

FACTORS AFFECTING METHANE PRODUCTION OF COMPOSITE AND CROSSBRED CATTLE GRAZING TROPICAL AND SUBTROPICAL PASTURES IN NORTHERN AUSTRALIA

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SUMMARY

Understanding the factors affecting variation in methane (CH₄) production rate among individual animals is an essential step in developing methane phenotypes to enable genomic selection for lower CH₄ emissions. GreenFeed units (C-Lock inc., Rapid City, SD, USA) are an increasingly popular method for recording methane production of grazing cattle. GreenFeed units take short-term breath measurements (of several minutes duration) when visited by cattle. Our aim was twofold 1) understand the factors associated with variation in CH₄ production records from grazing beef cattle across tropical and sub-tropical cattle grazing regions and 2) given these factors and frequencies of visitation, determine trial length necessary to derive accurate phenotypes for genomic prediction. In total 5 trials were conducted across 3 locations resulting in repeated measurements on 328 mixed sex cattle. Factors including test day, trial location, hour of visit and pipe temperature had a significant effect on CH₄ production. GreenFeed unit contributed a significant proportion of the total variation in methane emissions, across both intraday and daily methane production ($P < 0.05$). Testing the effect of trial length ranging from 5 and 52 days, showed that within-animal variation slightly decreased with repeated observations. However, the addition of cattle not previously recorded increased between animal variation, this slowed post 30 days. The optimal trial length balancing maximising cattle recorded, and repeated observations, was 28 days resulting in 20,440 visits from 324 cattle.

INTRODUCTION

Enteric fermentation from cattle accounts for 17 - 30% of CH₄ produced from human activities (Beauchemin *et al.* 2009). Methane production from cattle has been shown to be moderately heritable (Donoghue *et al.* 2016; Hayes *et al.* 2016), suggesting that selection for lower emitting animals to reduce emissions is possible. Collecting abundant and accurate phenotypic data on CH₄ is the initial phase in implementing genomic selection, however this has been very limited in tropical and sub-tropical environments. To address this, CH₄ production phenotypes have been collected utilising GreenFeed units (GFU) to measure CH₄ emissions of grazing cattle across both tropical and sub-tropical regions. The focus of this paper was to identify factors contributing to individual cattle CH₄ variation in tropical and sub-tropical grazing conditions and optimizing trial design by reducing length of future trials by focusing on reducing within animal variation whilst maximising visitation to GFU by cattle.

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MATERIALS AND METHODS

Background. Five trials were conducted across central and northern Queensland grazing regions between 25/7/2023 and 24/2/2024 to record CH₄ production from growing beef cattle. Trials were conducted in accordance with animal ethics approval from the University of Queensland animal ethics committee, Number; 2022/AE000657. The number of GreenFeed units per trial varied between 4 and 12 with cattle able to freely visit any of the units over the trial period. Units were clustered in groups of 4 towards the extremity of each trial paddock, typically close to water sources. Number of cattle per trial varied depending on available pasture and paddock area with the number of recorded trial animals varying between 36 and 127. Cattle were familiarised with the units over a period of 21 days preceding the initiation of CH₄ recording, this involved more frequent release of attractant and supplementary feeding in proximity to the GFU. Cattle that were observed repeatedly using the GFU were removed before being reintroduced after 5 days to allow other cattle to become accustomed to the units. Trials were conducted over varying periods between 28 and 51 days across the 3 trial sites all with varying pastures, soils and climatic conditions. Cattle were composite and mixed breeds representative of production animals suited to conditions. A full summary of the 5 trials is provided in Table 1.

Table 1. Summary data regarding trial location, length and cattle number, visitation

	T1	T2	T3	T4	T5
Trial location	Brian Pastures Research Station		Goldsborough Station	Spyglass Research Station	
Length (days)	51	45	35	32	28
Recorded cattle	36	40	127	68	62
Paddock area (ha)	44.47	48.05	693.51	115.07	115.07
Pre-trial weight (kg)	369 ± 30	358 ± 28	289 ± 30	197 ± 24	196 ± 19
Av. daily visits per animal (Max-Min)	5 (1-15)	7 (1-27)	4 (1-16)	4 (1-11)	4 (1-11)
Av. total visits per animal (Max-Min)	194 (35-292)	206 (39-355)	72 (6-184)	77 (14-144)	82 (6-135)

Statistical Analysis. Quality control resulted in the exclusion of observations with unknown radio frequency identification (RFID), cattle with less than five visits over each trial, observations with airflow rate below 20L/s and negative CH₄ values were removed. A linear mixed model was fitted to both CH₄ production per individual based on both intraday and averaged daily CH₄ production:

$$y = \mu + b + a + e \quad (1)$$

where y is the resulting vector of estimates for CH₄ production; μ is the mean; b is a vector of fixed effects including hour of record, visit duration, pipe temperature all nested within machine, total visitation and initial trial weight nested with in trial; a is the random animal effect, $\sim N(0, R\sigma^2)$, where $R\sigma^2$ is the between individual variance by test day; e is the random residual term. *Bos taurus indicus* (BI) percentage and sex (nested within trial) were excluded as non-significant factors contributing to CH₄ production. When averaging multiple visitations within the period of a day for an individual, all observations within that day were assigned the daily average CH₄ and were then merged with observational data within that day. Observations greater than 4 standard deviations of the mean of the residuals were excluded. Repeatability was calculated as between individual variance over the total variance as per equation 2:

$$Repeatability = \frac{\sigma_a^2}{(\sigma_a^2 + \sigma_w^2)} \quad (2)$$

where σ_a^2 is the between animal variance and σ_w^2 is the within animal variance. Proportion of variation for each factor was determined using the effectsize package (Ben-Shachar *et al.* 2020). Potential to decrease future trials was tested using an expanding window of additional test days between 5 and 52. Optimal trial length aimed to maximise the number of recorded cattle and observations.

