

Validation of ZELP Sense™ against Respiration Chambers: The Future of Scalable Methane Measurement

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Abstract

Accurate measurement of enteric methane (CH₄) emissions from cattle is essential for greenhouse gas mitigation but remains constrained by conventional methods such as respiration chambers (RC), which are costly, labour intensive and hard to scale. Wearable CH₄ measurement devices offer a potential alternative for continuous CH₄ emissions monitoring but require validation.

This pilot study evaluated the performance of ZELP Sense against RC measurements while assessing the baseline cattle response to the device. Six Kiwi-cross dairy cows underwent a 5-day adaptation period using non-functional devices, after which four adapted cows completed two 3.5-day measurement periods under ad libitum feeding and feed restriction. CH₄ emissions were recorded simultaneously by RC and ZELP Sense and compared at the daily level. Cattle exhibited a brief behavioural response to device fitting (< 90s), after which normal feeding and rumination resumed within 90 minutes, suggesting rapid habituation. Feed restriction significantly reduced dry matter intake (DMI) and CH₄ emissions ($p < 0.001$); however a strong day effect was also present ($p < 0.0001$). Agreement between methods was low to moderate during P2 (CCC = 0.496; RC = 227.5 ± 3.9 g/day vs ZELP Sense 216 ± 12.5 g/day) and moderate during P3 (CCC = 0.681; RC = 143.2 ± 2.1 g/day vs ZELP Sense 141.0 ± 9.4 g/day). When both P2 and P3 were combined, overall concordance increased to CCC = 0.807. A significant slope shift between measurement periods ($p = 0.019$) demonstrated that ZELP Sense captured DMI reduction-driven changes in CH₄ production. Overall, ZELP Sense showed moderate agreement with RC while tracking treatment induced trends in CH₄ emissions, showcasing the potential for scalable CH₄ estimation.

Introduction

Methane (CH₄) is a potent greenhouse gas (GHG) with a 100-year global warming potential (GWP) roughly 28 times that of carbon dioxide (CO₂) [1]. Given that global CH₄ emissions have increased at an average annual rate of 1.22% since 2001 [2], and that overall GHG concentrations have raised global surface temperatures by 1.1°C above pre-industrial levels [3], the urgency to limit further warming has become evident. Under the Paris Agreement, countries agreed to take measures to limit global warming to below 2°C above pre-industrial levels [4]. However, more recently CH₄ has been identified as the second largest contributor to climate change [2], with livestock enteric fermentation representing 46% of the industry's GHG emissions [5]. This underscores the need to develop scalable, accurate, low-labour, and cost-effective enteric CH₄ measurement technologies to enable reliable emission monitoring and support the advancement of CH₄ mitigation strategies [6]. Respiration chambers (RC) are the gold standard method for quantifying enteric CH₄ emissions from individual ruminants due to their high gas recovery rates, which are close to 100% [7]. Regardless, their application is limited by practical and biological constraints: animals housed in RC are unable to express normal patterns of movement and feeding behaviour, which may decrease dry matter intake (DMI) and consequently alter daily CH₄ production [8]. These behavioural and physiological adjustments are difficult to correct for as CH₄ output is influenced by an additional variety of interacting factors such as stress, activity levels (standing/lying bouts) and diet composition, which do not follow simple linear relationships [9]. Additionally, RC are costly and hard to scale for large-scale studies [10]. They also require an adaptation period to train the cattle and allow them to get used to the chamber environment [8]. Alongside RC, the Greenfeed system (GFS) (C-Lock Inc.) is one of the most commonly used CH₄ estimation tools and improves practical deployment as it can be used in both housed and pasture settings [11], with studies indicating it can generate average CH₄ daily emission totals reaching a correlation with RC of $r = 0.7$ after 17 days, $r = 0.9$ after 12 and $r = 0.93$ after 45 [12]. However, the system relies on short, spot sampling events and depends on animal interaction with the GFS. To obtain reliable CH₄ estimates, a minimum of approximately eight 2–6 minute sampling events per cow over 3 days is typically required [13]. Additionally, available reports suggest that differences in day/nighttime interactions and visit frequency may introduce bias in CH₄ estimation, as visits tend to decrease during the night [14]. Moreover, environmental factors, particularly wind, may affect the quality of the data [13, 15].

Other established techniques, such as SF₆ tracer methods and sniffer-based systems, present advantages, but remain limited by practical constraints. These technologies can be difficult to scale and are often labour intensive [16–18].

The sampling strategy can be configured for either continuous high-frequency data collection during research and validation phases, or periodic sampling protocols designed to balance data resolution requirements with power consumption constraints for extended deployment periods. The data collected by the device are offloaded via WiFi and processed by ZELP's Machine Learning (ML) models to generate CH₄ emission values (g/day), corrected for environmental factors. As a wearable device, ZELP Sense could be used to estimate in-situ CH₄ emissions from cattle across various operational settings, including confined and grazing systems. With

continuous sampling and the ability to deliver multiple CH₄ emission readings per day, it could also be used to explore correlations between physiological parameters and CH₄ emission patterns. The primary objectives of this study were to: (1) provide a baseline assessment of cattle's behavioural response to the fitting and wear of the ZELP Sense device, and any welfare related issues; (2) evaluate the performance of ZELP Sense in the estimation of daily enteric CH₄ emissions in cattle under controlled conditions, using RC as the reference method for validating the novel tool; (3) assess the agreement between ZELP Sense and RC CH₄ measurement under feeding restriction. It was expected that behavioural responses to the ZELP Sense device would be minimal and transient and not interfere with maintenance behaviours nor heavily influence CH₄ emissions from cattle. Additionally, it was hypothesised that ZELP Sense would provide CH₄ emission values in strong agreement with RC measurements. Finally, it was expected that the device would effectively detect the changes in CH₄ emissions under different feeding conditions, with a magnitude and direction comparable to those observed using RC.

Results

Behaviour, DMI and Liveweight Assessment

During P1, initial ZELP Sense device application prompted a transient behavioural response in most cattle, characterised by head-thrashing, running, and/or circling; this agitation did not exceed 90 seconds. Following this period, all animals exhibited rapid habituation, resuming normal feeding upon re-entry to their pens (see Table 1).

