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Article Title (within 20 words without abbreviations)	Comparative Analysis of Enteric Methane Emissions in Lactating Holstein Cows Using the GreenFeed Monitoring System and the Sniffer Method
Running Title (within 10 words)	Comparison of GreenFeed and Sniffer in lactating Holstein cow
Author	Kyunghwan Oh[1,8], Yoorae Kim[2], Janghoon Jo[3], Wonseob Kim[4], Yubeen Cho[1], Taketo Obitsu[5], Eunjoong Kim[6], Huseong Lee[7], Sanggun Roh[8], Jaesung Lee[1], Sungdae Lee[9], Honggu Lee[1]
Affiliation	[1] Department of Animal Science, Konkuk University, Seoul 05029, Korea, Republic of [2] Department of Animal Science, The Pennsylvania State University, University Park 16802, United States [3] Department of Animal Science, Purdue University, West Lafayette 47907, United States [4] Department of Animal Resources, Daegu University, Gyeongsan 38453, Korea, Republic of [5] Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima 739-8528, Japan [6] Department of Animal Science and Biotechnology, Kyungpook National University, Sangju 37224, Korea, Republic of [7] Institute of Agriculture, Academic Assembly, Shinshu University, Nagano 399-4598, Japan [8] Graduate School of Agricultural Science, Tohoku University, Sendai 980-8572, Japan [9] Precision Animal Nutrition Division, National Institute of Animal Science, Rural Development Administration, Wanju 55365, Korea, Republic of
ORCID (for more information, please visit https://orcid.org)	Kyunghwan Oh (https://orcid.org/0009-0001-9413-053X) Yoorae Kim (https://orcid.org/0000-0003-4154-1738) Janghoon Jo (https://orcid.org/0000-0001-8153-7165) Wonseob Kim (https://orcid.org/0000-0002-0234-5665) Yubeen Cho (https://orcid.org/0009-0008-6642-1795) Taketo Obitsu (https://orcid.org/0000-0003-4415-6181) Eunjoong Kim (https://orcid.org/0000-0002-5962-6994) Huseong Lee (https://orcid.org/0000-0003-1931-2007) Sanggun Roh (https://orcid.org/0000-0003-1092-5691) Jaesung Lee (https://orcid.org/0000-0001-8940-9862) Sungdae Lee (https://orcid.org/0000-0002-9167-4099) Honggu Lee (https://orcid.org/0000-0002-0679-5663)
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Authors' contributions Please specify the authors' role using this form.	Conceptualization: Oh K, Kim Y, Obitsu T, Kim E, Roh S, Lee S, Lee H Data curation: Oh K, Lee H Formal analysis: Oh K, Jo J, Lee J Methodology: Oh K, Kim Y, Obitsu T, Kim E, Lee H, Lee H Software: Oh K, Jo J Validation: Oh K, Jo J, Lee H Investigation: Oh K, Kim Y, Cho Y Writing - original draft: Oh K, Kim W Writing - review & editing: Oh K, Kim Y, Jo J, Kim W, Cho Y, Obitsu T, Kim E, Lee H, Roh S, Lee J, Lee S, Lee H
Ethics approval and consent to participate	All experimental procedures in this study were approved by the Institutional Animal Care and Use Committee of Konkuk University (Permit numbers: KU23156, KU23234, KU24172)

CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Honggu, Lee
Email address – this is where your proofs will be sent	hglee66@konkuk.ac.kr
Secondary Email address	
Address	Department of Animal Science, Konkuk University, Seoul 05029, Republic of Korea, Room 509
Cell phone number	+82-10-3307-4387
Office phone number	+82-2-450-0523
Fax number	

Abstract

This study aimed to evaluate two different spot-sampling methods—the sniffer-based method (SB) and the GreenFeed system (GF)—for estimating enteric methane (CH_4) emissions in dairy cows. Specifically, this study was performed to determine whether the SB using a gas hood system could serve as an acceptable alternative to the GF under practical on-farm conditions. A total of 24 lactating Holstein cows were used across three experimental phases (n = 24). CH_4 emissions were measured eight times over four consecutive days in each phase, with measurements taken twice per phase using both the GF and SB. CH_4 emissions from the SB were estimated by applying the CH_4-to-carbon dioxide ratio to a previously validated prediction equation. Results showed that the GF tended to report higher CH_4 emissions (312.9 ± 56.50 g/d) compared to the SB (297.1 ± 61.83 g/d). Bland–Altman analysis showed acceptable agreement between methods, with a bias of 15.78 g/d and 95% limits of agreement ranging from -96.12 to 127.68 g/d, corresponding to 7.1% of the total range. For the CH_4-to-carbon dioxide ratio, the SB produced consistently higher values (0.077 ± 0.012) than the GF (0.064 ± 0.011). Bland–Altman analysis for the CH_4-to-carbon dioxide ratio indicated a small mean bias (–0.012) and minimal proportional deviation (21.3%). Variance homogeneity testing using the Brown-Forsythe test indicated no significant method-dependent differences in CH_4 emission variability across experimental phases ($p = 0.104$). Phase-specific performance of the SB showed acceptable agreement with the GF, with mean absolute percentage errors ranging from 11.57% to 19.20% and confidence rates between 80.80% and 88.43% across phases. In summary, the SB provided CH_4 estimates comparable to those from the GF across all experimental phases. Given its advantages such as portability, flexible installation, and low operational cost, the SB represents a feasible and accessible alternative for on-farm quantification of CH_4 emissions in dairy cows. Together, these findings indicate that the SB provides acceptable estimates of CH_4 emissions from lactating Holstein cows under on-farm conditions, making it a feasible on-farm measurement method alternative to the GF.

Keywords (3 to 6): Dairy cow, GreenFeed system, Measurement technique, Methane emission, Sniffer

INTRODUCTION

Global assessments indicate that livestock production accounts for approximately 11%–12% of worldwide greenhouse gas **(GHG)** emissions, with cattle accounting for about 61.7% of the total GHG emissions from the livestock sector [1]. Among GHGs, methane (**CH_4**) is the second most abundant GHG and plays a key role in global warming owing to its high global warming potential [2, 3], which is approximately 28 times greater than carbon dioxide (**CO_2**) over a 100-year timescale [4]. Consequently, reducing CH_4 emissions is widely recognized as a preferred target and a primary focus of mitigation efforts [5, 6]. Therefore, mitigation strategies aiming to reduce enteric CH_4 emissions have focused on dietary treatments, nutritional feed additives, animal breeding, and genetic selection [7, 8]. However, reliable, accurate, and robust measurements of enteric CH_4 emissions are required to assess the effectiveness of mitigation strategies and to support national GHG inventories [9–11]. To meet these requirements, various methods have been developed and implemented for measuring enteric CH_4 emissions from ruminants [12–15].

Among the available methods, the respiration chamber (**RC**) is considered the "gold standard" owing to its high accuracy and repeatability under controlled conditions [16, 17]. However, RCs are expensive to construct and operate, require intensive labor, and cause behavioral disturbances due to confinement, making them impractical for large-scale applications [18, 19]. To overcome these limitations, non-invasive spot-sampling methods have been developed to estimate CH_4 emissions through short-term breath sampling, enabling high-throughput measurement of animals [14, 20]. One such method is the GreenFeed system (**GF**), a widely used gas-flux quantification system designed as an open-circuit head chamber combined with an overhead hopper that measures exhaled gases when animals place their heads inside [13, 14, 21]. It operates by integrating measurements of airflow, gas concentrations, and muzzle position during individual visits, continuously recording data, with daily CH_4 emissions calculated through an internal algorithm [17, 20, 22]. The GF is designed to collect and quantify gas emissions from ruminants, which are individually identified using a radio frequency identification tag when the animal visits for measurement. GF has demonstrated strong correlation with RC-based measurements ($R^2 = 0.96$) [23], making it a reliable method for use under on-farm conditions. Another approach is the sniffer method, first introduced by Garnsworthy et al. [24], which involves short-duration continuous analysis of exhaled breath from feed troughs in an automatic milking system (**AMS**) during milking or from concentrate feeding, allowing repeated measurements over prolonged periods [18, 25]. Specifically, the ‘with CO_2 measurements’ approach simultaneously measures CH_4 and CO_2 concentrations, using CO_2 as a tracer gas and the CH_4/CO_2 ratio as a proxy for prediction equations [26, 27], owing to the advantage that CO_2 in the sampled air is influenced only by the animal’s breath [28].

However, most validations of the sniffer method have been conducted in association with AMS, which are not universally implemented in commercial dairy farms. Consequently, there is a need for CH_4 emission measurement approaches that can be applied under conventional on-farm conditions without AMS. Similar approaches have shown that a fraction of exhaled breath can be sampled during feeding by partially enclosing feed troughs or individual feed bins [25, 29]. Therefore, we hypothesized that the adaptation of the sniffer method for floor-based feeding environments would yield CH_4 estimates comparable to those obtained with the GF, thus representing a viable alternative for measuring CH_4 emissions from dairy cows under on-farm conditions. The objective of this study was to validate the feasibility and accuracy of the sniffer method by comparing its performance with that of the GF in lactating Holstein cows under on-farm conditions.

