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Estimates of daily oxygen consumption, carbon dioxide and methane emissions, and heat production for beef and dairy cattle using spot gas sampling

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ABSTRACT

A simulation study was conducted to examine accuracy of estimating daily O_2 consumption, CO_2 and CH_4 emissions, and heat production (HP) using a spot sampling technique and to determine optimal spot sampling frequency (FQ). Data were obtained from 3 experiments where daily O_2 consumption, emissions of CO_2 and CH_4, and HP were measured using indirect calorimetry (respiration chamber or headbox system). Experiment 1 used 8 beef heifers (ad libitum feeding; gaseous exchanges measured every 30 min over 3 d in respiration chambers); Experiment 2 used 56 lactating Holstein-Friesian cows (restricted feeding; gaseous exchanges measured every 12 min over 3 d in respiration chambers); Experiment 3 used 12 lactating Jersey cows (ad libitum feeding; gaseous exchanges measured every hour for 1 d using headbox style chambers). Within experiment, averages of all measurements (FQALL) and averages of measurements selected at time points with 12, 8, 6, or 4 spot sampling FQ (i.e., sampling every 2, 3, 4, and 6 h in a 24-h cycle, respectively; FQ12, FQ8, FQ6, and FQ4, respectively) were compared. Within study a mixed model was used to compare gaseous exchanges and HP among FQALL, FQ12, FQ8, FQ6, and FQ4, and an interaction of dietary treatment by FQ was examined. A regression model was used to evaluate accuracy of spot sampling within study [i.e., FQALL (observed) vs. FQ12, FQ8, FQ6, or FQ4 (estimated)]. No interaction of diet by FQ was observed for any variables except for CH_4 production in experiment 1. No FQ effect was observed for gaseous exchanges and HP except in experiment 2 where CO_2 production was less (5,411 vs. 5,563 L/d) for FQ4 compared with FQALL, FQ12, and FQ8. A regression analysis between FQALL and each FQ within study showed that slopes and intercepts became farther from 1 and 0, respectively, for almost all variables as FQ decreased. Most variables for FQ12 and FQ8 had root mean square prediction error (RMSPE) less than 10% of the mean and concordance correlation coefficient (CCC) greater than 0.80, and RMSPE increased and CCC decreased as FQ decreased. When a regression analysis was conducted with combined data from the 3 experiments (mixed model with study as a random effect), results agreed with those from the analysis for the individual studies. Prediction errors increased and CCC decreased as FQ decreased. Generally, all the estimates from FQ12, FQ8, FQ6, and FQ4 had RMSPE less than 10% of the means and CCC greater than 0.90 except for FQ6 and FQ4 for O_2 consumption and CH_4 production. In conclusion, the spot sampling simulation with 3 indirect calorimetry experiments indicated that FQ of at least 8 samples (every 3 h in a 24-h cycle) was required to estimate daily O_2 consumption, CO_2 and CH_4 production, and HP and to detect changes in those in response to dietary treatments. This sampling FQ may be considered when using techniques that measure spot gas exchanges such as the GreenFeed and face mask systems.
Key words: gaseous exchanges, heat production, spot sampling, ruminant animals

INTRODUCTION

Measuring gaseous exchanges (i.e., daily O_2 consumption and CO_2 and CH_4 production) are of considerable interest in ruminant nutrition. Energy lost to CH_4 is needed for ME estimation, and heat production (**HP**) calculated from these gaseous exchanges is used to estimate net energy (Moe et al., 1972). Furthermore, due to the global concern about enteric CH_4 production from beef and dairy cattle (Niu et al., 2018), accurate

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measurement of enteric CH_4 production in research is needed to develop strategies to lower CH_4 production.

