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PROXIES IN PRACTICE: IMPLEMENTING RUMEN METAGENOMIC PROFILES FOR GENOMIC SELECTION OF METHANE EMISSIONS

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SUMMARY

Selective breeding for low-methane ruminants is a viable solution for reducing agricultural greenhouse emissions, but requires phenotypic measures of methane. Methods for obtaining on-farm methane emission measurements are available but are expensive and laborious, whereas proxy traits may provide a more efficient and cheaper alternative. A rumen metagenomic profile (RMP) has recently been shown to be one suitable proxy for methane, as it is both predictive of, and genetically correlated with, methane-related traits. We describe a two-step approach for using RMP as a proxy trait to generate genomic breeding values of methane related traits from portable accumulation chambers (PAC). We illustrate the utility of this approach on a commercially reared sheep flock. Our sequence-based, low-cost approach is extremely robust and could be extended to other ruminants (i.e., cattle) and would have greater utility for large ruminants where measuring methane emissions is more difficult compared to sheep.

INTRODUCTION

The global livestock industry is actively developing mitigation tools to reduce methane emissions from ruminants to meet international agricultural greenhouse gas reduction targets. One mitigation tool that is currently available for industry is selective breeding but requires on-farm measures of methane, but obtaining direct on-farm measurements of methane emissions is expensive and laborious. One measurement technology that has successively been used commercially on-farm is portable accumulation chambers (PAC) with over 5,000 commercial measures being collected per year in sheep across New Zealand (Archer *et al.* 2023). However, infrastructure constraints and logistic challenges are limiting access for breeders and farmers to measure their flocks using PAC. Proxy traits can provide an inexpensive alternate that enable a larger number of phenotypic methane measures to be collected. One proxy that has previously been shown to be predictive of and genetically correlated with PAC methane traits is RMP (Bilton *et al.* 2024). This study builds on Bilton *et al.* (2024) by presenting an approach for incorporating RMP information into the generation of PAC methane breeding values that can be used in a selection index for breeding.

MATERIALS AND METHODS

Experimental animals and protocols applied in this study were approved by the AgResearch Ruakura (Hamilton, NZ) Animal Ethics committees (application number 2383).

Animals and phenotypes. This study used 13,748 animals born between 2019 and 2023 from a commercial New Zealand sheep flock where all animals had been successfully genotyped (Table 1) and performance traits recorded. Methane emissions of a subset of these animals (1364 across all birth years) were measured using PAC at approximately 7-9 months of age following the standard protocol for PAC (Jonker *et al.* 2020). PAC data and additional animal information and measurements were downloaded from the Sheep Improvement Limited database (Newman *et al.* 2000). The PAC methane trait used in this study was methane emissions in grams per day (CH_4).

Table 1. Number of animals and phenotypes by year of birth

Phenotype(s)	Birth Year					Total
	2019	2020	2021	2022	2023	
PAC only	163[1]	2	236	252	233	886
PAC + RMP		250			228	478
RMP only					747	747
Neither	3118	2649	2657	2032	1181	11,637
Total	3281	2901	2893	2284	2389	13,748

[1] Born 2019 animals had two rounds of PAC measurements, where measurements were taken two weeks apart

Rumen sampling & metagenomic profiles. Rumen samples were collected from 975 born 2023 rams at approximately 6 months of age via stomach intubation across two days of sampling. Animals were removed from feed in morning and afternoon batches, where each batch of animals was sampled over a five-hour period by two teams of technical staff when the animals were between 1 hour and 6 hours off feed. Rumen samples were preserved using the TNx2 solution (Budel *et al.* 2022) and sequenced using restriction enzyme-reduced representation sequencing (Hess *et al.* 2020) with *Pst*I across multiple libraries. Libraries were sequenced with single end reads (1x100) on an Illumina NovaSeq6000 generating 26 Gb of data per 368 sample library. 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) and a cohort adjustment based on year of birth, time off feed group (hour intervals) and sample date was applied. An additional 250 animals (born 2020) also had RMPs that formed part of the dataset of the Bilton *et al.* (2024) study. A microbial relationship matrix (MRM) was constructed using the 4585 animals from the Bilton *et al.* (2024) study (that also had PAC and genotype data) plus the 975 rams born 2023 from this study using the approach described in Hess *et al.* (2020).

Animal genotyping. All animals were genotyped using a variety of nested SNP chips, where 13,232 common SNPs across all chips were retained for analysis. The software KGD (Dodds *et al.* 2015) was used to construct a genomic relationship matrix (GRM) based on VanRaden method 1, where each pairwise relationship was computed using only SNPs with non-missing data.