MATERIALS AND METHODS

All experimental procedures in this study were approved by the Institutional Animal Care and Use Committee of Konkuk University (Permit numbers: KU23156, KU23234, KU24172), in accordance with the "Guide for the Care and Use of Experimental Animals."

Animals, Feeding, and Management

This study was conducted at the Konkuk University Experimental Farm (Chungju-si, Republic of Korea) to compare two different methods for measuring enteric CH_4 emissions in dairy cows. The experiment consisted of three experimental phases, each involving eight dairy cows: Phase 1 from October to December 2023, Phase 2 from August to September 2024, and Phase 3 from September to October 2024. A total of 15 primiparous and 9 multiparous Holstein dairy cows were used during the study. All animals were housed in free stall barns, with two cows assigned per pen under the same environmental conditions. A total of four pens were used during each experimental phase. The farm environment allowed the animals to move freely, except during gas measurement periods. All cows had free access to drinking water. The diet was composed based on a total mixed ration (**TMR**) and bait feed as a form of commercial concentrate, which were offered separately to the cows (**Table 1**). In the first phase, feed was given once daily at 8:00 a.m., while in the second and third phases, it was offered twice daily at 8:00 a.m. and 5:00 p.m. Bait feed was available from both measurement methods, allowing access for one animal at a time. Each phase included at least 10 days of adaptation to dietary treatment and both two CH_4 emission measurement methods within the same adaptation period.

Measurements of Animal Production and Emissions

Data collection was performed twice for four consecutive days in each phase, resulting in a total of six measurement periods and forty-eight measurements. Individual feed intake, milk production and characteristics, body weight (**BW**), and gas measurement were recorded during data collection. During data collection, dairy cows were housed individually by dividing the housing pen. All feed refusals were weighed and recorded individually the day after the feed was offered and before providing the new diet to estimate individual feed intake and dry matter intake (**DMI**). Samples of TMR and bait feed were collected throughout the experiment for dry matter determination (oven-dried at 60℃ for 48h), and a sample was stored before chemical analysis. The chemical composition of TMR and bait feed in each phase (**Table 1**) was determined from oven-dried samples. Ether extract (Method 920.39), and ash (Method 942.05) were analyzed according to the procedure of the Association of Official Analytical Chemists [30]. Neutral detergent fiber, acid detergent fiber, and acid detergent lignin were analyzed following the procedure of Van Soest et al. [31]. Crude protein (**CP**), neutral detergent insoluble CP, and acid detergent insoluble CP were analyzed using the procedure of AOAC (Method 954.01). Non-fiber carbohydrates (**NFC**) and energy values including total digestible nutrients, digestible energy and metabolizable energy, were calculated according to the formulas proposed by NRC [32]. The BW was measured individually and recorded using an indicator (CI-200A, CAS Co., Ltd., Gyeonggi-do, Republic of Korea) on the last day (4th day) of data collection. Dairy cows were milked twice daily (at 07:00 and 16:00) in a tandem milking parlor, and individual milk yield (**MY**) was recorded at each milking. Milk samples were collected individually and were stored at 4℃ with the addition of a milk preservative (Broad Spectrum Microtabs II™, Advanced Instruments, MA, USA). Milk component analysis, including fat and protein percentages, was performed using an automatic milk analyzer (CombiFoss 7 DC, FOSS, Hillerød, Denmark). Energy-corrected milk (**ECM**, kg/d) was calculated according to Tyrrell and Reid [33] as follows:

$$\mathrm{ECM} = (\mathrm{MY\ (kg/d)} \times [376 \times \text{milk fat (\%)} + 209 \times \text{milk protein (\%)} + 948]) / 3138 \cdots [1]$$

Methane emissions were measured using the GF (C-lock Inc., Rapid City, SD, USA) and the sniffer-based method (**SB**) using a gas hood system. Gas measurements were performed individually at designated time points every 9 h, totaling 8 measurements over 4 days (twice per phase), resulting in 16 measurements per animal during a single phase to compare the difference between measurement methods. Gas measurement was carried out by individually luring the animals to the designated locations where the gas measurement methods were installed. Because it was not feasible to measure an individual animal using both methods simultaneously, measurements were conducted consecutively within the same session, with the GF applied first, followed immediately by measurement by the SB (**Fig. 1)**. To minimize potential variability arising from the time gap between consecutive measurements, paired GF and SB measurements were scheduled within the same measurement session, and measurements were distributed at 9 h intervals over four consecutive days to reduce short-term diurnal variance.

Gas Measurements by the GreenFeed System

CH_4 and CO_2 emissions from dairy cows were measured individually using a GF installed in a free-stall barn. Prior to gas measurements, animals underwent at least 7 days of training to familiarize them with the system. The training session was conducted to ensure the animals recognized the measurement station as a safe place where feed is supplied and to maintain proper head positioning during the measurement process. Air filters were replaced whenever the airflow rate dropped to 27 L/s. The accuracy of the system was calibrated through CO_2 recovery tests performed before and after each experimental phase, resulting in an average recovery rate of 100.1 ± 4.99% (mean ± standard deviation). To minimize interference from background (**BG**) gases, such as originating from manure or the floor surface, the ventilation fans in the barn were turned off prior to each measurement.

Gas measurements were conducted by luring dairy cows to the site using pelleted concentrate as bait. Once the animal entered, emitted gases were collected, dust was filtered, and the airflow rate was measured. The collected gas samples were then analyzed using a non-dispersive infrared sensor to determine gas concentrations. Upon entry, the individual’s radio frequency identification tag was recognized, and the gas measurement lasted for a minimum of 5 min per animal. During each measurement, the ‘head proximity’ value was continuously monitored to ensure the animal’s head position remained within the system. A waiting period of 2–3 min was given between measurements to prevent mixing of data. Sampled gas concentrations were logged at 1 s intervals and continuously recorded throughout every measurement. Simultaneously, entry and exit times, the number of bait feed drops, and feed refusals were recorded for each individual. After each measurement, the number of bait feed drops, and remaining bait feed was counted to adjust DMI calculations. The calculated

CH_4 and CO_2 emissions (g/d) data were transmitted via network connection to the C-Lock server, where the data were processed and presented to the laboratory.

Gas Measurements and Analysis Using the Sniffer-based Method

For estimating daily CH_4 emissions, a gas collection system was developed, comprising a Ceiling-Sealed Gas Collection Hood integrated with a feeding trough (**CS-GCH**) and the SB. The CS-GCH is designed to collect exhaled breath from individual dairy cows, inspired by previous studies [29, 34]. The SB was used to analyze gas concentrations in real-time with a Microportable GHG analyzer (Model: GLA131-GGA; ABB/LGR, Quebec, Canada). The analyzer operates based on Off-Axis Integrated Cavity Output Spectroscopy (OA-ICOS^{TM}) technology, a variant of cavity ring-down spectroscopy—a laser-based absorption technique that enables high sensitivity and precise quantification of trace gases. It provides a flow time response of 1 s and measurement precisions of 4 ppb for CH_4 and 0.6 ppm for CO_2. The measurable concentration ranges were 0–5% for CH_4 and 0–20,000 ppm for CO_2. During each phase, gas collection was performed 16 times per animal (twice per phase, at 9 h intervals over four consecutive days).

Given the specific conditions in the Republic of Korea—where dairy cows typically managed in floor-level feeding systems and the adoption rate of AMS remains low, the CS-GCH was designed with an adjustable structure to accommodate farm conditions and allow CH_4 measurements under floor-based feeding systems. To accommodate the natural feeding posture of dairy cows, the dimensions of the CS-GCH were based on actual on-farm measurements of head extension during feeding (W 100 × D 80 × H 140 cm), ensuring sufficient space for proper head positioning. All animals were acclimated to the CS-GCH for at least seven days before the experiment began. To encourage voluntary entry and remain inside the CS-GCH, bait feed was provided immediately before individual gas measurements. Once the stanchion secured the animal’s head in position, gas measurement was initiated and continued for a minimum of 360 s per animal. After each individual measurement, a 2–3 min waiting period was maintained before measuring the next animal to prevent cross-contamination of gas samples between individuals. During this interval, the hood was ventilated to ensure that no residual gas remained inside, and the gas concentrations were confirmed to return to the BG gas level. The frame of the CS-GCH was constructed of steel, with polycarbonate panels attached to minimize interference from external airflow and wind. Gas sampling inlets (6 × 4 mm polyurethane tubes) covered with 50-μm mesh filters were positioned at four locations – two at the middle section where the head is placed during measurement, and two in the lower section directly above the feeding trough to optimize gas collection. These gas collection tubes were enclosed and secured to prevent interference from the dairy cows. The four gas collection tubes were combined into a single line and fitted with an air filter before being connected to the SB. Gas inside the CS-GCH was drawn by a diaphragm pump (DAP-12S, Ulvac Inc., Chigasaki, Japan) at a flow rate of 6 L/min. The sampled gas then passed through a tube fitted with an air filter (ZFC-74, SMC, Tokyo, Japan) and a membrane-type gas dryer (SWF-M06-400 and SWC-M08-100, AGC Inc., Tokyo, Japan) before being connected to the SB. The layout of the gas collection system is illustrated in **Fig. 2**. The CH_4 and CO_2 concentrations of the sampled gas were logged at 1 s intervals and continuously monitored. To ensure that measured gas concentrations reflect exhaled breath exclusively, BG gas concentrations were measured and subtracted from the measured values. The BG gas concentration measurements were performed for at least 10 min before or after each measurement session. The average CH_4 and CO_2 concentrations recorded during BG gas measurements were used for background (BG) gas correction and to calculate the CH_4/CO_2 ratio. In estimations of CH_4 emissions, accurate calculation of the CH_4/CO_2 ratio requires a gas sample containing a reasonable breath concentration [35]. To remove the effect of low concentration of breath, corrected CO_2 concentrations exceeding 0.05%—after excluding the BG CO_2 concentration from the sampled gas—were included for further analysis because lower levels were considered insufficiently representative of exhaled breath, following the method of a previous study [35]. After background (BG) gas correction, average CH_4 (ppm) and CO_2 (ppm) concentrations per animal were calculated, and the CH_4/CO_2 ratio (ppm/ppm, v/v) was determined. Following data filtering, the refined CH_4/CO_2 ratio for each individual was used to estimate daily CH_4 emissions from dairy cows.