Various techniques have been used to measure gaseous exchanges in ruminant animals (Hammond et al., 2016). Each technique has advantages and disadvantages. Respiratory chambers (i.e., indirect calorimeters) housing the entire animal or a group of animals are considered the gold standard to measure gaseous exchanges. However, this system is expensive to construct (Zhao et al., 2020). In addition, feed intake may be negatively affected in the respiratory chambers (Vyas et al., 2016). A headbox-type respiratory system has also been used to obtain continuous measurements over 24-h periods. Although the headbox system may lead to more variation in measuring gaseous exchanges than respiration chambers, it offers a viable alternative to respiration chambers as they are less expensive and labor intensive (Foth, 2014). A disadvantage of the respiratory chamber system (whole-animal enclosures or headbox style) is that small numbers of animals are usually used in these experiments because of the limited numbers of chamber units.

As an alternative to respiration chamber systems, techniques that collect spot breath samples to estimate daily gaseous exchanges have been used such as the GreenFeed and face mask system (Hammond et al., 2016). The GreenFeed system (C-Lock Inc.) measures respired breaths when an animal visits the unit (Hristov et al., 2015a; Hammond et al., 2016). Because this technique collects spot breath samples several times a day over several days to estimate daily emissions for individual animals, a relatively large number of animals can be used in an experiment (Hristov et al., 2015b). Diurnal patterns of gas emission or consumption related to feed intake patterns (van Lingen et al., 2017) suggest that spot sampling of gaseous emission or consumption should be distributed over 24 h. The recommended spot breath sampling procedure using the GreenFeed system with animals in tie stalls (i.e., animals are forced to receive measurement at a certain time rather than voluntary access to the GreenFeed unit) is to collect breaths every 3 h after feeding (8 time points within a 24-h cycle) over 3 to 4 d (Hristov et al., 2015a,b). However, this spot sampling procedure needs validation for accuracy, and optimal frequency of sampling to estimate daily gaseous exchanges is currently unknown. In addition, the spot sampling technique has been focused on only CH_4 and CO_2 production, and it is not certain whether this spot sampling procedure can also be used for O_2 consumption to calculate HP.

A respiratory chamber system also uses the principal of spot gas sampling, but gas sampling frequency is high, leading to accurate quantification of daily gaseous exchanges. Therefore, data from respiratory chambers may be useful for simulating less frequent spot sampling, as data points of interest (different time points) can be isolated from the complete data set. Daily estimates from spot sampling can be compared with estimates using the full data set (i.e., observed) to validate accuracy of spot sampling and determine optimal sampling frequency. Such an exercise with respiration chambers could be used to develop the optimum sampling frequency for techniques that exclusively measure spot gaseous exchange such as the GreenFeed and face mask systems. Therefore, our objective was to use data from indirect calorimetry systems to simulate spot sampling and evaluate the accuracy of estimates of gaseous exchanges and HP. We hypothesized that (1) spot sampling could be used to estimate daily gaseous exchanges and HP, and (2) decreasing the frequency of spot sampling would increase prediction errors for daily gaseous exchanges and HP.

MATERIALS AND METHODS

Data Collection and Calculation

Three experiments were selected that examined energy metabolism using a respiratory chamber system (whole-animal enclosure or headbox style) with varying sampling frequency, air flow rate, feeding frequency, and animal breeds. Experimental procedures of Lee et al. (2015), Warner et al. (2017), and Judy et al. (2018) were reviewed and approved by Animal Care Committee at the Lethbridge Research and Development Centre, Institutional Animal Care and Use Committee of Wageningen University, and the University of Nebraska-Lincoln Animal Care and Use Committee, respectively. The first study selected was Lee et al. (2015) wherein 8 beef heifers receiving 4 dietary treatments (different levels of encapsulated nitrate) were used in a replicated 4 × 4 Latin square design with once daily ad libitum feeding. Individual animals were housed in whole-animal enclosed respiratory chambers for measurement of O_2 consumption and CO_2 and CH_4 production every 30 min over 3 d during each period. The detailed respiratory chamber design and sampling and measuring procedure are described in Romero-Perez et al. (2015). The second study was that of Warner et al. (2017) which used 56 lactating Holstein-Friesian cows in a randomized block design. The cows were either in early or late lactation, received 4 grass silages of varying qualities (harvested at different phenological stages) as dietary treatments, and were fed restrictedly (no more than 80% of individual ad libitum intake) twice a day. Gaseous exchanges were measured every 12 min over 3 d in whole-animal enclosed respiratory chambers. The detailed respiratory chamber structure and measuring procedure is described in van Gastelen et al. (2015). The third study selected was Judy et al. (2018) where 12 Jersey lactating cows were used in a crossover design where cows were fed a diet for ad libitum intake but given once or twice a day as the experimental treatment. Gaseous exchanges were measured every hour over 1 d in a headbox-type respiratory system. This system collected gas samples continuously from the chamber air exhaust for 1 h into sampling bags and the accumulated air sample was analyzed for gas components to calculate hourly gaseous exchanges. Details of the headbox-type chamber design and sampling and measuring procedures are described in Foth (2014) and Foth et al. (2015).