Statistical analysis. Predictions of methane from RMP for the 975 born 2023 rams were performed using a linear mixed model approach. The model used was of the form:

$$y_{ijkl} = \mu + cg_j + aod_k + brr_l + bdev_i + m_i + e_{ijkl} \qquad (1)$$

where μ is the overall mean, cg_j is the j^{th} contemporary group based on the combination of flock, birth year and sex, 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+), $bdev_i$ is the birth deviation from the flock and birth year mean, y_{ijkl} denotes the trait (CH_4), $\mathbf{m} \sim \mathrm{N}(\mathbf{0},\sigma_m^2\mathbf{M})$, $\mathbf{m} = (m_1,\ldots,m_n)'$, $\mathbf{e} \sim \mathrm{N}(\mathbf{0},\sigma_e^2\mathbf{I})$, $\mathbf{e} = (e_{1jkl},\ldots,e_{njkl})'$, $\mathbf{M}$ denotes the MRM, and $\mathbf{I}$ is the identity matrix. The microbial values, m_i, are referred to as the "RMP methane trait" as it provides an estimate of y_{ijkl} after adjusting for fixed effects. Model (1) was fitted on a training set consisting of all 4,585 animals from the Bilton *et al.* (2024) study and predictions of the microbial values ($\widehat{m}_i$) were made on the 975 born 2023 rams with RMP profiles.

To incorporate RMP information into the breeding values for the PAC methane trait, a bivariate model of the form:

$$\mathbf{y^*} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{e} \qquad (2)$$

was used, where $\mathbf{y^*} = (\widehat{\mathbf{m}},\mathbf{y})'$, $\mathbf{X} = ((\mathbf{X}_1,\mathbf{0})',(\mathbf{0},\mathbf{X}_2)')$, $\mathbf{b} = (\mathbf{b}_1,\mathbf{b}_2)'$, $\mathbf{Z} = ((\mathbf{Z}_1,\mathbf{0})',(\mathbf{0},\mathbf{Z}_2)')$, $\mathbf{u} = (\mathbf{u}_1,\mathbf{u}_2)'$, $\mathbf{e} = (\mathbf{e}_1,\mathbf{e}_2)'$, $\mathbf{X}_1$, $\mathbf{X}_2$ and $\mathbf{Z}_1$, $\mathbf{Z}_2$ are the incidence matrices for the fixed and random effects respectively, $\mathbf{b}_1$, $\mathbf{b}_2$ and $\mathbf{u}_1$, $\mathbf{u}_2$ are vector of fixed and random effects respectively, and $\mathbf{e}_1$, $\mathbf{e}_2$ are residual error terms. The model assumes that $\mathbf{u} \sim \mathrm{MVN}(\mathbf{0}, \mathbf{G}\otimes\mathbf{B})$, where $\mathbf{G}$ denotes the GRM and $\mathbf{B} = ((\sigma_{u1}^2,\sigma_{u12})'$,

$(\sigma_{u12},\sigma_{u2}^{2})')$, and $\mathbf{e} \sim \mathrm{MVN}(\mathbf{0}, \mathbf{I}\otimes\mathbf{C})$ where $\mathbf{C} = ((\sigma_{e1}^{2},\sigma_{e12})',(\sigma_{e12},\sigma_{e2}^{2})')$. Model (2) was fitted in ASReml 4.2 (Gilmour *et al.* 2015) using the qualifier "!BLUP 3" with prespecified variance and covariance values of $\sigma_{u1}^{2} = 0.243$, $\sigma_{u2}^{2} = 0.469$, $\sigma_{e12} = 0.242$, $\sigma_{e1}^{2} = 0.417$, $\sigma_{e2}^{2} = 1.802$, $\sigma_{e12} = 0.252$, which were obtained from the forward prediction analysis in Bilton *et al.* (2024). In addition, the fixed effects cg_j, aod_k, brr_l, and $bdev_i$ were included in $\mathbf{X}_2$ whereas $\mathbf{X}_1$ only contained the overall mean. The bivariate model was fitted using all 13,748 animals from the flock (Table 1) under four scenarios of differing levels of phenotypic information. These were (a) PAC data from born 2019 to 2022 (PAC (-b23)), (b) scenario (a) plus the RMP methane trait (PAC (-b23) + RMP), (c) all available PAC data (PAC all), and (d) all available PAC data and the RMP methane trait (PAC (all) + RMP). The genomic estimated breeding values (gEBVs) for the PAC methane trait ($\hat{\mathbf{u}}_2$) were extracted and examined across the four different scenarios.