The CH_4 emissions were estimated using the predictive equation proposed by Suzuki et al [27], which was developed for Holstein lactating cows based on RC and head-box measurements across a wide range of dietary conditions. Dairy production systems in Japan and the Republic of Korea share several fundamental characteristics, including herd structure, predominantly floor-level housing, and comparable feeding strategies, supporting the applicability of prediction models developed under similar management conditions and suggesting that measured values would be broadly comparable. Given the absence of nationally developed equations for estimating CH_4 emissions under on-farm conditions in the Republic of Korea, this externally validated model was selected as a practical approach for comparative analysis. Accordingly, CH_4 emissions were estimated as follows:

$$CH_4\ (\mathrm{L/d}) = -507 + 0.536 \times \mathrm{BW\ (kg)} + 8.76 \times \mathrm{ECM\ (kg/d)} + 5029 \times CH_4/CO_2 \cdots [2]$$

where BW represents the live weight of the individuals (kg), and ECM is calculated using Eq. 1 mentioned above. A conversion factor of 0.716 (g/L) was applied to convert CH_4 emissions from L/d to g/d, assuming standard temperature and pressure conditions.

Statistical Analysis

Statistical analyses were performed in Python (ver. 3.12.7; Python Software Foundation, Beaverton, Oregon) using pandas, NumPy, SciPy, statsmodels, pingouin, and fastdtw, while data visualization was conducted using matplotlib and seaborn to compare CH_4 emission estimates obtained from two different measurement methods. Outliers were identified using a z-score threshold, excluding data points with an absolute z-score greater than 2.0 from further analysis. This approach retains approximately 95.45% of the dataset, thereby minimizing the influence of extreme values and reducing noise within the dataset. Descriptive statistics—including mean, standard deviation (**SD**), standard error of the mean (**SEM**), and coefficient of variation (**CV**, %)—were calculated for the entire dataset as well as for each experimental phase. To evaluate the effect of the experimental phase on CH_4 emissions, DMI, MY, and ECM, a one-way ANOVA was performed using a general linear model, followed by Tukey's HSD test for pairwise comparisons. Differences were considered significant at $p < 0.05$, and a tendency was declared at $0.05 \leq p < 0.1$. Agreement between the two measurement methods was assessed using Bland–Altman analysis by calculating the mean difference as residuals (GF – SB, g/d) and the corresponding 95% limits of agreement (**LOA**). The LOA were calculated as ±1.96 SD from the bias. Residuals were plotted against GF values to evaluate potential proportional bias. Normality of residuals was evaluated using quantile–quantile plots. To assess variability between measurement methods, variance homogeneity across Method × Period was evaluated using the Brown–Forsythe test, which is robust to deviations from normality. In addition, absolute differences between methods (|GF – SB|) were calculated and analyzed using one-way ANOVA to examine potential phase-dependent effects, with Tukey's HSD test applied for pairwise comparisons. To evaluate the performance of the SB in each experimental phase, the mean absolute percentage error (**MAPE**) and confidence rate (**CR**) were calculated. MAPE represents the mean absolute error expressed as a percentage, calculated by averaging the absolute percentage errors. This metric indicates the magnitude of the error regardless of the prediction value. MAPE was utilized to verify differences between values obtained from the two different methods, while CR was calculated as 1 − MAPE (%) and represents the proportion of agreement. Temporal alignment between GF and the SB was analyzed using dynamic time warping (**DTW**) as a supplementary analysis. For the DTW analysis, outlier removal was not applied because all observed values, derived from repeated measurements collected at time points, were considered to represent valid within- and between-method comparisons and were necessary to preserve the integrity of the time series structure. The DTW analysis was performed at the animal level, treating repeated measurements over time as within-animal time series.

RESULTS

Descriptive Statistics of CH_4 Emissions and CH_4/CO_2 Ratio

The dataset obtained from the 24 lactating Holstein cattle during the data collection period was characterized by the following parameters (mean ± SD): parity of 1.6 ± 0.77, DMI of 25.1 ± 1.63 kg/d, BW of 641.6 ± 57.26 kg, ECM of 21.3 ± 2.82 kg/d, and daily MY of 24.5 ± 3.05 kg. These descriptive statistics are summarized in **Table 2**. Summary statistics for enteric CH_4 emissions (CH_4, g/d) and CH_4/CO_2 ratios measured using the GF and SB are presented in **Table 3**. Overall, GF recorded higher CH_4 emissions (312.9 ± 56.50 g/d) than the SB (297.1 ± 61.83 g/d) across all measurements. This difference was consistently observed across all experimental phases, with the largest difference occurring in Phase 3 (300.2 g/d and 278.4 g/d, respectively). Across all phases, the SD of daily CH_4 emissions ranged from 39.98 g/d to 76.18 g/d for GF and from 50.38 g/d to 77.78 g/d for the SB. The CV for CH_4 emissions was lower for the SB in Phase 2 than for the GF (16.67% vs. 24.19%). However, the opposite trend was observed in Phase 1 and Phase 3, with the SB showing 16.68% and 27.94%, and the GF showing 12.35% and 16.58%, respectively. For the CH_4/CO_2 ratio (*v/v*), the SB showed higher mean and median values overall (0.077 and 0.078, respectively) than the GF (0.064 and 0.064). The maximum value of the CH_4/CO_2 ratio recorded by the SB (0.103) was also higher than that of the GF (0.087), while the CV for the ratio was relatively similar between measurement methods, with the SB showing 15.960% and the GF showing 17.629%. Overall, these results indicate that the SB tended to produce slightly higher CH_4/CO_2 ratios while showing variability comparable to that of the GF across measurement phases. For measurements from the SB, BG CH_4 and CO_2 concentrations used for CH_4 emission estimations were 7.5 ± 2.68 ppm and 547.33 ± 42.99 ppm (mean ± SD), respectively, across all measurement periods.

Comparisons of Phase Effects on CH_4 Emission and Productivity Variables

Pairwise comparisons were performed to examine whether there was an association between phase-specific trends in productivity and CH_4 emissions. Methane emissions measured by both measurement methods did not differ significantly among experimental phases ($p = 0.31$ and 0.51, respectively), and the Tukey HSD test confirmed non-significance ($p > 0.05$) (**Fig. 3A**). In contrast, the CH_4/CO_2 ratio showed significant phase effects for both measurement methods ($p < 0.01$). For the SB, the ratio in Phase 3 was significantly lower than in Phase 1 ($p = 0.021$) and Phase 2 ($p = 0.003$), while for the GF, all pairwise comparisons among phases were significant ($p < 0.05$), with Phase 2 showing the highest value and Phase 1 showing the lowest (**Fig. 3B**). Among animal-related traits, body weight (BW) differed significantly across phases ($p < 0.01$), with higher values in Phase 3 compared to Phase 1 and Phase 2 ($p < 0.05$). DMI increased significantly from Phase 2 to Phase 3 ($p < 0.001$), while MY was lower in Phase 3 than in Phase 1 ($p = 0.012$).

