Validation of ZELP Sense™ for Quantifying Enteric Methane and Carbon Dioxide Emissions under Feed Additive Conditions in Cattle

Introduction:

Methane (CH_4) is a potent greenhouse gas (GHG) with a global warming potential approximately 28 times that of carbon dioxide (CO_2) over a 100-year period, and approximately 84 times greater over a 20-year period [1]. The largest anthropogenic source of CH_4 is enteric CH_4 produced by cattle [2]. As a result, there is considerable interest in measuring enteric CH_4 emissions and developing mitigation strategies to reduce CH_4 production from cattle [2]. With CO_2 production being directly influenced by metabolic rate and energy use, CO_2 measurement relative to liveweight and feed intake can help producers to identify the most efficient animals. These animals can have lower feed costs and support sustainable breeding problems [3].

Available CH_4 and CO_2 measurement methods tend to have trade-offs between accuracy, labour intensity, cost, scalability and behavioural effects. Respiration chambers (RC), typically seen as the gold standard in CH_4 and CO_2 measurement, are costly and restrictive to normal cattle behaviour [4–6]. Similarly, feeder-based spot samplers such as the GreenFeed system (GFS) (C-lock Inc, USA), although more cost effective and easier to deploy, are spot samplers that rely on animal-unit interaction, with data accuracy influenced by time of interaction and duration [7–9].

This pilot study aims to: [1] evaluate the agreement of CH_4 and CO_2 production (g/day) between RC and ZELP Sense™ at daily and 6-hourly intervals; [2] assess ZELP Sense's ability to detect and quantify the reduction in CH_4 emissions after the introduction of a CH_4 suppressing feed additive; [3] understand ZELP Sense's CH_4 and CO_2 sensitivity thresholds at lower emission levels.

We hypothesised that (i) the CH_4 and CO_2 data collected from ZELP Sense would show moderate to strong agreement with RC at both daily and 6-hourly intervals; (ii) ZELP Sense would detect a significant difference in post-treatment reductions in CH_4 emissions, in a time frame comparable to that of RC; (iii) the reduction in CH_4 post-treatment, if significant, would not significantly affect the agreement between the emission values provided by RC and those provided by ZELP Sense for both time resolutions.

Materials and Methods:

Animals and Experimental Design:

Four Holstein Friesian cattle were randomly allocated for the trial, which took place at a European-based faculty with RC.

The cattle comprised of a mix of dry cows (#40, #18) and heifers (#84, #27). The cattle's average liveweight prior to the trial was 632.5 ± 47.35 kg (± Standard Error of Mean).

All cattle involved were included in an adaptation period, which comprised of the cattle being introduced to RC use, by gradually introducing them to tie-stalls over 2-3 days, and to ZELP Sense use, by gradually fitting the device over 2-3 days (the headpiece and nosepiece were fitted first, before the gas sensing unit was added subsequently).

Cattle entered the open-circuit indirect RC at 9:00 on Day 1 and exited at 9:00 on Day 4, allowing for measurement to occur over four 24-hour periods. Cattle were fed at 9:30 and 15:00 every day.

There were two measurement periods: a control period (P1, the first two 24-hour periods) and a treatment period (P2, the second two 24-hour periods). During P1, all 4 cows received a maize silage and grass silage-based diet with supplemented calf concentrate and dairy minerals (11.7kg/d dry matter) to provide a baseline emissions profile. In P2, a CH_4 suppressing feed additive was supplemented in the same diet at 80 mg per kg/DMI. Water was provided *ad libitum* throughout both measurement periods.

ZELP Sense Set-Up:

The ZELP Sense device was a prototype version of all components, with firmware v4.1.0 and Machine Learning pipeline v3.0. It was made up of three main components: the headpiece, the nosepiece and the gas sensing unit (GSU).

The headpiece (1) was a breathable neoprene halter, which held the nosepiece near the cow's nostrils. The nosepiece (2) was made up of 2 baffle arms, 1 of which sampled the gas plume coming from the cow's nostril, directing it to the GSU. The GSU (3), housing batteries, sensors and a pump, processed the gas samples and offloaded the data via WiFi. The device sampled continuously, and data was processed by ZELP's Machine Learning model, which provided CH_4 and CO_2 values in grams per day, corrected for temperature, pressure and humidity.

Statistical Analysis:

All data was analysed and plotted using R (v4.6.0): main packages included "ggplot2" v4.0.3 and "dplyr" v1.2.1.

For the agreement analysis, both RC and ZELP Sense data were distributed into daily totals and four 6-hour windows (g/day) to reduce sampling frequency bias. The 6-hour windows were set at 9:00-15:00; 15:00-21:00; 21:00-3:00 and 3:00-9:00, with each value representing the average emission rate (g/day) across that window, plotted at the interval's start time. Daily totals were calculated by aggregating data across the 24-hour period spanning from 9:00 to 9:00 the following day. This resulted in 64 paired observations for the 6-hour analysis (n=4) and 16 paired observations for the daily agreement analysis (n=4). Four 6-hourly measurements and one daily measurement were then removed from the analysis due to a RC issue on Day 3 for Cow #40, yielding a total of 60 paired observations for the 6-hour analysis and 15 paired observations for the daily emissions agreement. The last 6-hourly data point was removed from the analysis due to the cows exiting RC prior to 9:00.

