

Factors affecting recording methane emission phenotypes of composite and crossbreed beef cattle grazing tropical and subtropical rangelands of northern Australia

Cameron Whistler*, Kieren McCosker*, Christie Warburton*, David Johnston‡, Tim Grant§, Bradley Taylor§, Kerry Goodwin§, Melissah Dayman§, Natalie Scott†, Scott Cullen†, Milou H Dekkers†, Sam Clark#, Ben J Hayes*

*University of Queensland, QAAFI, Centre for Animal Science, Brisbane, QLD 4000, Australia

‡Animal Genetics and Breeding Unit, University of New England, Armidale, NSW 2351, Australia.

§Department of Agriculture and Fisheries, Brisbane, QLD 4000, Australia

†University of Queensland, Queensland Animal Sciences Precinct, Gatton, QLD 4343, Australia

#University of New England, Armidale, NSW 2351, Australia

Lay Summary

Rearing cattle for beef production has a considerable contribution to global human related methane emissions. Cattle can be selectively bred to reduce methane production, but this requires accurate measurement of current methane emissions at an animal level. This study recorded short term breath measurements utilizing GreenFeed units (GFU) (C-Lock, USA) a commercially available system to observe methane production from cattle across 7 trials at 4 sites in northern Australia. This paper, undertaken to enable a broader genetic study, identifies and quantifies the main factors influencing methane production from individual cattle grazing tropical and sub-tropical rangelands using GFUs, also considering some environmental and location differences. Several factors were identified that contributed to variance in methane production these included the specific GFU, visitation frequency, air temperature, initial weight and available forage. Accurate recording of methane from individual grazing animals on subtropical and tropical rangelands identifies differences in production and selection for lower methane production in these environments to be undertaken.

Teaser Text

Methane emissions from beef cattle production grazing tropical and subtropical rangelands contribute a substantial proportion of global anthropogenic methane emissions. Producing methane production phenotypes from cattle grazing tropical and subtropical rangelands is possible using GreenFeed units; however, several sources of non-genetic variation need to be considered, including available forage, weights and visitation frequency.

Abstract

Globally agricultural emissions contribute 10-12% of all anthropogenic greenhouse gas emissions. Ruminant livestock account for a considerable proportion of these emissions, with cattle grazing in tropical and sub-tropical regions representing a major source. Enteric methane production has climatic

implications and represents a loss of energy from the animal that could be directed towards growth. This study focuses on cattle grazing tropical and subtropical rangelands in Australia, aiming to identify the factors that influence recording of methane emissions in these environments. Short term methane production was recorded from 453 mixed breed cattle across 4 trial sites utilizing GreenFeed units (GFU) (C-Lock, USA). Data was generated and combined from several sources including GFU, weather data, forage samples and estimated forage biomass. Using a linear mixed model the significance and variance of each factor on methane production was evaluated. Factors identified as explaining most of the variation for methane production included GFU ($P < 0.001$), total visitation ($P < 0.001$), daily visitation ($P < 0.001$), trial ($P < 0.001$), hour of visitation ($P = 0.03$), air temperature ($P < 0.001$), initial weight ($P < 0.001$), visit duration ($P < 0.001$) and forage biomass ($P < 0.001$). Four factors were tested and found to be not significant in explaining variation in methane production, including *Bos taurus indicus* (BI) content ($P = 0.99$), sex ($P = 0.06$), rain ($P = 0.07$) and temperature heat index ($P = 0.2$). A moderate coefficient of variation (CV) of 23.2% was observed in the raw observations of methane production. This study highlights important factors to consider when utilising GFU for methane measurement of grazing beef cattle. This study provides a foundation for future studies aiming to quantify individual animal methane emissions for use in genetic evaluation programs, to reduce industry emissions and improve the sustainability of beef production in tropical and sub-tropical regions.

Key Words: beef cattle, enteric fermentation, GreenFeed, methane, sustainability

Abbreviations: CH₄, methane; CO₂, carbon dioxide; IW, weight at start of trial; EW, weight at end of trial; GFU, GreenFeed unit; RFID, radio frequency identification; BI, *Bos taurus indicus*; BT, *Bos taurus taurus*; RC, Respiration Chamber; SF₆, sulphur hexafluoride tracer gas; Scientific Information for Landowners, SILO; Australian Grassland and Rangeland Assessment System, Aussie GRASS.

Introduction

Globally, enteric methane from ruminant livestock is responsible for contributing ~17% of global methane emissions (Garnsworthy et al., 2019). Therefore, any reductions that can be achieved in the production of methane from cattle would considerably decrease global methane emissions and reduce contributions to global warming (Johnson and Johnson, 1995). Cattle produce methane as a result of degradation and fermentation of feed components by microbial communities in the rumen (Kittelman et al., 2014). Methane production also represents an energy loss of approximately 2-12% to the host animal (Johnson and Johnson, 1995). The type and quality of feed provided to ruminant livestock has been shown to affect methane production (McCaughey, Wittenberg and Corrigan, 1999). The majority of methane emissions in the beef supply chain are produced during grazing, primarily due to consumption of low-quality forages, with this phase accounting for 70% of sector's greenhouse gas emissions (Basarab et al., 2013). Tropical rangelands, which often consist of lower quality forages with lower digestibility, tend to lead to increased quantities of acetate in the rumen when grazed and subsequently increased methane production (Cunha et al., 2017).

Multiple strategies are necessary to substantially reduce the environmental footprint of beef and dairy production, including the selection of animals with lower methane emissions. A moderate heritability of 0.19 and 0.28 for methane production has previously been reported (Herd et al., 2014; Donoghue et al., 2015; Hayes et al., 2016), suggesting that sustained reductions in methane emissions can be achieved through consistent selection for low-emitting cattle. However, to effectively select cattle with lower methane emissions, it is essential to collect accurate phenotypes for many individuals under varying commercial grazing production systems and environments. The in-pasture observation of methane production from grazing animals has several challenges which need to be identified and managed to provide suitable phenotypic merit. GreenFeed units (GFU) (manufactured by C-Lock inc., Rapid City, SD, USA) provides a platform that enables the measurement of methane and carbon dioxide mass flux of individual cattle in a free-grazing setting.

High concordance has been observed in previous studies comparing methane production measured by both GFU and respiration chamber (RC). In both systems variation, was observed in methane production with dry matter intake (DMI) (Huhtanen, Ramin and Hristov, 2019). Higher variation was observed in consecutive visits to GFU compared to RC, causing greater between-animal and temporal variation likely due to restriction of animal grazing choice, time of grazing and foraging behaviours in recording observation using RC (Hegarty, 2013). Ad libitum visitation of cattle introduces more variability than RC but may produce more accurate methane production of grazing cattle across time.