Agreement and Bias between the GF and the Sniffer-Based Method

Agreement between the GF and SB methods was evaluated using Bland–Altman analysis (**Fig. 4A, Fig. 4B**). For CH_4 emissions, the mean bias (GF − SB) was 15.78 g/d, indicating that the GF tended to measure slightly higher CH_4 emissions than the SB. The SD of the differences was 57.09 g/d, resulting in a 95% LOA ranging from −96.12 to 127.68 g/d. The 95% confidence intervals (**CI**) for the lower LOA ranged from −212.25 to 20.02 g/d, and for the upper LOA from 11.55 to 243.82 g/d, respectively. The overall span between the LOA was 223.80 g/d, with the bias representing approximately 7.1% of this range. When expressed as a proportion of the lowest and highest average CH_4 emission values, the bias accounted for 7.8% of the minimum average measurement and 3.4% of the maximum. These results demonstrate that the GF and SB provide comparable estimates of daily CH_4 emissions under on-farm conditions. In terms of the CH_4/CO_2 ratio, the mean bias (GF − SB) of −0.012 indicates that the SB tended to record slightly higher CH_4/CO_2 ratios than the GF on average. The SD of the differences was 0.013, resulting in a 95% LOA between −0.04 and 0.01. The 95% CI for the lower and upper LOA ranged from −0.065 to −0.011 and −0.013 to 0.040, respectively. The overall span between the LOA was 0.0518, and the bias accounted for approximately 24.0% of this range.

Residual analysis was performed to evaluate measurement differences across the observed range of values. For CH_4 emissions, residuals (GF − SB) plotted against the measurement range were distributed from −100 g/d to 100 g/d, without an evident systematic pattern across the range (**Fig. 4C**). Similarly, residuals for the CH_4/CO_2 ratio ranged from −0.04 to 0.02, indicating that the GF tended to record slightly lower CH_4/CO_2 ratios than the SB (**Fig. 4D**). To further assess the presence of proportional bias, linear regression analysis was conducted by regressing residuals against the mean of the two measurement methods. For CH_4 emissions, the slope of the regression line was not significantly different from zero ($\beta_1 = -0.117$, $p = 0.477$), and the same pattern was observed for the CH_4/CO_2 ratio, where the regression slope was also not significant ($\beta_1 = -0.114$, $p = 0.576$). Additionally, the quantile–quantile plots for both CH_4 emissions and the CH_4/CO_2 ratio showed close alignment along the 45-degree line, further supporting the assumption of normality. Overall, although the GF showed a tendency to record slightly lower CH_4/CO_2 ratios compared to the SB, the residuals for CH_4 emissions were evenly distributed without directional bias, suggesting that measurement errors were consistent and not substantially affected.

To further assess variability between methods, variance homogeneity across Method × Period was evaluated using the Brown–Forsythe test. For CH_4 emissions, no significant differences in variance were detected between the GF and the SB across experimental phases ($p = 0.104$). In addition, the absolute differences between methods ($|GF - SB|$) did not differ significantly among phases (ANOVA, $p = 0.206$), and no significant pairwise differences were identified by Tukey's HSD test ($p > 0.05$). In contrast, for the CH_4/CO_2 ratio, the Brown–Forsythe test indicated significant differences in variance across Method × Period ($p = 0.019$). Furthermore, the absolute differences between methods ($|GF - SB|$) showed a significant effect of the experimental phase (ANOVA, $p < 0.001$). Tukey's HSD test revealed significant differences between Phase 1 and Phase 2 ($p < 0.001$) and between Phase 1 and Phase 3 ($p < 0.001$), whereas no significant difference was observed between Phase 2 and Phase 3 ($p = 0.337$).

Consistency and Phase-Specific Performance of the Sniffer-Based Method

Phase-specific performance of the SB was evaluated using MAPE and CR (**Table 4**). Phase 1 exhibited the highest CR (88.43%) and the lowest MAPE (11.57%), whereas Phase 2 had the highest

MAPE (19.20%) and the lowest CR (80.80%). Phase 3 showed an intermediate level of agreement, with MAPE of 17.72% and a CR of 82.28%. The SEM for the GF ranged from 9.99 to 19.67 g/d across phases, whereas the SEM for the SB ranged from 12.59 to 19.44 g/d.

Time Series Comparison of CH_4/CO_2 Ratio to Assess Temporal Consistency

The DTW analysis was performed as a supplementary analysis to compare the temporal patterns of the CH_4/CO_2 ratio between the two measurement methods and to assess the similarity of temporal variation in a CH_4-related trait. The time series alignment of CH_4/CO_2 ratios between the GF and the SB is presented in **Fig. 5**. The mean DTW distance was 0.273 (ranging from 0.118 to 0.593), which decreased to 0.014 (ranging from 0.007 to 0.028) after normalization by the warping path length.

DISCUSSION

The increasing attention to accurate, large-scale estimates of enteric CH_4 emissions—for national GHG inventories, mitigation strategies, and genetic selection—has prompted the development of various measurement approaches [7, 9, 11]. These include spot-sampling techniques, which measure CH_4 emissions during short term by analyzing exhaled gases from individual animals during feeding or milking [11–14]. This study evaluated the SB in comparison with the GF to determine whether the two methods could yield comparable CH_4 emission estimates in dairy cows.

Agreement Between Measurement Methods in Estimating CH_4 Emissions and CH_4/CO_2 ratio

Among the CH_4 measurement methods currently developed and utilized, RC are widely recognized as the gold standard for CH_4 measurement owing to their high accuracy and precision, serving as a reference for evaluating alternative methods. While the GF has demonstrated relatively high correlations with RCs ($R^2 = 0.81$) [19], comparative studies between the GF and sniffer methods remain limited. For example, Troy et al. [29] evaluated CH_4 emissions using an infrared multi-gas analyzer coupled with a CH_4 hood system in beef cattle; however, a direct comparison between a feed trough-hood-based sniffer method and the GF in dairy cattle has not been previously reported, highlighting the need for systematic evaluation of these approaches.

In the present study, the GF reported significantly higher CH_4 emissions (312.9 ± 56.50 g/d) than the SB (297.1 ± 61.83 g/d), with a mean difference of 18.44 g/d. Such differences may arise from variations in MY, ECM, DMI, and stage of lactation, all of which are known to significantly influence CH_4 production [7, 16]. The average MY and ECM in this study (24.5 and 21.3 kg/d, respectively) were substantially lower than those reported in previous studies, ranging from 33.9–42.6 kg/d (MY) and 34.2–40.5 kg/d ECM (ECM), respectively [27, 36−38]. This relatively lower level of milk production likely contributed to the lower CH_4 emissions predicted by the SB in the present study, especially since SB-derived CH_4 estimates are based on a predictive equation incorporating productivity-related variables.

Nevertheless, despite the significant difference in absolute CH_4 outputs, the overall agreement in measurement range and variability suggests that both methods can yield comparable estimates under on-farm conditions. Bland–Altman analysis indicated close agreement between the GF and SB for both CH_4 emissions and CH_4/CO_2 ratios. Bland–Altman analysis is widely used to assess agreement by plotting the difference between two paired measurements against the average of the two measurements, thereby enabling evaluation of systematic bias and the range of differences represented by the 95% CI and LOA, which are considered acceptable when they fall within a range [39]. In the present study, the mean bias for CH_4 emissions (GF – SB) was 15.78 g/d, corresponding to 7.1% of the measurement range. For the CH_4/CO_2 ratio, the mean bias was –0.012, corresponding to 24.0% of the measurement range. The majority of data points fell within the 95% LOA, the level of agreement is considered acceptable for genetic or inventory applications.

Residual analyses further supported the absence of proportional bias between the GF and the SB for either CH_4 emissions or the CH_4/CO_2 ratio. Residuals were symmetrically distributed across the measurement range, and regression slopes of residuals against the average of the two methods were not significantly different from zero ($p = 0.477$ and $p = 0.576$, respectively). These results indicate that between-method differences were relatively consistent across low and high values, rather than increasing with measurement magnitude. Therefore, the observed disagreement represents a consistent offset between methods rather than a bias that changes with measurement scale, supporting the comparability of the GF and SB across the range of measurements in this study.

To further evaluate phase-specific consistency between the two measurement methods, MAPE and CR were assessed. MAPE is a scale-independent metric that expresses prediction errors as percentages by averaging the absolute differences between two values, thereby providing an easily interpretable measure of accuracy [40, 41]. The SB showed the highest agreement in Phase 1 (MAPE = 11.57%; CR = 88.43%) and the greatest variability in Phase 2 (MAPE = 19.20%; CR = 80.80%). Despite these phase-specific differences, overall performance consistently achieved a CR above 80%, demonstrating acceptable repeatability across phases. The CH_4/CO_2 ratio obtained from the SB (0.069–0.082) aligned well with those reported in previous studies, ranging from 0.06 to 0.09 [36–38].

This consistency with previously reported ranges supports the reliability of the feed-trough-based sniffer approach established in the present study.

