Phenotyping sheep in a portable accumulation chamber and using devices with different accuracies measured the same methane trait

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Abstract

Across countries and ruminant species, animal selection has been identified as a desirable method of reducing methane (CH_4) emissions. A possible strategy is to develop a reference population to enable genomic selection for emission traits. However, the high cost and slow throughput of phenotyping make it challenging to rapidly collect sufficient information for publishing CH_4 breeding values in sheep. This project intends to measure methane emissions from 10,000 animals.

The most common method of measuring CH_4 emissions from sheep is with a portable accumulation chamber (PAC) where CH_4, carbon dioxide (CO_2) and oxygen (O_2) are measured at a mid-point (20 or 25 minutes) and end point (40 or 50 minutes) in the PAC. Two measurement devices can be used, referred to as FID (for CH_4) and FoxBox (for CO_2 and O_2); a third device known as an 'Eagle' can measure CH_4, CO_2, and O_2. The Eagle device is both cheaper and simpler to use. However, there are concerns about the lower sensitivity and precision of the Eagle compared to FID and FoxBox. The aim of this study was to compare both the duration of measurement, and the devices used to measure methane traits in sheep.

Data from 3,729 lambs and ewes were fitted with a bivariate animal model for methane rate (mL/min) from different measurement durations or measured with different devices. The following significant fixed effects were fitted for Site.Day.Run, birth and rearing type, age, age of dam, sire breed, and sex. Estimates of heritability of CH_4 ranged from 0.15 to 0.19 and were not significantly different between CH_4 measurement device or measurement duration. The genetic correlation for CH_4 measured using FID or Eagle was 0.96 for the short duration and equal to one for the long duration, and the phenotypic correlation between the two devices was 0.94 for the short duration and 0.97 for the long duration. The genetic correlation for CH_4 measured at 20-min and 40-min was equal to one for both measurement devices, with a phenotypic correlation of 0.80 when CH_4 was measured with the with the Eagle and 0.82 when measured with the FID.

Among other factors, the accuracy of genomic prediction depends on the heritability of the trait and the number of animals measured. We used the heritability of CH_4 according to different measurement methods to predict accuracy of genomic prediction. Assuming a heritability of 0.17 from the Eagle long measurement and 10,000 animals measured, gave an accuracy of genomic prediction of 0.42. We assumed that shortening the measurement time from 40 mins to 20 mins would allow 40% more animals measured

(14,000 total), which resulted in a accuracy of genomic prediction of 0.48. We found that the heritability was not significantly different between measurement durations, however if it were lower for the shorter measuring period (0.15) the accuracy of genomic prediction would be 0.45.

By reducing the measurement duration with the PAC methodology, there is limited or no loss of precision indicated by heritabilities that are not significantly different. The time saved with shorter measuring periods can be used to phenotype more animals or reduce labour costs. The overall benefit is a lower cost per animal with potentially more animals measured and an overall increase in accuracy of genomic prediction. However more clarity is needed regarding how many additional animals can be recorded with shorter measurement durations.

Keywords: methane, sheep, small ruminant, phenotyping, protocol.
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Introduction

Globally, genetic selection for lower methane has been identified as a promising method of reducing the contribution ruminant species have on greenhouse gas output. Over the past two decades, several Australian studies measured methane (CH_4) on over 7,000 sheep and reported estimates of heritability (0.11 to 0.18) for various methane traits (Robinson *et al*., 2014, Goopy *et al*., 2016, Paganoni *et al*., 2017, Wahinya *et al*., 2022, Sepulveda *et al*., 2022). Additionally, selection line experiments in New Zealand have demonstrated genetic selection does lower methane production (Rowe *et al*., 2019). One of the main challenges to an industry-wide implementation of methane selection is the publication of reliable breeding values for a methane trait as the trait is currently not measured by breeders. A possible strategy is to develop a reference population to enable genomic selection for emission traits, but rapid collection of sufficient phenotypes for this purpose is not easy.

The majority of sheep production is based on pasture systems. Measuring many animals for methane output in pasture production systems is a challenge. One method used in sheep to measure methane production is the use of portable accumulation chambers (PACs). The use of PACs has improved the feasibility of measuring large numbers of sheep. Each PAC chamber is an airtight box, a sheep is placed inside the box for a period of time (less than one hour), the methane (CH_4), carbon dioxide (CO_2), and oxygen (O_2) concentrations are measured at multiple time points during the PAC occupation. As the volume of the box is known, the gas concentration accumulated over the measurement duration can be converted to methane rate (ml/min). Across studies and between protocol methods, different gas measuring devices have been used, it is important to determine if the different devices are measuring the same trait, if the various datasets are to be used in the same genetic evaluation. The typical protocol requires a large amount of experienced and technical labour, and throughput is limited by the number of chambers and the occupation duration.

