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REPEATABILITY AND HERITABILITY OF DAIRY COW METHANE CONCENTRATION USING SNIFFER SENSORS

F. Almasi[1], S.R.O. Williams[2], C.J. Vander Jagt[1], L.C. Marett[2,3], J.L. Jacobs[2,3] and J.E. Pryce[1,3]

[1] Agriculture Victoria Research, Agribio, Bundoora, VIC, 3083 Australia
[2] Agriculture Victoria Research, Ellinbank, VIC, 3821, Australia
[3] La Trobe University, Bundoora, VIC, 3083, Australia

SUMMARY

Large-scale phenotyping of methane emissions in dairy cows is necessary for genetic selection, yet gold-standard methods such as respiration chambers and the SF_6 technique are expensive and impractical for broader implementation. In this study, 50 Arcoflex (Arcoflex International Pty Ltd) sniffer sensors were installed one per bay in a herringbone dairy facility, with one per day. Methane concentrations (100 to 5,000 ppm) were recorded every 10 seconds between March and November 2024. Data were gathered from 532 genotyped cows during both morning and afternoon milkings, with an average of 227 visits per cow. Methane concentration measurements were summarised with two methods: (1) an arithmetic mean of all observations per visit, and (2) a "peak" average, defined as data points that exceeded their immediate neighbours. Univariate linear mixed models in ASReml were then applied to estimate heritability (h^2) and repeatability (r). Overall, arithmetic means per visit averaged 445 ppm, whereas peak-based visits averaged 639 ppm. The highest heritability (0.12±0.02) was estimated for peak-based measurements at the per-visit level, while the highest repeatability (0.38±0.01) occurred when peak data were aggregated weekly. While these findings highlight the potential of sniffer sensors for large-scale methane phenotyping, more data from additional herds and farm settings are needed to further validate this approach.

INTRODUCTION

Methane is a potent greenhouse gas, and its accurate measurement is essential for developing effective mitigation strategies to address climate change. Notably, enteric fermentation in ruminants, contributes approximately of 6% of the entire global greenhouse gas emissions (Beauchemin *et al.* 2020), highlighting the importance of targeting this source for emission reductions. While gold standard methods such as respiration chambers and the SF_6 tracer technique provide high-quality data (Deighton *et al.* 2014), their high costs and labour-intensive nature limit their feasibility for large-scale application which is required for reliable genetic selection. Alternatively, sniffer sensors installed in milking parlours offer a cost-effective, though spot-sampling, approach to monitor methane emissions under practical farm conditions. This study aims to evaluate the repeatability and heritability of sniffer-based measurements under various scenarios to identify the most reliable method for large-scale methane monitoring in herringbone Australian dairy operations.

MATERIALS AND METHODS

Sensor Setup and Data Collection. Fifty Arcoflex sensors (Arcoflex International Pty Ltd) were installed in a herringbone dairy, with one per feed bin (Ellinbank SmartFarm, Victoria, Australia). The heated electrode sensors continuously recorded methane concentrations in parts per million (ppm) at ten-second intervals while cows were being milked. The sensors function within a measurement range of 100 to 5,000 ppm. Data were extracted using Arcoflex Data Exporter V1.7.1.0 from March 1 to November 30, 2024.

Each side of the milking parlour (left and right) had 25 bays with individual feed bins; once milking concluded on a side, the bins were raised, allowing all cows in those 25 bays to exit simultaneously. This setup ensured a clear pattern of entry and exit that facilitated consistent data capture throughout the study period.

For genomic estimation, we utilised the XST-74K genotype set. This set comprises approximately 74,000 single nucleotide polymorphisms (SNPs) and is a combination of AVR's (Agriculture Victoria Research) custom DairyBio XT-50K chip and the standard Illumina Bovine 50K chip. The integration of these two chips enhances the density and coverage of genomic data, providing a more robust foundation for prediction models (Van Den Berg *et al*. 2024).