As a supplementary analysis, DTW was applied as a novel analytical approach to evaluate the similarity of CH_4/CO_2 ratio time series obtained from two measurement methods across comparable time points. DTW quantifies similarity between two time series by identifying an optimal alignment that minimizes cumulative distance within a distance matrix, producing a warping path that represents the best temporal correspondence, with lower DTW distances indicating greater similarity [42]. Although DTW analysis has been increasingly applied in ecological research, such as clustering foraging trajectories of grey seals or comparing animal movement patterns [43], its application to CH_4 measurement method comparison has not been previously reported. In the present analysis, the mean DTW distance across animals was 0.273. Thus, normalization was applied to account for differences in sequence length and alignment complexity, reducing the DTW distance to 0.014 after normalization by the warping path length. These results suggest that the GF and SB captured broadly consistent temporal patterns of the CH_4/CO_2 ratio, despite minor differences in absolute values. Importantly, DTW focuses on similarity in temporal dynamics rather than numerical agreement; therefore, these findings should be interpreted as concordance in temporal dynamics rather than pointwise agreement, complementing conventional analyses by revealing dynamic patterns that static comparisons cannot capture. Taking together, despite minor differences in absolute CH_4 values, the SB showed repeatable and stable CH_4 estimates under field conditions and demonstrated strong comparability to the GF; however, understanding the sources and structure of variability remains essential for interpreting method-specific differences.

Sources of Variability in Spot-Sampling Measurements

Regarding variability, the GF exhibited lower CV (%) for CH_4 emissions during phases 1 and 3 (12.35% and 16.58%, respectively) compared with the SB (16.68% and 27.94%, respectively), along with lower SD and SEM. However, when data were considered across the entire measurement period, no significant differences in overall variability were observed between the GF and SB (CV: 18.06%

vs. 20.81%; SD: 56.50 g/d vs. 61.83 g/d; SEM: 8.24 vs. 9.02), indicating comparable stability of CH_4 emission estimates despite phase-specific differences. For the CH_4/CO_2 ratio, the SB (0.077 ± 0.012) consistently showed higher mean values than the GF (0.064 ± 0.011). Across all periods, the CV was slightly higher for the GF (17.63%) than for the SB (15.96%), while SDs were similar (GF: 0.011; SB: 0.012). The CV ranges observed for the SB (7.91–18.89%) were consistent with previously reported values of 15%–20% [28, 36, 44, 45], suggesting that the variability observed in the present study falls within expected methodological and animal-related variation.

Despite similar levels of variability, the difference in absolute CH_4/CO_2 ratio values between methods is likely attributable to methodological factors rather than measurement instability. In particular, the GF employs a flux-based approach using a relatively high airflow (20–40 L/s) [21, 46 47], whereas the SB operates at a substantially lower sampling rate (approximately 6–7 L/min) at a specific sampling spot [35, 37]. Such differences can influence gas dilution and concentration profiles, potentially contributing to systematic differences in measured CH_4/CO_2 ratios. In addition, the GF estimates daily CH_4 emissions using internal algorithms that account for differences between exhaled breath and BG gas concentrations [10, 13, 20], which may further affect the comparability of absolute values. Although numerical differences in variability metrics were observed across phases, variance homogeneity tests indicated no significant method-dependent differences in CH_4 emissions. This suggests that the observed variability is unlikely to reflect methodological instability and is instead more likely driven by phase-related physiological or behavioral factors. In contrast, variability in the CH_4/CO_2 ratio exhibited clear phase dependence, consistent with the inherent sensitivity of gas sensors installed in ratio-based spot-sampling measurements to short-term fluctuations in breathing patterns.

Unlike RC, which provide continuous 24-h measurements and capture CH_4 emissions from both ruminal and hindgut fermentation, the GF and SB rely on short-term spot-sampling protocols. This limits their ability to capture diurnal variation in emission patterns [10, 48] or CH_4 produced via hindgut fermentation, which contributes up to 3% of total CH_4 emissions [7]. To minimize these potential effects, gas measurements in the present study were conducted at 9 h intervals over four consecutive days, covering different time points and enhancing temporal representativeness [49]. CH_4 emissions from dairy cows vary throughout the day and across the lactation period [19], and this variability could partly be influenced by rumen activity and animal behavior. Increased rumination time has been associated with reduced CH_4 emissions [50, 51]; however, rumination is highly dynamic, subject to momentary changes, voluntarily regulated by animals, and easily interrupted by environmental stimuli such as milking [52]. Because rumination behavior was not recorded in the present study, its contribution to short-term variability could not be quantified, but it likely explains part of the short-term variation in spot-sampling measurements. Previous studies have shown that increasing the number of measurement days or animals can reduce such variability and improve repeatability [44, 53–55].

Beyond methodological factors, phase-dependent differences in diet composition and productivity likely contributed to the variability observed in the CH_4/CO_2 ratio, particularly for the SB. In the SB, the CH_4/CO_2 ratio was lowest in Phase 3, coinciding with a shift toward a higher NFC, lower-fiber diet and with reduced ECM despite higher BW. Increased NFC intake has been associated with reduced CH_4 emissions per unit of ECM [56], and because SB-based CH_4 estimates are derived from BW, ECM, and the CH_4/CO_2 ratio, the combined effects of dietary composition and productivity are consistent with the lower CH_4/CO_2 ratio observed in Phase 3. In contrast, the CH_4/CO_2 ratio measured by the GF showed a different phase pattern, which is less likely to reflect productivity alone and may instead be influenced by visit-related factors such as measurement timing relative to feeding, diurnal variation, airflow dilution, and head position.

Practical Implications of the SB Under On-Farm Conditions

In the present study, a customized feed-hood system, defined as CS-GCH, was developed to enable the collection of exhaled breath from cows managed under floor-level feeding conditions. The conceptual foundation of the CS-GCH was adapted from previously reported hood-based gas collection systems [25, 29, 34], while incorporating modifications to account for the natural head position and the use of floor-based feeding systems. Although the sniffer method was originally introduced for collecting exhaled gas within AMS infrastructure in dairy cows [24, 35, 37], the development of CS-GCH was motivated by the limited adoption of AMS, which restricts the applicability of conventional sniffer-based approaches integrated within AMS infrastructure. Studies in beef cattle have reported gas measurements based on sniffer methods taken from feed troughs or electronic feeders [25, 29, 34]; however, these methods were positioned at elevated heights or enclosed using a polycarbonate hood, which may not reflect the feeding environment of dairy cows managed at floor level. Although the CS-GCH improves practicality, it operates in an open space without a sealed head enclosure, potentially allowing partial intrusion of ambient air and thereby influencing the concentration of the sampled gas and contributing to variability in the results. In addition, sniffer-based methods have been reported to be more sensitive to factors such as muzzle movement and head position relative to the feed trough, as well as individual breathing patterns, which can substantially affect the sampled CH_4 and CO_2 concentrations [18]. These characteristics necessitate not only additional effort for manual positioning of the measurement method, the recording of entry and exit times, but also careful background (BG) gas correction and repeated measurements to reduce short-term variability under on-farm conditions.

Interpretation of the SB-derived CH_4 estimates also requires consideration of the predictive equation applied. In this study, CH_4 emissions were estimated using the equation proposed by Suzuki et al. [27], which was developed for Holstein lactating cows based on RC and head-box data across diverse diets and validated by comparing predicted values with measurements obtained under varying dietary conditions using both head-boxes and sniffer method, as well as with a heat production unit-based equation. This equation was selected as a practical and externally validated model because there are no nationally established predictive equations for estimating enteric CH_4 emissions under on-farm conditions. However, this equation does not explicitly account for energy balance, which may lead to systematic bias under certain physiological conditions. Previous studies have shown that highly efficient cows or cows under negative energy balance may exhibit overestimated CH_4 emissions, whereas cows in positive energy balance during mid to late lactation may be underestimated [57]. In addition, efficient cows tend to produce less heat, BW and ECM, which may further contribute to an overestimation of CH_4 production [13]. While cows with greater feed efficiency tend to consume less feed for the same level of milk production and, given the positive relationship between CH_4 production and DMI, improvements in residual feed intake are therefore expected to reduce CH_4 emissions [58], empirical data from dairy cows indicate that reduced CH_4 yield is associated with reduced diet and fiber digestibility, suggesting that less efficient animals may produce less CH_4 output due to low efficiency at extracting energy from fiber [59]. In the present study, although the ranges of DMI, BW, and ECM in the present dataset fell within those used for equation development and the CH_4/CO_2 ratios (0.069 – 0.082) were comparable to those reported by Suzuki et al. (0.088) [27], whereas ECM production was lower (21.3 kg/d vs. 25.4 kg/d), while DMI (25.1 kg/d vs. 16.7 kg/d) and BW (641.6 kg vs. 589 kg) were higher, suggesting relatively lower production efficiency in the present study. These differences may contribute to an overestimation of CH_4 emissions when applying equations developed based on higher-efficient cows.

Despite these limitations, both methods produced comparable CH_4 estimates that were distributed within a similar range, indicating that the SB yields comparable results to those of the GF. Like the GF, the SB also requires an adaptation period and involves several indicators necessary for estimating CH_4 emissions beyond gas measurements. However, the main advantages of the SB lie in its portability, flexibility, and applicability in non-AMS infrastructures, which can overcome the spatial limitations of fixed installations and enable the collection of CH_4 emission data across multiple locations with relative ease. This flexibility enhances its applicability under diverse on-farm conditions, without reliance on permanently installed infrastructure. Although additional steps are required to derive ECM, the SB remains practical because it does not depend on variables such as DMI that are difficult to obtain routinely, thereby further enhancing its practicality for large-scale field applications. Overall, these findings demonstrate that the SB provides CH_4 estimates comparable to those of the GF, highlighting its feasibility and accessibility as a practical on-farm method for estimating enteric CH_4 emissions under field conditions.