Recording of methane production from beef cattle is already less common than in dairy cattle due to the ease of which recording can be undertaken. Due to this, considerable numbers of Holstien, Friesian cattle in the global north have been recorded (Difford et al., 2020; van Breukelen et al., 2022). The recording of beef cattle is generally undertaken within feedlot directly pre slaughter (Ryan et al., 2022; Crowley et al., 2024), or as part of performance recording, young cattle pre mating utilizing respiration chamber (Donoghue et al., 2016; Smith et al., 2022). These studies have been largely conducted in temperate regions and focus mainly on *Bos taurus taurus* (BT) cattle. Recently, some focus has shifted to methane production from grazing in tropical regions due to the large numbers of cattle in these regions, and forage differs in digestibility from those in temperate regions (Ku-Vera et al., 2020; Korir et al., 2022). The aim of this study was to assess the impact of several factors on recording of methane production from grazing cattle in both tropical and subtropical conditions, using GFU. This work was conducted as part of a broader research project focused on estimating the genetic control of methane production in cattle. Identifying these factors affecting methane production of cattle in tropical grazing situations, is an important step towards deriving accurate methane emission phenotypes and determining which sources of variation must be appropriately controlled in future genomic evaluations.

Materials and Methods

Data collection

Animal welfare and safety were paramount in trial design and execution. All experiments using cattle were undertaken in strict accordance and with prior approval provided by the University of Queensland animal ethics committee, Number; 2022/AE000657. Seven trials were undertaken across four sites in Queensland, Australia, between July 2023 and August 2024. GreenFeed units were used in all trials to measure and record methane (CH₄) and carbon dioxide (CO₂) emission under representative production conditions. Prior to each trial, a three-week acclimation period was undertaken to familiarise cattle to the GFU, during which supplementary feeding of lucerne pellets were provided in feed troughs near the GFU. Additionally, GFU were programmed to distribute pellets at an increased drop frequency to encourage animals to position their head within the machine in an adequate manner to provide a breath sample. Cattle observed repeatedly using the GFU and considered accustomed were removed from the trial to allow shy cattle to utilize and adjust to the units. After a period of five days, these excluded animals were reintroduced to the group. Trial animals had access to all GFU and were allowed to graze and visit the GFU *ad libitum*. Each animal could use any GFU system to provide a sample once every two hours. However, pellets were only distributed for a maximum of eight visits within a 24-hour period. Visits exceeding two minutes of continuous recording were retained as observations. Throughout the trial period animals had access to available forage with no other supplementary feeding provided.

Site description

The number of GFU and animals varied across sites and was based on what was practically achievable at the site. This was largely determined by the available forage resources and normal management practices. At the Brian Pastures Research Station site (-25°34'02"S 151°47'04"E), Southeast of Gayndah, Queensland, four GFU were used between July and October 2023 to assess the emission from two groups of trial animals. Trial animals co-grazed paddocks that were representative of blue gum on granite and alluvial plains land type, containing *Heteropogon contortus* dominated pasture augmented

with *stylo* legumes. The first trial group (T1) ran for 51 days and resulted in visitation from 33 yearling heifers. The second trial (T2) ran for a period of 45 days and recorded 38 yearling heifers. The second trial site was located at Goldsborough Station (-26°23'26"S 148°31'02"E), northwest of Roma, Queensland, hosted 12 GFU to measure a single cohort of animals over a 35-day period (T3). Grazing area was a mix of Brigalow Belah scrub and Poplar box sown predominately with *Cenchrus ciliaris*. In total, 127 cattle were recorded, 78 weaner heifers and 49 weaner males. Trials four and five were conducted from Spyglass Research Station (-19°29'27"S 145°42'12"E), north of Charters Towers, Queensland, hosting four GFU. Land type consisted of lancewood and rosewood ridges which descended to narrow leaved ironbark in the gullies and loamy alluvial soils in proximity to water courses. The first trial (T4) recorded 68 weaned heifers over a period of 32 days. The second trial group (T5) ran for 28 days and resulted in 62 weaned heifers being recorded. A further two trials were than undertaken at Nindooinbah station (-28°02'41.4"S 153°02'22.2"E) southeast of Beaudesert from April to July 2024. Trial 6 (T6) ran across 47 days and recorded 60 bulls, trial 7 (T7) recorded 65 heifers over a trial period of 41 days.

Factor definitions

GreenFeed units record wind speed, wind direction and pipe temperature during animal visitation to determine methane mass flux. Wind speed and direction were recorded by an anemometer fitted to the exterior of the GFU at an elevated position, and pipe temperature recorded the air temperature of sampled air measured by hot film anemometer from within the GFU (Huhtanen et al., 2015). These data were supplemented with additional interpolated weather data retrieved from Scientific Information for Land Owners (SILO) for each trial period retrieved from the Queensland government website the long paddock (Stone et al., 2019) using the geolocation of each trial site. The methodology for SILO weather data is fully described by Stephen et al. (2001). In short, interpolated data is systematically generated at a 5km² grid scale, providing daily minimum and maximum temperatures, rainfall, solar radiation and relative humidity at minimum and maximum daily temperatures, this was used to calculate temperature humidity index (THI) (Thom, 1959), which was used as the primary indicator of heat load experienced by cattle in this study.

Cattle weight pre-trial was recorded before and following a 12-15hr on-water off-feed overnight curfew, this process was repeated post-trial under the same procedure. Change in weight (ΔW) across the trial period was calculated from the difference in pre- and post-trial empty weights dividing ΔW by days between weighing. Weight data, GFU data and climate data were combined with *Bos taurus indicus* (BI) content estimated from individual genotypes as fully described in Hayes et al. (2023). Definition of abbreviations are fully described in Table 1.

The rangeland condition, type and forage at each trial was assessed monthly using FORAGE (Zhang and Carter, 2018). Forage biomass was compared by season, between trials, as well as within trial. Additionally, forage quality was measured directly using a modified botanical technique, with samples collected from 5 random sites prior to paddocks being grazed in T4, T5, T6 and T7. Forage within a 1m² quadrant was harvested to ground level and weighed. The forage samples collected from a paddock were pooled together to create a composite sample and subsampled. This subsample was analysed using a standard forage analysis package offered by Feed Central (Charlton, QLD, Australia), providing information on metabolizable energy (ME), non-detergent fibre (NDF), acid detergent fibre (ADF), crude protein, starch and lignin content. It is important to mention that these samples were not analysed further to identify site-specific differences in forage quality, such as measuring stem-to-leaf ratios or evaluating pasture composition.

Statistical Analysis

The combined dataset from across the seven trials included 46,738 visitations from 453 individual animals. Quality control of retrieved data involved the removal of unknown animal RFID recordings, removal of low visitation animals (<5) across at least 5 days, removal of observations with airflow under 20 (L/s) and removal of methane observations (<0 gCH₄/day). In total 26 observations were removed through quality control, and the number of trial cattle was reduced by 11, all excluded due to low visitation. To evaluate the effects of candidate variables on daily methane production estimates a general linear model was utilized on all remaining methane observations. Cattle which made multiple

visitations within the period of a day had visitation averaged to produce a daily mean.

$$\mathbf{y} = \mu + \mathbf{X}\mathbf{b} + \mathbf{Z}\mathbf{a} + \mathbf{e} \quad (1)$$