Simplifying the protocol by using cheaper and easier to use devices, could reduce the cost of measurements. However, cheaper and easier to use devices tend to have lower precision and can lower the accuracy of measurements. Additionally, the amount of time within a PAC chamber could potentially be reduced. While this would lower the accuracy of the measurement, it would allow for additional animals to be measured and/or reduce labour costs per animal measured. Reducing the accuracy either with less accurate devices or shorter measurement durations, will also lower the heritability for the same trait. It is important that the sheep industry is provided with accurate breeding values for methane, both heritability and number of animals phenotyped affect the

accuracy of genomic prediction. It is therefore important to investigate how potential changes to protocol will change the accuracy of genomic prediction.

This work aimed to demonstrate that reducing measurement duration, may decrease the accuracy of measurements, but the reliability of genomic prediction would increase, as more animals could be phenotyped. An additional objective of this analysis, was to determine if different measurement devices are measuring the same methane trait. This would help determine the feasibility of including all 17,000 records from the current and historic projects in a single genetic evaluation, especially as the historic data did not have access to the recent developments in measurement technology. This could allow the current protocols to be simplified by reducing the number of gas measuring devices and thereby reducing labour intensity.

Material and methods

Data collection

Methane phenotyping

Between March 2022 and February 2024, CH_4, CO_2, and O_2, was measured on 3,769 sheep across seven sites (Four research sites and three industry breeder flocks) in New South Wales (NSW), Australia. At one of the research sites, 501 lambs were measured in 2022, and another 504 lambs in 2023, all other sites measured mixed aged ewes and were only visited once. At each site, up to a maximum of 84 animals were measured each day, with the aim of phenotyping 500 sheep over consecutive days. Animals were placed in a holding paddock near to the site of PAC measurements, with access to feed and water. Animals were measured in up to seven batches (six batches per day is the current standard practice) across twelve PACs. The 12 chambers were occupied in a staggered order with animals taken off feed one hour earlier. The measurement of 12 sheep constituted one run, and after allowing air circulation the protocol was repeated with a new run of 12 sheep. The gases were measured within seconds of the set times, at a mid-point 20 minutes (25 minutes for lambs) and again at the end point 40 minutes (50 minutes for lambs). After the end point measure, the animal was released from the PAC. For each chamber, the three gases were measured using both the Eagle-2 device (Eagle) and a combination of FID (CH_4) and FoxBox (CO_2 and O_2) devices, hereafter this combination will be referred to as FID. The historic data (not used in this study) only measured with FID.

Genetic parameter estimation

Univariate animal models with restricted maximum likelihood (REML) were used to estimate all variance components, using WOMBAT (version 2022). The model can be summarised with matrix notation:

$$y = Xb + Za + e \quad (1)$$

Where y is a vector of trait observations, for methane (CH4). Four traits were considered, being different measures of methane output: mid-point (20min for ewes, 25min for lambs) and end-point measure (40min for ewes, 50min for lambs) and measured with either the Eagle or the FID. Only animals with both Eagle and FID measurements at both time points were included. Due to differences in means and variation between sites, each methane rate was centred to the site mean and standardised by site standard deviation. The matrices X and Z are incidence matrices associated with the fixed effects vector b (Site.Day.Run, birth and rearing type, age, age of dam, sire breed, and sex), and the vector of random additive genetic effects a ~ $(0,Hs^2_a)$, respectively. Heritability and genetic and phenotypic correlations between related pairs of methane

traits, i.e. between the two measurement devices and between the two time points, were estimated in bivariate analyses.

Power calculations

Different measurement protocols may result in different trait heritabilities and numbers of animals measured and these parameters were used to predict the accuracy of genomic prediction. The first equation of Daetwyler *et al*. (2008), was used for this purpose

$$r_{g\hat{g}} = \sqrt{\frac{\lambda h_o^2}{\lambda h_o^2 + 1}} \quad (2)$$

Where, λ is the ratio (Me/T) of number of observed phenotypes (*T*) to the number of effective chromosome segments and h^2 is the heritability. Assuming M = 50,000 SNP markers, Ne is effective population size = 150, L is average chromosome length of 1 Morgan and k is the number of chromosomes = 27, such that the effective number of chromosome segments was 8,100, calculated as Me = 2NeLk. The heritabilities used were from the genetic parameters estimates from the univariate analyses. The number of animals tested was based on measurements to be made during this project (10,000 animals), if historic data can be included (17,000 animals), if at least 2,500 animals are measured every year after the conclusion of this project (29,500, total animals after 5 years), and the previous scenarios repeated if an additional 40% more animals could be recorded with time saved with short measurement durations (14,000 with current project, 21,000 with historic added, 38,500 with future measurements).

