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USING RUMEN MICROBIAL PREDICTORS FOR GENOMIC PREDICTION OF FEED EFFICIENCY

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SUMMARY

Obtaining phenotypic measures of feed efficiency requires measuring intake levels and growth rates over a period of approximately 8 weeks (2 weeks of adaption and 6 of measurement), which is expensive and low-throughput. Rumen microbial community (RMC) profiles have shown to be associated with feed efficiency traits in ruminants and so may be a suitable proxy. Using a dataset of 1298 animals across 4 genetically linked flocks that were measured through a feed intake facility (FIF), we predicted feed efficiency from RMC profiles and obtained higher prediction accuracies compared to host genomic prediction. The genetic and phenotypic correlations between feed efficiency traits measured from the FIF and predicted from RMC profiles were estimated as 0.64 and 0.33 for mid-trial intake and 0.47 and 0.30 for residual feed intake (RFI). These results suggest RMC profiles have the potential to be used as a proxy for feed efficiency traits in ruminants.

INTRODUCTION

Feed efficiency relates to the amount of feed an animal consumes to produce a fixed amount of product. There are many economic and environmental benefits from breeding for more feed efficient animals, such as reduced feed costs and reduced greenhouse gas emissions per unit of product. Feed efficiency traits are likely to play an important role in future breeding programs as competition for land resources intensifies and targets for greenhouse gas emissions are introduced. Various traits have been proposed to quantify feed efficiency, but all generally require measuring intake levels over an extended period. Although specialised facilities have been developed to measure intake, they are expensive to operate and only a limited number of animals can be measured at a given time. A potential proxy for feed efficiency in ruminants is the rumen microbial community (RMC) profile, as the fermentation process in the rumen, responsible for breaking down feed to produce volatile fatty acids that provide the majority of energy to ruminants, is driven by the microorganisms in the RMC. Previous studies have found associations between the rumen microbiome and feed efficiency in cattle (Li *et al.* 2019) and sheep (Hess *et al.* 2022). RMC profiles have previously been shown by Bilton *et al.* (2022) to be a viable proxy for methane traits. In this study, we extend this work to investigate the feasibility of RMC profiles as a proxy for feed efficiency.

MATERIALS AND METHODS

Experimental animals and protocols applied in this study were approved by the AgResearch Grasslands (Palmerston North, NZ) AgResearch Ruakura (Hamilton, NZ) Animal Ethics committees (approvals 13563, 13892, 14221, 15047 and 15386).

Animals & phenotypes. Data from 4 genetically linked performance-recorded sheep flocks were obtained and consisted of 1298 ewe lambs that were born between 2014 and 2020 (Table 1). Feed efficiency traits were measured using a sheep Feed Intake Facility (FIF) based near AgResearch's Invermay campus, Mosgiel, New Zealand. The lambs were measured at approximately 9 months of age in cohorts of approximately 200 animals across 42 days after a 14-

day introductory period and were feeding on alfalfa pellets from automated feeders. The cohorts for the animals born in 2014 and 2015 were also separated into five pens of equal size. A full description of the experiment and data collection is given in Johnson *et al.* (2022). Feed efficiency traits that were calculated were mid-trial intake (MidIntake), mid-trial metabolic liveweight (MidLWT), and residual feed intake (RFI) was computed as described in Johnson *et al.* (2022). The mid-trial traits were obtained as predictions at day 21 of the measurement period from a linear model of the measured trait values. Additional animal information and measurements were downloaded from the Sheep Improvement Limited database (Newman *et al.* 2000). The animals used in this study are a subset of the animals used by Bilton *et al.* (2022).