Where \mathbf{y} is a vector for the trait daily CH₄ production; μ is the mean; \mathbf{b} is a vector of fixed effects with hour of visit, visit duration, pipe temperature nested within machine, initial trial weight, total visitation, forage biomass within trial, daily visits per animal, \mathbf{X} is a design matrix mapping records to fixed effects; \mathbf{a} is the random animal effect, $\sim N(0, \mathbf{R}\sigma_w^2)$ where \mathbf{R} is a matrix of RFIDs for cattle to account for repeated records and σ_w^2 is the variance at the individual level, varying by test day. \mathbf{Z} is a design matrix mapping records to animals; \mathbf{e} is the random residual term. Factors tested but excluded as non-significant ($P > 0.05$) were BI percentage, sex (nested with trial as only one trial had multiple sexes), rainfall and THI. Observations greater than 4 standard deviations from the mean of the residuals were additionally excluded, resulting in the removal of 157 observations but the number of trial cattle was not further reduced by this process. Non-significant factors were excluded one at a time by lowest F value until only significant factors were retained for further analysis. Forage analysis of samples collected in T4-T7 were not included in model analysis due to incompleteness; however, correlation analysis was undertaken. Proportion of variance for each significant fixed effect was calculated utilizing effectsize package in R (Ben-Shachar, Lüdtke and Makowski, 2020). Repeatability was calculated as variance between individuals divided by the total variance within and between individuals as per Eq 2, utilizing the model described above (Eq. 1.) using the rptR package (Stoffel, Nakagawa and Schielzeth, 2017) in R.

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

Where σ_b^2 is the between animal variance and σ_w^2 is the within animal variance obtained from Eq.1.

Results

Across the 7 trials a total of 453 cattle were successfully recorded for methane production. Raw data means, standard deviations and coefficient of variation for methane and carbon dioxide from across the

seven trials are presented in Table 2. A moderate coefficient of variation (CV) for daily CH₄ production was produced from this study, 23.17%. A lower value was observed for CO₂, 14.95%. After fitting (Eq 1), conditional R² was 0.69 using method described in Nakagawa and Schielzeth (2013). Repeatability as per (Eq 2) was 0.34 (95% CI, 0.31-0.37). P values for each factor are provided in Table 3. Model factors retained which had a significant effect on methane production (P < 0.05) included total visitation, daily visitation, hour of visit, GreenFeed unit, visit duration, pipe temperature, initial weight, forage biomass and trial. Non-significant (P > 0.05) factors identified included BI percentage, rain, THI and sex.

Visitation pattern

Analysis of visitation data from all trials generally indicated a diurnal pattern, where there was significantly (P < 0.001) higher visitation (34,081) occurring between 5am and 4pm daily, compared to outside these hours (12,663). All trials displayed a higher visitation frequency during these times, with the average peak visitation across all trials occurring between 10 and 11am. Visitation outside these hours was less frequent especially in T1 the first trial conducted at Brian Pastures, and T3 which was undertaken at Goldsborough Station, as shown in Fig 1. This caused a slightly negative correlation (P < 0.05) between hour of visit and methane production to be observed. Clustering of visitation over the length of the trial for individual trials was also common with higher visitation observed at specific periods early and midway through T3, before visitation decreased.

The decline in visitation towards the end of T3 coincides with increased rainfall in that trial and is likely responsible for the decreased visitation observed. Similar effects of rain were captured in T4 in the first trial conducted at Spyglass research station and T6, at Nindooabah station southern Queensland, which showed decreased visitation on days where rainfall occurred. Visitation within trial across the test days is presented in Fig 2. Peak visitation for all other trials trended later occurring towards the end of each trial. The exception to this was T6 which had a peak in visitation around 30 days and a smaller relative peak towards the end of the trial. Trial 2 showed the most consistent daily visitation and had some visitation outside typical diurnal expectation. Both T6 and T7 undertaken at Nindooabah station

experienced the most varied visitation outside the expected diurnal visitation to that observed in T1 and T3. This pattern of visitation also directly effects the observed methane production of individual cattle as shown in Fig 3, where the highest visitation animal for each trial and the corresponding hourly methane production is displayed. The condensed visitation in T1 and T3 resulted in less evenly distributed methane production observations over the length of the trial. Visitation in T1 peaked around test-day 38 which was the only rainfall event exceeding 10mm. This was contrary to what was observed in T3 with high rainfall resulting in reduced visitation.

Trial 3 benefited from the use of 12 GFU which meant a greater number of total observations were possible (7,517) in a short 32-day trial period. Additionally, recording of high numbers of cattle was possible with 30 visits per hour occurring on 82 occasions this was far higher than other trials, likely due to the number of GFU available. The increased number and availability of machines likely decreased competition between cattle for usage and, in turn, resulted in the concentrated visitation pattern. However, a relatively low number of cattle were recorded with only 42% of trial cattle repeatedly making visits across at least 5 days. Visitation to individual GFU were significantly different ($P = 0.004$), with machines in T3 recording between 26 and 1078 observations. Machines in other trials recorded a minimum of between 964 and 2414 visits, with high visitation machines capturing 1,154 and 3,004 observations. Unit 357 in T7 recorded far higher visitation 3004 compared to other trials. For instance, T1 and T6 both ran for a slightly longer duration and only recorded 2,119 and 1,948 visits from the highest visited unit. The high usage of some machines in T3 and low usage of others indicates that there is strong preferential selection of units by cattle with the same GFU being utilized repeatedly within a day. Across trial usage of GFU is presented in Fig 4, high usage of available GFU is observed in all trials except for T3 which showed some selectivity due to the increased number of GFU available.

The spread between highest and lowest usage units was between 190 and 1,052, with T3 having the largest difference in visitation between GFU. The lowest difference in visitation between units occurred in T5 with a spread of only 190. The average spread across all trial was a difference in visitation of 496. Increased visitation from 12pm in T2, T6, T7 and to a smaller extent T5 may be indicative of animals being overly accustomed to the lucerne pellet reward provided as attractant by the GFU. Increased

visitation shortly after 12pm occurs due to animals expectation for attractant reward upon GFU reset which occurs at 12pm daily (C-Lock, 2020). However, a small peak in midnight feeding of cattle was observed by O'connell, Giller and Meaney (1989) in a grazing trial this may suggest this behaviour is unconnected to the use of GFU.

Visitation was highly variable between animals ($P < 0.001$), with the highest visitation for a single animal across a trial of 355 visits whilst the lowest number of visits made by an animal was 6. Daily visitation, like total visitation, was significantly different between individuals ($P < 0.001$), highest visitation by an animal per day was 27 visits recorded in T2, other trials had similar high visitation animals making between 11 and 21 visits in a single day of recording. During the trial a maximum of 8 total releases of between 7 and 8 drops occurred with a minimum interval of 2 hrs between releases. A comparison of statistics between trials is provided in Table 4.

Effect of factors on methane production

Visitation, both absolute and daily, across the trial showed a negative correlation ($P < 0.001$) with pipe temperature (-0.31, -0.18), THI (-0.42, -0.22), and rainfall (-0.1, -0.08), respectively. A correlation matrix of interactions between factors is presented in Fig 5. This is a strong indication that higher daily temperatures reduce GFU visitation. This has a substantial impact on the number of observations that can be generated from GFU under tropical conditions when increased summer temperatures and humidity seemingly resulting in reduced visitation. Higher numbers of cattle (300) in T3 and more GFU (12) compared with other trials did not result in more observations per animal, rather the inverse was observed with lower visitation compared to other trials, with observations also being restricted in time to approximate daytime hours. Individual visitation was low for T4 and T5 however a higher proportion of cattle visited the GFU generating a similar number of observations. Early trials showed low proportional visitation of cattle with around 40 – 50% of cattle visiting the GFU. However, this increased with successive trials with high numbers (>75%) of cattle being successfully recorded in T4 to T7.

