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BRIEF COMMUNICATION: Adaptation of grazing sheep to GreenFeed emission monitoring units under New Zealand ryegrass-white clover pastures

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Abstract

GreenFeed (GF) emission monitoring units have been extensively used in cattle, but few studies have been conducted using sheep in pastoral grazing situations. This study aimed to investigate ewe visiting behaviour in the presence of GF units and the successful collection of methane (CH_4) measurements. Commercial sheep pellets were introduced to 156 naïve two-tooth Romney ewes at an allocation of 130 g/ewe/day. From those, 76 frequent pellet consumers were selected for introduction to three GF units. Out of those, 22 (28.9%) exhibited daily visits and a good stay duration (≥2 minutes) per visit at the GF units, allowing for valid CH_4 mass flow calculations. In total, the GF units were visited an average of 42.8 ± 14.9 times daily, making 4.6 ± 1.12 visits/ewe/day by the 22 frequent users. The average duration of successful CH_4 emission measurement was 5:32 ± 3:21 minutes/visit. The calculated average total CH_4 emissions were 30.02±5.52 g/d. The study provided valuable insights into the behavioural variability and engagement levels of ewes with GF units, along with functionality and feeding aspects of the three GF units. Further, the study highlighted key challenges in training ewes and pellet consistency, which should help improve the understanding of measurement variability to support more reliable use of GF systems in grazing settings.

Keywords: sheep, enteric methane, GreenFeed units, pellet standardisation, ewe behaviour

Introduction

Enteric methane (CH_4), the predominant greenhouse gas (GHG) produced by ruminant livestock, represents the largest agricultural GHG emission source in many countries, including New Zealand (NZ) (Ministry for the Environment 2024). The enteric CH_4 emissions from sheep significantly contribute to agricultural GHGs, which have been less researched in a grazing setting.

Previously, respiration chambers and the sulphur hexafluoride (SF_6) method have been the main techniques for measuring CH_4 emissions from sheep (Della Rosa et al. 2021). However, these methods require trained animals, restricted animal movements, and high labour and monetary inputs. The GreenFeed (GF) monitoring system (C-lock, Rapid City, United States) is a portable automated flux measurement unit that offers a non-invasive approach for estimating enteric CH_4 and CO_2 emissions. It has been primarily validated in indoor or controlled settings, with limited application and validation in NZ's pasture-based grazing conditions (C-Lock Inc. 2024). Notably, a review on GF unit usage conducted by Della Rosa et al. (2021) highlighted that over 77% of the research was carried out under confinement conditions. Further, GF units have been extensively used in cattle, but few studies have been carried out using sheep (Nguyen et al. 2018).

Previous research highlighted that individual use and visiting patterns (frequency, duration and the time of the day) have a significant effect on the predicted daily CH_4 emission estimates by GF units (Waghorn et al. 2016; Jonker et al. 2016). Enteric CH_4 emission measurements from sheep under grazing conditions are likely to be more accurate, as they reflect their diet and natural grazing behaviour. However, sheep are generally more difficult to train than cattle, particularly under NZ's intensive pastoral grazing setting (Goopy et al. 2015; Ulyatt et al. 2005). Limited information is available on the behavioural response of sheep to GF units under grazing. This study aimed to investigate ewe visiting behaviour in the presence of GF units and the successful collection of CH_4 measurements over 20 days under pastoral grazing conditions. It also focused on identifying key factors that affect sheep engagement with GF units and identifying practical considerations for effective implementation of GF units under NZ's intensive pastoral grazing setting, to help optimise measurement outcomes.

Materials and methods

Study design

This study was conducted over 73 days at Massey University's Keeble's farm, 5 km south of Palmerston North, with approval under Massey University animal ethics protocol 24/18.

Between 13 December 2024 and 4 February 2025, 156 naïve two-tooth Romney ewes, with an average body weight of 51.2 ± 4.79 kg, were allocated 130 g/ewe/day commercial pellets (Meal Time Stock Feed All Rounder multi pellets, Denver Stock Feeds, Palmerston North) in troughs while grazing a mixed perennial ryegrass (*Lolium perenne* L.)/white clover (*Trifolium repens* L.) pasture. Ewes had *ad libitum* access to pasture. Initially, direct observation was used to identify the number of ewes consuming pellets for an hour after the pellets were distributed each morning. Over a three-week period, the number of pellet-consuming ewes increased. Seventy-six ewes who consumed pellets regularly for a minimum of five days were identified as frequent pellet consumers and were selected for the study (Figure 1).

