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Comparing carbon dioxide, oxygen, and methane exchanges, and heat production measured using GreenFeed and respiration chambers

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ABSTRACT

The aim of this study was to evaluate the precision and accuracy of GreenFeed (GF) Emission Monitoring system in measuring O₂, CO₂, and CH₄ exchanges, and heat production (HP) when compared with respiration chambers (RC). Thirty-two lactating Nordic Red cows (634 ± 60.4 kg BW, 145 ± 63.1 DIM, 28.9 ± 6.7 kg milk/d, and parity 1.6 ± 1.16) allocated to 8 blocks based on BW, milk yield, DIM, and parity were used to compare CO₂, O₂, and CH₄ exchanges measured by GF units (n = 2) versus RC (n = 4) in a switch-back design. The experimental period for every block was 5 wk; the cows were measured by GF during 2 wk, the third wk in RC, and another 2 wk by GF. The cows were fed a grass silage-based diet with constant forage-to-concentrate ratio for each cow during the study, ranging from 42:58 to 67:33, depending on their milk production at the beginning of study. For GF data, the records outside of the range of mean ± 2.5 × SD of all measurements over 4 wk for each cow were identified as outliers. Altogether, 214 out of 3,115 data points were removed as the outliers. Average CO₂, O₂, and CH₄ exchanges, and HP in addition to their SD measured by GF during 4-wk periods (12,778 ± 1,137, 8,795 ± 773, 462 ± 57 g/d, and 131 ± 11 MJ/d) were close to those measured by RC (13,103 ± 1,421, 8,817 ± 941, and 453 ± 56 g/d, and 132 ± 14 MJ/d), respectively. Repeatability of O₂, CO₂, and CH₄ exchanges, and HP for GF was 0.88, 0.88, 0.82, and 0.88, respectively, when calculated based on the averages of 2-wk periods. Accuracy of 0.90 was achieved in 6, 8, and 12 d for O₂, CO₂, and CH₄, respectively. Based on mixed model linear regression analysis, GF (averages of 4-wk data) estimated O₂ exchanges of cows with a strong relationship with RC data (averages of 3-d; R² = 0.89, root mean square

prediction error [RMSPE] = 3.1% of mean, concordance correlation coefficient [CCC] = 0.94), followed by CO₂ (R² = 0.84, RMSPE = 3.5% of mean, CCC = 0.88) and CH₄ (R² = 0.79, RMSPE = 4.6% of mean, CCC = 0.87). Heat production estimated by GF was strongly related to the estimations made by RC (R² = 0.88, RMSPE = 3.2% of mean, CCC = 0.93). The intercept and slope were not biased for O₂, CO₂, and HP, whereas for CH₄, intercept and slope were greater than 0 and less than 1, respectively. Therefore, GF can be used as an alternative to measure respiration gas exchanges and HP of dairy cows, which paves the way for large-scale measurements of energy metabolism and feed efficiency.

Key words: respiration gas exchange, measuring techniques, energy metabolism, dairy cow

INTRODUCTION

Ruminants contribute to global food security by converting forages, crop byproducts, and other feed ingredients not suitable for human consumption to high-quality animal-based products. Worldwide, ruminant livestock are raised mainly (i.e., 86%) on the feed which is not human edible (Mottet et al., 2017). Global demand for meat and milk is anticipated to rise by 35% to 56% between 2010 and 2050 (van Dijk et al., 2021). However, the criticism of contributing to climate change via emitting GHG, especially enteric CH₄ emissions, should be considered and resolved to benefit from ruminants' contribution to global food security in a sustainable manner. Improving feed efficiency (FE) means producing more meat and milk with the same amount of feed. Better FE is shown to improve feed utilization and reduce CH₄ emissions simultaneously (Connor et al., 2015; Guinguina et al., 2021).

Measuring CO₂ and O₂ may enable us to estimate FE without the need for measuring DMI which is a major challenge under farm conditions. Huhtanen et al. (2021) indicated that residual CO₂ production (RCO₂) can be used to rank the cows according to FE without measur-

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ing DMI. Furthermore, measurement of CH₄ is required for testing the nutritional options and genetic approaches aiming at mitigation of CH₄ emissions. For instance, a variety of newly developed feed additives have been recently introduced (Honan et al., 2021) that need to be tested under different conditions before implementation on a farm. Therefore, the possibility of using gas exchanges for improving FE and the need to test the newly developed feed additives for mitigation of enteric CH₄ emissions urges the availability of low-cost and accurate techniques for O₂, CO₂, and CH₄ measurements. In 2012, the GreenFeed (GF) emission monitoring system (C-Lock Ltd., Rapid City, SD) was introduced for measuring CO₂ and CH₄ when the animals are in their natural environment using a spot sampling technique (Hammond et al., 2015). In recent years, the GF system has been equipped with O₂ sensor that enables measuring energy metabolism and FE of the cows based on gas exchange data, which is independent from DMI (Huhtanen et al., 2021). The GF system is a noninvasive and web-based system that measures respiration gases of the animal when visiting the unit (Hammond et al., 2015). Methane emissions measured by the GF system indicated a good relationship with values predicted by equations derived from respiration chamber (RC) data (Huhtanen et al., 2019), as well as direct comparisons with RC (Manafiazar et al., 2016; Doreau et al., 2018; Garnsworthy et al., 2019). However, to our knowledge, there are no direct comparisons of the GF and RC for measuring CO₂ and O₂ exchanges and heat production (HP) which are needed for studying energy metabolism and FE.

The objective of this study was to evaluate the precision and accuracy of the GF system in measuring O₂, CO₂, and CH₄ exchanges, and HP when compared with RC. We hypothesized that there would be minimal differences in gas exchange and HP measurements of lactating dairy cows, indicating reasonable precision and accuracy of the GF system. For this purpose, daily averages, variability, repeatability, and diurnal variation of the gas exchanges and HP measured by both methods were compared. In addition, the gas exchange values generated by the GF system were compared with those generated by RC using mixed model regression analysis to evaluate how accurately and precisely the GF predicts RC values.

MATERIALS AND METHODS

Animals, Experimental Design, and Diets

The experiment was approved by regional State Administrative Agency (ESAVI/20853/2021) in accordance

with the guidelines of the European Community Council Directive 2010/63/EU (EU, 2010) for animal experiments complying with the ARRIVE guidelines (Kilkenny et al., 2010).

The animal experimentation was conducted during September 2021 and February 2022 at the Natural Resources Institute Finland (Luke) research facilities (Jokioinen, Finland). Thirty-two Nordic Red dairy cows (634 ± 60.4 kg BW, 145 ± 63.1 DIM, 28.9 ± 6.7 kg milk/d, and parity 1.6 ± 1.16) blocked based on BW, milk yield, DIM, and parity were used. Each block of 4 cows (8 blocks in total) entered the study at least 1 wk after the previous block. The cows were trained for using the GF systems for 2 wk before entering the experiment. The gas exchanges (CO₂, O₂, and CH₄) of cows were measured for 2 wk in the GF emission monitoring system (C-Lock Ltd.), 1 wk in RC, and another 2 wk in the GF system, as is described in the next section.