Emissions were modelled using linear mixed models (LMM) fitted by restricted maximum likelihood (REML), including method and day as fixed effects and animal ID as a random effect, allowing for heterogeneous variances between methods. For Bland-Altman analysis, paired daily values were utilised to determine the mean of the two methods and their difference (ZELP Sense - RC), with agreement summarised using the mean bias and 95% limits of agreement (bias ± 1.96 SD).

Individual animal emission patterns were plotted at six-hour intervals and averaged across cattle (with standard error of the mean ±SEM) for both CH_4 and CO_2 in order to assess closeness in pattern of emissions.

In the six-hour interval data, the fitted LMM was visualised by overlaying it on the raw data per cow in order to assess the CH_4 reduction. As a white paper, this present study focuses solely on ZELP Sense's ability to detect the CH_4 reduction, as opposed to assessing the efficacy of the feed additive or any DMI- driven CH_4 changes between P1 and P2.

To evaluate agreement between methods, mean absolute percentage errors (MAPE, %), mean percentage error (MPE, %), Pearson's correlation (r) and Lin's Concordance Correlation Coefficient (CCC) [10] were calculated for the paired daily values and the 6-hour values for both measurement periods (P1; P2) and overall (P1+P2) for both CH_4 and CO_2.

Results:

Daily CH_4 Agreement between RC and ZELP Sense:

Daily CH_4 emissions measured by RC and ZELP Sense are summarised in Table 1, with agreement statistics presented in Table 2.

Table 1: Mean ± SEM daily CH_4 emissions measured by respiration chambers (RC) and ZELP Sense across the control measurement period (P1), the feed additive treatment measurement period (P2) and both periods combined (P1+P2)

Period	RC (g/day)	ZELP Sense (g/day)
P1 (Control)	288 ± 34	280 ± 55
P2 (Treatment)	243 ± 25	244 ± 41
P1 + P2 (Overall)	267 ± 37	262± 51

Mean CH_4 emissions decreased during the feed additive period for both measurement systems. RC measurements declined from 288 ± 34 g/day during the control period (P1) to 243 ± 25 during treatment (P2), while ZELP Sense measurements declined from 280 ± 40 g/day to 244 ± 41 g/day.

Table 2: Daily CH_4 Method Agreement Statistics between respiration chamber (RC) and ZELP Sense, using RC as reference method across the control measurement period (P1), the feed additive treatment measurement period (P2) and both periods combined (P1+P2).

Period	Pearson's r	CCC	MAPE (%)	MPE (%)	Bias (g/day)
P1 (Control)	0.885	0.780	7.98	-3.18	-7.68
P2 (Treatment)	0.885	0.805	6.86	-3.48	-7.93
P1+ P2 (Overall)	0.899	0.844	7.45	-3.32	-7.80

Agreement between methods was consistently strong. Pearson's correlation coefficients exceeded 0.88 in both periods and increased to 0.899 when all observations were combined. Lin's concordance correlation coefficient (CCC) ranged from 0.780-0.844, while mean absolute percentage error (MAPE) remained below 8% across all analyses. ZELP Sense showed a small negative bias relative to RC (-7.68 to -7.93 g/day), indicating consistent slight underestimation of CH_4 emissions.

Bland-Altman analysis (Figure 1) demonstrated minimal systematic bias and acceptable limits of agreement across both measurement periods.