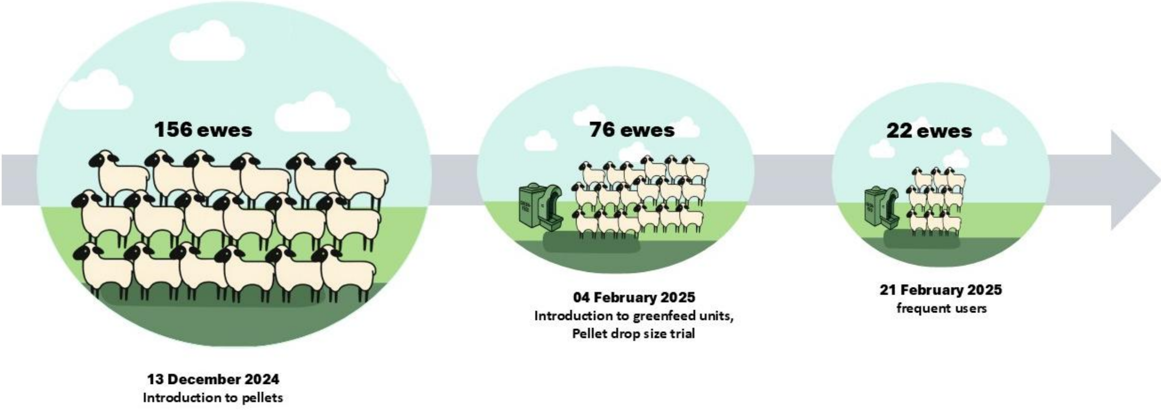

Figure 1. Timeline of the experiment, including the pellet adaptation phase (53 days) and pellet drop size standardisation period (5 days).

On 4 February, three small ruminant GF units (GF 286, 287 and 372) were moved into the paddock, providing the selected ewes with free access. A trial was conducted to standardise the pellet drop size, as initial observations using 6 mm-diameter pellets, which were within the manufacturer's recommended size (<7 mm, C-Lock Inc., 2024), showed inconsistencies in the amount and weight dispensed/drop. To address this, both 6 mm and 4 mm pellets were tested, and the 6 mm pellet was selected for the experiment as the 4 mm pellet delivered excessive feed/drop resulting in bait amounts that exceeded 10% of the ewe's expected daily intake (~30 g), which is undesirable. The drop size of the 6 mm was 4.91 (± 0.26)g, and the 4 mm was 5.08 (±0.74)g. As the pellet dispense issues remained unsolved, from Day 12 of the experiment with GF units, 6 mm diameter pellets were manually broken into smaller pellets that had no effect on diameter. For quality control, a sample of 10 pellets from each batch was measured using a calliper to ensure size consistency. The two pellet sizes (whole and broken) were alternated daily to assess their impact on each GF unit for 5 days. The weights of the first 10 pellet drops were measured each time the pellet type was changed. All GF units were configured with the following settings: drop dispense interval (12 seconds), minimum time between feeding periods (10,800 seconds), maximum drops per feeding period (15) and maximum feed periods (8). Calibrations and maintenance were conducted as per manufacturer recommendations, including automatic standard calibrations, and initial CO_2 recovery calibrations (99.64 ± 4.98%).

Data Analysis

The daily probability of animals visiting a GF unit was modelled using a generalised linear mixed model (GLMM) with a binomial distribution and a logit link function. The fixed effects in the model included the GF unit and calendar date. The animal identifier was included as a random intercept to account for repeated measurements within individuals across the trial. The model was fitted using the PROC GLIMMIX procedure in SAS (SAS Institute Inc., Cary, NC, USA). Predicted probabilities of visitation were back-transformed from the logit scale using the *ilink* option and plotted over time by GF unit. To visualise temporal trends, smoothed probability curves were generated using PROC SGPLOT. No interactions were included due to limited degrees of freedom. The visit event was defined as any recorded interaction with the GF unit with a minimum stay duration of 2 minutes on a given day (visit=1). Observations with missing dates or visit values were excluded from the analysis.

As the data were non-normally distributed and included small sample sizes, a non-parametric Mann-Whitney U test was used to compare visit frequency and visit duration between the two pellet types, pooling data across all GF units.

