$y_{pqrs}^{LW6} = \mu + cg_p + bdev_q + brr_r + aod_s + a_i + e_{ipqrs} \quad (2)$$

$$y_{pqrsu}^{CH_4} = \mu + cg_p + bdev_q + brr_r + aod_s + paclwt_u + a_i + e_{ipqrsu} \quad (3)$$

Bivariate models. Bivariate models were computed with ASReml v4.2ni (Gilmour *et al.*, 2015) using the following multiple trait model:

$$\begin{bmatrix}\boldsymbol{y}_1\\ \boldsymbol{y}_2\end{bmatrix} = \begin{bmatrix}\mu_1\\ \mu_2\end{bmatrix} + \begin{bmatrix}\mathbf{X}_1 & 0\\ 0 & \mathbf{X}_2\end{bmatrix}\begin{bmatrix}\boldsymbol{\beta}_1\\ \boldsymbol{\beta}_2\end{bmatrix} + \begin{bmatrix}\mathbf{Z}_1 & 0\\ 0 & \mathbf{Z}_2\end{bmatrix}\begin{bmatrix}\boldsymbol{a}_1\\ \boldsymbol{a}_2\end{bmatrix} + \begin{bmatrix}\boldsymbol{e}_1\\ \boldsymbol{e}_2\end{bmatrix} \quad (4)$$

where $\boldsymbol{y}_k$ is the trait, μ_k is the mean, $\mathbf{X}_k$ and $\mathbf{Z}_k$ are design matrices for the fixed and random genetic effects, $\boldsymbol{\beta}_k$ is the fixed effects, $\boldsymbol{a}_k$ is the random genetic effect such that $(\boldsymbol{a}_1, \boldsymbol{a}_2)' \sim MVN(0, \mathbf{G}\otimes\mathbf{B})$, $\boldsymbol{e}_k$ is the residual term such that $(\boldsymbol{e}_1, \boldsymbol{e}_2)' \sim MVN(0, \mathbf{I}\otimes\mathbf{C})$, where $\mathbf{B}=((\sigma_{a1}^2, \sigma_{a12})', (\sigma_{a12}, \sigma_{a2}^2)')$, $\mathbf{C}=((\sigma_{e1}^2, \sigma_{e12})', (\sigma_{e12}, \sigma_{e2}^2)')$, and k=1 for fatty acid trait and k=2 for PAC trait. Fixed effect terms were the same as in the univariate models described above with $cgwt_t$ or $paclwt_u$ as a covariate for fatty acid composition or CH_4 measures, respectively. Heritabilities were computed using the genetic (σ_{ak}^2) and environmental variances (σ_{ek}^2) for each respective response variable. Genetic correlations ($\sigma_{a12}/((\sigma_{a1}^2\sigma_{a2}^2)^{-0.5})$) and phenotypic correlations ($((\sigma_{a12}+\sigma_{a12})/((\sigma_{a1}^2+\sigma_{e1}^2)(\sigma_{a2}^2+\sigma_{e2}^2))^{-0.5})$) were also computed from this model.

Results & Discussion

The combined dataset of 2253 genotyped animals had 1625 animals measured by PAC. A total of 780 fatty acid samples (including some technical duplicates) were collected from animals previously measured for CH_4. After analysis, 686 had animals with both PAC measurements and matching fatty acid profiles. This dataset was used to investigate relationships between genetics, fatty acid composition, and CH_4 emissions in sheep.

Univariate heritabilities for LW6, FAs, and CH_4. Heritability estimates for live weight at 6 months of age (0.30 ± 0.034) were consistent with prior studies from similar New Zealand sheep populations (Pickering *et al.*, 2012). Meat fatty acids have broad variation in heritability (ranging from 0.01 to 0.76). This may be due to differences in synthesis direct from nutritional contribution versus biohydrogenation under genetic control and age of deposition. Heritability estimates for live weight adjusted CH_4 grams per day from scaled PAC measurements (0.32 ± 0.090) and live weight at PAC measurement (0.25 ± 0.033) were consistent with prior studies (Jonker *et al.*, 2018; Hickey *et al.*, 2018; Hickey *et al.*, 2022).