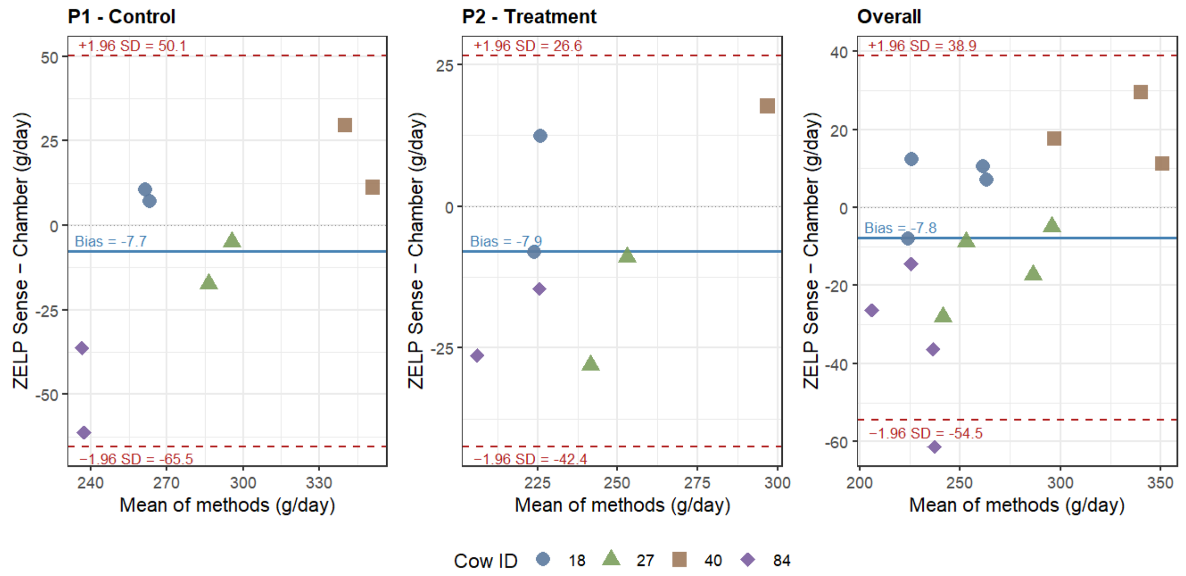

Figure 1: Bland-Altman plots showing agreement between CH_4 emissions values from respiration chambers (RC) and ZELP Sense produced during the control feeding period (P1), treatment period (P2), and across all observations combined (P2+P3). The blue continuous line represents the mean difference (bias) between the two methods, and the dotted lines represent the 95% limits of agreement (LoA)

CH_4 Agreement at six-hour intervals between RC and ZELP Sense:

Agreement statistics for six-hour interval observations are summarised in Table 3. Mean emission profiles are shown in Figure 2, individual animal CH_4 emission profiles in Figure 3, and LMM trajectories in Figure 4.

Table 3: Mean ± SEM CH_4 emissions (g/day) estimated from six-hour interval mean observations measured by respiration chambers (RC) and ZELP Sense during control feeding measurement period (P1) and CH_4 reducing feed additive treatment measurement period (P2), and both periods combined (P1+P2).

Period	Pearson's r	CCC	MPE (%)	MAPE (%)
P1 (Control)	0.844	0.838	-1.30	11.30
P2 (Treatment)	0.789	0.772	-2.90	8.40
P1+ P2 (Overall)	0.855	0.848	-2.00	10.00

Agreement remained strong during the control period (CCC = 0.838; r = 0.844) and decreased during treatment (CCC = 0.772; r = 0.789). When both periods were combined, Pearson's correlation was 0.855 and CCC was 0.848, with a MAPE of 10%.

Mean six-hour emission profiles (Fig.1, Fig.2) showed that ZELP Sense closely followed the CH_4 emission pattern measured by RC throughout both measurement periods.

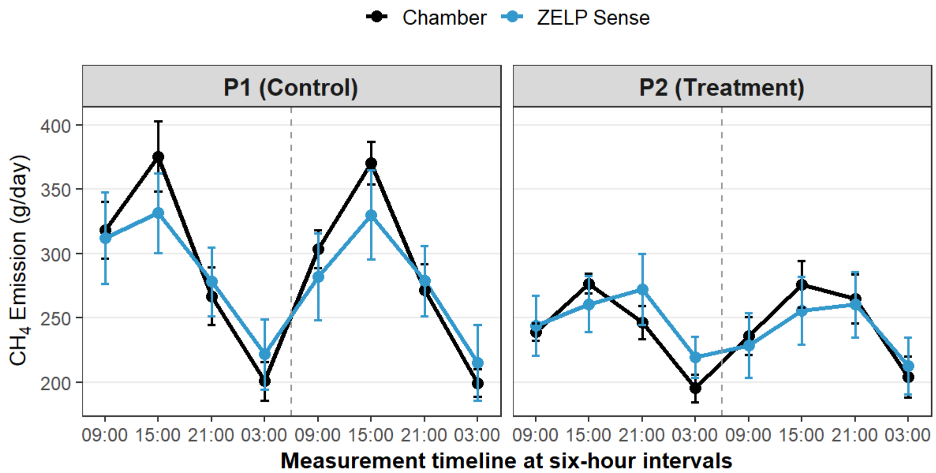

Figure 2: Emissions pattern showing six-hourly mean enteric methane (CH_4) emission rate measured by respiration chambers (chamber= black) and ZELP Sense (blue) during the control feeding measurement period (P1) and CH_4 reducing feed additive treatment measurement period (P2). Data points represent the mean emission rate (g/day) at each six-hour interval, with error bars depicting the standard error of the mean (±SEM). Vertical dashed lines are at 24-hour daily cycle.