Change in trial weight had a small positive correlation with total (0.29, $P < 0.001$) and daily visitation frequency (0.08, $P < 0.001$). There is some evidence that weight gain may therefore be attributed to consumption of the lucerne attractant. Average change in weight across trials was 0.14 kg/day. Variation between trials was considerable with two of the seven trials having a mean decrease in weight over the corresponding trial period. Low or negative growth is likely a result of environmental conditions and resulting poor forage quality and availability. For both T2 and T3 change in weight was considerably positively correlated with the frequency of daily (T2 = 0.31, T3 = 0.16, $P < 0.001$) and total visitation (T2 = 0.65, T3 = 0.30, $P < 0.001$) in contrast to the combined dataset. For this group of cattle, the attractant distributed by the GFU may have contributed substantially to total feed intake because of low forage availability. Cattle in T2 were, on average, a year older and averaged -0.06 kg/day loss in weight for the 45-day trial in comparison to their counterparts in T3 and T5, as cattle in T2 were older the mean initial weight at beginning of the trial period, 357.4 kg. Animals in T3 had a similar negative ΔW of -0.142 kg/day over a slightly shorter period of 35 days. Cattle in T7 successfully achieved a positive gain in weight of 0.137 kg/day these cattle were of a similar age and starting weight as T2 cattle with a mean weight of 322 kg. The conflicting growth between trials suggest that cattle age and weight contributed less to weight gain than trial conditions.

Forage biomass varied between trials. Trial 2 had the lowest with 206 kg ha⁻¹ and T5 the highest with 2736 kg ha⁻¹ on offer. The forage samples collected from the four trials ranged from 73.1% to 83% for nondetergent fibre (NDF). More variance was observed for acid detergent fibre (ADF), ranging from 42.7% to 56.7%, with T4 having the highest ADF component. Trial 4 as a result had the lowest hemicellulose fibre fraction, the difference between NDF and ADF at 24.1% with T7 having the highest available, at 30.5%. Results from forage samples for T4-T7 and estimated forage biomass are provided in Table 4. A significant positive correlation ($P < 0.001$) between forage biomass and nutritional components such as lignin (0.54), protein (0.48) and starch (0.62) was observed. Methane production was negatively correlated with both crude protein (-0.17) and metabolizable energy (-0.08) ($P < 0.001$). The opposite association was observed between methane production and forage biomass (0.25), lignin

(0.17) and starch (0.27) ($P < 0.001$). It is worth mentioning that these correlations reflect the forage on offer during each trial and potentially do not reflect the actual diet consumed by animals.

Variance in methane production explained by factors

Three factors explained most of the variation observed for methane production. Of these initial weight accounted for the greatest amount of variance, explaining 11.8%. Total visitation explained 5.6%, while trial accounted for 4.0% of observed variation, making them the next two most influential determinants. Other factors, such as daily visitation frequency (1.5%), visit duration (0.5%), pipe temperature (0.3%) and machine effect (0.2%) explained only a small proportion of variation. Combined visitation factors machine variation, visit duration daily and total visitation contributed approximately 7.8% of variance observed. The proportion of variation in methane production attributed to GFU was small at only 0.2%. The same 4 GFU (356, 357, 564, 565) were utilized across T1, T2, T4, T5, T6 and T7 with cattle having free access to all the units. However, methane production per animal varied by at most 4.76 g/day between units with the smallest variance between units being only 0.67 g/day. All GFU least square means are presented in Table 5 with considerable differences between those in T3 (457 - 472) being observed. Hour of visit contributed less than 1% of observed variation but remained significant ($P < 0.05$).

Discussion

This study successfully identified the key determinants and quantified their effect on methane production from cattle in tropical and subtropical environments. Visitation and usage of GFU was found to influence daily methane production estimates. Daily visitation, total accumulated visits, hour of visit and duration of visit all were significant in defining cattle methane production. Visitation was indirectly affected by rainfall, temperature and available forage at each trial location which contributed to variation in methane production. While sex and BI percentage were non-significant in describing variation in the whole dataset in this study, results in select trials indicate further research is required to determine the effect of these factors. Slight variation in methane production due to GFU measurement

was observed. The variation due to GFU was reduced with increased usage with the least frequented machines having the highest variation.

Visitation pattern

Suitable visitation of the GFU over the trial period and across days is necessary so that the accumulation of short-term observations of methane production accurately reflect true methane production rates. A minimum of 5 visits across at least 5 days was used as a threshold in this study. This reflects minimum visitation suggested in similar studies making use of the GFU system for methane production measurements (Arthur et al., 2017). Dressler et al. (2023) reported that 40 visits to GFU produced highly similar results as 100 visits. Cattle in this study recorded on average ~ 145 visits which far exceeds the suggested 40 visits. However, 55 cattle recorded fewer than 40 visits approximately 12% of cattle recorded, these low visitation cattle displayed more variable methane production due to sampling. A negative association of -0.63 ($P < 0.001$) between total visitation and CV of methane production was observed, with increasing visitation by cattle across the trial period resulting in a lower CV for methane production. However, cattle that made between 5 and 90 visits to the GFU produced a similar CV. This aligns with findings from other studies, where 30 visits to a GFU was found to produce similar averages to those of higher-visitation animals, with as many as 80 visits (Herd et al., 2016). A slight decrease in CV was observed in cattle that made over 100 visits suggesting that a far higher visitation frequency would be needed to reduce variation in observations by a significant margin and would be impractical to produce. Future trials can utilise the same minimum visitation threshold of 5 visits across 5 days to maximise cattle recorded.

The CV for CH₄ production in this study was moderate at 23.17%. These results are comparable to those reported by Ryan et al., (2022), who found a CV for methane production for steers and heifers of 25.08% and 25.33%, respectively, under feedlot conditions. The CV of CO₂ observed in this study was 14.95%, which is similar but slightly higher than the value reported by Ryan et al. (2022), who reported a CV for CO₂ of 11.93%. This finding may indicate that methane production from short-term breath measurement collected from continual and free access to GFU under both feedlot and grazing produce

similar phenotypic data. However, the higher variation observed for CO₂ in grazing cattle is likely to reflect to higher levels of activity exhibited by grazing cattle, when compared to those measured under feedlot conditions. This variability may also be influenced by differences in grazing behaviour and the timing of assessments at the GFU, as grazing cattle may visit the unit at various times throughout the day depending on individual grazing patterns. Carbon dioxide is produced by metabolic activity and variation is likely to reflect differences in respiration rate, whilst variability for CH₄ production is likely reflecting intermittent eructation. Consistent with existing literature, this study observed a strong correlation between CO₂ and cattle's pre-trial weight, providing further evidence that increased body weight is associated with higher baseline metabolic activity. Additionally, dietary intake greatly influences both metabolic rate and CO₂ emissions, with variations in forage quality and quantity directly impacting both CO₂ and methane production. This highlights the importance of considering not just animal weight but also diet composition and intake levels when assessing metabolic outputs and greenhouse gas emissions in livestock.