Bivariate relationships between CH_4 and FAs. Heritability estimates for live weight adjusted CH_4 grams per day (μ=7.59, σ=1.45) in bivariate models were consistent with the above results, ranging from 0.21 to 0.28 with an average of 0.24 ± 0.089. For the OBCFAs, the genetic correlations ranged from -0.53 to 0.46, and the phenotypic correlations ranged from -0.12 to 0.08 (Table 1). Amongst the highest positive genetic correlations was for C17:1 while the strongest negative genetic correlation was for iso-C17.

Table 1. Genetic parameters computed from bivariate analyses of daily CH_4 and OBCFAs.

Fatty Acid	*CH_4 h^2*	*FA μ*	*FA σ*	*FA h^2*	*r_g*	*r_p*
iso-C14	0.23 ± 0.090	0.04	0.039	0.36 ± 0.093	-0.02 ± 0.239	**-0.09 ± 0.043**
iso-C15	0.24 ± 0.089	0.13	0.028	0.22 ± 0.084	-0.25 ± 0.286	-0.04 ± 0.042
anteiso-C15	0.24 ± 0.089	0.18	0.039	0.18 ± 0.083	-0.16 ± 0.300	-0.06 ± 0.042
C15:0	0.24 ± 0.088	0.35	0.067	0.24 ± 0.089	-0.29 ± 0.271	**-0.10 ± 0.042**
iso-C16	0.22 ± 0.086	0.12	0.025	0.19 ± 0.078	-0.53 ± 0.307	-0.02 ± 0.042
iso-C17	0.24 ± 0.088	0.39	0.058	0.39 ± 0.091	**-0.45 ± 0.210**	**-0.12 ± 0.042**
anteiso-C17	0.24 ± 0.089	0.44	0.083	0.26 ± 0.084	-0.26 ± 0.258	-0.02 ± 0.043
C17:0	0.25 ± 0.088	0.87	0.085	0.52 ± 0.097	-0.34 ± 0.204	-0.05 ± 0.045
C17:1	0.24 ± 0.086	0.41	0.047	0.50 ± 0.101	**0.46 ± 0.212**	0.08 ± 0.044

[1] Live weight adjusted CH_4 heritabilities were computed for each bivariate model
[2] Mean (μ) and standard deviation (σ) for fatty acids are percentages (%) of total fat content
[3] Correlations significantly different from 0 are highlighted in bold

These results indicate that fatty acid profiles are heritable and linked to variation in methane profiles. In particular, OBCFAs are specific to microbial metabolism which implicates the rumen microbiota (Fievez *et al.*, 2012; Toral *et al.*, 2020). This indicates that fatty acids reflect changes in the rumen microbial composition and could be a viable proxy trait to use for selection. These genetic correlations imply that host factors influence the microbial composition and fatty acid metabolism of the rumen. Rumen microbial composition, immunological, and gene expression studies would complement the above results.
This dataset is the foundation for further investigations, including a multi-country study. Results will facilitate investigation into biological mechanisms unpinning methane production in ruminants and inform feasibility studies of breeding programmes at a wider scale. Animal breeding has been demonstrated to be a feasible approach to reduce methane emissions from ruminants. To develop a selective breeding strategy, a cost-effective on-farm approach is needed at-scale. We have investigated the utility of fatty acid profiles as a predictor in New Zealand sheep. We plan to validate this result in Irish sheep as part of the Methane Predict research program which will also improve the precision of the estimates. Future work will be assessment of implementation in a breeding programme and comparison of alternative proxy traits to determine which are suitable for application in the pastoral agricultural industry.

Conclusions

This study generated a large dataset from 3 New Zealand sheep flocks for joint meat fatty acid profiles and CH_4 measurements. Bivariate analyses showed that OBCFAs were genetically correlated with CH_4 measured using PAC. These results show that meat fatty acids may be a viable proxy trait to estimate CH_4 emissions in livestock ruminants. These results also indicated that rumen microbiome metabolism plays a key role as fatty acids that cannot be produced by human or animal metabolic pathways were genetically correlated with CH_4 emissions.

Acknowledgements

This project is funded by the Irish Department of Agriculture, Food and the Marine (DAFM) and New Zealand Ministry for Primary Industries (MPI) via a joint research initiative (2022IRLNZ121) awarded to Fiona McGovern and Suzanne Rowe.

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