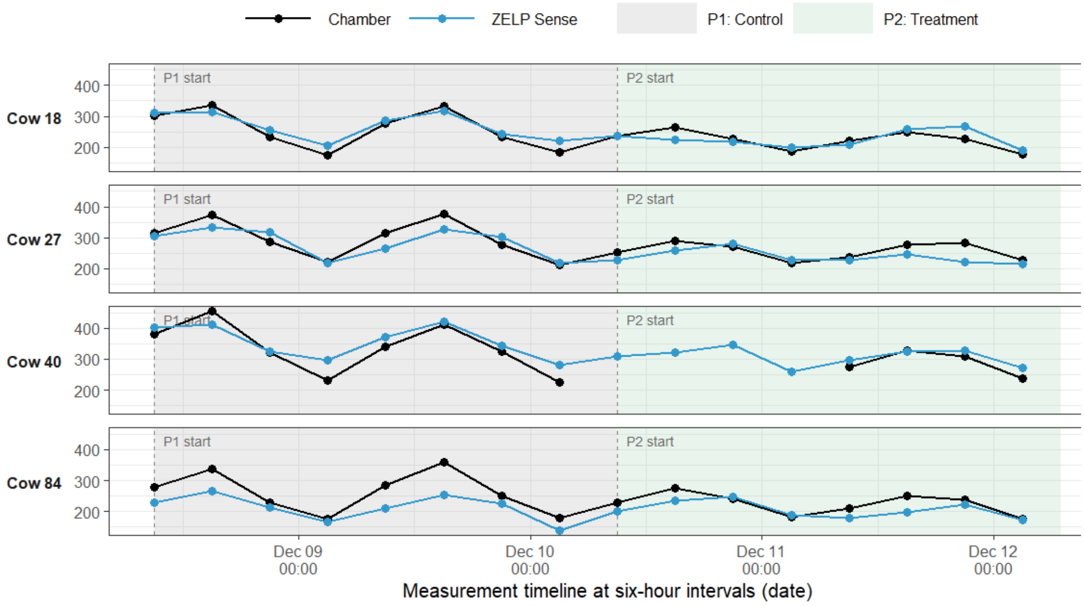

Figure 3: Individual emission profiles showing six-hourly enteric methane (CH_4) emissions measured by respiration chambers (chamber= black) and ZELP Sense (blue) across the four cows. Background shading shows control feeding measurement period P1 (Days 1-2, grey) and reducing feed additive treatment measurement period P2 (Days 3-4, green).

LMM in Figure 4 depicts the ability of ZELP Sense in detecting the CH_4 reduction post-treatment introduction.

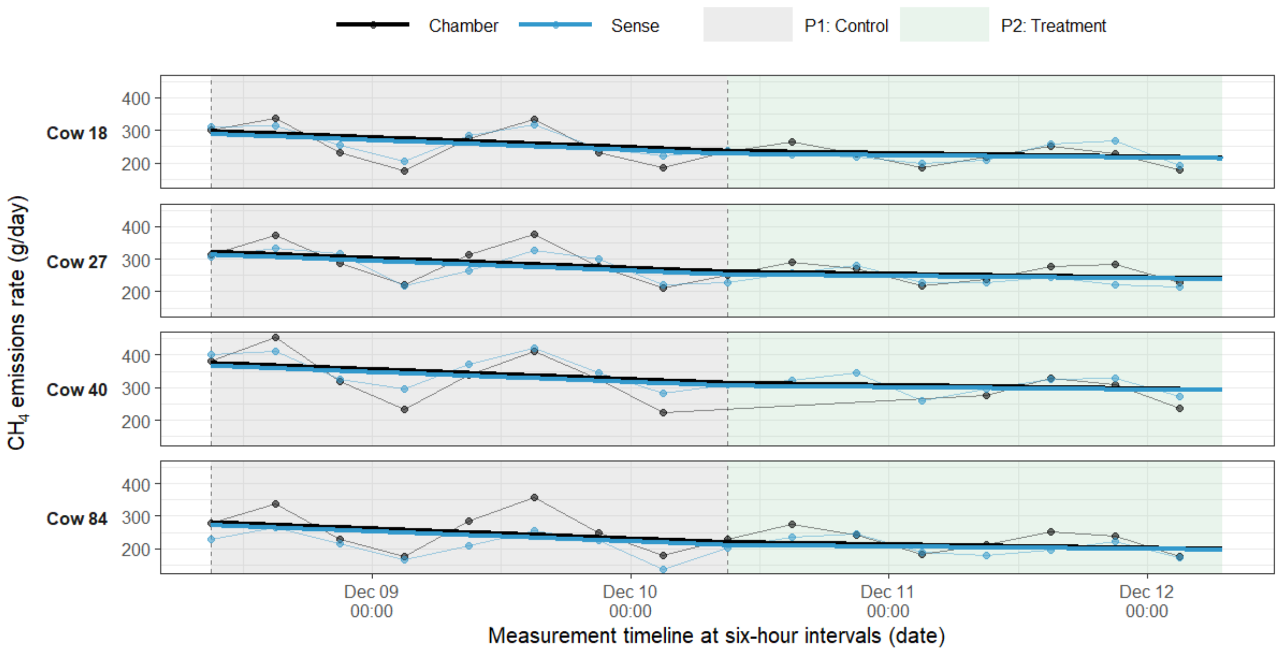

Figure 4: Linear mixed model (LMM) trajectories showing six-hourly enteric methane (CH_4) emission rates measured by respiration chambers (chamber= black) and ZELP Sense (blue) across the four cows. Faded points and thin lines represent six-hourly emission rates (), while bold lines depict the piecewise LMM fit (fixed + random intercept). Background shading shows control feeding measurement period P1 (Days 1-2, grey) and reducing feed additive measurement period P2 (Days 3-4, green).