Table 1
Cattle Behavioural Response Scores in Adaptation Period (P1). Behavioural criteria range from 1–5, with 1 corresponding to "Highly Tractable", and 5 to "Violent/Aggressive". E= Eating, R= Ruminating, S= Settled

Cow (n)	Main device fitting	0.5 h post-fitting	1.0 h post-fitting	1.5 h post-fitting	2 h post-fitting	4 h post-fitting	Notes: Main Fitting
C1	1	E	E	R	S	S	Settled well to crush and device fitting.
C2	2	E	E	R	S	S	Transient (1 min) jumping, head swinging, running, minimal bellowing.
C3	2	E	E	R	S	S	Transient (1.5 min) bellowing and swinging head, spinning post- dummy device fitting.
C4	2	E	E	R	S	S	Transient (1 min) head swings, running and spins then settled
C5	1	E	E	R	S	S	Calm with dummy device, weary of crush.
C6	3	E	E	R	S	S	Head-swinging towards handlers, spinning and agitated for 1 min.

Throughout the rest of the trial, twice-daily observation showed that animals remained calm and had no issues eating or drinking while wearing the ZELP Sense device. No abrasion or localised hair loss was observed by AgResearch staff in skin-device contact areas (head, neck, muzzle) post-visual assessment at the end of P1.

The following behaviours resulted in the exclusion of C5 and C6 from P2 and P3, and their designation as spares: C5 displayed significant distaste of tie-stalls and a lack of caution toward animal-handlers, increasing safety risks due to aggressive pushing towards handlers. C6 also presented a safety concern, head-swinging towards handlers during ZELP Sense fitting due to over-familiarity. To note, C2 occasionally exhibited defensive hind-limb lifting or kicking upon personnel entry to the RC during P2 and P3.

Average liveweight at the beginning of the trial (P1, Day 1) was 430.8 ± 12.7 kg. During P1, where cows were housed in the barn with non-functional devices, average liveweight increased to 447.0 ± 12.0 kg by the start of P2. After the cows entered the RC for P2, average liveweight declined reaching 438.0 ± 11.9 kg by the end of P2 (Day 5). During the rest period, liveweight remained stable (P3 D1 = 438 kg ± 13.6) (See Fig. 1).

As shown in Fig. 1, during P3 a significant downward trend was observed. A reduction in body weight was observed from D1, P2 = 447.0 ± 12.0 to D4, P2 = 438.0 ± 11.9 , with a mean weight reduction of 32.25 kg per cow. Similarly, the reduction in liveweight between the *ad libitum* baseline (P2 entry) and the end of the feed restriction period (P3 end) was statistically significant ($p = 0.001$). During the P2 baseline period, the average daily DMI was 8.84 kg (range: 7.4–10.1 kg/day). In P3, FO was restricted based on individual P2 intake levels. On the first day of restriction, the mean DMI decreased from 9.03 kg (D1, P2) to 6.53 kg (D1, P3), representing 73.8% of the P2 baseline, which aligned closely with the experimental target of 75%. As the restriction continued, intake further declined on D2 from 8.12 kg (P2) to 5.10 kg (P3), which is 57.7% of the baseline. By Day 3, the mean DMI reached its lowest point at 2.80 kg, corresponding to only 31.7% of the D3 P2 (9.35 kg) baseline values. Comparing the average DMI of P2 (8.84 kg) against the average DMI of P3 (4.81 kg), there was a significant overall reduction in intake ($p < 0.001$).

Respiration Chamber and ZELP Sense Data Comparison and Evaluation

Table 2 shows mean ($\pm$ Standard Error Mean, SEM) daily CH₄ emissions (g/day) values from both measurement methods across cattle during P2 and P3. Please refer to Appendix 2 for daily CH₄ values from both RC and ZELP Sense for individual cattle during P2 and P3.

Table 2
Mean ($\pm$ SEM) daily methane emissions (g/day) measured using respiration chambers (RC) and ZELP Sense across cattle during the *ad libitum* feeding period (P2) and feed restriction period (P3).

Period	Day	RC CH ₄ (g/day)	ZELP Sense CH ₄ (g/day)
P2	1	237.9 $\pm$ 9.2	254.4 $\pm$ 30.6
	2	222.7 $\pm$ 6.4	206.4 $\pm$ 18.0
	3	222.0 $\pm$ 3.1	185.5 $\pm$ 12.3
P3	1	188.3 $\pm$ 4.4	170.7 $\pm$ 23.0
	2	147.1 $\pm$ 3.9	141.9 $\pm$ 9.4
	3	94.2 $\pm$ 2.6	110.3 $\pm$ 13.1

Daily CH₄ emissions (g/day) measured by both RC and ZELP Sense followed similar downward trends across the measurement periods (P2: RC = 227.5 ± 3.9 g/day vs. ZELP Sense = 216 ± 12.5 g/day, P3: RC = 143.2 ± 2.1 g/day vs. ZELP Sense = 141.0 ± 9.4 g/day).

During P2, average emissions from both methods remained relatively stable, with slight declines observed from Day 1 to Day 3. RC mean emissions decreased from 237.9 ± 9.2 on D1 to 222.0 ± 3.1 g/day on D3, while ZELP Sense measurements recorded a decline from 254.4 ± 30.6 to 185.5 ± 12.3 g/day.

The feed restriction in P3 resulted in a marked reduction in CH₄ emissions across both measurement methods. RC average emissions declined by approximately 50%, going from 188.3 ± 4.4 g/day on D1 to 94.2 ± 2.6 g/day on D3. Similarly, ZELP Sense recorded a reduction of approximately 35% in CH₄ production from Day 1 to Day 3 of P3, going from 170.7 ± 23.0 to 110.3 ± 13.1 g/day. These declines in CH₄ production coincided with the progressive reduction in DMI recorded in P3.

While ZELP Sense captured the dietary induced reduction in CH₄ patterns observed in the RC, its data exhibited higher variability. This is evidenced by the larger SEM values compared to the RC measurements throughout both measurement periods (Table 2).

Agreement between the RC and ZELP Sense data was evaluated using concordance correlation coefficients (CCC), Bland-Altman analysis, and LMM. Daily CH₄ emissions recorded by RC and ZELP Sense for each animal are shown in Fig. 2, together with daily DMI.

Across all animals, CH₄ emissions measured by both systems followed similar patterns throughout the trial. During the *ad libitum* feeding period (P2), CH₄ emissions remained relatively stable, with only minor day-to-day variation observed in both RC and ZELP

Sense measurements (Fig. 2). Following the introduction of feed restriction (P3), CH₄ emissions declined progressively across the remaining trial days for all animals. This reduction was detected by both measurement methods and occurred concurrently with the decline in DMI, which is represented by the bar plots in Fig. 2. DMI decreased substantially after the start of the restriction phase, falling from approximately 7–10 kg/day during P2 to approximately 1–5 kg/day during P3, depending on the individual animal. As intake declined, CH₄ emissions measured by the RC and ZELP Sense decreased correspondingly, falling to approximately 70–130 g CH₄/day by the end of P3.