The cows were fed a grass silage-based diet supplemented with concentrates. The amount of concentrate supplied and consequently the forage-to-concentrate ratio was adjusted based on milk yield of the cows (Luke, 2022) when entering the study (ranging between 42:58 and 67:33, with the average 51:49 on DM basis), which was kept constant for every cow during the whole 5 wk of study to avoid any confounding effect between diet and gas exchange measuring techniques. Experimental silages were made from primary growth of a mixed timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) sward at Jokioinen, Finland (60°48'N, 23°29'E). The composition of the forage was 73% timothy and 27% meadow fescue on DM basis. The grass was wilted under good drying conditions for 1 to 3 h, harvested using a precision chopper (JF FCT 1350, JF-Fabriken-J Freudendahl A/S, Sonderborg, Denmark), and ensiled in horizontal bunker silos. Silage additive (AIV 2 Plus, Eastman Ltd., Oulu, Finland) was applied to the harvested grass by an applicator attached to the chopper at a target rate of 5 L/ton fresh matter.

The cows (26 primiparous and 6 multiparous) were kept in a freestall barn fitted with transponder collars allowing for identification at the feeding bins, milking parlor, and weigh scales. The grass silage was delivered 4 times a day at 0630, 1300, 1530, and 1730 h by an automatic feeding wagon (TR Feeding Robot, Pellon Group Ltd., Ylihärmä, Finland) that weighed the amount provided to each individual bin in the freestalls (during GF measurements). The individual cows were allowed to access their silage using the roughage intake control system (Insentec B.V., Marknesse, the Netherlands). The grass silage was weighed and offered manually at 0700, 1300, 1700, and 1900 h in the RC. At least 5% more silage compared with the silage consumed during previous days was offered to ensure ad libitum feed-

Table 1. Formulation of dietary concentrates and their chemical composition¹

| | Basal concentrate | Milking parlor concentrate ² |
|---|-------------------|---|
| Ingredient, g/kg as fed | | |
| Barley | 160 | 362 |
| Wheat | 140 | 170 |
| Oat | 220 | — |
| Molassed sugar beet pulp | 100 | 140 |
| Rapeseed meal | 350 | 298 |
| Mineral and vitamin premix ³ | 30 | 30 |
| Chemical composition, g/kg DM unless stated | | |
| DM, g/kg as fed | 873 | 873 |
| OM | 920 | 922 |
| CP | 202 | 187 |
| NDF | 230 | 206 |
| GE, MJ/kg DM | 18.4 | 18.1 |

¹Grass silage was fed at a ratio ranging from 42:58 to 67:33 on DM basis depending on the milk yield of the cow. The grass silage was characterized with 255 ± 8.2 g/kg DM content, (g/kg on DM basis) 931 ± 1.5 OM, 104 ± 3.9 CP, 561 ± 9.8 NDF, 21 ± 5.3 water soluble carbohydrates, and 17.5 ± 0.07 MJ/kg DM GE. Fermentation quality included pH = 3.92, (g/kg on fresh basis) formic acid 16.1, acetic acid 16.7, propionic acid 0.52, butyric acid, 0.72, lactic acid 52.2, ammonia N in total N 43.0, and total VFA 18.1.

²Milking parlor concentrate (0.6 kg/d) was provided to all cows in the milking parlor and in chambers.

³Lypsykivennäinen Tiineys+, Hankkija Oy, Finland; free access to LypsyMelli (Mg), Raisio, Finland.

ing. Basal supplementary concentrate in pelleted form (Table 1) was fed at 0335, 0700, 1130, 1630, and 1930 h via automatic feeding kiosks in the freestalls and 1 h before feeding silage in the RC (i.e., 0600, 1200, 1600, and 1800 h). The cows were allowed to visit the GF units every 4 h 42 min, and during each visit they received maximum 8 drops of 50 g basal concentrate at 40 s intervals to attract them to the units. In addition, the cows received 0.3 kg milking parlor concentrate (Table 1) during each milking (0.6 kg/d). In the RC, equal amount of concentrate fed in milking parlor (0.6 kg/d milking parlor concentrate) and the GF unit (about 1.4 kg/d basal concentrate) was provided in addition to the main concentrate portion to keep similar feeding conditions for the GF and RC periods. Cows were milked 2 times a day at about 0645 and 1645 h in a 2 × 6 auto tandem milking parlor (Herringbone SAC, Kolding, Denmark) during the GF measurements (wk 1, 2, 4, and 5) or inside chambers (can milking system, SAC, Kolding, Denmark) during RC measurements (wk 3). Water and salt blocks were freely available throughout the experiment.

Measurements, Sample Collection, and Chemical Analyses

Carbon dioxide, O₂, and CH₄ exchanges were measured during wk 1, 2, 4, and 5 of the experiment using 2 GF units (C-Lock Inc., Rapid City, SD). The details of GF system are described elsewhere (Hammond et al., 2015). Briefly, the GF system estimates respiration gas exchanges during each cow's visit to the unit (usually between 3 and 5 min) by measuring air flow and gas con-

centrations (by infrared sensors for CO₂ and CH₄, and paramagnetic sensor for O₂) corrected for background gas concentrations (i.e., when the cows are not visiting the unit). The GF system was equipped with an Auto-Calibration system, and the calibrations were performed automatically according to the manufacturer's specifications. The CO₂ recovery test was performed for every GF unit at the beginning of the experiment, every 4 wk during the experiment, and at the end of the experiment. The gas exchanges were calculated by subtracting the recorded background gas concentration from the gas concentrations recorded during the animal's visit in the GF. The difference in concentration was subsequently multiplied by the airflow to calculate the gas exchange rates in gram/day corrected for standard temperature and pressure.

During experimental wk 3 for every block, the cows entered the RC ($n = 4$, 21.5 m³) for gas measurements, and total fecal and urinary collection (4 d), with the first day being considered as acclimatization as described in detail by Bayat et al. (2022). Briefly, the cows were restrained within the transparent chambers by a neck yoke with a resting area covered with a rubber mat (180 × 126 cm). Gas analysis was configured to allow automatic measurements at 3.5-min intervals from each RC and the reference air. The zero and span calibrations of analyzers were conducted at the beginning of every 4-d measurement for each block of cows using standard gases (AGA Ltd.). The concentration of measured gases and airflow (corrected based on standard temperature and pressure) was recorded and monitored (Oxymax v. 4.86, Columbus Instruments, OH), and the data captured were used for further calculations. Environmental control of tempera-

ture across a range of 15 to 22°C and relative humidity of 50% to 70% was maintained through an adjustable air conditioning system (air flow 500–2000 L/min; cooling/heating capacity of 5.0 kW).