CONCLUSION

This study evaluated the performance and applicability of the SB, adapted for floor-based feeding environments without AMS, in comparison with the GF. Although the SB yielded slightly lower CH_4 emission estimates than the GF, the differences between the two methods were small and within an acceptable range of measurement errors. Both methods demonstrated comparable variability, while the SB yielded CH_4/CO_2 ratios consistent with those reported in previous studies, confirming its analytical reliability. These results indicate that the SB can provide estimates of enteric CH_4 emissions that are comparable in accuracy and repeatability to those obtained with the GF under on-farm conditions. Given its portability, flexible installation, and low operational cost, the SB represents a practical approach for CH_4 measurement in dairy cows, particularly in farms lacking AMS infrastructure. Continued validation under diverse diets and environmental conditions will further strengthen its reliability and broaden its applicability for both research and large-scale data collection purposes. Future work should evaluate the performance of the SB across a wider range of diet compositions and breeds to strengthen its applicability under diverse field conditions. In addition, testing different sampling frequencies and measurement timings may help refine implementation strategies and support broader on-farm application across a wide range of environments.

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Table 1. Chemical composition of total mixed ration (TMR) and bait feed during the experiments (expressed as % of dry matter (DM), unless otherwise noted)

Parameters	***Phase 1***		***Phase 2***		***Phase 3***	
	TMR	Bait feed[4]	TMR	Bait feed	TMR	Bait feed
Chemical compositions, % of DM basis						
CP	13.47	21.79	8.68	18.45	8.84	18.60
NDF	52.09	35.09	32.37	30.05	32.34	30.00
NDICP	4.05	5.64	5.16	2.97	6.01	3.00
ADF	32.69	17.71	18.41	14.87	18.58	14.74
ADICP	1.38	1.50	3.08	1.76	3.88	1.79
ADL	9.79	8.79	6.73	10.51	7.09	10.36
EE	3.34	5.20	1.91	4.71	2.27	4.75
Ash	7.65	7.54	5.30	7.96	5.15	7.24
NFC	27.50	36.02	46.90	41.80	55.27	55.54
TDN[1]	52.96	62.27	64.55	61.24	63.34	66.40
DE, Mcal/kg DM[2]	2.34	2.75	2.85	2.70	2.79	2.93
ME, Mcal/kg DM[3]	1.91	2.32	2.43	2.28	2.37	2.51

[1] TDN, total digestible nutrients. Calculated as mentioned in NRC (2001). TDN = tdNFC + tdCP + (tdFA × 2.25) + tdNDF – 7.

[2] DE, digestible energy. Calculated as mentioned in NRC (2001). DE = 0.04409 × TDN(%).

[3] ME, metabolizable energy. Calculated as mentioned in NRC (2001). ME = 1.01 × DE (Mcal/kg) – 0.45.

[4] Bait feed are given additionally with the TMR, not included inside the TMR.

TMR, total mixed ration; CP, crude protein; NDF, neutral detergent fiber; NDICP, neutral detergent insoluble crude protein; ADF, acid detergent fiber; ADICP, acid detergent insoluble crude protein; ADL, acid detergent lignin; EE, ether extract; NFC, non-fiber carbohydrates.

Table 2. Descriptive statistics of animals used for method comparison across experimental phases

Phase	Animals, n[1]	Parameter	Mean	SD	Median	Min	Max
Overall	24	Parity	1.6	0.77	1.0	1.0	3.0
		DMI, kg/d	25.1	1.63	25.2	20.9	29.3
		BW, kg	641.6	57.26	645.5	527.5	791.5
		ECM[2], kg/d	21.3	2.82	21.7	15.4	26.0
		MY, kg/d	24.5	3.05	24.8	18.7	34.1
Phase 1	8	Parity	1.2	0.40	1.0	1.0	2.0
		DMI, kg/d	25.5	2.06	25.8	20.9	29.3
		BW, kg	627.8	43.54	632.5	565.5	725.0
		ECM, kg/d	22.1	2.18	23.0	17.6	24.6
		MY , kg/d	25.7	3.44	24.9	21.6	34.1
Phase 2	8	Parity	1.8	0.86	2.0	1.0	3.0
		DMI, kg/d	23.9	0.46	24.0	22.4	24.3
		BW, kg	619.3	63.47	613.0	527.5	741.0
		ECM, kg/d	21.7	2.09	22.3	17.7	25.3
		MY , kg/d	25.1	1.68	25.3	21.6	27.4
Phase 3	8	Parity	1.8	0.86	1.5	1.0	3.0
		DMI, kg/d	25.9	1.19	25.8	23.6	27.9
		BW, kg	676.3	49.12	670.3	603.0	791.5
		ECM, kg/d	19.9	3.55	19.2	15.4	26.0
		MY, kg/d	22.7	2.97	22.8	18.7	26.5

[1] n, number of individual dairy cows used. A total of 24 dairy cows were used in the study, with different animals assigned to each experimental phase (n = 8 per phase).

[2] ECM, energy-corrected milk. Calculated as mentioned by Tyrrell and Reid (1965). ECM = (MY (kg/d) × [376 × milk fat (%) + 209 × milk protein (%) + 948]) / 3138.

DMI, dry matter intake; BW, body weight; MY, milk yield; SD, standard deviation.

Table 3. Descriptive statistics of enteric methane emissions (g/d) and CH_4/CO_2 ratio from dairy cows measured using the GreenFeed system (GF) and the sniffer-based method (SB)

	Overall		***Phase 1***		***Phase 2***		***Phase 3***	
Techniques	GF	SB	GF	SB	GF	SB	GF	SB
Animals, n[1]	24	24	8	8	8	8	8	8
CH_4, g/d								
Mean[2]	312.9	297.1	323.6^{a}	302.0^{a}	314.9^{a}	311.7^{a}	300.2^{a}	278.4^{a}
Median	311.0	304.8	324.1	303.4	301.1	324.4	291.8	271.3
Min	215.0	172.8	241.5	222.1	234.5	193.3	215.0	172.8
Max	521.8	433.3	378.9	415.7	521.8	403.4	384.3	433.3
CV, %	18.06	20.81	12.35	16.68	24.19	16.67	16.58	27.94
SD	56.50	61.83	39.98	50.38	76.18	51.98	49.77	77.78
CH_4/CO_2 ratio, v/v								
Mean	0.064	0.077	0.055^{c}	0.079^{a}	0.073^{a}	0.082^{a}	0.065^{b}	0.069^{b}
Median	0.064	0.078	0.052	0.079	0.073	0.083	0.066	0.070
Min	0.043	0.050	0.043	0.061	0.059	0.072	0.049	0.050
Max	0.087	0.103	0.069	0.103	0.087	0.093	0.078	0.090
CV, %	17.629	15.960	13.921	15.024	13.171	7.909	14.631	18.894
SD	0.011	0.012	0.008	0.012	0.010	0.007	0.009	0.013

[1] n, number of individual cows used. A total of 24 dairy cows were used in the study, with different animals assigned to each experimental phase (n = 8 per phase).

[2] Mean, Average values calculated after outlier removal based on z-score threshold ($|z| > 2.0$)

[abc] Mean Values are presented with superscript letters indicating significant differences among experimental phases within each measurement method based on Tukey's HSD test ($p < 0.05$).

GF, GreenFeed system; SB, sniffer-based method; CV, coefficient of variation; SD, standard deviation.

Table 4. Summary of phase-specific mean absolute percentage error (MAPE), standard error of the mean (SEM), and confidence rate (CR) for GreenFeed system and the sniffer-based method (SB).

	Animals, n[1]	SEM	MAPE[2], %	CR[3], %
Overall				
GF	24	8.24		
SB	24	9.02	16.10	83.90
Phase 1				
GF	8	9.99		
SB	8	12.59	11.57	88.43
Phase 2				
GF	8	19.67		
SB	8	13.42	19.20	80.80
Phase 3				
GF	8	12.44		
SB	8	19.44	17.72	82.28

[1] n, number of individual cows used. A total of 24 dairy cows were used in the study, with different animals assigned to each experimental phase (n = 8 per phase).

[2] MAPE (%), mean absolute percentage error. MAPE, % = $(1/n) \times \Sigma\ |(x_i - y_i) / x_i| \times 100$. Where x_i represent values obtained from GreenFeed system and y_i represent values obtained from the sniffer-based method.

[3] CR (%), confidence rate (CR, % = 1 − MAPE, %).

GF, GreenFeed system; SB, sniffer-based method; SEM, standard error of the mean; MAPE, mean absolute percentage error.