Average CH₄ emissions measured by RC and ZELP Sense within each measurement period, and the corresponding CCC and MAPE values are presented in Fig. 3.

During P2, agreement between RC and ZELP Sense measurements were poor to moderate, with a CCC of 0.496 (95% CI 0.23–0.68) and a MAPE of 13.8% (Fig. 3). During the feed restriction period (P3), agreement between the two systems improved. The CCC increased to 0.681 (95% CI 0.21–0.89), while the MAPE rose to 18.3% (Fig. 3). When observations from both P2 and P3 were combined, the overall agreement between the two measurement systems increased, with a CCC of 0.807 (95% CI 0.61–0.91) and a MAPE of 16.1%.

Agreement between the two measurement systems varied among animals. Individual CCCs ranged from 0.734 to 0.925, while MAPE ranged from 10.6% to 19.9%, as shown in Fig. 2. The highest agreement between methods was observed for C1 (CCC = 0.925), whereas lower agreement was observed for C3 (CCC = 0.734) (Fig. 2).

Agreement between CH₄ emissions measured by the RC and ZELP Sense was further evaluated using Bland-Altman analysis, presented in Fig. 4.

Figure 4: Bland-Altman plots showing agreement between methane emissions values from respiration chambers (RC) and ZELP Sense produced during the *ad libitum* feeding period (P2), the feed restriction period (P3), and across all observations combined. The dashed line represents the mean difference (bias) between the two methods, and the dotted lines represent the 95% limits of agreement (LoA).

During P2, the mean difference between the two measurement methods was – 20.4 g (–9.0%) CH₄/day, indicating that ZELP Sense tended to estimate slightly lower CH₄ emissions compared with the RC measurements (Fig. 4). The limits of agreement ranged from – 88.5 to 47.7 g CH₄/day (–38.9% to 21.0%). During P3, the mean difference between the two methods was smaller (–4.6 g CH₄/day; –3.2%), indicating improved agreement between the systems under restricted feeding conditions. Limits of agreement during this period ranged from – 67.9 to 58.7 g CH₄/day (–47.4% to 41.0%) (Fig. 4). When measurements from both periods (P2, P3) were combined, the overall mean difference between the two methods was – 12.5 g (–6.7%) CH₄/day, with limits of agreement ranging from – 78.8 to 53.7 g CH₄/day (–42.5% to 29.0%), as shown in Fig. 4.

Results from the LMM analysis show that CH₄ estimates did not differ significantly between the measurement methods during either P2 or P3, P2 ($p = 0.080$), P3 ($p = 0.616$). In contrast, measurement day had a significant effect on CH₄ emissions in both periods, with p -values of 0.0002 in P2 and < 0.001 in P3. This reflects the changes in CH₄ emissions observed across the trial days. A significant slope shift ($p = 0.019$) was detected during the feed restriction phase (P3), indicating a significant change in CH₄ emission trends across the restriction period. No slope shift was tested for P2 because of the *ad libitum* feed protocol during that phase.

Discussion

The immediate behavioural response to the ZELP Sense device application was characterised by a transient period of agitation, including head-thrashing, circling and bellowing. This response was found to remain for less than 90 seconds in all cattle. The short duration suggests that the behaviours were likely a response to the novelty of the GSU around the neck, rather than a pain response or prolonged behavioural distress [19]. All cows resumed eating within 0.5 h and were observed ruminating within 1.0–1.5 h post-fitting. Notably, rumination is recognised as a low arousal state that would typically reduce under prolonged pain or stress [20]. The rapid progression from transient agitation to feeding and rumination therefore indicates quick habituation to the device.

These findings are consistent with studies evaluating other methane measurement and halter-based systems' effect on behaviour and welfare. Pereira *et al.* [21] reported that SF₆ halter equipment drove no major changes in eating time, lying bouts, or

social/discomfort behaviours post-adaptation (ruminating time increased during measurement: 25.2% vs 17.9% pre-equipment; $p < 0.001$; idling decreased: $p < 0.01$), concluding minimal behavioural impact after habituation. The rapid return to normal feeding and rumination observed with ZELP Sense is therefore consistent with this “ephemeral resistance during fitting followed by habituation” [21], as well as aligning with RumiWatch halters’ minimal and transient effect on dairy cows’ behaviour [22], supporting the interpretation that ZELP Sense does not induce sustained behavioural signs of discomfort.

Liveweight is a useful parameter for establishing both animal welfare status and emissions baselines, as heavier animals have greater feed capacity and DMI, which drives higher absolute CH₄ emissions (19, 20). The cattle's initial average weight of 430.8 ± 12.7 kg aligns with standard mature weights for Kiwi cross cattle [25]. The stable liveweight throughout P1, with P1 D1 = 430.8 ± 12.7 and P1 D5 = 438.0 ± 12.4 suggests consistent DMI, and therefore that the device did not restrict feeding behaviours, supporting the interpretation that animals adapted well to the device. Again, this interpretation parallels Pereira *et al* [21], where DMI did not differ between equipped/non-equipped cows during measurement (23.1 against 22.0 kg/d; $p = 0.56$).

The subsequent liveweight loss during P2, despite *ad libitum* feeding, is more plausibly related to RC conditions than to the ZELP Sense device. Short term liveweight fluctuations of 5–10% in ruminants often reflect changes in gut fill and excrement rather than tissue loss [26],[27], particularly under changing housing or feeding regimes. The significant reduction in liveweight during P3 ($p < 0.001$) is likely driven primarily by the imposed reduction in FO, as DMI fell to roughly 32% of baseline, leading to a decline in body mass. Although the protocol targeted a 50% reduction in feed offered, the larger realised drop in DMI suggests that, alongside restricted availability, secondary factors such as RC-related stress and restlessness due to hunger contributed to increased feed refusals [8, 28].

GFS tie-stall work [29] and head-mounted CH₄ simulators [30] likewise show early behavioural changes minimised by habituation/training, with minimal welfare impacts when designs avoid feeding interference. While we cannot rule out ZELP Sense contributing to P2/P3 refusals, P1 patterns and these parallels suggest chamber/feed restriction as primary drivers, with the device a possible but probably minor contributor [30].

