Evaluating ZELP Sense™ and GreenFeed™ Performance for Measuring Enteric Methane in Lactating Cattle

Introduction:

Greenhouse gas emissions have increased substantially over recent decades, heavily contributing to rising temperatures and emphasising the urgency of meeting the targets set under the Paris Agreement [1].

Methane (CH_4) is of particular concern because of its strong 20-year warming potential, which is approximately 84 times greater than that of carbon dioxide (CO_2). As a result, mitigation of CH_4 emissions has become a key focus in efforts to reduce the climate impact of livestock production.

Accurate quantification of enteric CH_4 emissions remains challenging. Currently available CH_4 measurement tools typically involve trade-offs between accuracy, scalability, cost, and labour requirements. Highly accurate methods such as respiration chambers are expensive, labour-intensive, difficult to scale, and require cattle to be removed from their natural environment [2–4]. In contrast, field-based approaches such as spot sampling systems, like Greenfeed (GF, C-Lock Ltd, Rapid City, South Dakota, USA) are more practical and cost-effective in certain conditions, but may sacrifice accuracy be hard to deploy on pasture, or require a proportional amount of interaction between the animal and the measurement device [5].

Aims:

The aims of this study were to measure CH_4 emissions in lactating cows and [1] compare CH_4 estimates obtained using ZELP Sense with those measured using GF, and [2] to investigate sensitivity of ZELP Sense at higher and lower emission levels by evaluating its response to diets with and without additive treatment effects.

Materials and Methods:

<u>Experimental design:</u>

The study was conducted at a research facility in the United Kingdom, between the 1^{st} and 11^{th} of December 2025, using eleven lactating Holstein-Friesian cows housed indoors.

The study consisted of two phases. During the first phase, cattle underwent a three-day adaptation period wearing complete non-functional ZELP Sense devices to familiarise the animals with the equipment. Immediately following fitting, animals were continuously observed for two hours to monitor for any persistent abnormal behaviours. Then, they were observed twice a day during the remaining adaptation period for unusual behaviours.

The second phase consisted of simultaneous CH_4 measurements using ZELP Sense and GF. Measurements were conducted over two four-day monitoring periods. Six cows were monitored from 1-4 December, while the remaining five cows were monitored from 8-11 December. A ZELP Sense device remained fitted to each cow continuously throughout each monitoring period.

Animals were divided into three dietary treatment groups. Four control animals received a standard Total Mixed Ration (Con TMR). Four animals received Total Mixed Ration supplemented with a CH_4 reducing feed additive (treatment TMR), while three animals received the additive only during parlour feeding (parlour treatment). One cow assigned to the Parlour treatment group was excluded prior to analysis because of insufficient interaction with GF during the study.

<u>Animals and Site:</u>

The 11 cows were in stage 4 of lactation with a mean body weight was 706 ± 63.34 kg, excluding two cows for which weight measurements were unavailable.

All cattle were fitted with medium or large ZELP Sense devices, predominantly medium, with material trimmed around the ears where required to allow adequate mobility while maintaining correct nosepiece alignment around the muzzle. A ZELP WiFi gateway was installed to support device connectivity.

<u>ZELP Sense Set-Up:</u>

The ZELP Sense device comprised three main components: a breathable neoprene headpiece available in extra small, small, medium, and large sizes; a nosepiece, available in small and medium sizes, with two baffle arms, one of which is designed to direct the exhaled plume towards the gas sensing unit (GSU); and the GSU itself, which houses the electronic components, including batteries, infrared sensors, and the pump.

The system operates by sampling a portion of the exhaled plume at 2 Hz (every 0.5 seconds), alongside background environmental air and additional contextual parameters. Measurements of CH_4 and CO_2 are corrected for dilution using a proprietary algorithm (version 3.01) developed by ZELP. A representative image of the ZELP Sense device fitted to a Holstein Friesian cow is shown in Figure 1.

Figure 1: Cow wearing ZELP Sense. 1- Headpiece; 2- Nosepiece; 3- Gas Sensing Unit (GSU).

For this study, outputs were generated both as daily CH_4 emissions (g/day) and as consecutive 6-hour interval emission-rate estimates showing the average CH_4 emission rate (g/day) over each interval (T to T + 6 h, T = timestamp).

Statistical analysis

To evaluate whether the six-hour interval aggregation of ZELP Sense values influenced agreement with GF, ZELP Sense CH_4 estimates were assessed using both daily aggregated values and values from the 6-hour intervals.

Completeness was assessed to determine usability, with 0 indicating good completeness and 1 indicating data that were not usable. ZELP Sense data collected during GF sampling visits were excluded from analysis, as the GF pump may not allow the exhaled gas plume to be adequately collected.