Figures

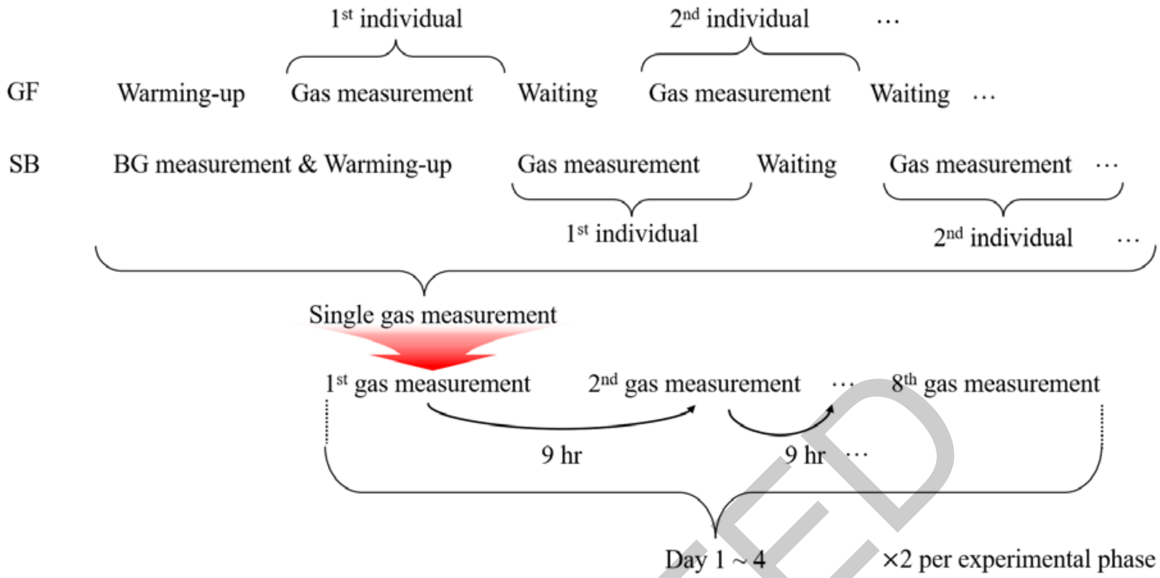

Figure 1. Schematic timeline of gas measurements using the GreenFeed system (GF) and the sniffer-based method (SB) during an experimental phase. Each animal was measured twice per phase at 9-h intervals over four consecutive days, resulting in a total of 16 measurements per animal per phase. A 2–3 min waiting period was applied between individual measurements to prevent cross-contamination. The SB included background (BG) gas measurements before and after each measurement.

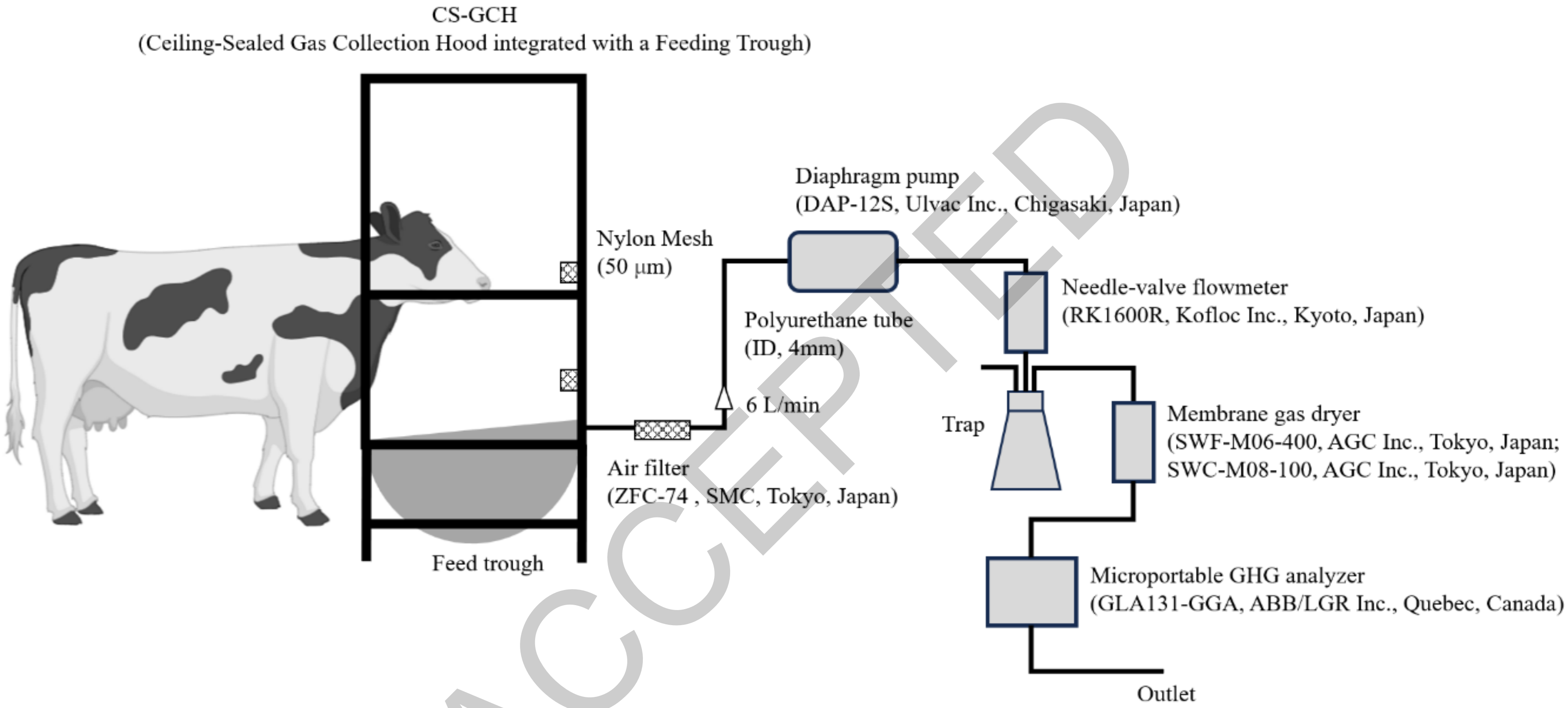

Figure 2. Schematic diagram of the gas collection system for measuring the methane to carbon dioxide ratio in exhaled breath during feeding, using a ceiling-sealed gas collection hood integrated with a feeding trough (CS-GCH). Exhaled breath was collected from the head of cows during feeding a bait feed using a ceiling-sealed hood installed above a floor-level feed trough. Sampled gas was drawn through a nylon mesh and air filter at a constant flow rate (6 L/min) using a diaphragm pump to filter particulate matter, passed through a trap and membrane gas dryer to reduce moisture inside gas, and continuously analyzed for CH_4 and CO_2 concentrations using a Microportable greenhouse gas analyzer.