Together these behavioural, DMI and liveweight patterns suggest ZELP Sense induces only transient welfare effects similar to SF₆/GFS/RumiWatch systems under proper habituation [21, 29, 30].

Across both measurement methods, daily CH₄ emissions declined over time within each measurement phase and reduced markedly when feed was restricted, reflecting the central role of DMI in driving CH₄ production. During *ad libitum* feeding (P2), emissions remained relatively stable in the RC but showed a steeper decline when measured by ZELP Sense. Under feed restriction (P3), both methods captured a great drop in emissions, approximately 50% for RC and 35% for ZELP Sense, closely tracking the forced reduction in DMI.

This ability of ZELP Sense to reproduce the trajectory of emissions across diets aligns with recommendations that new methods be judged primarily on their capacity to detect treatment effects rather than to replicate absolute RC values exactly [31, 32].

Comparison showed low to good concordance between methods, with CCC values of 0.49 in P2 and 0.68 in P3 and 0.807 when data from both measurement periods were combined. Although these CCCs fall below the ≥ 0.90 thresholds suggested for high absolute agreement in method comparison studies [31, 33, 34], they indicate that ZELP Sense captures the pattern of emissions reasonably well. Mean absolute percentage error remained relatively low (13.8% in P2; 18.3% in P3; 16.1% overall), suggesting that average daily estimates from the wearable and RC are comparable at the population level.

LMM results support this interpretation: no significant method effect was detected in either measurement period (p -value P2 = 0.080; P3 = 0.616), while measurement day had a strong effect ($p < 0.001$ in P2; $p < 0.001$ in P3), reflecting the diet induced decline in emissions. The piecewise mixed model showed a significant slope shift between P2 and P3 ($p = 0.019$), demonstrating sensitivity to the DMI-driven reduction in emissions. These results align with findings from GFS validations, where McGinn *et al.* [35] reported no method difference (1% bias, $p = 0.019$) and Ma *et al.* [13] reported correlation coefficient of $r = 0.84$ between daily CH₄ GFS and RC values.

Bland-Altman analysis revealed that ZELP Sense consistently underestimated CH₄ relative to RC, with mean biases of -20.4 g/day (-9.0%) during P2, -4.6 g/day (-3.2%) during P3, and -12.5 g/day (-6.7%) overall. The 95% limits of agreement were wide, particularly

in P2 (P2: -88.5 to + 47.7 g/day; -38.9% to + 21.0%), narrowing during feed restriction (P3: -67.9 to + 58.7 g/day; -47.4% to + 41.0%). Approximately 85–90% of observations fell within these limits, indicating that while the two methods are not fully interchangeable for single-day absolute measurements, agreement improved under restricted feeding conditions and when data was aggregated. The scattering on the plots revealed proportional bias [36], with larger negative differences at higher emission levels and tighter clustering around zero during P3, further supporting this.

These results position ZELP Sense as statistically comparable to validated alternatives. In Hammond *et al.* [37], GFS showed near-identical mean emissions to RC (198 vs 218 g/day Exp 1; 208 vs 209 g/day Exp 2), but poor daily concordance (CCC = 0.1043 combined), while SF₆ produced higher emissions than GFS during grazing (186 vs 164 g/day, $p < 0.001$) with moderate concordance (CCC = 0.6017, $p < 0.01$). ZELP Sense's CCC values (P2 = 0.49; P3 = 0.68, P2 + P3=0.81) are therefore in line with other validated CH₄ measurement tools, with non-significant method effects ($p = 0.080, 0.616$) and strong treatment sensitivity (slope $p = 0.019$) demonstrating robust statistical performance.

Some larger deviations occurred at higher emission levels: several factors may explain this variability. First, the physical blockage of the sampling baffle arm during P2, noted throughout the study as well as in the post-deployment assessment of the device, may have impacted the accuracy of the ML pipeline dilution model (as a result of the blockage affecting the dilution model signal). During P2, this blockage had not yet been identified as the cause of reduced data completeness. In contrast, during P3, the baffle arm was manually cleared twice a day throughout the measurement period, restoring signal clarity and improving data completeness, likely leading to the higher CCC. Similarly, in the SF₆ technique, not accounting for drifting permeation rate may result in an error of up to 15% in the inferred CH₄ emission rate [38].

Second, the difference in sampling frequency between the two systems may also contribute to the variability [39]. ZELP Sense sampled CH₄ concentrations every 0.5 seconds, capturing rapid fluctuations associated with breathing cycles and eructation peaks. In contrast, the RC recorded gas concentrations at 10-minute intervals, effectively smoothing short-term fluctuations and providing a more averaged signal. When high-frequency signals are aggregated into daily totals, variability associated with short-term concentration spikes may propagate into the final emission estimates [39]. Third, the two methods differ in how CH₄ production is measured. RC directly measure net gas flux, whereas the ZELP Sense relies on a model-based conversion from ventilation rate and breath concentration to emission rate. Any uncertainty in dilution, or environmental corrections may therefore affect the CH₄ output values for ZELP Sense.

Although ZELP Sense in this small pilot study underestimated overall CH₄ production, it responded very well to decreases in CH₄ emissions in response to lower DMI, indicating that relative changes in CH₄ within a group are well predicted.

In conclusion, the behavioural, intake and physiological indicators suggest that ZELP Sense has had a minimal and transient impact on cattle welfare, with animals habituating rapidly post-fitting. Future research should incorporate longer term production and welfare metrics, such as milk yield, milk composition and continuous quantitative behavioural monitoring, to exclude subtle or longer-term effects [40–42].

There are limitations identified from this study which lie in the potential for systematic bias arising from differing calibration and recovery protocols. RC and ZELP Sense utilised different standard gases, calibration intervals, and methodologies, which may introduce baseline discrepancies.

Further validation across larger populations, production systems, diets, and environmental conditions are required to establish standard operating procedure for the ZELP Sense device for accurate CH₄ emissions estimation from cattle in both confined and grazing systems.

Materials and Methods

Experimental Design

Six Kiwi cross (Holstein-Friesian x Jersey) dry cows were sourced from Massey University Dairy Farm 4, Palmerston North and were randomly allocated to the trial, which took place at the Bioeconomy Science Institute Grasslands Research Centre, Tennent Drive,

Palmerston North 4410, New Zealand. All the manipulations adhered to the guidelines of the 1999 NZ Animal Welfare Act and AgResearch Code of Ethical Conduct and were approved by the AgResearch Animal Ethics Committee (AE approval #2568).