Both measurement methods detected a significant reduction in CH_4 emissions in the treatment period. RC measured a reduction of 40.7 g/day ($p = 0.004$), while ZELP Sense detected a comparable reduction of 36.9 g/day ($p < 0.001$). LMM identified a significant decline in CH_4 emissions across the study ($p < 0.001$), but no significant additional change in slope was detected at the treatment transition ($p = 0.191$). No significant method-by-time interaction was detected ($p = 0.776$), indicating that ZELP Sense reproduced the temporal response observed by RC.

Daily CO_2 Agreement between RC and ZELP Sense:

Daily CO_2 emissions are summarised in Table 4, with Bland-Altman plots shown in Figure 5.

Table 4: Mean ± SEM daily CO_2 emissions measured by respiration chambers (RC) and ZELP Sense across the control measurement period (P1), the feed additive treatment measurement period (P2) and both periods combined (P1+P2)

Period	RC	ZELP Sense
P1 (Control)	9487 ± 465	9461 ± 1705
P2 (Treatment)	9162 ± 300	9200 ± 1285
P1+ P2 (Overall)	9335 ± 418	9330 ± 1465

Mean CO_2 emissions were broadly comparable between methods during the control and overall periods, although greater variability was observed for ZELP Sense measurements. During treatment, RC measured lower CO_2 emissions than ZELP Sense, resulting in reduced agreement.

Table 3: Daily CO_2 Method Agreement Statistics between respiration chamber (RC) and ZELP Sense, using RC as reference method across the control measurement period (P1), the feed additive treatment measurement period (P2) and both periods combined (P1+P2).

Period	Pearson's r	CCC	MAPE (%)	MPE (%)	Bias (g/day)
P1 (Control)	0.90	0.50	11.30	-0.80	-26.10
P2 (Treatment)	0.50	0.30	7.80	-2.80	-250.10
P1+ P2 (Overall)	0.80	0.40	9.70	-1.70	-130.60

Pearson's correlation decreased from 0.90 during P1 to 0.50 during P2, while CCC declined from 0.50 to 0.30. Across all observations, overall agreement was weak ($r = 0.80$; CCC = 0.40), despite an overall MAPE of 9.70%.

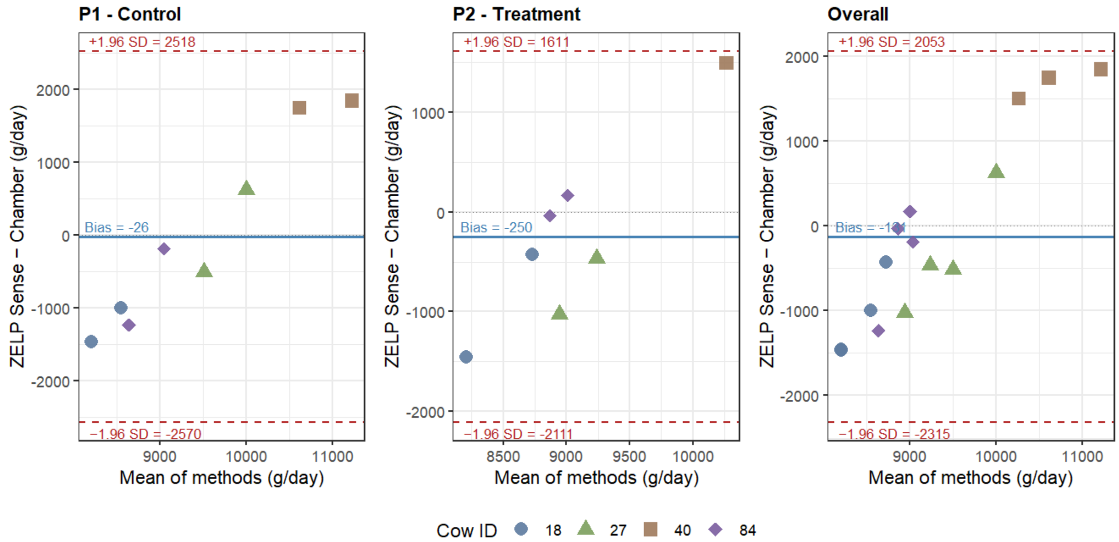

Figure 5: Bland-Altman plots showing agreement between CO_2 emissions values from respiration chambers (RC) and ZELP Sense produced during the control feeding period (P1), treatment period (P2), and across all observations combined (P2+P3). The blue continuous line represents the mean difference (bias) between the two methods, and the dotted lines represent the 95% limits of agreement (LoA).

CO_2 Agreement at six-hour intervals between RC and ZELP Sense:

Agreement statistics for six-hour CO_2 observations are presented in Table 6, with corresponding emission profiles shown in Figures 6-8.