Representative samples of silage and concentrates were taken on a weekly basis and stored at –20°C until chemical analysis. Feed refusals were weighed daily throughout the study and were subtracted from the offered diet to calculate daily feed intake. Silage samples were prepared for pH measurement and analysis of VFA, formic acid, lactic acid, ethanol, water-soluble carbohydrate, soluble N, and ammonia N concentrations, as described by Ahvenjärvi et al. (2018). Concentrate and silage samples were dried in a forced-air oven at 55°C for 48 h, ground through a 1-mm screen (Sakomyly KT-120, Koneteollisuus Oy, Klaukkala, Finland), and analyzed for DM, NDF, ash, ether extract, and CP, and silage DM content and consequently DMI were corrected for the loss of volatiles in the oven, as described in detail by Ahvenjärvi et al. (2018). Gross energy of feed samples was determined using a Parr 6200 Oxygen Bomb Calorimeter (Parr Instrument Co.) with benzoic acid (CAS 65-85-0, cat. no. 3415, Parr Instrument Co.) as a standard.

The amount of milk was gravimetrically recorded (Pellon SAC, Kolding, Denmark) at every milking during the GF measurements (28 d). During RC measurements, milk yield was measured in situ 2 times per day. Daily milk samples were taken during 4 consecutive milkings from the evening of the second d to the morning of the fourth d in each week and preserved with 2-bromo-2-nitropropane-1,3-diol (bronopol) for fat, protein, and lactose determination using an infrared analyzer (MilkoScan FT+; Foss Electric A/S, Hillerød, Denmark) at a commercial laboratory (Valio Ltd., Seinäjoki, Finland). Daily milk component concentrations were determined by average concentrations in the morning and evening milkings weighted by milk yield at each milking. The cows were weighed every time they left the milking parlor by a walk-through static scale (Pellon Group Ltd., Ylihärmä, Finland). The animals were monitored daily for health problems, and any abnormalities and infections were recorded and treated according to the general barn guidelines. In the current study, all cows completed the whole experiment without major symptoms affecting the results.

Calculations and Statistical Analyses

The ECM yield was calculated using milk fat, protein, and lactose yields based on the equation presented by Sjaunja et al. (1991). Crude protein of diet was calculated as $N \times 6.25$, and for milk it was calculated as $N \times 6.38$. Energy loss as CH_4 was calculated using the factor 55.24 kJ/g (Kriss, 1930). Heat production was calculated

according to Brouwer (1965), using volumes of O_2 , CO_2 , and CH_4 , as well as urinary N excretion. Respiratory quotient (**RQ**) was calculated as CO_2/O_2 (L/L).

Normality of analyzed variables was checked using box plot and scatter plot of residuals and fitted values generated using the Mixed procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). Outlier data were identified using Influence option in Proc Mixed of SAS when regressing observations from the GF system to RC. One cow was identified as an outlier for CO_2 , O_2 , CH_4 , and HP, which was removed from the dataset. For GF records, the records outside the range of mean $\pm 2.5 \times$ SD of all measurements over 4 wk for each cow were identified as outliers and removed (214 out of 3,115 data points, i.e., 6.9% were removed). The linear regression analysis was performed using Proc Mixed of SAS (Littell et al., 1996) to evaluate the relationship between the GF and RC observations applying the following model:

$$Y_{ij} = b0 + b1 \times X_{ij} + C_i + B_j + e_{ij},$$

where Y_{ij} is a cow's daily average of either O_2 , CO_2 , CH_4 , or HP measured by the RC, $b0$ is the intercept, $b1$ is the linear regression coefficient, X_{ij} is the cow's daily average of either O_2 , CO_2 , CH_4 , or HP measured by the GF, C_i is the random effect of chamber, B_j is the random effect of block, and e_{ij} is the random residual.

In addition to the regression analysis, the methods were compared by ANOVA using Proc Mixed of SAS when block and chamber were considered as random and method, hour of the day, and their interaction was considered as a fixed effect. Repeatability of every variable was calculated as $Rep = \sigma^2 \text{ between cow} / (\sigma^2 \text{ within cow} + \sigma^2 \text{ between cows})$, where σ^2 is the variance calculated using Proc Mixed of SAS with a model including cow and chamber as random variables for data generated by RC, and cow as a random variable for data generated by the GF system. Accuracy of the gas values over n days was calculated as $n \times Rep / [1 + (n - 1) \times Rep]$, where n is number of days and Rep is estimated repeatability. Lin's concordance correlation coefficient (**CCC**) between parameters measured by the GF and RC was calculated according to Lin (1989). The CCC consists of 2 components, the Pearson correlation coefficient and a bias correction factor, providing measures of precision and accuracy, respectively. Root mean square error of prediction (**RMSPE**) was calculated as $\sqrt{[\sum (\text{observed} - \text{predicted})^2 / n]}$. Mean squared error was decomposed into random error, error due to the deviation of regression slope from unity (linear bias), and error due to overall bias, as described by Bibby and Toutenburg (1977).

Two different methods for calculating the average gas exchanges for every cow were considered as follows: (1) the "arithmetic averaging method," as a straightforward

averaging of the visit fluxes defined as the sum of the visit fluxes divided by the number of measurements, which does not take into account the timing of the visits during the day, and (2) the “time of day method,” which considers the hourly visit times for calculating the gas exchanges. In the latter, first the hourly averages of gas exchanges for every cow were estimated using a mixed model considering block as a random effect and hour of the day as a fixed effect. The daily average for each cow was calculated based on the least square hourly averages estimated from the mixed model.

RESULTS

Dry matter intake of the cows during the GF periods was similar ($P = 0.60$) to that during the RC measurements (Table 2). The average and SD of milk and ECM yields, milk composition, and BW of the cows were rather similar for both methods.

Average O_2 and CO_2 exchanges were slightly lower but not significantly different ($P \geq 0.16$) for the GF compared with the RC (Table 2). In contrast, average CH_4 production was slightly higher, even though not significantly different ($P = 0.21$) for the GF compared with RC. Methane yield ($P = 0.29$) and HP ($P = 0.84$) were similar for both methods. Ratio between CH_4 and CO_2 as gram/kilogram was higher ($P < 0.01$), whereas RQ was lower ($P < 0.01$) for the GF than RC.

Visit Frequency, Diurnal Variations and Calculation Methods

The cows visited the GF units on average 3.59 ± 0.78 times per day (min = 1.71 and max = 5.76 visits/d per cow). The average visit time was $4:07 \pm 0:38$ min. Visits of cows to the GF units are presented in Figure 1. The visits were more frequent at 0100 h, followed by 0700 and 2000 h. However, there was a considerable variation in the number of visits indicated by the large SD for each visiting hour, especially for 0100, 0700, 0900, and 2000 h.