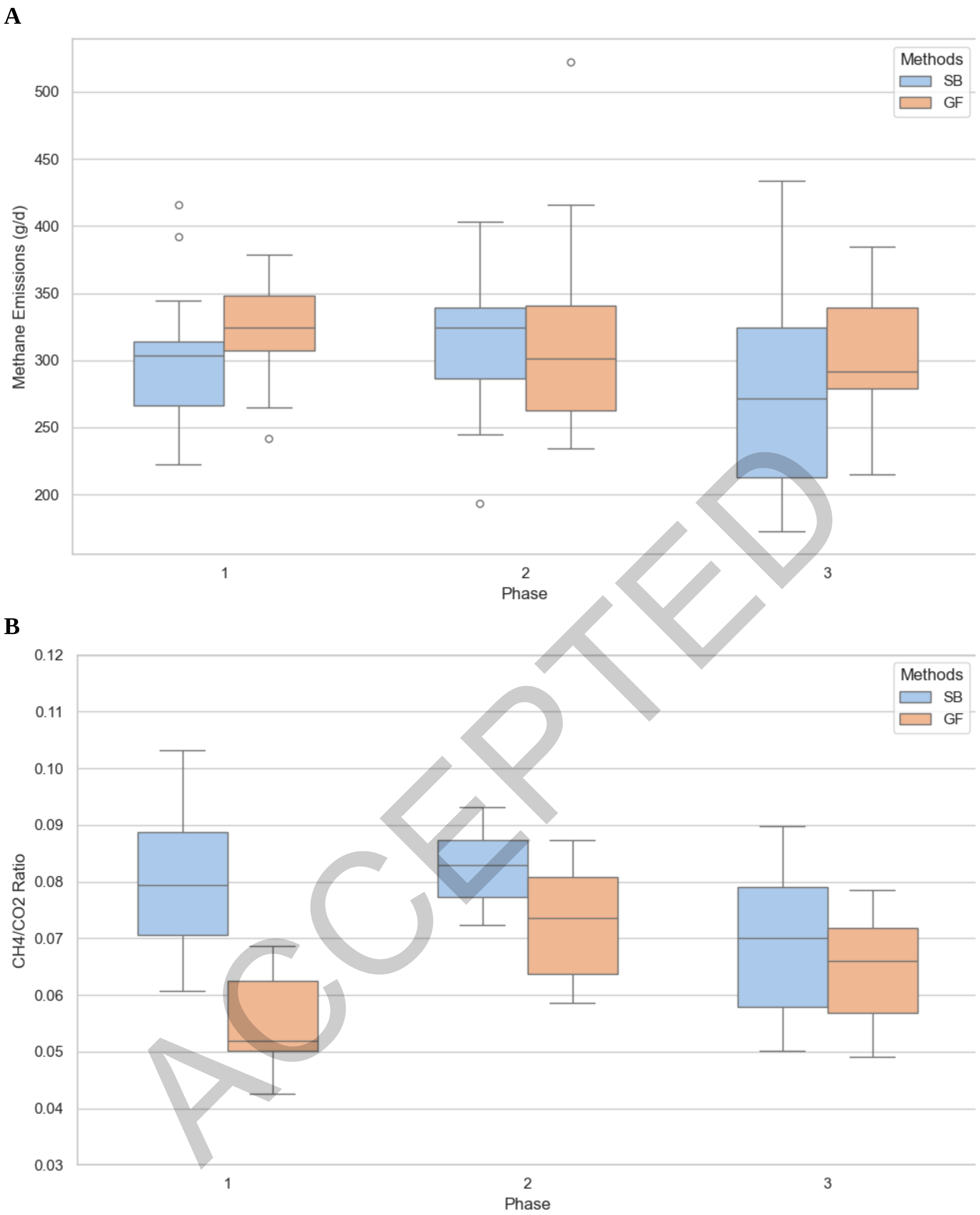

Figure 3. Boxplot visualization of the comparisons of methane emissions (g/d) and CH_4/CO_2 ratio (*v/v*) measured using the sniffer-based method (SB) and Greenfeed system (GF) across three experimental phases. Each phase comprised a distinct group of dairy cows (n = 8 per phase). Each box represents the interquartile range, with the median shown as a horizontal black line. Whiskers indicate 1.5 × interquartile range. **A** Methane emission across the experimental phases measured via the GF and the SB. **B** CH_4/CO_2 ratio across the experimental phases measured via the GF and the SB.

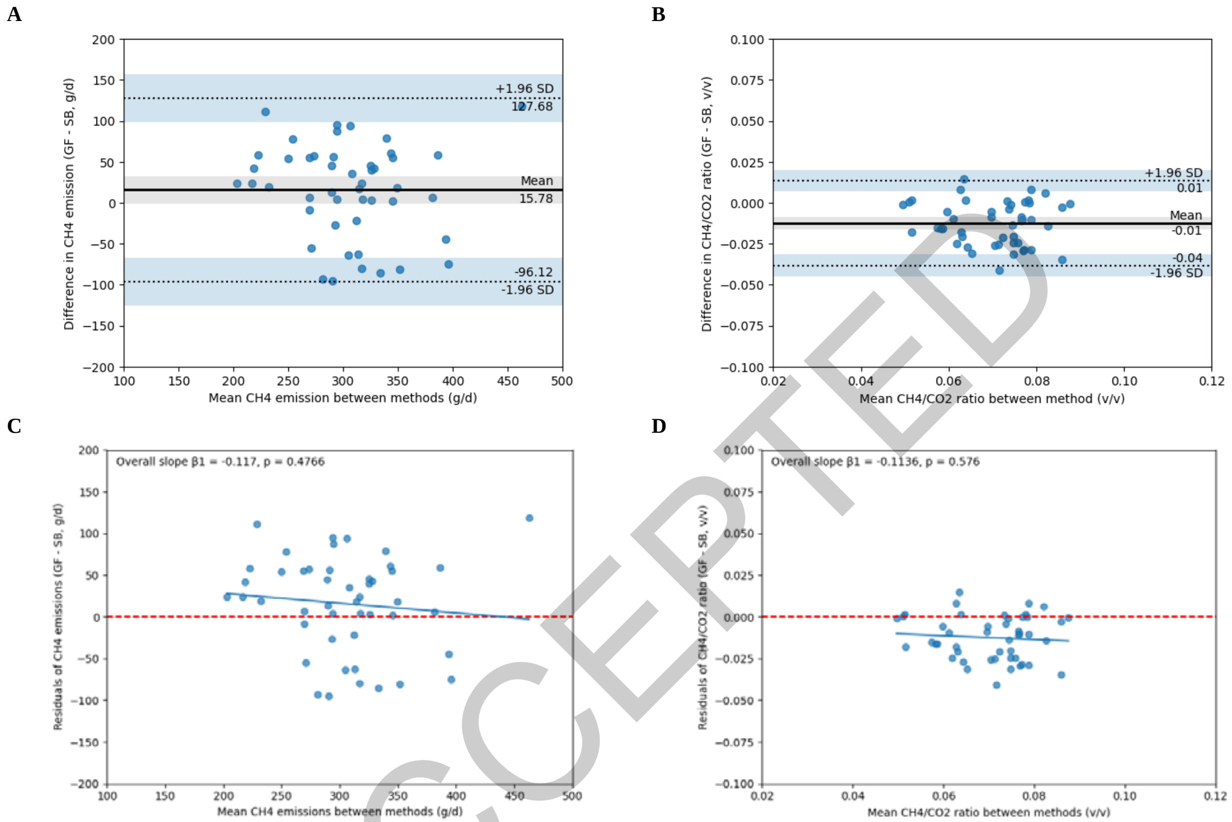

Figure 4. Comparison of methane (CH_4) emission estimates between the GreenFeed system (GF) and the sniffer-based method (SB) using Bland–Altman and residual analyses (n = 24). **A** Bland–Altman plot for CH_4 emissions showing mean bias (15.78 g/d) and 95% limits of agreement (LOA; –96.12 to 127.68 g/d). **B** Bland–Altman plot for CH_4/CO_2 ratio showing mean bias (–0.01) and 95% LOA (–0.04 to 0.01 v/v). **C** Residual plot of CH_4 emissions (GF – SB) against the mean of the two methods, with the fitted regression line indicating the relationship between residuals and measurement magnitude (slope β_1 = –0.117, *p* = 0.477). The red dashed line indicates y = 0. **D** Residual plot of CH_4/CO_2 ratio (GF – SB) against the mean of the two

methods, with the fitted regression line indicating the relationship between residuals and measurement magnitude (slope $\beta_1 = –0.114$, $p = 0.576$). The red dashed line indicates y = 0.

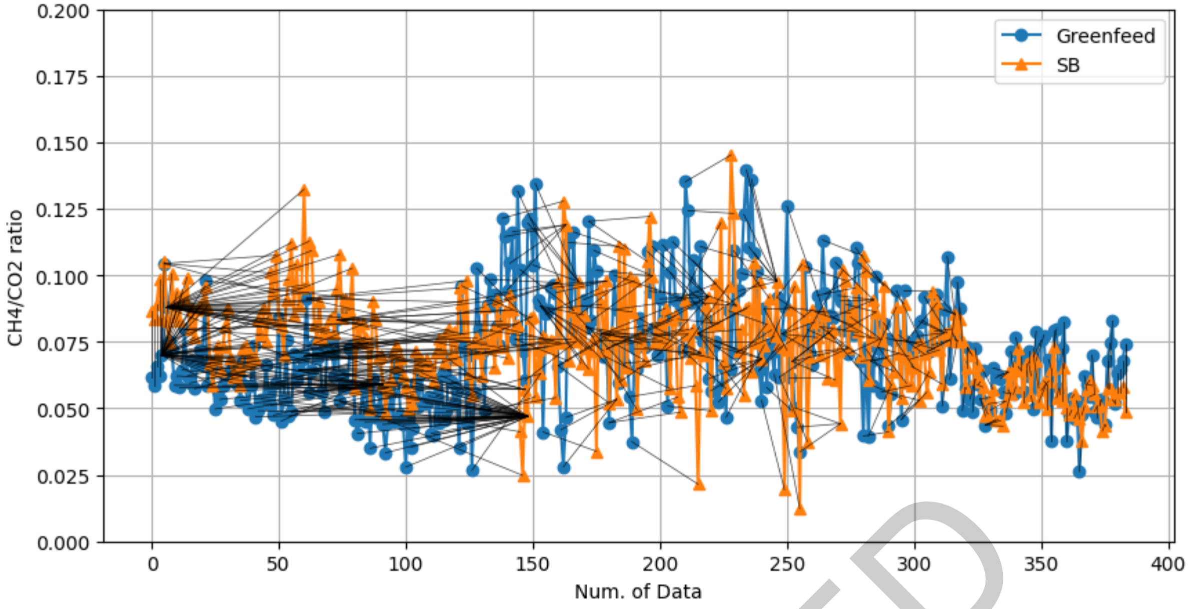

Figure 5. Time series comparison of CH_4/CO_2 ratio measured by GreenFeed (blue) and the sniffer-based method (SB, orange) by dynamic time warping (DTW) analysis (n = 384). Values were not excluded by outlier removal because they reflect the valid between comparison. A total of n = 384 observations were included, derived from 24 cows × two measurements per phase × eight repeated measurements per measurement. The black lines indicate dynamic alignment between the two measurement methods using dynamic time warping (DTW). Num = Number.