Results and discussion

Phenotypic comparison

The relationship between short measurements (20, and 25 minutes) and long measurements (40 and 50 minutes) was very strong for both Eagle and FID devices. (Figure 1). This is an indication that the methane production during the time in the PAC is relatively constant. It also suggests the duration of measurement in the PAC could potentially be decreased. Historic projects used a range of measurement durations, these results suggest that the current and historic datasets could be combined regardless of the measurement duration used.

The relationship between measurement devices (Eagle and FID) was also very strong for both the short measurement and long measurement durations (Figure 2). The reason for measuring with both devices in this project, was to ensure that the Eagle

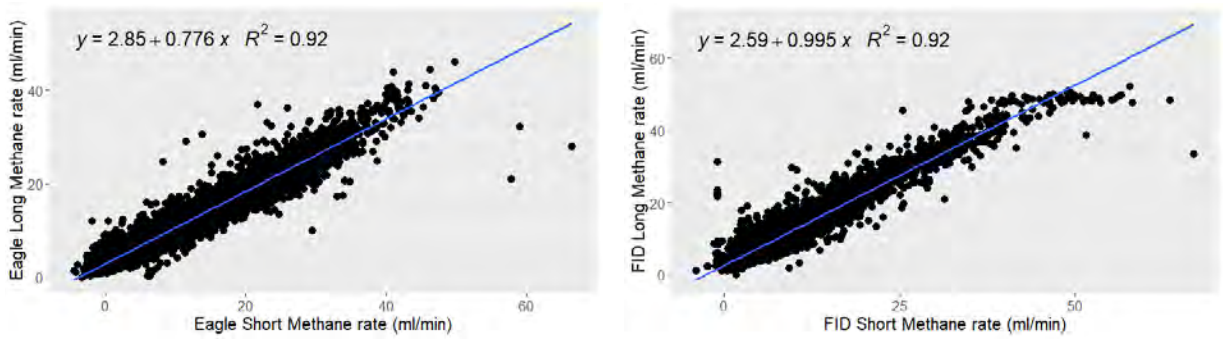

Figure 1. Relationship between methane rate (ml/min) measured with a short duration (mid-point 20 or 25 minutes) and long duration (end-point 40 or 50 minutes) with either the Eagle (Left) or FID (Right).

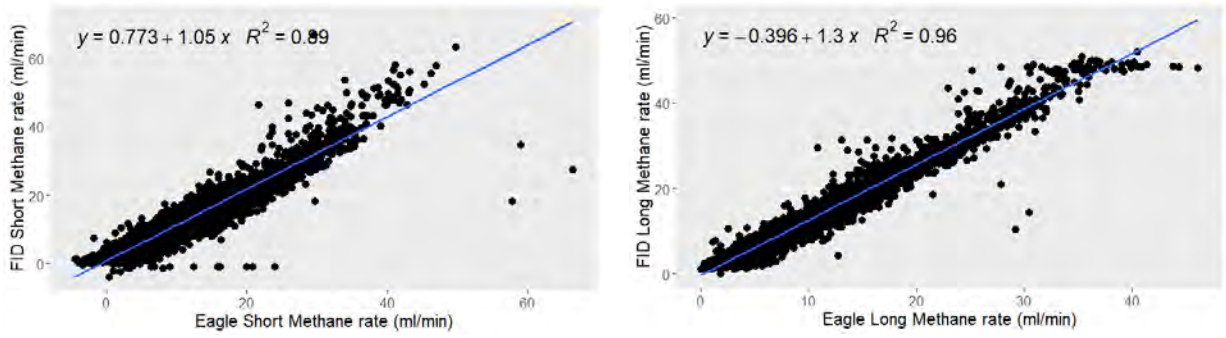

Figure 2. Relationship between methane rate (ml/min) measured with either Eagle or FID for a short duration (20 or 25 minutes) (Left) and long duration (40 or 50 minutes) (Right).

device was measuring the same trait as the historic data which used the FID. These results support that both devices are measuring the same trait. Only measuring with one of the devices for the remainder of the project could simplify the protocol, reduce labour, and consumable costs.