The trial had a total duration of 12 days and was split into 3 phases: Phase 1 (P1), the 5-day habituation period; Phase 2 (P2), the 3.5-day *ad libitum* measurement period and Phase 3 (P3), the 3.5-day feed-restricted measurement period (P3). During P1, non-functional (non-sampling) ZELP Sense devices were fitted and worn by the cattle for 5 days to assess, identify and minimise wearable-induced stress and behavioural effects that could influence CH₄ emission patterns [8]. The cows were fitted with the device in a crush and were subsequently released into the barn. Following adaptation, in P2, a subset of four animals that responded well during P1 were selected for a 3.5-day measurement period. At the start of the period, each animal was fitted with a functional ZELP Sense device, before being released into a RC. The animals were provided with feed and water *ad libitum*. Food offered (FO) and feed refusal (FR) data were recorded to analyse the CH₄ emission data in relation to DMI. A 4-day rest period, during which the animals were returned to the barn device-free, was implemented between P2 and P3. Following the rest period, the cows were refitted with the same ZELP Sense device and placed back in RC for the 3.5-day P3. For the first two days of this measurement period, FO was restricted by 25% relative to the intake recorded per cow in P2. On the third and fourth day (with the fourth being a half day), an additional 25% restriction in FO (i.e. a total reduction of 50% compared to the feed intake per cow in P2) was imposed. The cattle were in the same chambers for both P2 and P3.

This simultaneous measurement approach allowed for the direct assessment of intra-animal day-to-day variability and provided a direct, minimally limited comparison between the ZELP Sense data and the RC measurements.

Table 3
Experimental design and timeline of the trial, showing the study phases, dates, duration, number of cows, housing environment, and purpose of each phase. RC = respiration chamber; FO = feed offered.

Phase	Dates	Duration	Cow Number	Environment	Purpose
P1	September 8-September 12	5 days	6 cows	Barn	Gradual introduction of ZELP Sense using non-functional devices and adaptation to tie-stalls.
P2	September 15-September 18	3.5 days	4 cows	RC	Baseline CH ₄ measurement period with ad libitum access to fresh pasture feed.
Rest Period	September 18-September 21	3.5 days	4 cows	Barn	No devices: return to barn between chamber phases.
P3	September 22-September 25	3.5 days	4 cows	RC	Sensitivity testing with feed restriction. FO reduced to 75% of P2 individual feed intake for the first 2 days; FO reduced to 50% of P2 individual feed intake for the last 2 days.

Animals, Adaptation and Animal Behaviour Monitoring

All cows taking part in the study were Kiwi cross dry cows (parity ≥ 2, mean weight 430 ± 15 kg; mean age 79 ± 9 months) and were initially included in a 5-day adaptation phase.

For the sake of trackability, each cow was numbered as Cow 1 (C1), Cow 2 (C2), Cow 3 (C3), Cow 4 (C4), Cow 5 (C5) and Cow 6 (C6). In P1, the 6 cows were restrained in a crush and fitted with the ZELP Sense devices, before being released back into the barn. It is important to note that these were non-functional devices. The headpiece and nosepiece of the devices were of standard issue, but the gas-sensing unit was non-functional. Instead of containing the typical electronic components, it housed weights to mimic their weight. P1 served as both an acclimatisation phase to mitigate potential stress induced by the ZELP Sense device and by the restraint conditions of RC, and as the preliminary screening phase to select cattle demonstrating optimal adaptation for subsequent inclusion in P2 and P3. In order to adapt cattle to the RC confined environment, all cows were temporarily housed in tie-stalls during P1. This acclimatisation process occurred up to twice between days 3 and 5 post-fitting of the ZELP Sense device. Each tie-stall period lasted a maximum of three hours and helped simulate the chamber environment while the device was worn. Animals that demonstrated optimal adaptation to both the ZELP Sense device and the tie-stalls were subsequently included in P2 and P3. The cow's adaptation was assessed by a member of AgResearch staff intermittently monitoring and scoring cattle during both the initial fitting of the device and the remainder of the adaptation phase. For the fitting of the device, the animals were scored during device

fitting, using a 1–5 scale, where Score 1 was “Highly Tractable” and Score 5 was “Violent/Aggressive”, see Table 2. Their activity was then observed at 0.5 hours post-fitting, 1-hour post-fitting, 1.5 hours post-fitting, 2 hours post-fitting and 4 hours post-fitting. For the remainder of the adaptation phase, and the rest of the trial, the cattle were monitored twice-a-day for stress indicators via the same member of AgResearch staff. The animals were scored on the same 1–5 scale where Score 1 was “Highly tractable” and Score 5 “Violent/ Aggressive”, see Table 4.

Table 4

Behavioural scoring criteria used by AgResearch staff to assess animal temperament and response during and after ZELP Sense device fitting. Scores range from 1 (highly tractable) to 5 (violent/aggressive).

Score (n)	Descriptor	Behavioural Criteria
1	Highly Tractable	Exhibits a stationary, relaxed posture. Movement during restraint is slow and controlled. Handling requires little to no effort.
2	Mildly Responsive	Displays intermittent displacement activity and frequent shifts in footing. Handling requires minor, intermittent correction, but the animal remains fundamentally manageable.
3	Moderately Agitated	Exhibits nervousness and impatience, including moderate physical struggling and pronounced tail flicking. Requires consistent restraint effort; may actively resist crush and device.
4	Highly Uncooperative	Displays erratic, uncontrolled movements, muscle tremors (quivering), and violent struggling. Escape attempts and distressed vocalisation (bellowing) are common.
5	Violent/ Aggressive	Behaviour ranges from severe agitation to overt aggression toward personnel/ equipment. Manifested by forceful jumping, thrashing, and frequent bellowing.

On day one of P1, when the 6 animals were weighed, controlled internal drug release (CIDR) devices were inserted in order to suppress oestrous cycling. CIDRs were replaced during the rest period between P2 and P3.

Diet and Feeding Schedule

Cattle received fresh pasture, divided into 30 kg bins, twice daily (4 PM and 8 AM) during both measurement phases (P2 and P3). Pasture nutrient analysis, performed at Massey University, included: Ash (550°C furnace, AOAC 920.153), Crude protein (Dumas, AOAC 968.06), Fat (Soxtec, AOAC 991.36), NDF (Fibertec, AOAC 2002.04), and ADF (Fibertec, AOAC 973.18). Refer to Appendix 1 for the complete chemical composition pasture table offered throughout both P2 and P3. All cattle were also provided with access to water, *ad libitum*, during both periods.