Figure 2 shows the diurnal variations of O_2 , CO_2 , and CH_4 exchanges calculated by the mixed model described previously. The peak of O_2 was 22.6% higher than its nadir for RC, while the difference was much smaller (9.5%) for the GF (Figure 2a; $P < 0.01$ for the interaction of hour of day and method). For CO_2 , the diurnal variation measured by RC was greater than that for O_2 , as indicated by a 42.8% higher peak of CO_2 compared with the nadir, while the difference was much smaller for GF (i.e., 16.0%; Figure 2b; $P < 0.01$ for the interaction of hour of day and method). The diurnal variation was even greater for CH_4 (82.7% and 30.7% greater peak than the nadir for RC and GF, respectively;

Table 2. Summary statistics of respiratory chambers and GreenFeed emission monitoring system measurements of experimental cows (n = 31)

| | Respiration chambers (daily) | | | | | GreenFeed (2-wk periods) | | | | | P-value ¹ | |
|------------------------|------------------------------|-------|--------|--------|---------------|--------------------------|-------|--------|--------|-----------------------|----------------------|----------------------|
| | Mean | SD | Min | Max | Repeatability | Mean | SD | Min | Max | Repeatability (daily) | | Repeatability (2-wk) |
| DMI, kg/d | 21.2 | 2.9 | 16.2 | 28.8 | 0.93 | 21.5 | 2.8 | 17.1 | 29.2 | 0.82 | 0.85 | 0.60 |
| Milk yield, kg/d | 27.2 | 6.8 | 16.4 | 42.6 | 0.98 | 27.7 | 6.4 | 15.0 | 41.5 | 0.95 | 0.96 | 0.63 |
| ECM yield, kg/d | 32.1 | 7.0 | 22.2 | 49.7 | 0.97 | 31.9 | 5.9 | 20.2 | 46.2 | 0.92 | 0.96 | 0.48 |
| Milk composition, g/kg | | | | | | | | | | | | |
| Fat | 52.2 | 5.4 | 44.1 | 67.2 | — | 50.3 | 5.2 | 41.7 | 66.7 | 0.86 | 0.90 | 0.053 |
| Protein | 40.3 | 4.3 | 31.6 | 47.9 | — | 40.7 | 4.0 | 33.1 | 47.4 | 0.94 | 0.93 | 0.75 |
| BW, kg | 628 | 57 | 499 | 715 | — | 633 | 57.0 | 503 | 722 | 0.98 | 0.99 | — |
| Gas exchanges | | | | | | | | | | | | |
| O_2 , g/d | 8,817 | 941 | 6,623 | 11,361 | 0.97 | 8,795 | 773 | 7,126 | 10,185 | 0.63 | 0.88 | 0.95 |
| CO_2 , g/d | 13,103 | 1,421 | 10,215 | 16,983 | 0.98 | 12,778 | 1,137 | 10,171 | 15,365 | 0.54 | 0.88 | 0.16 |
| CH_4 , g/d | 453 | 56 | 347 | 565 | 0.97 | 462 | 57 | 375 | 631 | 0.44 | 0.82 | 0.21 |
| CH_4 , g/kg DMI | 21.3 | 1.6 | 16.8 | 25.1 | 0.62 | 21.6 | 1.5 | 18.4 | 26.1 | 0.54 | 0.61 | 0.29 |
| CH_4/CO_2 , g/kg | 34.6 | 1.6 | 29.6 | 38.1 | 0.90 | 36.1 | 2.3 | 31.4 | 43.1 | 0.38 | 0.85 | <0.01 |
| RQ ² | 1.08 | 0.04 | 0.99 | 1.15 | 0.76 | 1.06 | 0.03 | 0.98 | 1.13 | 0.23 | 0.67 | <0.01 |
| Heat production, MJ/d | 132 | 14.0 | 100 | 170 | 0.96 | 131 | 11.4 | 106 | 153 | 0.63 | 0.88 | 0.84 |

¹Analyzed by a mixed model (Proc Mixed of SAS), including Method as a fixed effect, and Block and Chamber as random effects.

²RQ = respiratory quotient (CO_2/O_2 on volume basis).

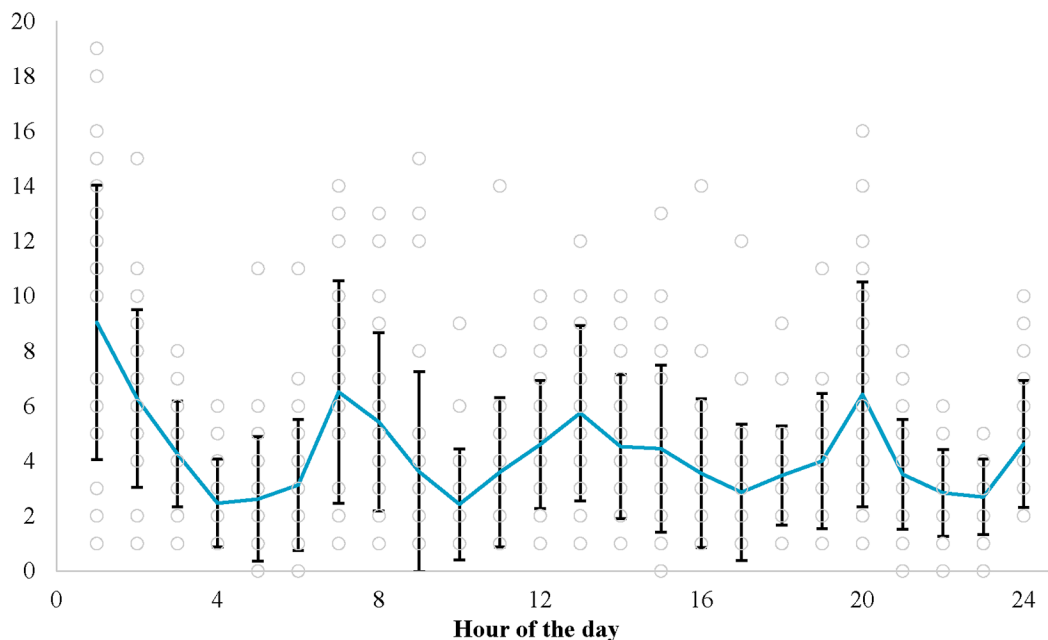


Figure 1. Hourly visits of the cows ($n = 31$) during 2 experimental periods (2×2 wk) to GreenFeed system. Every circle indicates the number of visits by different cows during every hour ($n = 660$).

Figure 2c; $P < 0.01$ for the interaction of hour of day and method). For daily HP, the diurnal variation was between those for O_2 and CO_2 exchanges, with 26.4% and 10.5% higher peaks than nadirs for RC and GF, respectively (Figure 3; $P < 0.01$ for the interaction of hour of day and method).

The average gas exchanges from the GF system calculated based on the “arithmetic method” and “time of day method” were not significantly different ($P \geq 0.91$) from each other (CO_2 $12,780 \pm 1,466$ and $12,870 \pm 1,288$ g/d; O_2 $8,769 \pm 857$ and $8,792 \pm 840$ g/d; CH_4 462 ± 85 and 465 ± 76 g/d, respectively; data not presented).