Table 6: Mean ± SEM CO_2 emissions (g/day) estimated from six-hour interval mean observations measured by respiration chambers (RC) and ZELP Sense during the control measurement period (P1), the feed additive treatment measurement period (P2), and both periods combined (p1+P2)

Period	Pearson's r	CCC	MPE (%)	MAPE (%)
P1 (Control)	0.712	0.642	-0.30	11.7
P2 (Treatment)	0.441	0.421	-2.30	9.40
P1+ P2 (Overall)	0.642	0.593	-1.20	10.60

For CO_2, six-hour agreement was again weaker than CH_4. P1 showed moderate agreement (CCC = 0.642; r = 0.712), while P2 showed reduced concordance (CCC = 0.421; r = 0.441).

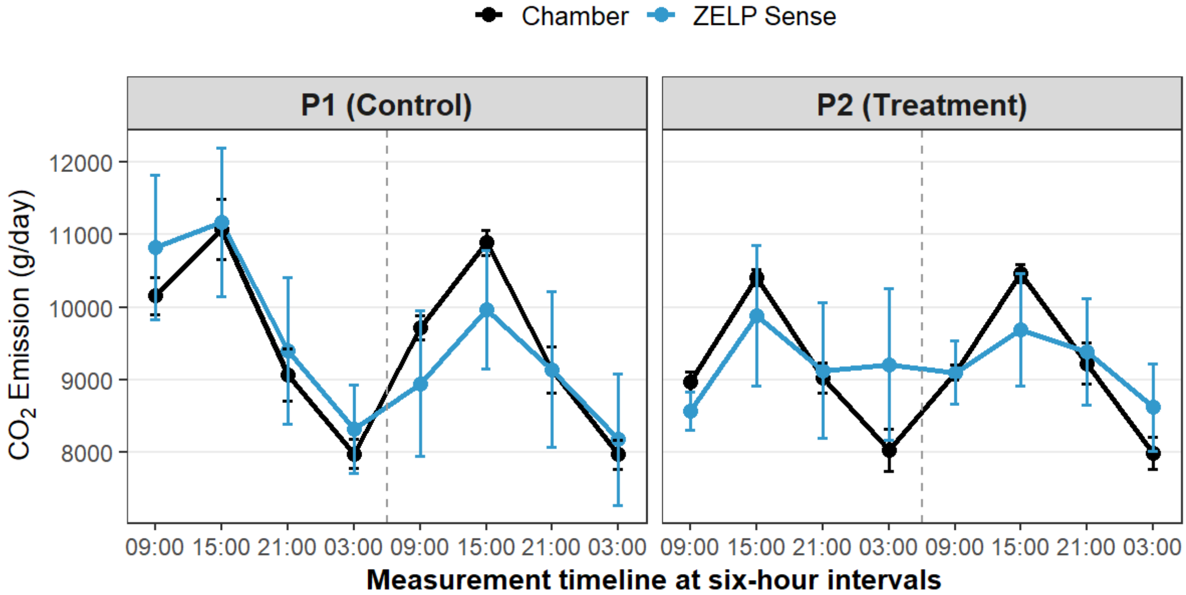

Figure 6: Emissions pattern showing six-hourly mean carbon dioxide (CO_2) emissions measured by respiration chambers (chamber= black) and ZELP Sense (blue) during the control feeding period (P1) and CH_4 suppressant treatment period (P2). Data points represent the mean emission rate (g/day) at each six-hour interval, with error bars depicting the standard error of the mean (±SEM). Vertical dashed lines are at 24-hour daily cycle.