DMI was monitored throughout the trial. DMI was recorded daily across P2 and P3 by weighing FO and FR in the RC. The dry matter content of the FO and FR was determined by drying samples at 105°C for 24 hours.

The liveweight of the cattle in the trial was recorded before and after each period (P1, P2, P3). As liveweight influences feed intake capacity, it was measured alongside DMI and CH₄ emissions production. Previous research indicates a correlation of -0.18 (SE 0.08) between live weight and CH₄ production (g/day) [43].

Respiration Chamber and Camera Set-Up

The open-circuit respiration chambers used during the trial were custom designed for AgResearch, with dimensions of 1945 mm (W) x 2275 mm (H) x 3900 mm (L). The flow rate was maintained between 1680 and 1800 L/min. On average, the temperature was kept at 21°C and the relative humidity was at 57%, yielding an average temperature humidity index (THI) value of about 67, calculated as per the NRC formula [44]. The CH₄ measurements were taken at 10-minute intervals, and the daily emissions of the gas was calculated from the difference between the concentration flowing in and that flowing out of the chamber, corrected for temperature, pressure, and humidity. To assist with monitoring, Wi-Fi-enabled cameras were installed inside the chambers using suction cups on the plexiglass front. This system provided real-time footage uploaded to a server, allowing for remote monitoring of the cattle and enabling footage review in the event of device or RC failures.

Overview of ZELP Sense Device and Experimental Set-up

The devices' gas sampling strategy was set at 2Hz frequency (1 sample every 0.5 seconds). The collected data was displayed on Grafana (Version 12.4), an open-source analytics platform that allows for real-time data monitoring and plotting. To ensure data collection independent of network availability, a memory card within the GSU stored all collected data, which was downloaded post-study. The ZELP Sense pipeline and firmware versions used during this trial were 2.2.0 V and v2.0.2 respectively, both pre-production prototype versions.

As pictured in Fig. 5, the nosepiece (2) has a base and two baffle arms. The baffle arms house 1-micron (μm) filters to prevent ingress of particles that could obstruct the gas sampling pathways. The neoprene headpiece (1) is available in different sizes to accommodate a range of animals, from approximately 330 kg to 900 kg cattle. Sizing is adjustable at the occipital bone and muzzle to conform to individual animal morphology and maintain optimal positioning of the sampling apparatus (nosepiece) within the breath trajectory. All 4 cows were fitted with a small sized headpiece. The adjustable neck strap incorporates a breakaway mechanism designed to deploy when subject to a pre-determined force, to prevent entanglement. The 2.8 kg, 18x24 cm GSU (3) housed below the neck, is lighter in weight to typical cow bells and has been deemed acceptable for bovine use based on other studies [45, 46].

The data collected by the device are offloaded via WiFi and processed by ZELP's ML model. CH_4 emissions are calculated by tracking the cow's ventilation rate and the CH_4 concentration (ppm) in the eructed breath, with additional data and ZELP's Machine Learning models used to correct for dilution and environmental conditions (temperature, humidity and pressure). CH_4 sensor zero-drift is accounted for by incorporating floor levels of ambient CH_4 .

The ZELP Sense devices were disassembled post-trial and tested in-house to assess possible breakages and failures. Device identification (IDs) and cow IDs were linked and recorded manually.

Statistical Analysis: Descriptive Statistics and Modelling

All data was analysed using R and plotted using Python and R. The primary Python (3.12.0) packages used were Pandas 2.3.2 and NumPy 1.26.4. Chamber values served as the baseline to validate ZELP Sense data. For liveweight analysis, a paired t-test was used to assess the difference in liveweight between the start of P2 and the end of P3, with individual cow as the unit of analysis ($n = 4$). The analysis was performed in R (v4.6.0), with significance set at $p < 0.05$.

For CH_4 analysis, RC and ZELP Sense measurements were aggregated into daily CH_4 totals per cow in order to reduce sampling frequency bias. 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 random effect, and allowing for heterogeneous variances between groups. When relevant, post-hoc pairwise comparisons between factor levels were conducted from the LMM results, with the corresponding p-values adjusted for false discovery rate. Daily mean CH_4 emission totals per cow were calculated for both the RC and ZELP Sense methods, though the fourth day of each measurement phase was excluded from all analysis to ensure data consistency across full 24-hour periods. To evaluate agreement between methods, the Concordance Correlation Coefficient (CCC) was calculated at the paired daily level for P2, P3, and the combined dataset (P2 + P3). Individual animal accuracy was assessed using the Mean Absolute Percentage Error (MAPE) calculated across both sampling periods. For Bland-Altman analysis, paired daily values were utilized to determine the mean of the two methods and their difference (ZELP Sense - RC), with agreement summarized using the mean bias and 95% limits of agreement ($\text{bias} \pm 1.96 \text{ SD}$). The DMI of each cow (FO-FR) was plotted to examine trends in CH_4 totals and assess the effect of the restricted-feed treatment. Finally, a custom piecewise mixed model was formulated to assess the slope shift in emission totals from P2 to P3, allowing for an evaluation of ZELP Sense's sensitivity to dietary-induced reductions in CH_4 emissions. Liveweight trends and period averages were extrapolated and used as a marker to evaluate the successfulness of the restrictive diet period.

Declarations

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The authors thank the engineers and machine learning team at ZELP for supporting the trial, AgResearch staff for the resources and collaboration, and finally the Global Methane Hub (GMH) for the support and the provision of funding.

Competing Interests

S.S., J.P., H.K. and R.B. are employees of ZELP Ltd., the company that develops the ZELP Sense device evaluated in this study. S.M. declares no competing interests. The Global Methane Hub (GMH) indirectly provided funding support for this research through the provision of a grant to ZELP Ltd. The funders had no role in the study design, data collection, data analysis, interpretation of the results, manuscript preparation, or the decision to publish.

Author Contribution

S.S. for the formal analysis, investigation and writing, J.P. for the data analysis and modelling, S.M. for the trial design conceptualisation, reviewing and project administration, H.K. for the data manipulation, R.B for the trial design conceptualisation and supervision. All authors reviewed the manuscript

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The authors thank the engineers and machine learning team at ZELP for supporting the trial, AgResearch staff for the resources and collaboration, and finally the Global Methane Hub (GMH) for the support and the provision of funding.

Data Availability

The datasets generated and analysed during the current study are not publicly available due to commercial confidentiality but may be made available from the corresponding author subject to reasonable request and approval.