For GF data, the frequency of visits by cattle in each group was examined as a potential source of bias. Visit duration was also evaluated in relation to treatment group, and daytime and nighttime GF visits were characterised.

Statistical analysis was performed using R for statistical computing (version 4.5.2). The comparison of CH_4 emission measurements using GF and ZELP Sense, considering both daily and six hourly measurements separately, was conducted by linear mixed modelling (LMM), with measurement method entered as fixed effect and animal ID entered as random effect, while controlling for the potential effect of DMI and diet additive. Given that some outlying measurements were identified in initial exploratory analysis, the model fit was based on the robust scoring equations estimator to downplay their influence on the results. Estimated marginal means were obtained from the model and statistically compared between methods at average values of the control variables. Statistical significance was assessed at the 5% level. For ZELP Sense data at different resolutions, separate LMMs were fitted for each temporal resolution.

Estimated marginal mean (EMM) was calculated from the final fitted model to provide adjusted mean CH_4 emissions for each measurement method after controlling for the effects of dry matter intake (DMI) and dietary treatment. The EMM was visualised with corresponding 95% confidence intervals for comparison between GF and ZELP Sense.

Results:

ZELP Sense device wear was well tolerated, with no removals required during the adaptation nor measurement periods. The only mechanical issue observed during the study was occasional blockage of the sampling nosepiece baffle arm, which reduced gas plume capture and consequently affected dilution model performance. No additional datasets required exclusion beyond measurements collected during active GF visits.

Prior to the primary analysis, agreement between GF and ZELP Sense was evaluated using both daily CH_4 estimates and 6-hour interval estimates. LMM analysis demonstrated that both data resolutions produced highly comparable results, with similar estimated marginal means, pairwise comparisons and statistical significance. Consequently, only the 6-hour interval analysis is presented here, as it provides greater temporal resolution while reaching the same conclusions as the daily values analysis.

LMM demonstrated no statistically significant difference between CH_4 emissions estimated by ZELP Sense and those estimated by GF, after accounting for DMI and dietary treatment. Using the 6-hour interval estimates, measurement method was not a significant source of variation ($X^2 = 2.48$, $p = 0.116$). Likewise, neither DMI ($p = 0.124$) nor dietary treatment ($p = 0.275$) significantly influenced the comparison between methods within the fitted model.

Estimated marginal means derived from the LMM showed that GF measured CH_4 emissions approximately 22.5 g CH_4/day higher than ZELP Sense on average after adjustment for DMI and dietary treatment. However, this difference was not statistically significant (standard error, SE = 14.3 g/day; $t = 1.57$; $p = 0.246$), indicating good agreement between the two measurement systems (see Fig. 2).

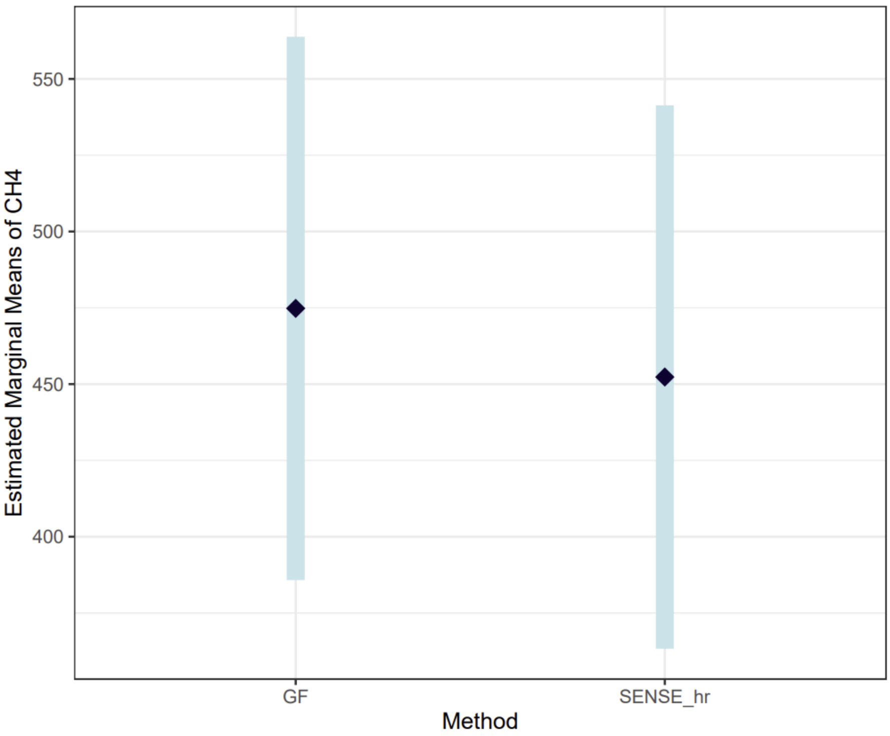

Figure 2: Estimated marginal means (EMMs) of CH_4 emissions predicted by the linear mixed model for GF and ZELP Sense at 6-hour interval estimates (ZELP Sense_hr). Means were adjusted for dry matter intake (DMI) and dietary treatment, with vertical bars representing the 95% confidence intervals.