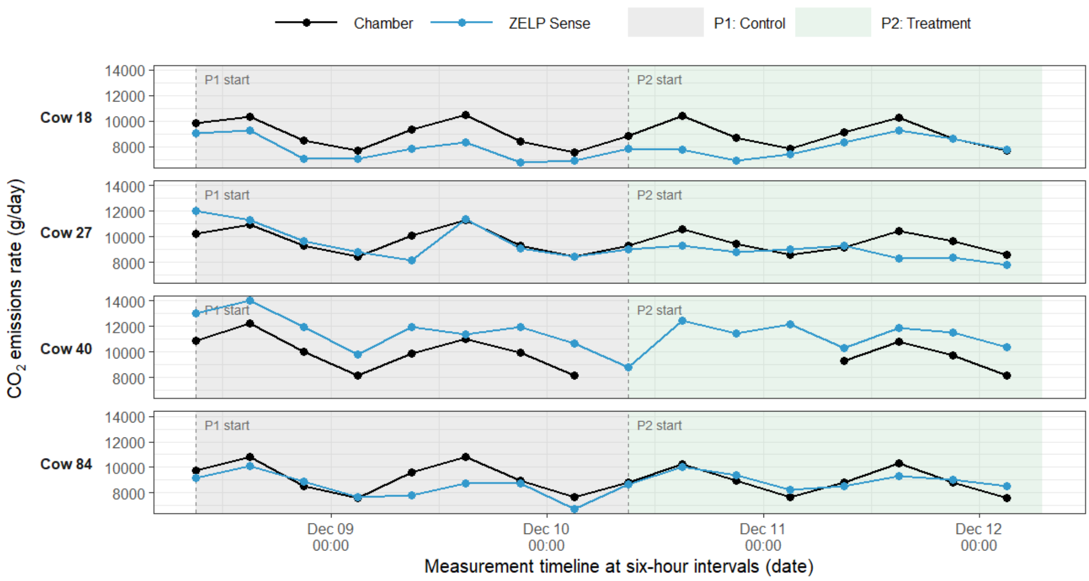

Figure 7: Individual emission profiles showing six-hourly carbon dioxide (CO_2) emissions measured by respiration chambers (chamber= black) and ZELP Sense (blue) across the four cows. Background shading shows control feeding period P1 (Days 1-2, grey) and suppressant treatment period P2 (Days 3-4, green).

Across both periods, CO_2 six-hour agreement was moderate to low (CCC = 0.593; r = 0.642), although mean percentage error remained close to zero (-1.20%) and MAPE was 10.60%. These results suggest that ZELP Sense captured average CO_2 magnitude reasonably but showed reduced precision in reproducing RC-derived temporal variation, particularly during the treatment period.

For CO_2, treatment-associated reductions were less consistent.

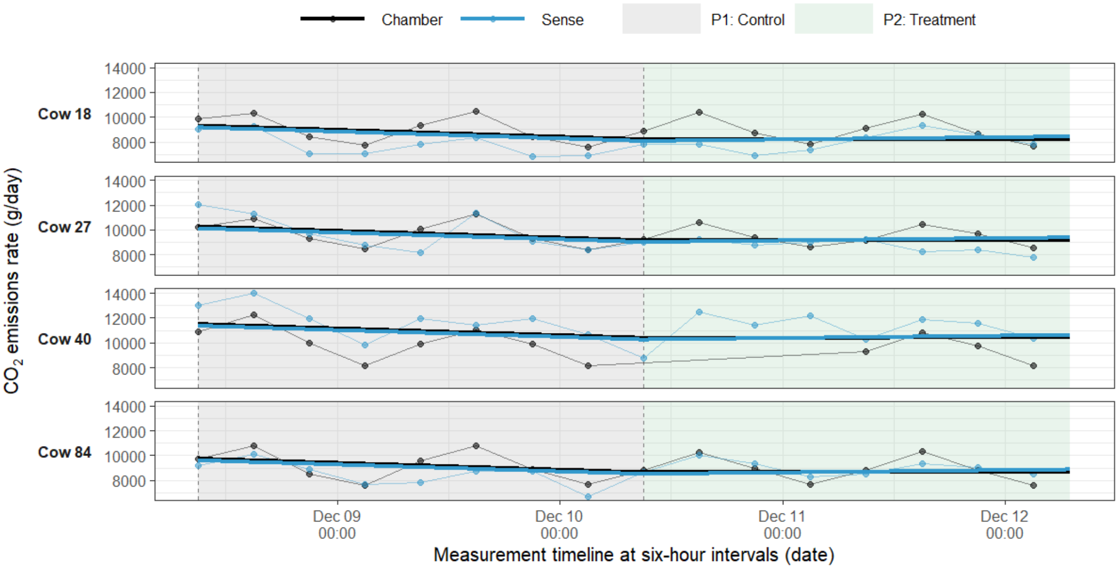

Figure 8: Linear mixed model (LMM) trajectories showing six-hourly carbon dioxide (CO_2) emissions measured by respiration chambers (chamber= black) and ZELP Sense (blue) across the four cows. Faded points and thin lines represent six-hourly emission rates (), while bold lines depict the piecewise LMM fit (fixed + random intercept). Background shading shows control feeding period P1 (Days 1-2, grey) and suppressant treatment period P2 (Days 3-4, green).

Both RC and ZELP Sense detected a reduction in CO_2 emissions in the treatment period, though neither reached statistical significance. RC estimated a reduction of 319 g/day (p = 0.253) and ZELP Sense a reduction of 294 g/day (p = 0.273). No significant method time interaction was detected (p = 0.598).

Discussion:

This pilot study evaluated the agreement between ZELP Sense and RC for the quantification of enteric CH_4 and CO_2 emissions in cattle under control and CH_4-suppressing feed additive conditions. Across both daily and six-hour analyses, ZELP Sense performed better for CH_4 than for CO_2, with high daily agreement, low error, small negative bias, and successful detection of the reduction in CH_4 emissions post-treatment introduction. These findings suggest that the device can emulate RC CH_4 estimates under controlled conditions, while CO_2 estimation remains less reliable.