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Figures

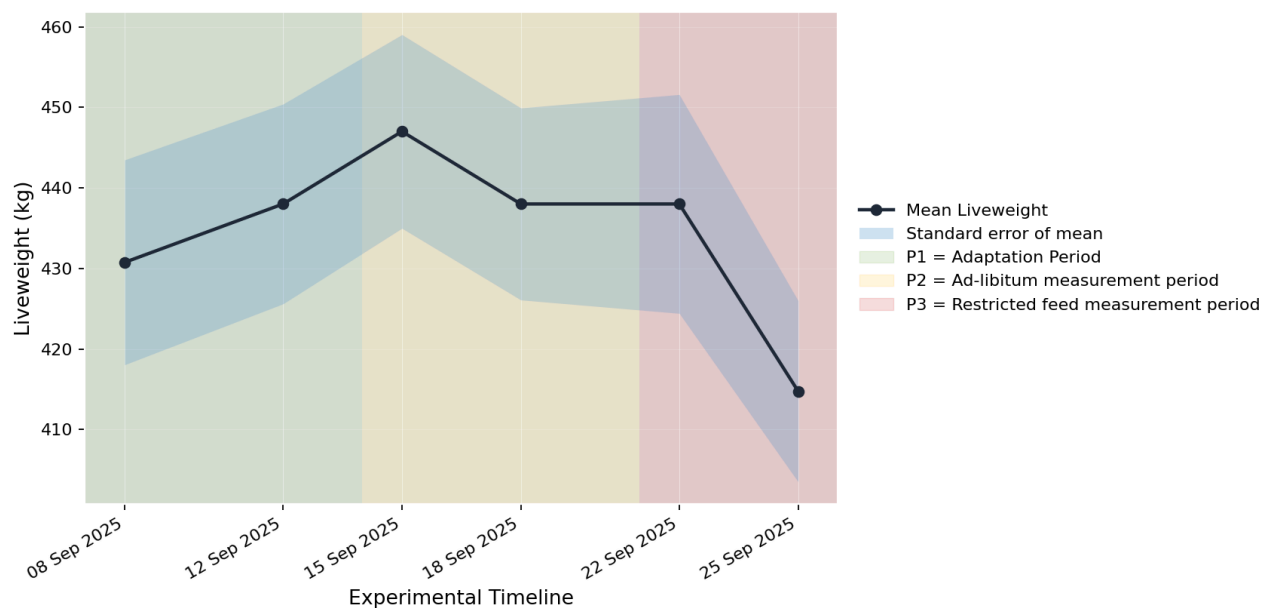


Figure 1

Average liveweight ($\pm$ SEM) (kg) of cows during adaptation (P1), ad-libitum feeding (P2), and restricted feed (P3) periods. Data points show mean values at the beginning and end of each experimental period.

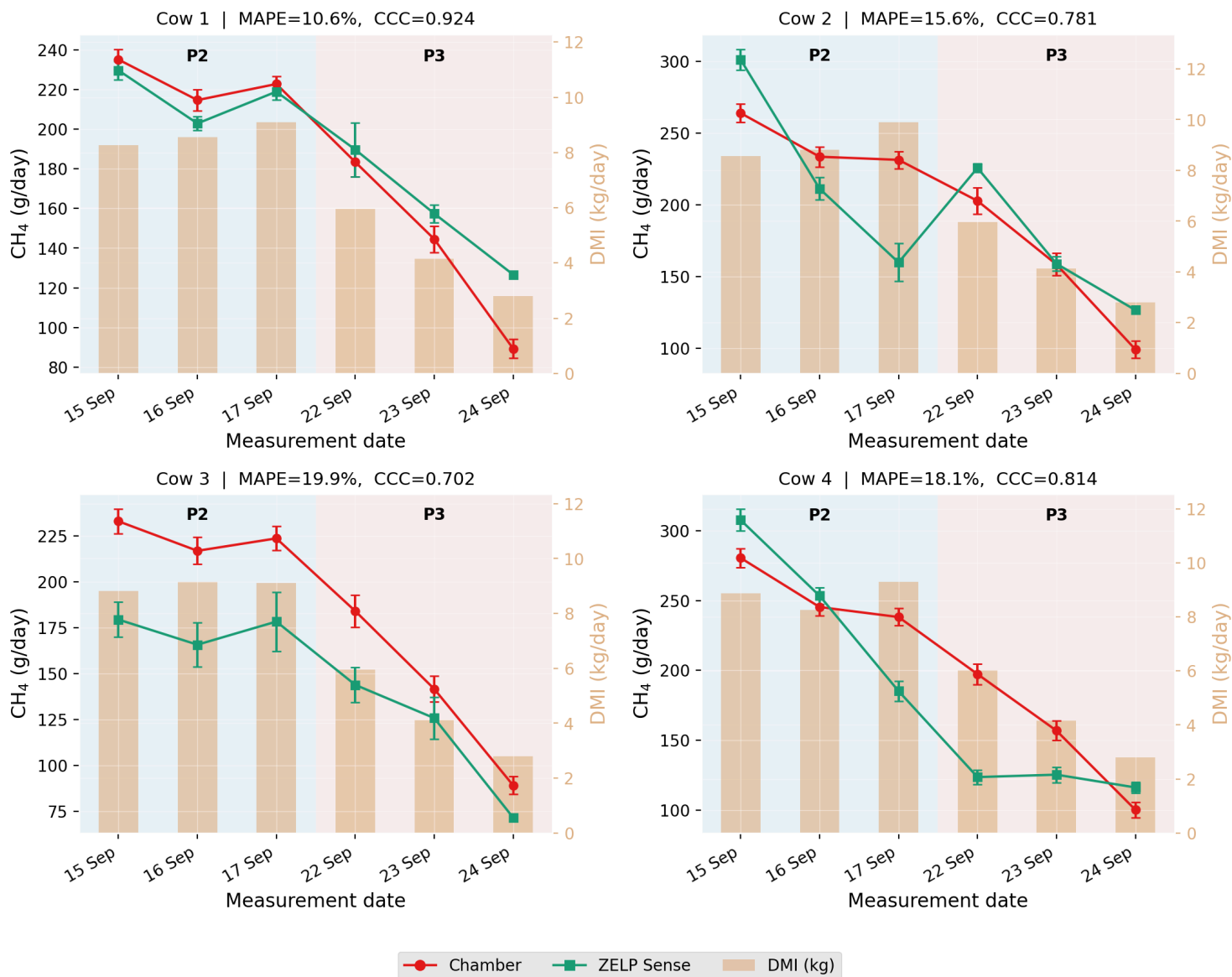


Figure 2

Daily methane emissions (CH₄ g/day) measured using respiration chambers (RC) and ZELP Sense for each cow across the experimental trial with standard error of the mean bars (SEM). Line plots represent CH₄ emissions recorded by each measurement method, while the bar chart shows daily dry matter intake (DMI). The change in colours (blue to pink) indicates the transition from the ad libitum feeding period (P2) to the feed restriction period (P3).