Table 1. Sample numbers by flock and year of birth

Flock	Dataset	Year of Birth					Total
		2014	2015	2016	2019	2020	
1	Training	87	145	154			386
2	Training	103	141	140			384
3	Training		95	93			188
4	Validation				158	182	340
Total		190	381	387	158	182	1298

Rumen microbial sampling & profiles. Rumen samples were collected from all animals via stomach intubation after the animals had been in the FIF for at least 4 weeks (2-week introductory period and 2 weeks of measurements). The protocol described in Bilton *et al.* (2022) was used to preserve, process and sequence the samples. The freeze-dried method (Kittelmann *et al.* 2014) was used for all samples except for the born 2020 samples from flock 4, which were preserved using the TNx2 solution (Budel *et al.* 2022). Sequencing was performed using a restricted enzyme-reduced representation sequencing approach (Hess *et al.* 2020) using *Pst*I and run across multiple flowcells on an Illumina HiSeq2500 or NovaSeq6000. The reference-free pipeline developed by Hess *et al.* (2020) was used to generate a count matrix of tags (unique raw sequences trimmed to 65 bp) from which a microbial relationship matrix (MRM) was computed.

Animal genotyping. To investigate prediction of feed efficiency traits from host genomics and comparing to the RMC profiles, a subset of the genomic relationship matrix (GRM) computed in Bilton *et al.* (2022) for animals included in this study was used. This GRM was computed in KGD (Dodds *et al.* 2015) using VanRaden method 1 with non-missing SNPs for each matrix entry and assuming missing data is at random. Animals were genotyped on a variety of nested SNP arrays. SNPs with a call rate of 70% were retained, resulting in 14,923 SNPs in the combined dataset.

Statistical models. Data was split into (a) a training set consisting of the 958 ewes from flocks 1 to 3, and (b) a validation set consisting of the 340 animals from flock 4. Univariate mixed models fitted to the training data were of the form:

$$y_{ijkl} = \mu + cg_j + aod_k + brr_l + a_i + e_{ijkl} \quad (1)$$

$$y_{ijkl} = \mu + cg_j + aod_k + brr_l + m_i + e_{ijkl} \quad (2)$$

where μ is the overall mean, cg_j is the j^{th} contemporary group based on the interaction of flock, birth year, cohort and pen, aod_k is the effect of the k^{th} age of dam (2, 3, 4+), brr_l is the effect of the l^{th} birth/rear rank group (1/1+, 2/2, 2+/1, 3/2, 3+/3+), y_{ijkl} denotes the feed efficiency trait (MidIntake, MidLWT, RFI), $m_i \sim N(\mathbf{0},\sigma_m^2\mathbf{M})$, $a_i \sim N(\mathbf{0},\sigma_g^2\mathbf{G})$, $e_{ijkl} \sim N(\mathbf{0},\sigma_e^2\mathbf{I})$, **M** denotes the MRM, **G** denotes the GRM and **I** is the identity matrix. We refer to the microbial values, m_i, as the "RMC feed efficiency trait" since it provides an estimate of the feed efficiency trait y_{ijkl} (MidIntake, MidLWT, RFI) from the RMC profiles. Predictions of the microbial values ($\hat{m}_i$) and the animals direct genomic breeding values ($\hat{a}_i$) were made for the animals in flock 4 (validation set). Prediction accuracies

were computed as the correlation between $\hat{m}_i$ or $\hat{a}_i$ and the adjusted phenotype (y_i^*) defined as the residuals from the linear model:

$$y_{ijkl} = \mu + cg_j + aod_k + brr_l + e_{ijkl} \quad (3)$$

fitted using both the training and validation sets. The microbiability (the proportion of variance of feed efficiency trait explain the RMC profiles) was computed as $\hat{\sigma}_m^2/(\hat{\sigma}_m^2 + \hat{\sigma}_e^2)$, and the heritability was computed as $\hat{\sigma}_g^2/(\hat{\sigma}_g^2 + \hat{\sigma}_e^2)$ using all 1298 animals from both the training and validation sets.