Methane production measured by sulphur hexafluoride (SF₆) and respiration chamber (RC) in adult cattle produced a CV of 18.7% and 22.1% respectively (Grainger et al., 2007). Variation in methane production measured by GFU in this study was slightly higher. This is likely due to RC and SF₆ recording methods both eliminating visitation as a source of variation (as they continuously measure methane production over 24 hours or more). Based on our results, including a measure of visitation frequency in the linear model for GFU observations is suggested to account for difference in record number between high and low usage cattle. A moderate positive correlation was observed between visitation frequency and both methane production and carbon dioxide production which further suggests that visitation frequency contributes to determining methane production for grazing cattle. Additionally, both daily and total visitation frequency were significant in explaining variation in methane production when modelled. Two methods for improving precision of GFU results have been previously suggested, either increasing trial length or the increasing the number of trial participants are suggested over increasing daily visitation (Cottle et al., 2015). Over periods of 7 and 14 days it was found that animals would visit the greenfeed throughout most hours of the day, accounting for visitation time and

arithmetic averaging produced no significant difference in mean methane production (Manafiazar, Zimmerman and Basarab, 2016). Directly increasing daily and total visitation is not feasible under *ad libitum* feeding however, training procedures can increase recording percentage as seen in the improved outcomes seen in T4 onwards. Cattle visitation within day is highly variable for both time of visit and visit duration which contribute to a higher CV using daily averaged methane production reduces CV by reducing this within day variation.

Trial length and total number of trial cattle are both restricted by management and environmental conditions. This resulted in trial lengths varying between 28 and 51 days and the number of cattle per GFU fluctuating around 20. The lowest visitation animals retained made just 6 visits and were present in both T3 and T5. Trial 5 was tied with T7 as the shortest duration trial at 28 days in length. Generally, cattle increased visitation frequency provided a longer trial period, two possible reasons for this were that cattle became more accustomed to the GFU over time or as available forage quantity and quality reduced, and the attractant became more appealing. Visitation frequency to the GFU under free grazing grassland trials are as expected lower than those produced under confinement within feedlot with Ryan et al. (2022) reporting that a minimum of 20 visits was achieved per individual over a 60 day period. Our study found that minimum visitation generally increased relative to trial length with T1, T2 and T6 all having minimum visitation exceeding 30 and conducted over trial periods exceeding 45 days. However, recording percentage was obviously lower in T1 and T2, which may have resulted from poor familiarisation with GFU or a more prominent dominance structure in the herd due to increased age of trial cattle. Animal to machine ratio was the same between T1, T2, T4 and T5 with ~ 19 animals per unit. However, recording percentage was higher in T4 and T5 compared to T1 and T2. And although T3 had the lowest recording percentage with the highest animal to GFU ratio T6 and T7 both achieved reasonably high recording percentage with a slightly higher animal per GFU ratio. The low recording percentage in T3 suggests that a ratio of 25 cattle per GFU may be too high, reducing shy animal visitation. Trials with higher recording percentage generally included cattle with low close to minimum threshold visitation. Based on our results altering the proportion of animals per GFU will be unlikely to

increase visitation or spread visitation across hours of the test day, however, exceeding 20 animals per unit may reduce recording percentage.

Initial weight of cattle upon commencement of trials was moderately positively correlated with total and daily visitation. Increased visitation therefore may be due to herd dynamics with body weight and condition both significantly contributing to social hierarchy (Sołtysiak and Nogalski, 2010). When a consistent concentrate reward was offered as an attractant to visit a fixed system, high dominance animals were found to visit significantly more often than lower dominance animals (Ketelaar-de Lauwere, Devir and Metz, 1996). Dominant animals determine where and when grazing occurs, while less dominant animals either follow leaders or disperse independently (Harris et al., 2007). This is likely to cause visitation to GFU to occur in concentrated groupings when dominant cattle decide to seek the attractant and visit the GFU. This is a possible explanation for the definitive peaks in visitation. Additionally, as dominant cattle exhaust their GFU allocation they may disperse and return to grazing, drawing submissive cattle away with them. This may contribute to the restricted visitation across hours of the day. Dominance and hierarchy were not observed in this study but may contributed to the daily variation in visitation pattern observed.

24hr methane emission patterns

Cattle are supposedly a diurnal species undertaking grazing activity preferentially during daylight hours and resting overnight. However, in tropical regions during warmer conditions intense periods of grazing occur in the early evening between 17 and 21 h and grazing beginning earlier in the morning ~4am (Jochims et al., 2020). The high variability observed in visitation in T4 and T5 tropical region trials may reflect this behaviour with animals visiting the GFU earlier and later each day than other trials. Additionally, variation in grazing between breed composition has been observed, with BI content animals displaying a higher preference for nocturnal grazing in southern hemisphere tropical regions (Giannetto et al., 2022). This behaviour is likely to better manage daytime thermal loads, when higher temperatures, atmospheric pressure and solar radiation intensity typically occurs. Altered grazing in

response to environmental cues may produce more dispersed visitation to GFU for cattle observed over summer.

Rainfall and methane emissions

Overall and compared to other factors observed in this study, rainfall was not found to have a dominating influence on how precisely GFUs estimated methane production. This finding is possibly due rainfall events being infrequent across trials and the effect of rainfall being represented by other parameters contained in the final model, such as trial location, test day and visitation. Rainfall was observed to influence visitation, which was found to be a strong determinant of methane production. A slight negatively correlation was found between total visitation and rainfall (-0.1), whilst a much weaker correlation was observed between daily visitation and rainfall (-0.08). Total rainfall events exceeding 10 mm occurred infrequently on 12 of the 260 test days. No rainfall events greater than 10 mm occurred in T4 compared to 6 days in T5, both these trials were undertaken at Spyglass Research Station, highlighting the difference between conditions even at the same location. Cattle are continuously exposed to environmental conditions when grazing and small variations may negatively affect GFU visitation.

Temperature and methane emissions

In our study, temperature humidity index was negatively correlated with CH₄ and CO₂ production, along with both daily and total visitation. Cattle grazing in northern Australia are regularly exposed to high heat load conditions, likely to result in behavioural and physiological responses. Under high heat load daily conditions, cattle display reduced feed intake to lower metabolic temperature and reduce the impact of heat stress (Mader et al., 2002) and alter their grazing times to predominantly nocturnal grazing (Eckhardt et al., 2025). Onset of heat stress was observed under feedlot condition in Queensland by behaviour changes such as shade seeking at THI in excess of 75, this behaviour resulted in both lowered feed intake and subsequent methane production (McGinn et al., 2008). For animals observed on rangelands, higher temperatures result in decreased average travel distance and congregating near water points which reduces grazing time and feed intake (Creamer and Horback, 2024). Over the course

of the 7 trials 60 days exceeded a THI of 75, between late October through till the end of February, temperatures likely to cause “strong” heat stress conditions according to Hahn et al. (2009). Additionally, T4 and T5 experienced severe heat stress conditions with 15 days exceeding a THI of 80. Temperature heat index of greater than 75 causing strong heat stress in cattle, 79 and above classified as severe and THI exceeding 84 were an emergency (Hahn et al., 2009). Lower visitation observed in these trials for individual animals may reflect lower desire for attractant due to reduced appetite because of higher temperatures. Additionally earlier sunrise and later sunset due to increased day length might explain the earlier and later visitation observed in T4 and T5 and the more varied use of GFU across the whole day to avoid temperature extremes.