RESULTS AND DISCUSSION

The pairwise comparison of the gEBV values for the 975 rams is shown in Figure 1a across the four scenarios with different phenotype information available for the born 2023 rams. The correlation between gEBVs with no phenotype measures (PAC (-b23)) with the gEBVs including the PAC measures (PAC (All)) for the born 2023 rams was 0.7, which was larger than the gEBV correlation of 0.63 between using only RMP (PAC (-b23) + RMP) with only PAC (All). However, gEBVs generated using only RMP (PAC (-b23) + RMP) or only PAC (PAC All) for the born 2023 rams had a similar correlation (0.87-0.89) to the gEBVs generated using all the phenotypes (PAC (All) + RMP). Here, we are assuming PAC (All) + RMP provides the best gEBVs as it has the most phenotype information included.

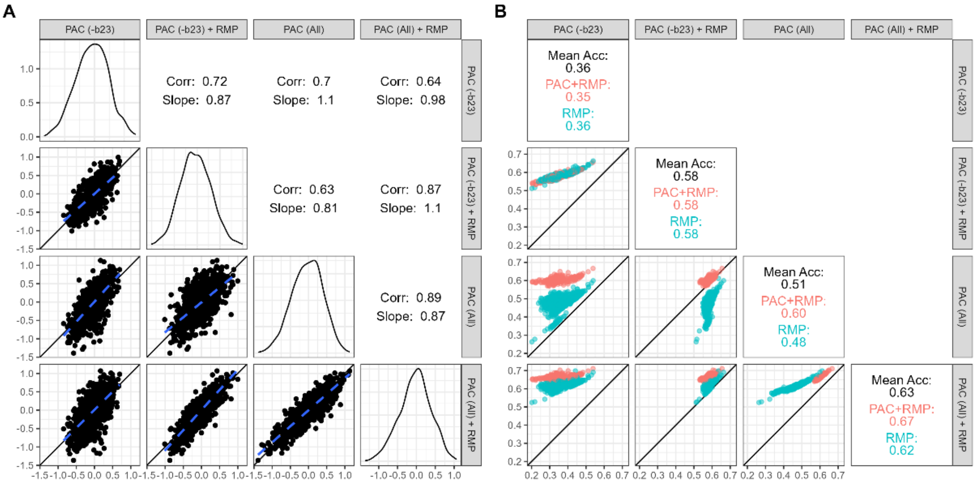

Figure 1. Pairwise comparison of gEBVs (A) values and (B) accuracies computed from the bivariate model using ASReml for the 975 born 2023 rams under four different scenarios of phenotype information available. The four scenarios are PAC data from born 2019 to 2022 (PAC (-b23)), PAC data from born 2019 to 2022 plus the RMP methane trait (PAC (-b23) + RMP), all available PAC data (PAC all), and all available PAC data and the RMP methane trait (PAC (all) + RMP). For plot A, the black solid line is the line of identity, the blue dashed line is the regression line, and the upper diagonals contain the correlation (Corr) and regression slope (Slope). For plot B, points are coloured based on if a ram only had a RMP (blue) or both a PAC and RMP measure (red), and the diagonals provide the mean accuracies.

The gEBV accuracies computed from ASReml are given in Figure 1B and show that using RMP information without PAC measures improves the mean gEBV accuracies from 0.36 (PAC (-b23)) and from 0.48 (PAC (All)) to 0.58 (PAC (-b23) + RMP) for the born 2023 rams with no PAC measures but were slightly lower than the mean accuracy of 0.62 when PAC measures were included (PAC (All) + RMP).

These results indicate that RMP profiles would greatly improve gEBV accuracy for animals without PAC measures but only modest improvement for animals with a PAC phenotype. Hence, RMP could be used as an alternative method for obtaining PAC measures, enabling larger numbers of animals to be phenotyped across the industry. Nevertheless, a sufficient number of animals would need both a RMP and PAC measure for training and calibrating the prediction models.

CONCLUSION

In this study, we have provided a strategy for incorporating RMP information into the generation of gEBVs for PAC methane traits based on using a two-step bivariate modelling approach. RMP could provide farmers with another option for generating phenotypic methane measures to breed for low methane animals that is lower cost (approximately \$60NZD per animal) compared to PAC (\$100NZD per animal). This would facilitate obtaining phenotypic measures of methane emissions for large number of animals and potentially enable faster genetic progress for methane traits.

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