Repeatability of Measurements and Relationship of Methods

Repeatability values calculated based on daily values for RC were higher than that for DMI (0.93 vs. 0.85) but similar for milk yield (0.98 vs. 0.96) and ECM yield (0.97 vs. 0.96) than the GF measurements (Table 2). The repeatability of gas exchanges, CH_4/CO_2 ratio, and HP were higher for the RC than GF measurements (0.90–0.98 vs. 0.61–0.88). However, the repeatability of CH_4 yield (g/kg DM) was similar between the RC and GF (0.62 vs. 0.61). Repeatability values for milk and ECM yields were higher than those for DMI (0.96–0.98 vs. 0.85–0.93). Repeatability values of O_2 consumption and CO_2 production between the 2 GF periods were high (0.88) and marginally higher than that of DMI (0.85),

whereas repeatability of CH_4 (0.82) was slightly lower than that for DMI (Table 2).

Repeatability for daily averages of gas exchanges measured by the GF was moderate (0.44–0.63; Table 2) and was improved further by averaging over 2-wk periods (0.82–0.88). Repeatability of RQ daily averages was low (0.23) and was improved further by 2-wk averaging of the GF data (0.67). Similarly, the repeatability of CH_4 yield increased from 0.54 to 0.67 for daily averages and 2-wk averages, respectively. The number of days required to reach 0.90 accuracy was 6, 8, and 12 d for O_2 , CO_2 , and CH_4 , respectively (Figure 4). Only minor changes in accuracy were found beyond 14 d, especially for CO_2 and O_2 .

Based on mixed model linear regression analysis, the GF estimated O_2 exchanges of cows with a strong relationship with the RC ($R^2 = 0.89$, RMSPE = 3.1% of mean, CCC = 0.94; Figure 5), followed by CO_2 ($R^2 = 0.84$, RMSPE = 3.5% of mean, CCC = 0.88; Figure 6), and CH_4 ($R^2 = 0.79$, RMSPE = 4.6% of mean, CCC = 0.87; Figure 7). The mean bias (41 g/d, 0.47% of mean) contributed to 12% of total RMSPE for O_2 exchanges with 9% slope bias and a major contribution from random variation (79% of RMSPE). The mean bias (–360 g/d, 2.7% of mean) contributed to 42% of total RMSPE for CO_2 production with a negligible slope bias (4.7%) and a major contribution from random variation (53%). For CH_4 production, mean bias (–9 g/d, 2.1% of mean) contributed to 21% and slope contributed to 32% of total

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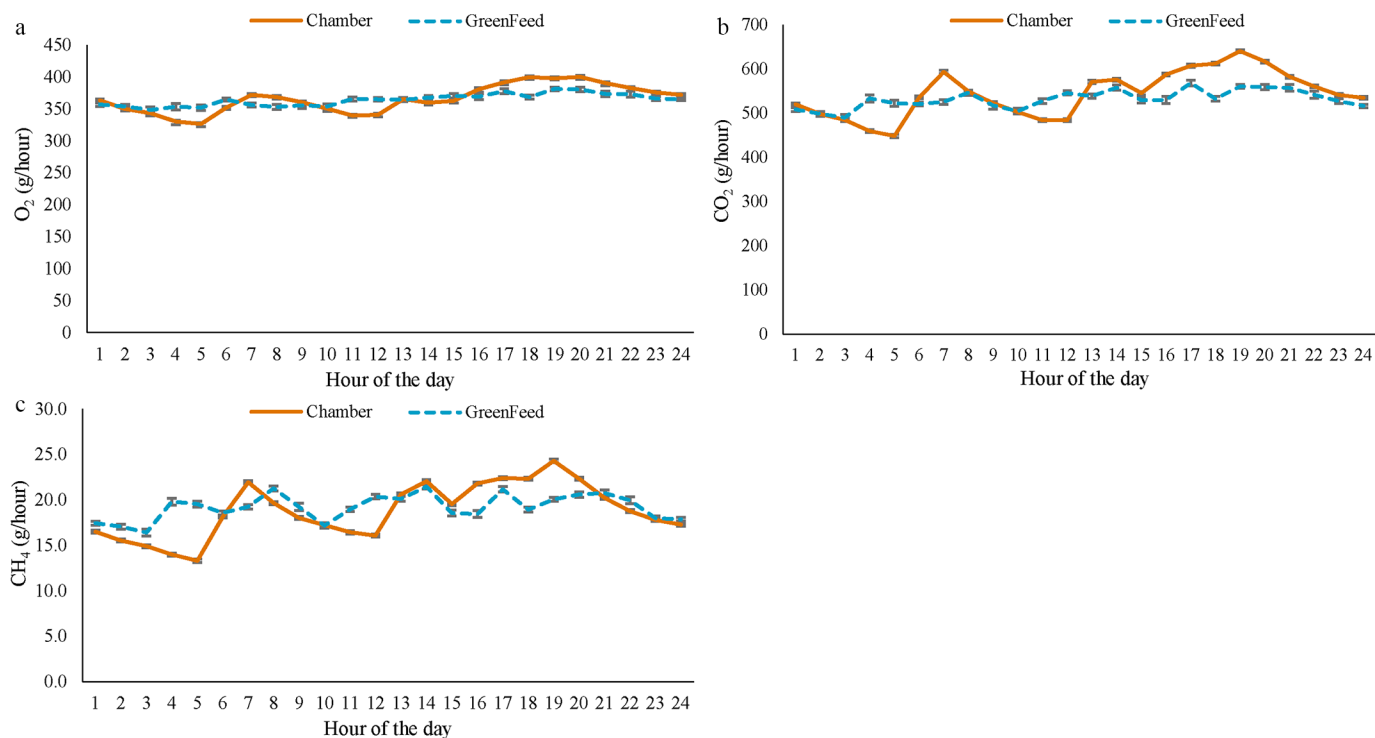


Figure 2. Diurnal variation of O_2 consumption (a) and CO_2 (b) and CH_4 (c) production of experimental cows ($n = 31$) using GreenFeed system and respiratory chambers over 4 wk and 3 d, respectively. The effects of method, hour of day, and their interactions for all gases were significant ($P < 0.01$) with the exception of method effect for O_2 ($P = 0.05$).

RMSPE, with the random variation contributing to 47% of total RMSPE. The intercept ($P \geq 0.41$) and slope ($P \geq 0.57$) in O_2 , CO_2 , and HP equations were not different from 0 and 1, respectively. For CH_4 , both intercept and slope were different ($P < 0.01$) from 0 and 1, respectively. Daily HP was estimated with a strong relationship with RC ($R^2 = 0.88$, RMSPE = 3.2% of mean, CCC = 0.93; Figure 8). The mean bias (1 MJ/d, 0.9% of mean) contributed to 22% of total RMSPE for HP with only 2% slope bias and a major contribution from random variation (76%).