Forage availability and quality

Forage biomass was variable between trial locations, likely reflecting historical management, and seasonal and environmental conditions. Temporal variation in forage quality, such as protein content and dry matter digestibility, has known impacts on metabolic rate and dietary intake, responsible for seasonal variation in methane production (Lassey, 2007). Measuring the intake of individual animals under extensive grazing conditions remains a substantial challenge. Additionally, free-grazing cattle possess the ability to select a diverse diet from the forage available within their grazing environment, and variation between individual animals may exist in their dietary selection. However, recent advancements in livestock tracking technology potentially offer a method to address these challenges in future research. The use of global positioning system and accelerometer sensors can provide detailed insights into livestock movement and grazing behaviours, potentially allowing individual variation in grazing behaviours associated with dietary intake and grazing patterns to be assessed (Augustine et al., 2022). The available forage in T1-T3 was substantially lower (down to ~200 kg ha⁻¹), compared to the four later trials. Such low forage availability is expected to constrain daily feed intake and were also likely associated with reduced digestibility. Although forage samples were not collected from these sites, the grazing resource was unable to support animals selecting a diet that met maintenance requirements, as evidenced by the observed negative weight change.

The findings of this study support previous observations that protein intake is negatively associated with methane production. Niu et al. (2016) observed that protein intake had a minimal effect on methane production, however dietary energy in the form of carbohydrates substantially contributed to methane production. Methane production is likely to decline in response to increased forage protein as the amount of carbohydrate, fermentable material is reduced proportionally to protein (Ellis et al., 2011). Nitrogen (N) is a key compound for microbial protein synthesis however in low protein diets cattle show an ability to scavenge N from urea in the blood to utilize in the rumen in place of dietary N (Kristensen, Storm and Larsen, 2010). Excess N is excreted as urea in urine or as pass through in manure (Dijkstra, Oenema and Bannink, 2011), and therefore does not directly contribute to methane production.

Forage quality samples showed that protein and metabolizable energy (ME) were slightly higher in T7 than any other trial. However, higher quantities of lignin present in the samples collected from T5, indicating forage quality was lower than T7. A strong association between lignin content and methane production exists because lignin reduces digestibility. In the current study, a positive association between ME and starch concentration in forage samples was observed, indicating that pastures with higher energy density contained greater amounts of readily fermentable carbohydrate. Diets with high ME have shown to result in higher quantities of voluntary intake than comparatively lower energy density diets (Waghorn, 2007). Methane production is heavily dependent on DMI (de Haas et al., 2011), and lower ME in available forage in the current study may be associated with unobserved reduced DMI. Feed intake from grazing cattle contributes a substantial proportion of variance in SF₆ based trials (Lassey, 2007). As such it is possible that the forage samples collected were not fully representative of the diet consumed by the animals.

Effect of GreenFeed unit on methane emissions

Cattle interaction with the GFU and machine effect are important considerations in model development as a small variation due to GFU was observed in this study. Cattle showed varied visitation preferentially choosing certain machines during morning and afternoon periods in some trials. This

pattern of visitation could be due to environmental cues such as sun position or wind direction. This likely contributed to the average variation of 5.26 g/day observed between units. The number of units used across T3 means that observation number is low contributing to the variation in those GFU. Previous studies have indicated that peak methane occurs shortly post feeding and decreases thereafter (Pinto et al., 2020), so animal choice in GFU during this time may cause variation between machines to be observed. Outright lower visitation overall may occur if a single unit is utilized in a position not desirable for cattle visitation. The use of multiple GFU is the simplest solution to account for the slight variation observed due to both machine preference and machine variation effect. However, the high number of units used in T3, and the low utilization of some GFU indicate that cattle will selectively choose units if allowed. Subdividing T3 into small trials likely would have improved GFU usage, so an upper limit on both cattle and GFU per trial is recommended. Results from this trial suggest not exceeding 25 animals per GFU is practical.

Conclusion

This paper is one of a few which have utilized GFU to observe methane production from individual cattle under representative grazing condition in tropical and subtropical regions. Our analysis demonstrates forage abundance, temperature and rainfall are all factors which affect methane production from cattle grazing tropical and subtropical rangelands. Differences in cattle behaviour (particularly visitation rates) has a considerable effect on variation in these measurements and should be included in models aiming to produce methane phenotypes for future genetic evaluations. Short term breath measurement by GFU requires frequent and adequately distributed visitation across the day to provide a representative methane phenotype for cattle. The current threshold used in this paper of 5 visits across five days produces suitably low CVs for methane production. Trial forage availability contributed significantly to methane production; however, forage quality is likely a considerable factor impacting the unexplained variability in the production of methane by cattle. Unobserved feed intake from cattle will contribute to variation within individual methane production, and including quantitative forage data is one method to improve model predictions. Improved visitation (for example with

increased prior training) and high daily visitation both resulted in less variable observations and better reflected true methane production of cattle. It is recommended that future research account for individual animal differences in intake, or appropriate proxy indicators such as grazing behaviour, as these factors are likely to improve the accuracy of methane phenotypes for genetic and management purposes and enhance our understanding of their influence on methane production.

Disclosures

After completion of this project contributing author Scott Cullen has since been employed by C-Lock the manufacturer of methane monitoring system GreenFeed used within this study, no other conflicts exist.

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Tables and Figures

Figures

Usage of GreenFeed units by hour of day for each trial

Figure 1 Counts of visit per hour across trial and by Greenfeed unit (GFU)

Distribution of visitation across trials

Figure 2 Relative visitation across trial day and hour of visit per trial

Distribution of visitation by most frequent visitation animal for each trial

Figure 3 Methane production of highest visitation animal in each trial across 24 hours

Visitation to separate GFU by individuals per trial

Figure 4 Percentage of cattle per trial which used unique GreenFeed units (GFU), all trials had free access to 4 GFU except T3 which included 12 GFU.

Correlation matrix of numeric factors

Figure 5 Correlation matrix between all significant numeric parameters at $P < 0.05$ blank squares indicate no-significant correlation was identified, 48007 observations representative of 453 individuals.

Tables

Table 1 Summary of traits

Trait	Abbreviation	Unit	Definition
Methane	CH ₄	g/day	Single observation from GFU as grams of methane emitted per day.
Carbon dioxide	CO ₂	g/day	Single observation from GFU as grams of carbon dioxide emitted per day.
Initial weight	IW	kg/day	Empty weight of animal pre-trial.
End weight	EW	kg/day	Empty weight of animal post-trial
Change in weight over trial period	ΔW	kg/day	Difference between pre- and post-trial weight divided by days between measurement.
<i>Bos taurus indicus</i> percentage	BI	%	Percentage of the genome which is <i>Bos taurus indicus</i> by descent based on chromosome segments.