Matching Methane Records to Individual Cows. Because the Arcoflex sensors were not linked to cow radio-frequency identification (RFID), a unique system was developed to associate methane concentration (MeC) readings with individual cows. The milking system captured each cow's National ID and milking order, as animals entered and exited each bay. These data were used alongside information from the milking parlour's gate system, which recorded the presence or absence of cows in the bays. By aligning the sequence in which cows entered the bays (entry order) with gate status (confirming occupancy), a unique identifier was generated for each cow during each milking session. This identifier provided a reliable link between the milk production data and the methane concentration measurements once the timestamps from both datasets were synchronised. Cross-checking these matched records ensured that methane concentration readings were correctly attributed to the right cow.

Data Cleaning. Prior to analysis, any cows receiving a methane mitigating feed additive(s) were excluded from the dataset. Records were also removed if the cow's days in milk (DIM) exceeded 450. Sensor records of 0 ppm were discarded because these often occur when an animal's head position is too far away from the sensor or when ambient methane concentrations are too low to be detected. Cows were milked twice daily, in the morning and afternoon, and recorded visits that were shorter than 2.5 minutes or longer than 25 minutes were also excluded (due to uncertainty of correct identified cow). The remaining visits had a minimum duration of 2.6 minutes, a maximum of 25 minutes, and a mean of 14.2 minutes (median 13.8 minutes). Although the sensors can measure up to 5,000 ppm, preliminary analyses indicated that values exceeding 3,000 ppm were rare and significantly skewed the distribution, therefore values above 3,000 ppm were removed.

Overall, 127,669 valid records remained (79% of total observations), with individual cows having a minimum of 1 visit, a maximum of 452 visits, and an average of 227 visits (median 226), over an average of 138 days. After all exclusions, data from 532 cows from 74 sires remained in the final dataset.

Statistical analysis. To determine the most repeatable time frame for MeC, 1,260 scenarios were created to examine repeatability estimates and compare Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) values. Each scenario varied in two ways: (i) window length of recording (from a minimum of 100 seconds to a maximum of 500 seconds) and (ii) start time of recording (ranging from 10 seconds to 1500 seconds after milking began). The response variable was the average MeC recorded per visit. A linear mixed-effects model was then specified, with milking session (morning vs. afternoon), bay number, and polynomial for both cow age (months) and days in milk (DIM) as fixed effects, and individual cows fitted as a random effect to account for repeated measurements within cows. Model estimation employed restricted maximum likelihood (REML) using the nlme package (Pinheiro *et al*. 2024) in RStudio (R Core Team 2023).

Based on the highest repeatability and lowest AIC and BIC values, the optimal interval was identified as second 90 to second 410, spanning 320 seconds. All subsequent analyses therefore focused on measurements collected within this time window, and data outside of it were excluded. Two averaging methods were then defined for further investigation: (1) calculating the simple arithmetic mean of all data points within the selected interval, and (2) identifying a "peak" within each visit (an observation exceeding its immediate neighbours) and computing the average of these.

Heritability and repeatability for each methane trait (average and peak average) were estimated using univariate linear mixed models in ASReml 4.2.1 (Gilmour *et al.* 2021). The general model is described by:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{Wc} + \mathbf{e}, \qquad (1)$$

where $\mathbf{y}$ is the vector of the response variable (either the average MeC or the average of MeC peaks). The vector $\mathbf{b}$ contains the fixed effects, which include the overall mean, bay number, milking session, and polynomial for both cow age (months) and DIM. In this framework, $\mathbf{u}$ represents the random additive genetic effects for each animal ($\mathbf{u} \sim N(0, \mathbf{A}\sigma_a^2)$) where A is the relationship matrix derived from the genotypes of 532 animals. The vector $\mathbf{c}$ denotes the random permanent environmental effects ($\mathbf{c} \sim N(0, \mathbf{I}\sigma_c^2)$) and $\mathbf{e}$ is the random residual effect ($\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2)$). Matrices X, Z, and W link observations to the corresponding fixed or random effects.

When data were summarised on a daily or weekly basis, the variables for bay number and milking session were removed from the model. This adjustment was made because the daily or weekly aggregated data no longer allowed for meaningful differentiation by bay or milking session.

Narrow-sense heritability (h^2) was calculated as the ratio of the additive genetic variance (σ_e^2) to the total phenotypic variance ($\sigma_a^2+\sigma_c^2+\sigma_e^2$). Repeatability was estimated by dividing the sum of the additive genetic (σ_e^2) and permanent environmental (σ_e^2) variances by the total phenotypic variance.