Results and discussion

Ewe visiting pattern and adaptation

During the study period, 70 ewes had their tag read at least once by a GF unit. Twenty-two of the 76 ewes (28.9%) consistently visited the GF units and remained for at least 2 minutes/visit, which is the minimum duration required for a valid CH_4 measurement. The remaining ewes (n=54) exhibited insufficient stay durations for valid CH_4 measurements. The number of daily visits and the number of visits/ewe/day were variable (Table 1).

Table 1: Descriptive statistics on daily spontaneous visit patterns, stay durations and pellet consumption of 76 two-tooth Romney ewes using 3 GreenFeed units over 20 experimental days.

Item	Mean ± SD	Range
Daily visits[a]	42.8 ± 14.9	28-58
Visits/ewe/day[a]	4.6 ± 1.12	3 - 7
Stay duration/visit[b], minutes	5:32 ± 3:21	2:00 - 12:45
Pellet consumption[b], g/d	24.9 ± 21.0	6.00 - 92.9

[a] Calculated for all 76 ewes used during the GreenFeed adaptation period.

[b] Represent data from a subset of 22 ewes that showed adequate stay durations (≥2 minutes/visit) for valid methane measurements, as recorded by the C-clock system.

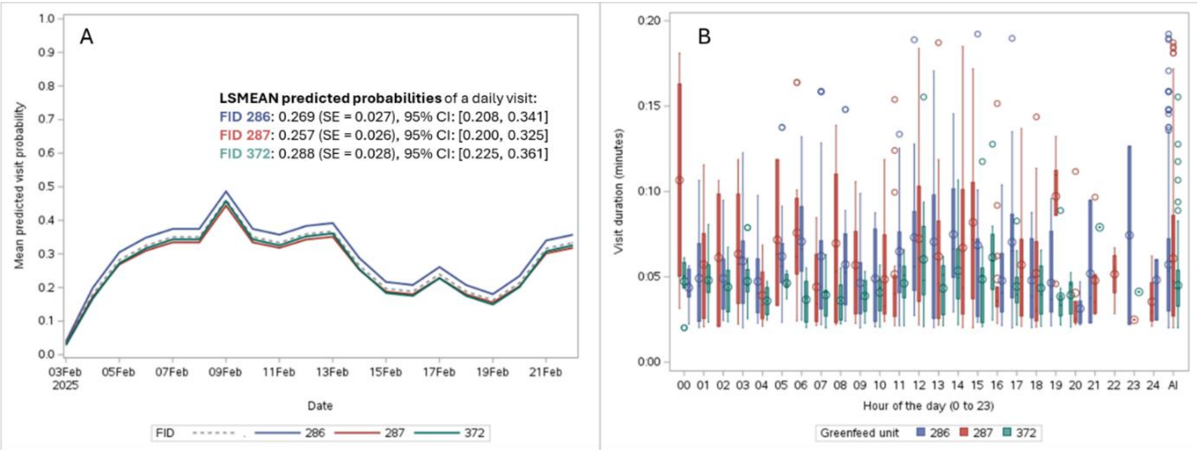

Figure 2. Temporal and diurnal patterns of 22 two-tooth Romney ewes that showed adequate stay durations (≥2 minutes/visit) interaction with GreenFeed Units (GF 286, GF 287 and GF 372). *A: LSMEAN (least square means) predicted daily visit probability by GreenFeed unit (FID) from February 3 to February 21, 2025 (SE = standard error; CI = confidence interval). B: Distribution of visit duration (minutes) by hour of day and FID (censored at 2 minutes).*