DISCUSSION

The main objective of the current study was to evaluate if the GF system is accurate and precise enough for measuring respiration gases to be used for evaluating individual cows for FE traits. A previous study (Huhtanen et al., 2019) demonstrated that CH_4 emissions measured by the GF system were in good agreement with models developed from RC data. However, greater accuracy and precision are needed for ranking individual cows according to FE than for determining diet or additive effects on CH_4 emissions. The study based on RC data (Huhtanen et al., 2021) showed that RCO_2 production is a promising tool for evaluating the cows for FE traits. Improving

FE would also be a sustainable method to mitigate CH_4 emissions per unit of product, as the most efficient cows ranked according to residual feed intake (Guinguina et al., 2020) or RCO_2 (Huhtanen et al., 2021) produced less CH_4 per kg ECM than the least efficient cows.

Similar averages for O_2 , CO_2 , and CH_4 exchanges when measured by the GF compared with RC indicate the suitability of the GF for group comparisons when measured over 4 wk. The lower diurnal variability of O_2 , CO_2 , and CH_4 exchanges when measured by the GF compared with RC (hourly peak divided by nadir 1.09 vs. 1.23 for O_2 , 1.16 vs. 1.43 for CO_2 , and 1.31 vs. 1.83 for CH_4 measured by the GF and RC, respectively; Figure 2) may be due to the smoother eating behavior when the cows were in their normal barn condition (i.e., the GF measurements). Diurnal variability of HP (hourly peak divided by nadir 1.13 vs. 1.33 for HP measured by the GF and RC, respectively; Figure 3) followed the same trend similar to O_2 and CO_2 exchanges. This is expected, as HP is calculated mainly from O_2 and CO_2 exchanges via the regression equation proposed by Brouwer (1965) with minor effects from CH_4 and urinary N excretion. The difference in variability of daily CH_4 emissions when measured by the GF compared with RC (CV 16.3% vs. 12.4%) was greater than the variability of O_2 (CV 9.6% vs. 10.7%) and CO_2 (CV 10.0% vs. 10.8%) exchanges.

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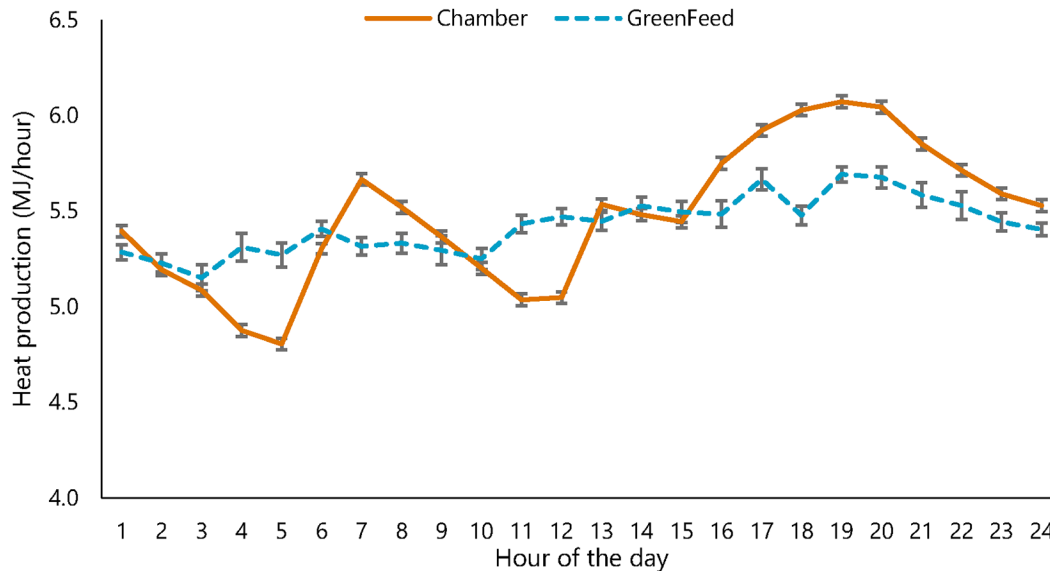


Figure 3. Diurnal variation of heat production of experimental cows ($n = 31$) using GreenFeed system and respiratory chambers over 4 wk and 3 d, respectively. The effects of method, hour of day, and their interactions were significant ($P < 0.01$).

This might be due to different visiting patterns of the cows to the GF units over different days which confounds the effects of natural hourly variation and daily variation increasing the between-day variability for measured daily CH_4 production. Feeding pattern was slightly different between the GF and RC systems, however, the feeding was frequent for both systems which minimizes the effect of feeding pattern on daily fluctuations of exchanged gases. Crompton et al. (2011) indicated that increasing the feeding frequency from 1 to 4 times per day reduces the max-to-min ratio of daily CH_4 production from 2.8 to 1.45. Ma et al. (2024) reported an interaction between hour of the day and technique when comparing the GF

(a rotating fixed spot sampling) and RC using 16 cows. Methane emission is more variable within a day than CO_2 emission, and O_2 consumption has the least fluctuations within a day (Lee et al., 2022). The greater daily variability in CH_4 yield (g/kg DMI) when measured by the GF compared with RC can be attributed to the nature of spot and less frequent sampling in the GF. However, similar averages and variability for DMI and yields of milk and ECM during the GF and RC measurements confirm the similar conditions (management, feeding, temperature, and so on) for the cows during the experiment.

High correlation of 0.81 between gas exchanges measured by the GF and RC has been reported earlier (Garnsworthy et al., 2019) by analyzing the original data from Hammond et al. (2016). Methane was measured by RC during wk 5, whereas it was measured during wk 10 to 12 by the GF, so that the effects of time and method were confounded (Hammond et al., 2016). Doreau et al. (2018), comparing the GF and RC using 8 cows measured for 2 wk in RC and 4 wk in the GF, reported correlations of 0.37 and 0.57 for daily CH_4 and CO_2 production, respectively. In addition, Hammond et al. (2015) comparing the GF and RC found a very weak relationship ($R^2 \leq 0.06$) between the methods in 2 experiments. The weak relationships between the GF and RC in both studies might be due to the very low number of cows, that is, 8 cows in Doreau et al. (2018) and 8 and 16 data points in experiments 1 and 2 of Hammond et al. (2015), respectively.