Genetic parameters

The variance components and estimates of heritability (0.15 to 0.19) were not significantly different regardless of measurement duration or measurement device (Table 1). The Eagle device tended to have lower estimates of heritability, the shorter measurement durations also tended to have lower estimates of heritability. This could be due to the lower sensitivity of the Eagle device, and the higher precision of the FID capturing more variation between animals, similarly the longer measurement duration allows for more variation to be captured. The genetic correlation between measurement durations was not different from one, with phenotypic correlations of 0.80 ± 0.01 (Eagle) and 0.82 ± 0.01 (FID). This indicates that the measurement durations are genetically the same trait. Furthermore, the genetic correlation between Eagle and FID was 0.96 ± 0.02 for the short duration and not different to one for the longer durations, indicating that the two devices are also measuring the same trait. This suggests that the protocol could be simplified by only measuring with the Eagle. This also implies that datasets with measurements with different devices or with different measurement durations could be combined in a single genetic evaluation. This provides the confidence that the historic data only measured with the FID is measuring the same trait as more recent projects that use the Eagle, and that future projects only need to use the Eagle. Further investigation which includes both recent and historic datasets is needed.

Table 1. Parameter estimates (σ^2_e, residual variance; σ^2_a, , additive variance; h^2, heritability) for methane traits recorded with the Eagle and FID.

Trait (ml/min)	N	$\bar{x}$ ± SD[1]	σ^2_e	σ^2_a	h^2
Eagle Short	3,729	0.00 ± 1.00	0.43	0.08	0.15 ± 0.04
FID Short	3,729	0.00 ± 1.00	0.44	0.09	0.18 ± 0.04
Eagle Long	3,729	0.00 ± 1.00	0.39	0.08	0.17 ± 0.04
FID Long	3,729	0.00 ± 1.00	0.40	0.09	0.19 ± 0.04

[1]Each site was centred to the mean and standardised by standard deviation.

Power calculations

If higher heritabilities with more accurate devices or longer recording periods are realised, higher accuracies of genomic prediction would be achieved by measuring more animals but with shorter measurement durations (Table 2). Assuming the protocol continues to measure with FID and the heritability is higher (0.19), an accuracy of genomic prediction of 0.44 would be achieved at the completion of this project (10,000 animals measured). However, if only the Eagle is used and a lower heritability of 0.17 is realised, the accuracy of genomic prediction will also be slightly lower at 0.42, and with the shorter measurement duration the accuracy would be 0.40 due to the lower heritability of 0.15. If the extra time from measuring for only 20 minutes was used to measure 40% more animals (14,000) the accuracy of prediction would be higher at 0.45. The current estimates of heritability are not significantly different, if we assumed the Eagle long and Eagle short both had a heritability of 0.17, the accuracy of genomic prediction is further increased for Eagle short to 0.48. This trend continues if historic data is added or with expected measurement goals in the future.

While the shorter protocols allow for more animals to be measured and to increase the accuracy of genomic prediction, it does not consider the logistical issues that come with phenotyping more animals. As it is not possible to retrospectively measure for longer durations it is recommended that the current protocol not be changed. However, labour is a key limiting factor and the shorter measurement duration would significantly reduce these costs per animal, if the extra time is not used to measure additional animals.

Table 2. Accuracy of genomic prediction using different protocols of recording.

Device	h^2	Duration	Number of animals	Accuracy of prediction
FID	0.19	40min	10,000	0.44
FID	0.19	40min	17,000	0.53
FID	0.18	20min	10,000	0.43
FID	0.18	20min	17,000	0.52
Eagle	0.17	40min	10,000	0.42
Eagle	0.17	40min	17,000	0.51
Eagle	0.17	40min	29,500	0.62
Eagle	0.15	20min	10,000	0.40
Eagle	0.15	20min	14,000	0.45
Eagle	0.15	20min	21,000	0.53
Eagle	0.15	20min	38,500	0.65
Eagle	0.17	20min	14,000	0.48
Eagle	0.17	20min	21,000	0.55
Eagle	0.17	20min	38,500	0.67

Conclusion

The largest challenge for prediction of breeding values for methane based on genomic testing is the phenotyping of enough animals to form a reference population. We demonstrated that measurement with the Eagle is sufficiently accurate to replace the FID and FoxBox devices. The amount of time each animal is in the portable accumulation chamber can be shortened to about 20 min without losing measurement accuracy and could be considered to allow phenotyping of additional animals.