In CH_4 measurement, Pearson's r exceeded 0.88 in both periods and reached 0.899 overall, while CCC ranged from 0.780 to 0.844 and MAPE remained below 8%. The observed bias was small and negative, indicating slight underestimation relative to RC, but the magnitude was low enough that it is unlikely to disallow for interpretation of treatment effects. This level of agreement is comparable to, and in some cases stronger than, that reported in previous validation studies of scalable CH_4 measurement methods [7]. The SF6 tracer technique has been regarded as accurate, with comparisons against RC showing similar mean emissions but greater day-to-day variability and occasional method-dependent bias. For example, Grainger *et al.*[11] reported that SF6 CH_4 emissions values were similar to RC measurements in lactating dairy cows, but between-day variation was greater for SF6 than for RC. Additionally, Muñoz *et al.* [12] reported that the SF6 method overestimated CH_4 emissions by about 11% and showed higher variability than RC. Therefore, the daily MAPE and high CCC observed in this study indicate that ZELP Sense compares well with established on-animal methods, such as the SF6 tracer technique, at least under the present controlled conditions.

At six-hour resolution, CH_4 agreement remained strong but was lower than the daily values, consistent with previous work showing that CH_4 estimates are sensitive to sampling duration, protocol, and short-term temporal variability [12]. Even so, the overall six-hour CH_4 CCC of 0.848 and Pearson's r of 0.855 indicate that ZELP Sense tracked the CH_4 pattern measured by RC over time well enough to preserve intraday variation [13].

An objective of this study was to determine whether ZELP Sense could detect the reduction in CH_4 emissions after the introduction of a CH_4-suppressing feed additive. Both systems showed a reduction in daily CH_4 emissions during the treatment period and no significant method-by-time interaction was detected. This suggests that ZELP Sense matched RC averages and reproduced the direction and timing of the treatment response. In comparison, GFS's performance depends heavily on visit frequency and temporal sampling coverage. Hammond *et al.*[7] found that GFS could approximate mean daily CH_4 emissions, but concordance with RC data was poor overall and treatment ranking differed when sampling was sparse or uneven. In this study, ZELP Sense achieved stronger daily concordance and preserved the treatment response, suggesting that the continuous sampling may aid in individual-level estimation and CH_4 change detection[7,13].

Daily CO_2 agreement was only moderate in P1 and poor during P2, with overall Pearson's r of 0.80 and CCC of 0.40. The small overall MPE (-1.70%) and relatively small bias during P1 suggest that the reduced concordance was not mainly due to a consistent directional bias, but instead to greater variability in the ZELP Sense estimates, particularly during P2. Similar challenges in CO_2 gas measurements have been reported, where CO_2 has been used as a tracer but has shown sensitivity to method and protocol differences [14].

At six-hour resolution, CO_2 agreement was weaker than CH_4, with agreement declining during the P2. Overall, the results indicated moderate agreement between methods, but reduced precision in reproducing the temporal variation measured by RC, particularly during P2. Both measurement methods detected a reduction in CO_2 emissions during P2, although neither reduction reached statistical significance, and there was no significant method by time interaction. These findings suggest that ZELP Sense was able to capture the general magnitude and direction of CO_2 change but was less consistent in reproducing the short-term variation measured by RC.

Weaker CO_2 concordance may also reflect the way ZELP Sense estimates emissions from breath and ML model correction. Changes in plume capture due to nosepiece positioning, dilution correction, sensor response are likely to have a larger effect on CO_2 than on CH_4 because CO_2 is produced continuously at higher absolute rates [14,15]. The fact that the MPE stayed near zero and the Bland Altman shows variability in higher and lower emission levels, suggests that the issue was not a strong directional bias, but rather reduced precision and greater within-period variability. This pattern is consistent with the broader literature on indirect gas measurement systems, where CO_2 often proves harder to estimate robustly than CH_4 under dynamic conditions [14,15].

This prototype version of ZELP Sense appears more suitable for CH_4 monitoring than for CO_2 monitoring, as the present data show that the device captured both absolute CH_4 levels and treatment-related change with acceptable accuracy. That places it in the same practical space as SF6 and GFS, but with the added advantage of continuous individual animal data sampling rather than intermittent sampling [7,8,13].

The results reflect the trade-off between accuracy and scalability in CH_4 measurement. RC are the most precise but are costly and restrictive, while SF6 tracer systems and GFS are more practical but still depend on sampling and animal interaction. If validated further, ZELP Sense could offer a useful middle ground with more continuous monitoring, no reliance on animal interaction and less behavioural disruption [7,13].

Several limitations should be considered when interpreting these findings. The study used only four cattle, which limits statistical power and generalisability across breeds, diets, and management systems. The trial was short, so longer-term repeatability and robustness across wider emission ranges were not tested. In addition, the work was conducted under RC, where airflow, posture and other variables are more controlled than in commercial housing or grazing environments [13,16].

To conclude, ZELP Sense showed strong agreement with RC for CH_4, captured the reduction in CH_4 after the introduction of the feed additive, and produced weaker but still informative CO_2 estimates. Compared with established tools such as SF6 tracer and GFS, the device looks promising for scalable CH_4 quantification, particularly where continuous individual-animal monitoring is needed. Further work should focus on larger cohorts, repeated measures across diets and production stages, and validation under commercial conditions.

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