In the current study, the average visit number to the GF units was 3.59 times per cow per day (ranging from

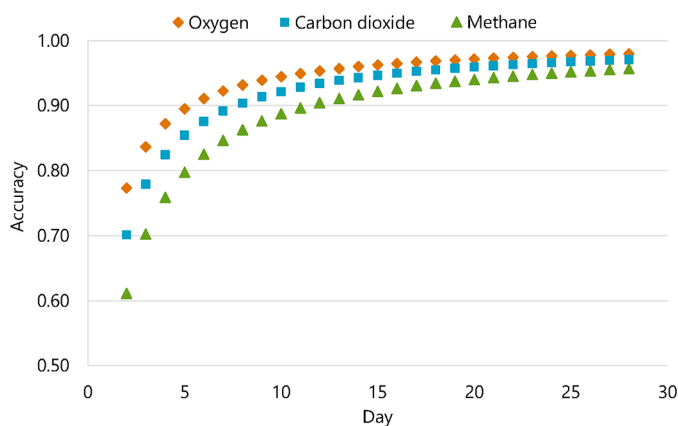


Figure 4. The relationships between the number of days of gas recording by the GF system and the accuracy of the means.

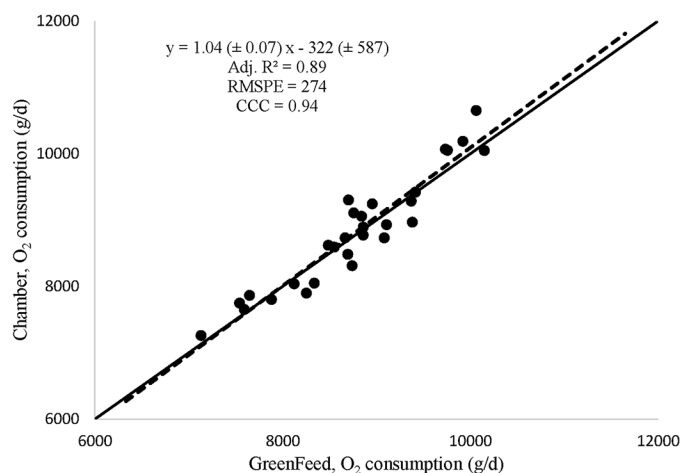


Figure 5. Relationships between estimated daily O₂ consumption (g/d) of experimental cows (n = 31) measured using GreenFeed and respiratory chambers using a mixed model measured over 4 wk and 3 d, respectively. The intercept and slope were not different from 0 and 1 ($P = 0.59$ and $P = 0.57$, respectively). The solid line represents 1:1 line.

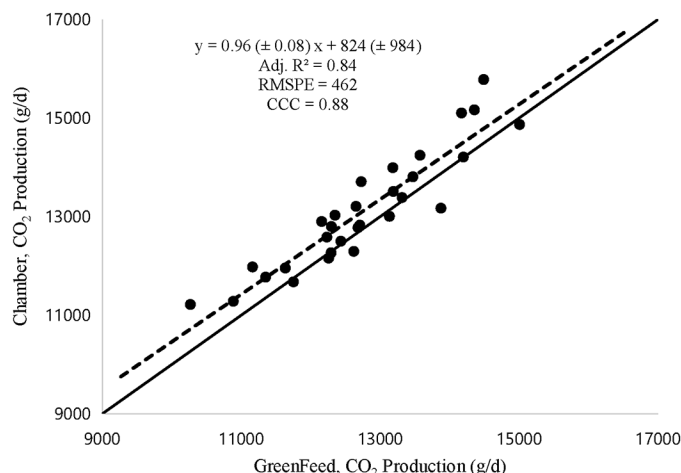


Figure 6. Relationships between estimated daily CO₂ production (g/d) of experimental cows (n = 31) measured using GreenFeed system and respiratory chambers using a mixed model measured over 4 wk and 3 d, respectively. The intercept and slope were not different from 0 and 1 ($P = 0.41$ and $P = 0.62$, respectively). The solid line represents 1:1 line.

1.71 to 5.76). This variation indicates clearly the advantage of comparing the methods on measurements from a large number of animals for a suitable timeframe to avoid randomness associated with measurements from a small number of animals. Ryan et al. (2022), using a big dataset of 1,099 beef, heifers, and bulls, reported a huge variation in visit frequency (between 0.33 to 4.95 visits per day). The duration of visits to the GF was on average 3.47 min, and the variation was not reported (Ryan et al., 2022). However, steers and heifers visited the GF system more frequently than bulls (on average 2.6 and 2.4 per day vs. 0.63 per day). By reviewing 30 studies conducted

using the GF system, Della Rosa et al. (2021) reported the mean visit duration was 3.4 min ranging from 2.0 to 6.6 min in 30% of reviewed studies reporting the visit duration.

The different visit times might have an effect on the calculated daily gas exchanges, especially when the diurnal variation due to feeding frequency is high. We estimated the LSM of hourly gas exchanges by a mixed regression model and compared it with the arithmetic averages of all visits to calculate the daily averages of gas exchanges (data not presented). There was no major influence from

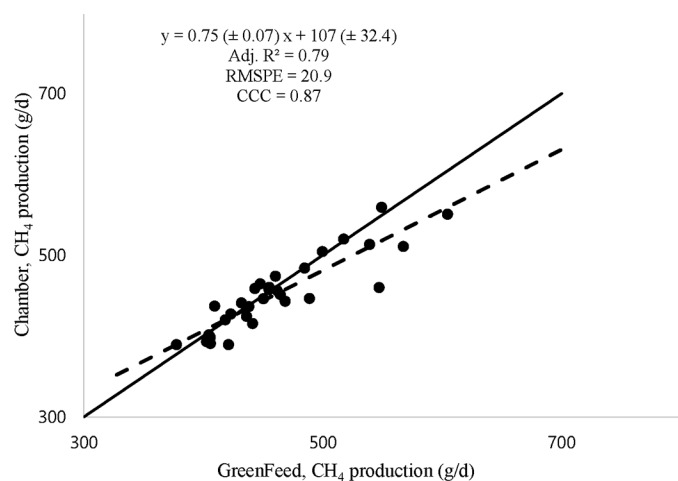


Figure 7. Relationships between estimated daily CH₄ production (g/d) of experimental cows (n = 31) measured using GreenFeed system and respiratory chambers using a mixed model measured over 4 wk and 3 d, respectively. The intercept was higher ($P < 0.01$) than 0 and the slope was smaller ($P < 0.01$) than 1. The solid line represents 1:1 line.