Discussion:

This study's main finding is that, after adjustment for DMI and dietary treatment, CH_4 estimates from ZELP Sense did not differ significantly from those measured by GF. The estimated marginal means indicated that GF measured slightly more CH_4 than ZELP Sense on average, but this difference was not statistically significant. Together with the lack of a significant method effect in LMM, these results support good agreement between the two methods under the conditions tested.

RC often remains the reference method for enteric CH_4 quantification because they capture total exhaled gases in a controlled environment, but they are costly, have low-throughput, and can alter animal behaviour and feed intake during isolation. GF is a practical alternative that samples eructated and exhaled gases during voluntary visits to a feed-baited unit, and multiple studies have reported reasonable agreement with RC measurements at the group level, though individual-animal ranking can be more variable and sensitive to visit frequency and training. In this context, the non-significant mean difference between ZELP Sense and GF observed here is encouraging.

The magnitude of the observed bias is small relative to typical daily emissions from lactating cows and lies within the range of method-to-method differences reported for other GF comparisons. For example, GF has shown differences of around 1-3% versus RC and mass-flow controllers over short periods [6], and larger between-animal variation when visit frequency is low or short-lasting [3,7] . The present study's design, with multiple days of simultaneous measurement and statistical control for DMI and diet, likely helped to stabilise these estimates and reduce method effects.

With no significant difference found in this study between ZELP Sense data at 6-hour intervals and daily totals, the higher resolution is valuable for capturing diurnal patterns linked to feeding, milking, and rumination, which are difficult to resolve with infrequent GF visits or short chamber runs [6,8,9]. Continuous monitoring also reduces reliance on animals' voluntary interaction with a fixed unit, which can be a limiting factor for GF in some systems and can introduce bias if visit frequency or duration differs across treatments or individuals [8].

Some limitations should be acknowledged. First, the study was conducted with 11 lactating Holstein-Friesian cows over two 4 day monitoring windows in a single housing system. While sufficient to detect large method effects, larger multi-site studies across lactation stages, diets, and management systems will be needed to generalise these findings and to evaluate individual-animal ranking performance. Second, GF data inherently depend on visit frequency and duration; although visit patterns were analysed and no major biases were detected, residual variability in GF sampling could affect the precision of the comparison.

The lack of a significant method effect between ZELP Sense and GF, together with the device's continuous sampling and no obvious effect on animal welfare, suggests that ZELP Sense can be used to evaluate dietary mitigation strategies and management interventions in settings where GF or chambers are impractical or too costly to deploy at scale.

References:

1. Rogelj, J. *et al.* Paris Agreement climate proposals need a boost to keep warming well below 2 °c. *Nature* **534**, 631–639 (2016).

2. Llonch, P. *et al.* Changes in feed intake during isolation stress in respiration chambers may impact methane emissions assessment. *Anim. Prod. Sci.* **58**, 1011–1016 (2018).

3. Della Rosa, M. M., Jonker, A. & Waghorn, G. C. A review of technical variations and protocols used to measure methane emissions from ruminants using respiration chambers, SF6 tracer technique and GreenFeed, to facilitate global integration of published data. *Anim. Feed Sci. Technol.* **279**, 115018 (2021).

4. Troy, S. M. *et al.* A comparison of methane emissions from beef cattle measured using methane hoods with those measured using respiration chambers. *Anim. Feed Sci. Technol.* **211**, 227–240 (2016).

5. Ma, X. *et al.* Evaluating GreenFeed and respiration chambers for daily and intraday measurements of enteric gaseous exchange in dairy cows housed in tiestalls. *J. Dairy Sci.* **107**, 10913–10931 (2024).

6. McGinn, S. M., Coulombe, J. F. & Beauchemin, K. A. Technical note: validation of the GreenFeed system for measuring enteric gas emissions from cattle. *J. Anim. Sci.* **99**, (2021).

7. Arthur, P. F. *et al.* Optimizing test procedures for estimating daily methane and carbon dioxide emissions in cattle using short-term breath measures. *J. Anim. Sci.* **95**, 645–656 (2017).

8. Coppa, M. *et al.* Repeatability and ranking of long-term enteric methane emissions measurement on dairy cows across diets and time using GreenFeed system in farm-conditions. *Methods* **186**, 59–67 (2021).

9. Zhao, Y. *et al.* A Review of Enteric Methane Emission Measurement Techniques in Ruminants. *Animals (Basel).* **10**, 1–15 (2020).