RESULTS AND DISCUSSION

Average-based measures recorded mean values of around 445 ppm, with SD ranging from 122 to 217 ppm (Table 1). By contrast, peak-based measures had higher means, approximately 639 ppm, and displayed larger SD (202 to 364 ppm), indicating greater variability in maximum emissions. In a study, Van Breukelen *et al.* (2022) reported a mean of 367 ppm, which is considerably lower than the 444 ppm observed here. This discrepancy may reflect differences in sensor technology and farm practices (e.g., automated milking systems versus herringbone). Additionally, Van Breukelen *et al.* (2022) attributed some of their lower measurements to sensor drift toward zero which was not observed in our data as the Arcoflex sensors do not detect values below 100 ppm.

The heritability estimates ranged from 0.03 to 0.12, depending on how visits were defined, with the highest value (0.12) achieved when peaks were averaged within each visit and the model included bay, milking session (morning vs. afternoon), DIM, and cow age as fixed effects (Table 1). This aligns relatively well with the 0.13 heritability reported by Van Breukelen *et al.* (2022) for mean MeC per visit. However, whereas Van Breukelen *et al.* (2022) observed a considerable increase in heritability (up to 0.32) when data were aggregated weekly, our analysis showed a decrease to 0.05. One likely explanation is the differing recording systems of automated milking systems (AMS) often rely on a single sensor for the entire herd, making the effect of "bay" irrelevant, whereas our herringbone setup employs multiple sensors, one per bay. Although 532 cows were phenotyped in this study, this sample size may not fully capture the genetic variation present in the broader population, suggesting future work should encompass more animals and potentially multiple herds to strengthen parameter estimates. While the bay effect was significant ($P < 0.05$), we chose not to pre-adjust for it prior to aggregating daily or weekly measurements. Instead, we accounted for bay effects directly in the model, which was feasible only at the per-visit level. Furthermore, in a separate analysis (unpublished), we observed that bays located closer to the shed entrance, where they were more exposed to wind, had lower repeatability than bays situated farther inside the facility. Consequently, pooling observations over time may have introduced additional, unaccounted-for variability at the bay level, lowering our heritability estimates for aggregated traits.

Table 1. Mean, variance components, repeatability, and heritability of MeC. V_A is additive genetic variance, V_{PE} is permanent environment variance, and V_P is phenotypic variance

Trait	**Mean±SD**	**V_A**	**V_{PE}**	**V_P**	**h^2±SE**	**r±SE**
Average	445±217	3484	3770	7254	0.09±0.02	0.19±0.01
Average of peaks	639±364	13442	9437	89213	0.12±0.02	0.2±0.01
Daily Average	444±188	1671	4180	35617	0.05±0.01	0.17±0.00
Daily Average of Peak	637±314	6480	10639	98669	0.07±0.01	0.17±0.01
Weekly Average	445±122	347	4652	14376	0.03±0.02	0.35±0.01
Weekly Average of Peaks	638±202	1888	12582	38501	0.05±0.02	0.38±0.01

The repeatability estimates in this study increased when methane measurements were aggregated over longer periods, reflecting how weekly averages can reduce day-to-day fluctuations in the data. Although the weekly repeatability values we obtained were lower than some previously published findings, the general pattern aligns with the results of Van Breukelen *et al.* (2022), who observed a repeatability of 0.30 per visit and 0.68 for weekly means. The rise in repeatability with temporal aggregation highlights the benefit of smoothing out short-term noise that can arise from factors such as cow movement or sensor proximity. However, in our herringbone system, the effect of bay-specific variability could no longer be accounted for once measurements were collapsed into weekly averages, potentially limiting the full advantage of data aggregation. In future work, we plan to correct for bay effects before aggregation to minimise unaccounted variability and improve the accuracy of weekly methane estimates.

CONCLUSION

This study revealed higher heritability and repeatability for peak-based measurements than for arithmetic means, suggesting that peaks may better capture genetic variation in methane output. From a practical standpoint, it remains to be established whether peak- or average-based approaches align more closely with total daily methane production and thus offer greater potential for mitigation through genetic selection. Overall, while these findings indicate that sniffer sensors can supply practical and sufficiently robust data, additional records from more cows and different herds are needed to further validate and refine methane measurement approaches in dairy operations.

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