To assess the heritability and genetic correlation of the FIF and RMC feed efficiency traits for the validation animals, a bivariate model of the form:

$$\hat{m}_i = \mu_1 + a_{1i} + e_{1i}$$
$$y_i^* = \mu_2 + a_{2i} + e_{2i}$$

was fitted using only the animals from the validation set, where μ_1, μ_2 are the overall means, $a_{1i} \sim N(\mathbf{0},\sigma_{1g}^2\mathbf{G})$, $a_{2i} \sim N(\mathbf{0},\sigma_{2g}^2\mathbf{G})$, $e_{1i} \sim N(\mathbf{0},\sigma_{1e}^2\mathbf{I})$, and $e_{2i} \sim N(\mathbf{0},\sigma_{2e}^2\mathbf{I})$. All models were fitted in ASREML v4.2 (Gilmour *et al.* 2015). The estimated heritability was computed as $\hat{\sigma}_{1g}^2/(\hat{\sigma}_{1g}^2 + \hat{\sigma}_{1e}^2)$ for the RMC traits and $\hat{\sigma}_{2g}^2/(\hat{\sigma}_{2g}^2 + \hat{\sigma}_{2e}^2)$ for the FIF traits.

RESULTS AND DISCUSSION

Prediction accuracies of the feed efficiency traits for each birth year of flock 4 and overall from RMC profiles and host genomics is given in Table 2. The RMC profiles yielded higher accuracies for the individual cohorts for all three traits compared to host genomics with accuracies ranging between 21% and 42%. These accuracies were similar to those observed for methane traits predicted form RMC profiles in sheep (Bilton *et al.* 2022). The microbiability estimates, which ranged between 0.41 and 0.68, were also larger than the corresponding heritability estimates for all traits when computed using both the training and validation animals.

Table 2. Prediction accuracies for feed efficiency traits predicted from RMC profiles (M) and host genomics (G) for the animals in flock 4

Trait	Model	Equation	Accuracy			Microbiability	Heritability
			b19	b20	b19 & b20	(All; n=1298)	(All; n=1298)
MidIntake	M	1	0.410	0.257	0.316	0.68 ± 0.06	
	G	2	0.096	0.145	0.123		0.34 ± 0.06
MidLWT	M	1	0.312	0.210	0.244	0.41 ± 0.08	
	G	2	0.230	0.101	0.163		0.39 ± 0.06
RFI	M	1	0.417	0.220	0.313	0.54 ± 0.07	
	G	2	-0.047	0.058	0.007		0.32 ± 0.05

Table 3 reports the genetic parameter estimates from the bivariate analysis using the validation animals from flock 4. Heritability estimates for feed efficiency from FIF were slightly higher than previous reported (Johnson *et al.* 2022) and roughly double the heritability estimates of the equivalent RMC feed efficiency trait. The genetic correlation between the FIF and RMC feed efficiency traits were moderate at 0.64 (MidIntake) and 0.46 (RFI), while the phenotypic correlations were lower at around 0.32 (MidIntake) and 0.30 (RFI). These results are very similar to those reported by Bilton *et al.* (2022) for methane traits, except that the genetic correlations are lower for the feed efficiency traits. A bivariate analysis for MidLWT trait was also performed but the heritability estimate for the RMC trait was close to zero and so the results are not reported here. Nevertheless, these results suggest there is potential for using RMC profiles as a proxy for feed efficiency traits, although the small number of animals used in this study means follow-up studies are needed to confirm these results.

Table 3. Heritability, genetic and phenotypic correlations and phenotypic variances from a bivariate analysis of feed efficiency measured from the FIF and predicted from RMC profiles using flock 4 validation animals

Parameter	MidIntake		RFI	
	FIF	RMC	FIF	RMC
Heritability	0.44 ± 0.16	0.15 ± 0.11	0.45 ± 0.14	0.26 ± 0.13
Phenotypic variance	76785 ± 6610	7772 ± 606	19166 ± 1625	1094 ± 87
Genetic correlation	0.64 ± 0.30		0.46 ± 0.26	
Phenotypic correlation	0.33 ± 0.05		0.30 ± 0.05	

CONCLUSION

Our results provide evidence that microbial predictors are a suitable proxy for feed efficiency. As determining feed efficiency in ruminants via direct phenotypic measures is difficult and expensive, RMC profiles provide opportunities for ranking animals based on their feed efficiency for application in breeding programs.

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