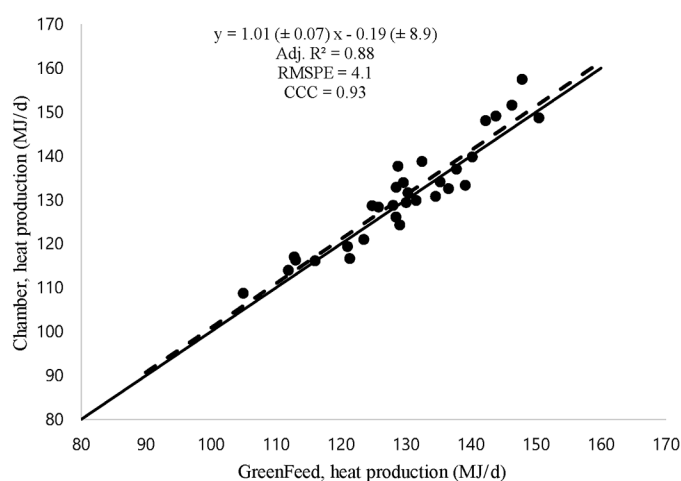


Figure 8. Relationships between estimated daily heat production (MJ/d) of experimental cows (n = 31) measured using GreenFeed system and respiratory chambers using a mixed model measured over 4 wk and 3 d, respectively. The intercept and slope were not different from 0 and 1 ($P = 0.96$ and $P = 0.89$, respectively). The solid line represents 1:1 line.

hourly calculations, perhaps due to sufficient length of the GF measurements (i.e., 28 d) and rather mild diurnal variation in O₂, CO₂, and CH₄ exchanges. The lack of difference between both methods of calculating the gas exchanges, that is, the “arithmetic averaging method” and “time of day method,” indicates that arithmetic averaging and considering the hourly averages in calculation of final data did not affect the final gas exchange data under the conditions of this experiment. The feed was delivered several times (silage, 4 times per day, and concentrates, 5 times per day), which reduces the diurnal variation in O₂ consumption, and especially in CO₂ and CH₄ production. In addition, the concentrate baits were given in a rotational pattern (to spread the visits of cows to the GF units over 24 h). Results of Arbre et al. (2016) suggest that measurements longer than 2 wk seem to improve the quality of data proportionally less, which is supported by our observation of less improvement in rolling averages of gas exchanges after 2 wk of measurements (data not presented). Arthur et al. (2017) recommended a minimum of 30 flux records, with each record obtained from a minimum GF visit duration of 3 min. In our study, total number of visits was 92 ± 20 during the 4-wk (2 + 2) measurements. Only 2 cows had less than 30 visits per period, but removing them from the data did not affect repeatability. Good accuracy of all gas measurements was obtained in 14 d with minor improvements with extended measurement periods. Frequent feeding resulting in minor diurnal variation in gas exchanges resulted in relatively high repeatability of daily measurements, especially for CO₂ and O₂. With 1 time daily feeding, longer periods might be needed, as diurnal variation in gas data are likely to be greater. However, diurnal variation is less in CO₂, and especially in O₂ is less, than in CH₄; therefore, it is likely that high accuracy is reached in 14 d in cows fed 1 time daily for gases related to FE.

High repeatability of the gas data is a prerequisite of using it for phenotypic ranking of the cows according to FE traits. In the present study, the repeatability values of all gases and HP when aggregated over 2-wk periods were high. In a RC study with sheep, repeatability of CH₄ emissions measured during 2 d across 2 rounds that were 2 wk apart was 0.55 (Pinares-Patiño et al., 2013). In the study of Ryan et al. (2022), repeatability of CO₂ and CH₄ emissions was 0.80 and 0.74 for growing steers and 0.82 and 0.69 for heifers with 15-d repeated periods, respectively. In their study, repeatability improved with the length of periods and was lower in poor compared with average and good GF visits. In agreement with Ryan et al. (2017), higher repeatability values were found by Manafiazar et al. (2017) with longer sampling periods. They also found decreased between-animal variability as the length of sampling period increased. Cabezas-Garcia

(2017) reported average repeatability of 0.69 for CH₄ production from 10 change-over studies in dairy cows. The higher value (0.82) in the present study can partly be because of longer measurement periods (2 wk vs. 1 wk) and because the cows were on the same diet during the whole experiment.

The lower repeatability of CH₄ compared with CO₂ is consistent with earlier studies (Huhtanen et al., 2015; Manafiazar et al., 2016; Ryan et al., 2022). Eructation of rumen fermentation increases variation of CH₄ concentration compared with other gases, which may result in greater random variation in CH₄ compared with other gases. This, together with more stable background concentration, may also explain the slightly lower repeatability of CO₂ production compared with O₂ consumption. All CH₄ is derived from rumen fermentation, whereas most of CO₂ and all O₂ are related to body energy metabolism, and, therefore, differences in feed intake and visit pattern to the GF have stronger influences on CH₄ than on CO₂ or O₂. Repeatability of CO₂ production was higher in our study compared with Manafiazar et al. (2016) and Ryan et al. (2022). In their studies, the animals were fed 1 time daily, whereas in our study, the cows were fed grass silage 4 times daily and concentrates 5 times from automatic concentrate feeders and 2 times daily during milking. Repeatability of both CO₂ production (0.88) and O₂ consumption (0.88) was marginally greater than that of DMI (0.85). Similar to our study, Manafiazar et al. (2016) found a higher repeatability for CO₂ production than for DMI, but Ryan et al. (2020) reported a higher value for DMI than for CO₂ production.

The mixed model linear regression analysis indicated a strong relationship between the GF and RC in measuring O₂ consumption, which was slightly weaker for CO₂, and especially for CH₄, as indicated by lower R² and greater RMSPE. The regression fit statistics are in line with the fact that O₂ is the metabolic gas having the least diurnal variability, and the variability increases for CO₂ and further for CH₄, as discussed previously. Relatively low and insignificant intercepts for O₂, CO₂, and HP indicate the lack of intercept bias for these measurements, which are very important for energy metabolism and FE studies. The slopes for O₂, CO₂, and HP were not significantly different from 1.0, and the magnitudes of the deviation from 1.0 was minor (+ 0.04, -0.04, and + 0.01 for O₂, CO₂, and HP, respectively). For CH₄, the regression fit statistics not being as satisfactory as for other gases (significant differences in intercept and slope, relatively high intercept of 107 g/d, and -0.25 deviation of slope from 1.0) probably indicates that more data points and measurements are needed to obtain reliable CH₄ emissions with the GF system. Overall, it seems 4 wk of measurements by the GF is providing reliable estimation of

gas exchanges and HP, however, the results for CH₄ were inferior to those obtained for O₂, CO₂, and HP.

CONCLUSIONS

Very similar averages of CO₂, O₂, and CH₄ exchanges and HP, in addition to their rather high repeatability measured by GF during a 4-wk period and in chambers, imply the suitability of using this system for comparing treatment means. Strong linear relationships between CO₂ and O₂ exchanges and HP, and the lack of mean and slope biases when measured over 4 wk by GF, indicate its potential to be used for ranking the dairy cows for FE. However, due to the spot sampling nature of the method, sufficient length of measuring periods needs to be considered to cover within- and between-day variations to have reasonable accuracies for gas exchanges and HP. This is more important for CH₄ than for O₂, CO₂, and HP due to its greater diurnal variation.

NOTES

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Nonstandard abbreviations used: CCC = concordance correlation coefficient; FE = feed efficiency; GE = gross energy; GF = GreenFeed; HP = heat production; RC = respiration chamber; RCO₂ = residual CO₂; RMSPE = root mean square error of prediction; RQ = respiratory quotient.

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