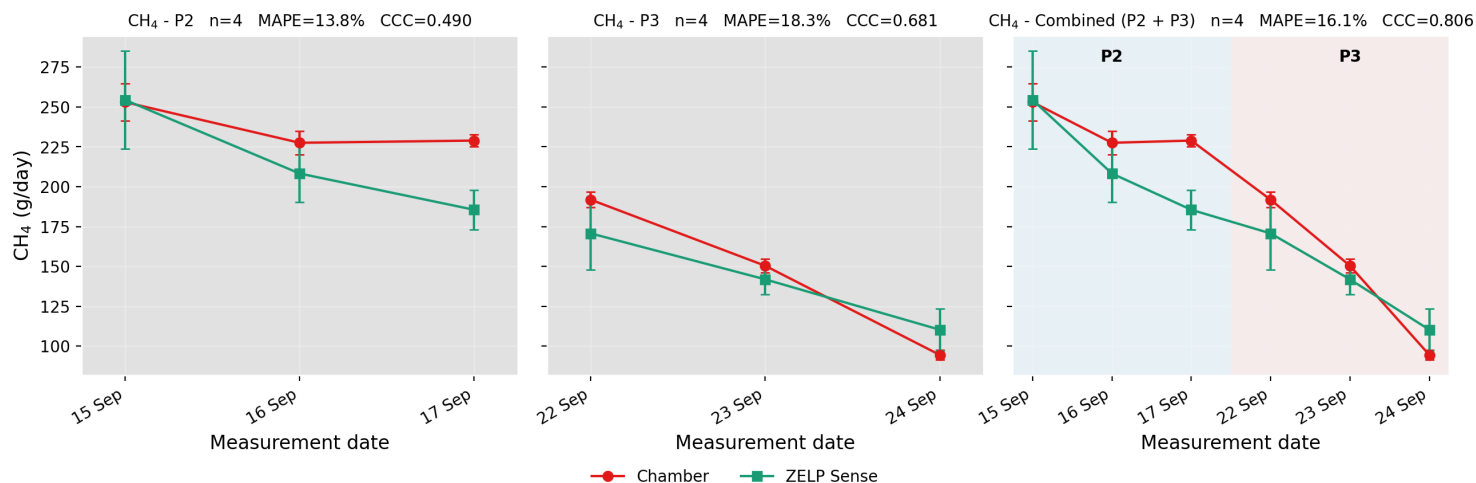


Figure 3

Agreement between respiration chamber (RC) measurements and ZELP Sense CH₄ emission estimates across feeding periods. Mean daily CH₄ emissions (g/day) measured using respiration chambers and estimated by ZELP Sense during the ad libitum feeding period (P2), the feed restriction period (P3), and across all trial days combined. Points represent daily mean emissions averaged across the four cows, with error bars indicating standard error of the mean (SEM). Concordance correlation coefficients (CCC) and mean absolute percentage error (MAPE) values are displayed for each period. The different colour backgrounds are representative of the different measurement periods (P2= Blue; P3= Pink)

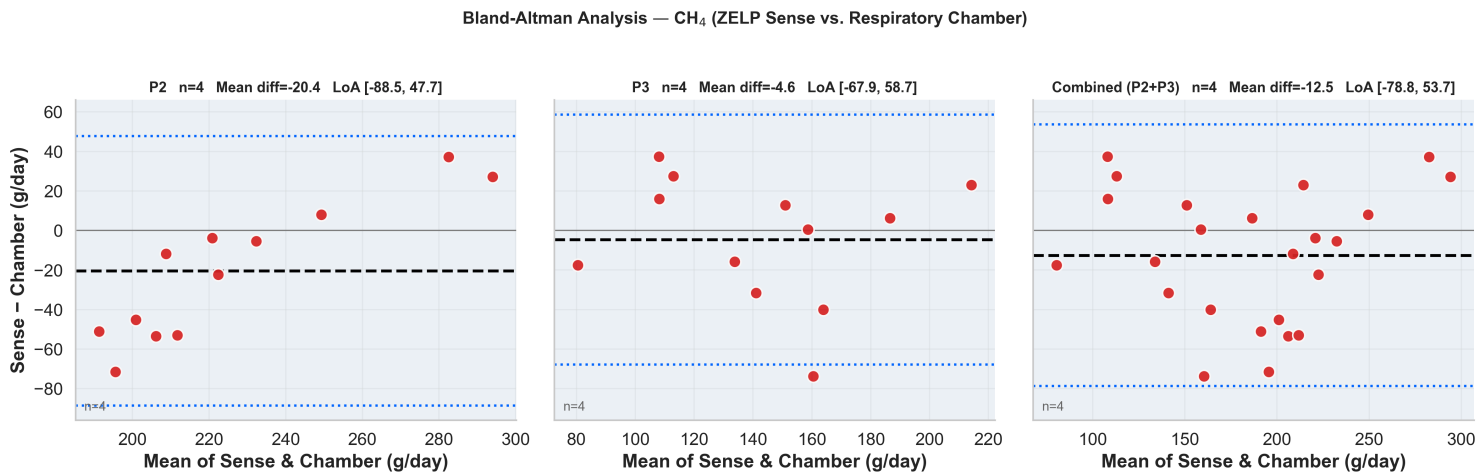


Figure 4

Bland-Altman plots showing agreement between methane emissions values from respiration chambers (RC) and ZELP Sense produced during the ad libitum feeding period (P2), the feed restriction period (P3), and across all observations combined. The dashed line represents the mean difference (bias) between the two methods, and the dotted lines represent the 95% limits of agreement (LoA).



Figure 5

The ZELP Sense device set-up on a Kiwi cross dry cow at AgResearch. 1- Headpiece; 2- Nosepiece and Baffle Arms; 3- Gas Sensing Unit (GSU) with adjustable neck strap

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [ValidationofZELPSenseagainstRespirationChambersTheFutureofScalableMethaneMeasurementSupplementaryInformation.docx](#)