Table 2 Raw data for average daily CH₄ and CO₂ production with coefficient of variation (CV) by each trial and combined values for all 7 trials standard deviation in parentheses.

Trial	CH ₄ , g/day (SD)	CH ₄ , g/day CV	CO ₂ , g/day (SD)	CO ₂ , g/day CV
T1	181.94 (25.86)	14.22	5379.69 (539.09)	10.02
T2	162.97 (25.31)	15.53	4998.24 (512.51)	10.25
T3	120.89 (34.21)	28.29	4468.15 (810.40)	18.14
T4	149.22 (33.81)	22.66	4742.74 (682.79)	14.40
T5	163.92 (34.55)	21.08	4842.35 (654.79)	13.52
T6	173.08 (23.90)	13.81	5711.23 (552.15)	9.67
T7	127.16 (22.17)	17.44	4765.45 (527.68)	11.07
All	154.27 (35.74)	23.17	5052.92 (755.36)	14.95

Table 3 Summary output of type 3 ANOVA with P values fitting factors to Eq 1, Greenfeed unit (GFU).

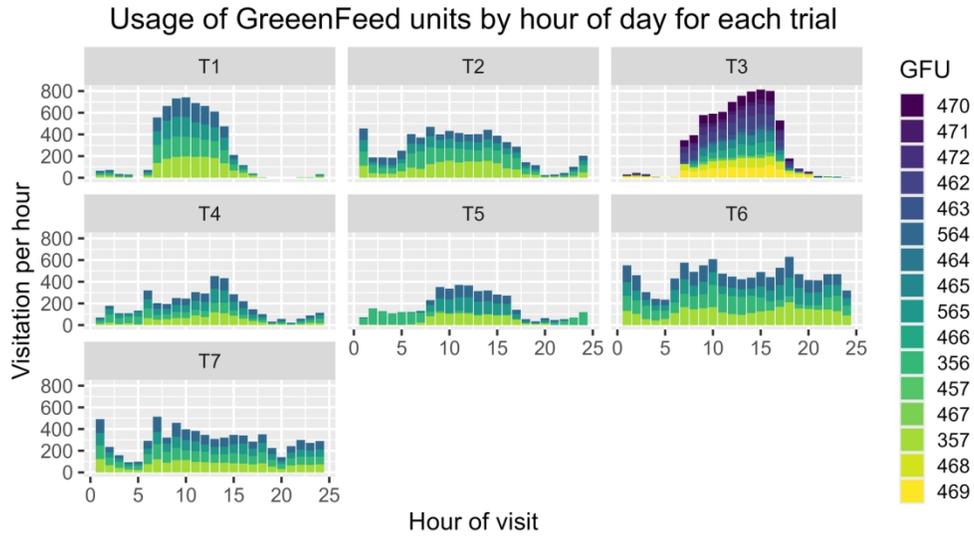
Fixed Effects	Sum Square	Mean Square	F value	P-value
Daily visitation	492973.65	492973.65	1078.25	P < 0.001
Trial: Forage biomass	77393.96	11056.28	24.18	P < 0.001
GFU: Visit duration	112856.90	7053.56	15.43	P < 0.001
Trial	36965.75	6160.96	13.48	P < 0.001
Trial: Initial weight	26367.70	3766.81	8.24	P < 0.001
GFU: Pipe temperature	56592.73	3537.05	7.74	P < 0.001
GFU	34385.72	2456.12	5.37	P < 0.001
Trial: Total visitation	12154.79	1736.40	3.80	P < 0.001
GFU: Hour of visit	16413.37	1025.84	2.24	0.003

Table 4 Summary statistics of trial differences, forage biomass, forage quality, visitation, weight and BI percentage.

Trial	T1	T2	T3	T4	T5	T6	T7
Trial location	Brian Pastures Research Station		Goldsboroug h Station	Spyglass Research Station		Nindooibah Station	
Trial period	25/07/202 3 - 13/09/202 3	15/09/202 3 - 29/10/202 3	25/10/2023 - 28/11/2023	04/12/202 3 - 04/01/202 4	28/01/202 4 - 25/02/202 4	06/04/202 4 - 21/05/202 4	18/06/202 4 - 15/07/202 4
Total trial visits	5,842	6,692	7,447	4,510	4,289	10,913	7,202
Trial length, days	51	45	35	32	28	46	28
Recorded cattle	35	38	127	67	61	60	65
Trial cattle	75	75	300	75	75	80	80
Recording %	0.44	0.51	0.42	0.91	0.83	0.75	0.81
Mean visits per animal	194	206	72	77	82	197	123
Lowest visitation animal	35	39	6	14	6	62	11
Mean initial weight, kg (Min-Max)	369 (294 - 457)	358 (290 - 444)	289 (210 - 359)	197 (146 - 265)	196 (156 - 238)	331 (271 - 432)	322 (223-458)
Mean end weight, kg (Min-Max)	403 (322 - 487)	355 (286 - 436)	283 (157 - 349)	212 (163 - 273)	274 (227 - 411)	361 (299 - 449)	330 (231 - 452)
ΔW , kg/day (Min-Max)	0.67 (0.12 - 1.10)	-0.05 (-0.47 - 0.31)	-0.17 (-3.23 - 0.51)	0.32 (0.01 - 0.66)	0.79 (0.43 - 2.12)	0.54 (0.22 - 0.95)	0.14 (-0.64 - 0.75)
Mean <i>Bos taurus indicus</i> % (Min-Max)	0.58 (0.39 - 0.93)	0.53 (0.42 - 0.94)	0.44 (0.34 - 0.69)	0.73 (0.45 - 0.95)	0.76 (0.41 - 0.95)	0.45 (0.39 - 0.50)	0.44 (0.24 - 0.50)
Animal/GFU Ratio	19	19	25	19	19	20	20
Forage biomass, kg ha ⁻¹	382	206	425	1395	2736	1885	1632
Metabolisable energy, MJ/kg	-	-	-	6.46	6.49	7.80	7.99
NDF, %	-	-	-	83	73.1	73.4	73.2
ADF, %	-	-	-	56.7	49	46.7	42.7
Lignin, %	-	-	-	8	9.2	6.0	5.2
Starch, %	-	-	-	0.2	0.9	0.9	0.7
Protein, %	-	-	-	3.2	7.4	5.7	7.5