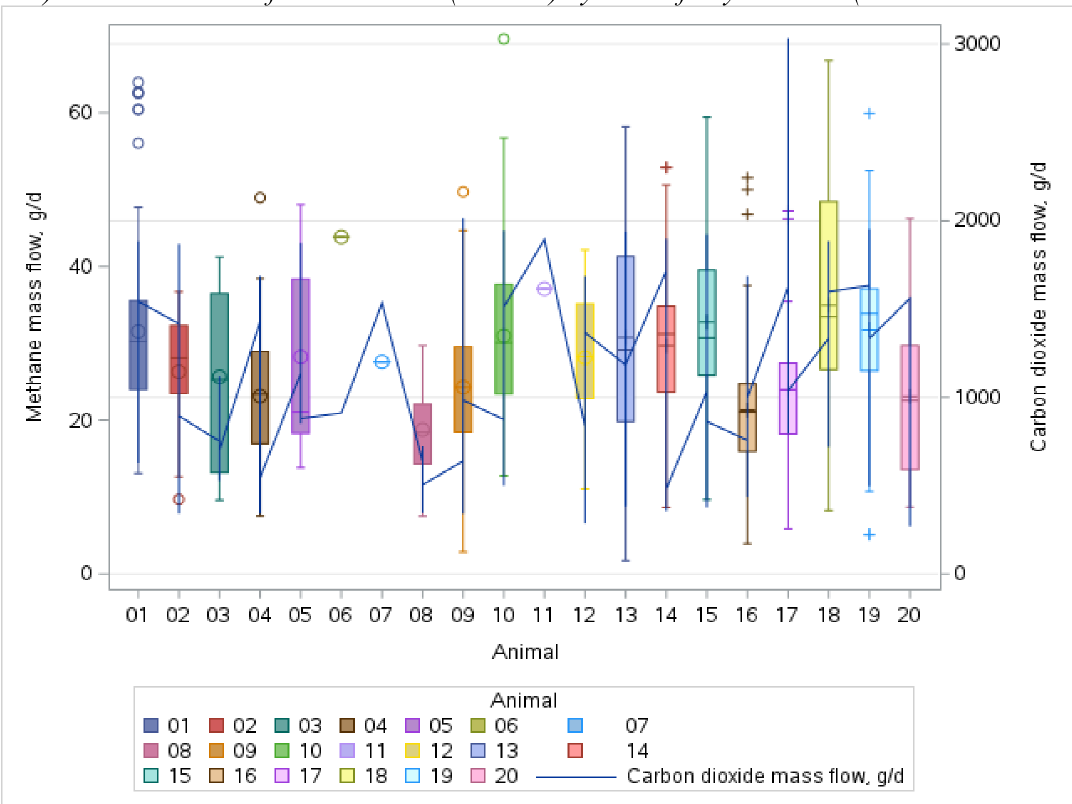

Figure 3: Valid CH_4 and CO_2 emission measurements (g/d) of 20 individual ewes (individual ewe ID numbers shown) meeting minimum stay duration ≥ 2 minutes from 5 to 21 February 2025 (n = 882 visits). Note: Two of the 22 ewes could not be included because their tags were unreadable by the GreenFeed units.

Over the trial period, ewe engagement with the GF units was variable but followed identifiable patterns of adaptation (Figure 2A). The predicted probability of an ewe visiting a GF unit on any given day ranged from 0.20 to 0.35, depending on the date and GF unit. These probabilities indicate that, on average, an individual ewe had a 20-35% chance of interacting with a GF unit on a given day once adapted to the system.

The initial period of exposure showed a gradual increase in visit frequency, indicating a learning or adaptation phase. By the second week, there was a subset of ewes consistently visiting the GF units, particularly those ewes associated with GF 372, which had the highest average predicted visit probability across time. Nevertheless, differences in predicted daily probabilities among GF units were minor and not statistically significant, indicating broadly similar levels of engagement across units and therefore, researchers may not need to be concerned about unit variation. The random intercept variance for animals was estimated at 1.02, confirming substantial between-animal variability in visit behaviour. The hourly visit duration patterns (Figure 2B) illustrate behavioural differences, indicating that while group-level trends were comparable, individual ewes varied in their likelihood and timing of engagement with the GF system. Temporal trends revealed fluctuations in visit probability that may reflect environmental conditions or variations in individual animal behaviour. There was no significant difference in either the number of visits ($p = 0.14$) or visit duration ($p = 0.13$) between whole and broken pellets across all GF units.

Enteric CH_4 *and carbon dioxide* (CO_2) *emission variability of ewes*

There was a substantial inter-individual variation among ewes (Figure 3). The majority of ewes exhibited median CH_4 mass flow rates ranging from 20-40 g/d, with interquartile ranges usually varying from 10-15 g/d. Notable individual differences were observed, with some ewes showing numerically higher median emissions (30-40 g/d) and wider distributions, while some demonstrated numerically lower and more consistent emission patterns. Many ewes (47.6%) displayed significant outlier measurements, which recorded maximum emissions exceeding 50 g/d/visit.

The CO_2 mass flow variations of individual ewes during their visits followed a similar pattern of variation as observed in CH_4 mass flow (Figure 3). Most ewes showed median CO_2 mass flow rates ranging from 500-1500 g/d.