RESULTS AND DISCUSSION

Most cattle made repeated visits within a 24hr period, to produce daily CH₄ estimates. Variation between intraday observations and daily averaged values were investigated to determine how the important of factors changed between the two measures, Table 2. High variation between intraday short-term breath measurements was observed which made predicting daily CH₄ production unreliable. Both repeatability (0.11) and the coefficient of determination (0.36) of the intraday breath measurements were lower than daily averaged data. This was due to considerable within animal variation observed in the intraday period.

Table 2 R² of model, within animal variance and between animal variance for methane measurements. Percentage of variance explained by factors in model, for factors P < 0.05

	Intraday	Daily
Cattle in estimates	328	328
Total observations	28746	28684
R ² of model	0.36	0.64
Within animal variance	1859.21	515.06
Between animal variance	239.42	276.84
Repeatability (CI)	0.11 (0.09-0.12)	0.31 (0.27-0.35)
<i>Percentage of variance explained</i>		
Hour of day	0.39	0.10
Duration	1.31	0.59
Initial weight	9.60	8.20
Pipe temperature	1.13	0.15
Total visitation	9.69	8.51
Trial	6.29	3.73
Machine ID	0.51	0.20

No relationship was found between CH₄ production and either BI percentage or sex ($P > 0.05$). Total visitation frequency and initial weight had the largest effect on CH₄ production ($P < 0.05$), in both intraday (9.7%, 9.6%) and daily (8.5%, 8.2%) respectively. Trial explained slightly less variation (6.3%, 3.7%). All factors explained less variation using the daily average method than intraday observations. Variance attributed to GFU based on machine ID was higher in the intraday period at 0.5% decreasing to just 0.2%, whilst remaining significant ($P < 0.05$) only a small proportion of variation is observed from different GFU. Inclusion of multiple GFU to ascertain and attribute variation is therefore necessary in trial design to ensure variation between machines is captured.

Between-animal variation was similar for both intraday and daily observations. However, within-animal variation was lower for daily averaged data. Repeatability for intraday data per individual is presented in Figure 1, repeatability was higher as a result. Repeatability trended upwards over the expanding period with high variability observed pre 20 days. Recorded cattle increased rapidly from only 211 at 5-day trial length to 305 after 14 days of recording, successfully recorded cattle increased slowly thereafter reaching 315 at 21 days.

Within-animal variation increased across the expanding trial periods but was highly variable and reflected visitation, cattle returning multiple times a day and for consecutive days had lower variation than cattle with less frequent visitation. Post 28 days only 4 additional cattle were recorded and 8,315 additional observations. Generally, repeatability could be improved by reducing within animal variation by more consistent and earlier visitation. Early adoption and consistent visitation could reduce trial length however ad libitum visitation necessitates longer trials. Dressler *et al.* (2023) reported that a minimum of 40 visits were required to produce a significantly correlated result as with 100 visits, this occurred at 29.5 ± 8.7 days. Our study shows that cattle make ~ 2 visits to GFU per day resulting in a mean of 40 visits falling earlier around day 20 however, recording for at least 28 days is recommended.

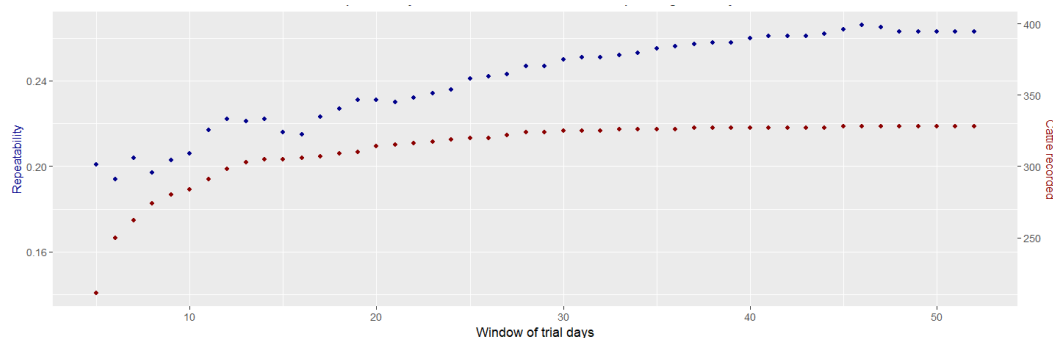


Figure 1 Total cattle recorded across an expanding number of trial days and the effect on repeatability

CONCLUSION

Total visitation and initial weight were significant and influential predictors of individual CH₄ emission estimates for both intraday and daily averaged data. Although duration of visitation, hour of visit and pipe temperature were also statistically significant predictors of CH₄ production, their proportional contributions to the model were comparatively smaller. Inclusion of multiple GFU per trial is suggested to observe between unit variation. A minimum trial length of 20 days is required to reduce variation in repeatability and produce approximately 40 repeated observations per animal. Based on the results from this study a slightly longer trial length of at least 28 days is suggested if the goal is to maximise the number of repeated observations and cattle recorded.

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