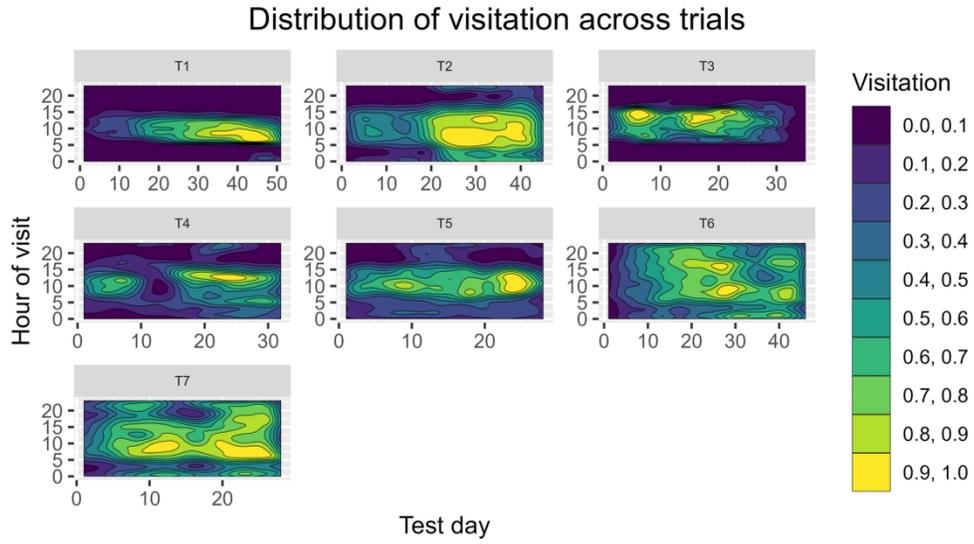
Table 5 Least square means and standard errors for methane measured by GreenFeed units

GreenFeed Unit	Trial	N	Least Square Mean	Standard Error
356	1-2, 4-7	10520	155.06	1.30
357	1-2, 4-7	10496	155.88	1.30
457	3	209	133.33	2.60
462	3	1077	127.87	2.21
463	3	382	145.25	2.39
464	3	175	116.53	2.69
465	3	691	126.33	2.27
466	3	804	129.66	2.24
467	3	26	128.73	4.79
468	3	841	121.78	2.24
469	3	1065	119.25	2.21
470	3	966	123.77	2.22
471	3	296	133.70	2.48
472	3	863	121.64	2.23
564	1-2, 4-7	9321	158.46	1.30
565	1-2, 4-7	9006	160.16	1.30



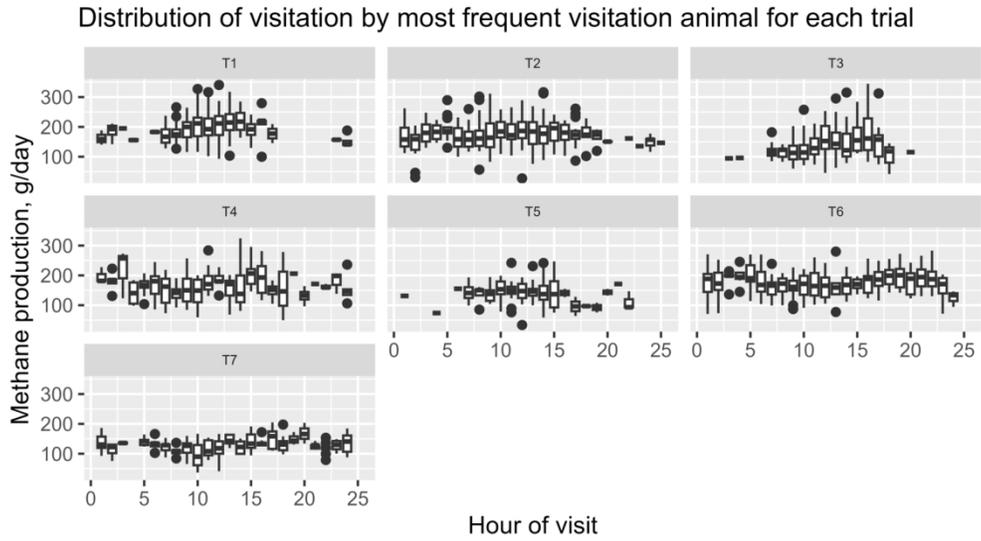
Counts of visit per hour across trial and by Greenfeed unit (GFU)

156x87mm (300 x 300 DPI)



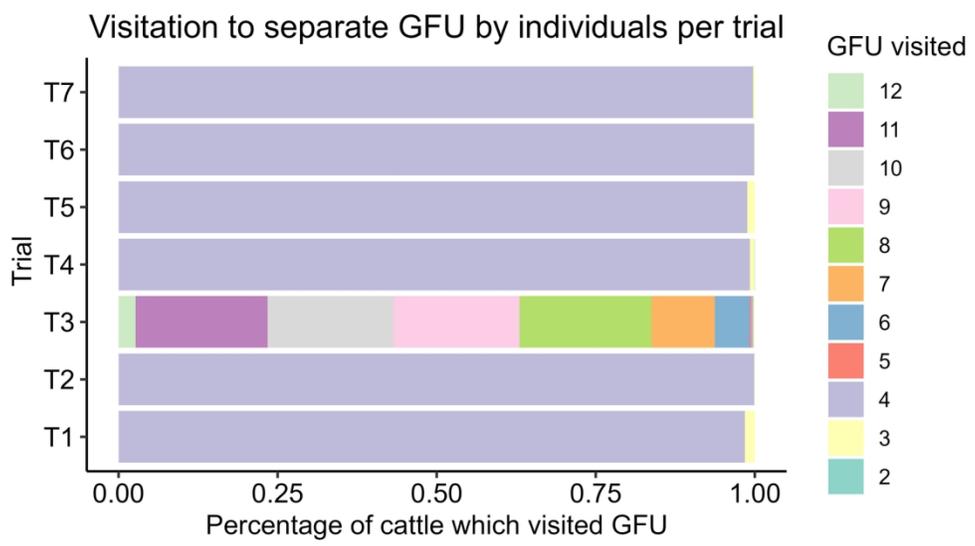
Relative visitation across trial day and hour of visit per trial

156x87mm (300 x 300 DPI)



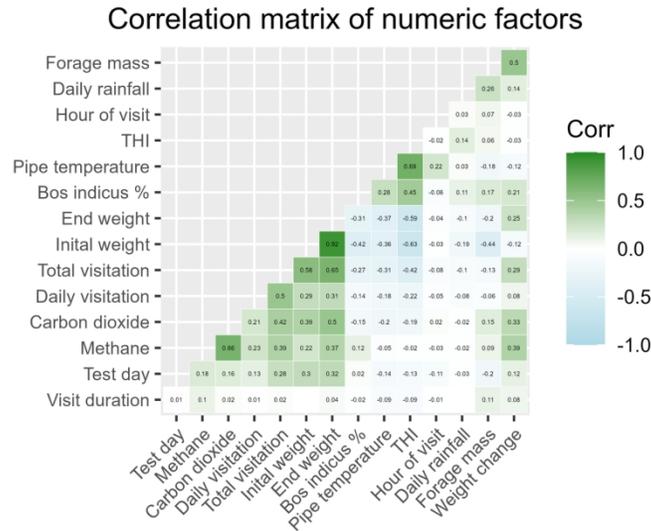
Methane production of highest visitation animal in each trial across 24 hours

156x87mm (300 x 300 DPI)



Percentage of cattle per trial which used unique GreenFeed units (GFU), all trials had free access to 4 GFU except T3 which included 12 GFU.

156x87mm (300 x 300 DPI)



Correlation matrix between all significant numeric parameters at P < 0.05 blank squares indicate no-significant correlation was identified, 48007 observations representative of 453 individuals.

156x87mm (300 x 300 DPI)