Comparison of the GF units' performances

(i) GF Feeder operation

Whole pellets had a diameter of 6 mm and an average length of 14.0 ± 7.20 mm, while broken pellets had the same diameter and an average length of 9.95 ± 2.53 mm. The average weight of broken pellet drops was 4.60 (±0.10) g, 6.12 (±0.57) g, and 4.51 (±0.12) g for GF 372, GF 286, and GF 287, respectively. For whole pellet drops, the average mass was 4.64 (±0.76) g for GF 372 and 5.17 (±0.01) g for GF 286. The drop size delivered from GF 286 was bigger (5.74±0.261 g) than that delivered by GF 287 and 372 (4.51±0.413 g and 4.62±0.261 g, respectively).

(ii) CH_4 and CO_2 measurements

The daily CH_4 emission averages over the measurement period revealed distinct patterns among the three GF units. All three GF units showed day-to-day variability. Carbon Dioxide measurements showed more consistent patterns for all three GF units, with a similar relative ranking between the GF units as for the CH_4 emissions.

Although numerous studies have assessed CH_4 emissions in cattle using GF units (e.g., Waghorn et al., 2016), there is a lack of published research involving sheep. Most CH_4 emission measurements in sheep have been conducted using alternative techniques, such as the SF_6 tracer method. To date, no peer-reviewed literature has reported the visiting pattern of ewes to GF systems for quantifying enteric CH_4 emissions in sheep grazing pasture.

Ewe visiting behaviour and adaptation success

The adaptation period used in GF studies varies across research, typically ranging from 3 to 16 days for cattle (Hammond et al. 2016). Although the ewes used with GF units were initially identified as regular pellet consumers, some displayed a low preference for pellets when fresh pasture was abundantly available. Although a proportion of ewes successfully adapted to the GF units, the overall predicted probability of daily visits, ranging from 0.20 to 0.35, indicates low to moderate engagement. This level of visitation may be insufficient for high-resolution intake or emission monitoring. Past research has also highlighted the irregular visiting pattern of livestock ruminants to GFs (Jonker et al. 2016). This study suggests that under traditional pastoral grazing systems, ewes require an extensive time period for adaptation to GF units compared to other ruminants. The review conducted by Hammond et al. (2016) on CH_4 emission testing using GF units in cattle grazing systems has also indicated that longer sampling periods and more animals are required compared to respiration chamber method and SF_6 tracer techniques, due to the high variance of animal visits. Therefore, these findings suggest that while the units were functional and accessible, further strategies (e.g., extended training periods, improved baiting, or unit repositioning) may be necessary to improve consistency and sampling density.

Enteric CH_4 *and Carbon dioxide* (CO_2) *emission variability of ewes*

In the study, each ewe contributed approximately five valid visits/day over a 20-day measurement period, providing a robust dataset. There was considerable variation between individual ewes in both CH_4 and CO_2 measurements. This variation is likely due to the measurement uncertainty associated with irregular visiting patterns. It also reflects that emissions are not constant throughout the day and depend on the timing of visiting relative to feeding.

Comparison of GF units' performances

Pellets with a 6 mm diameter were initially selected for the study to ensure bait consumption remained below 10% of daily dry matter intake, which could influence the natural grazing behaviour and affect ewes' enteric fermentation patterns. It highlights the importance of maintaining natural feeding behaviour to obtain representative CH_4 measurements. Both whole and broken pellets aligned with the manufacturer's recommendation that pellet length should be less than 20 mm (C-Lock Inc. 2024). The weight/drop varied between feeders (GF 372, GF 286, and GF 287; $P < 0.01$) despite using pellets of the same length, highlighting the need to measure the drop size, report it in publications and rotate units between experimental treatments to avoid confounding results caused by differences in pellet consumption. Furthermore, it is important to allow sufficient time in the adaptation period for troubleshooting in pastoral grazing settings.

This study is the first to quantify the adaptation of sheep to GF emission monitoring units under NZ's pastoral grazing conditions. The findings highlight the behavioural variability and moderate engagement levels of naïve sheep, offering a realistic benchmark for future trials. By identifying key challenges in training, pellet standardisation, and measurement consistency, this work provides valuable guidance for researchers and farmers planning to implement GF units in grazing settings. We encourage the research community to share their adaptation protocols and outcomes to build a collective evidence base that supports more efficient and informed deployment of GF technology in small ruminant systems.

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