In all experiments, O_2, CO_2, and CH_4 concentrations in air intake and exhaust of the chambers were measured and O_2 consumption and CO_2 and CH_4 production were calculated at standard temperature and pressure (0°C and 1 atm) to calculate HP at all measured time points using the following equation (Brouwer, 1965):

$$\text{HP (Mcal/d)} = 3.866 \times O_2 \text{ (L/d)} + 1.200 \times CO_2 \text{ (L/d)} - 1.431 \times \text{urinary N (g/d)} - 0.518 \times CH_4 \text{ (L/d)},$$

where Lee et al. (2015) and Judy et al. (2018) used average daily excretion of urinary N for urinary N in the equation and Warner et al. (2017) used the equation without urinary N.

In Lee at al. (2015), individual animals in each period had a total of 144 measurements of gaseous exchanges (every 30 min over 3 d). Individual cows in Warner et al. (2017) had about 330 measurements (every 12–14 min over 3 d). In Judy et al. (2018), individual cows in each period had a total of 24 measurements (every hour over 1 d). All measurements were averaged by animal (Warner et al., 2017) or animal within period (Lee et al., 2015; Judy et al., 2018) and labeled as (**FQALL**) within study to represent observed daily gas exchange and HP values. Then, gaseous exchanges and calculated HP measured at various time points were selected to simulate spot gas sampling with various frequencies (**FQ**) to compare with FQALL. The spot sampling FQ and sampling intervals in this simulation are based on the procedure proposed by Hristov et al. (2015a). In that study, a spot breath sampling protocol was proposed to estimate daily CH_4 production using the GreenFeed system (i.e., spot breath sampling with 8 time points over 3 d representing sampling every 3 h in a 24-h feeding cycle). In the current simulation of spot sampling, we chose to evaluate sampling FQ of 12, 8, 6, and 4 time points representing sampling every 2, 3, 4, and 6 h, respectively, in a 24-h cycle. For a spot sampling FQ of 12 (**FQ12**) data (i.e., gaseous exchanges and HP) at the following time points after morning feeding were extracted from the full set of data points in Lee et al. (2015) and Warner et al. (2017): 0, 6, 12, and 18 h on d 1; 2, 8, 14, and 20 h on d 2; 4, 10, 16, and 22 h on d 3 (i.e., sampling every 2 h in a 24-h cycle). The spot sampling frequency of 8 (**FQ8**) collected data points at the following time points after morning feeding: 0, 9, and 18 h on d 1; 3, 12, and 21 h on d 2; 6 and 15 h on d 3 (i.e., sampling every 3 h in a 24-h cycle). The spot sampling frequency of 6 (**FQ6**) collected data points at the following time points after morning feeding: 0 and 12 h on d 1; 4 and 16 h on d 2; 8 and 20 h on d 3 (i.e., sampling every 4 h in a 24-h cycle). The spot sampling frequency of 4 (**FQ4**) collected data points at the following time points after morning feeding: 0 and 18 h on d 1; 6 h on d 2; 12 h on d 3 (i.e., sampling every 6 h in a 24-h cycle). When gas measurements were collected at the designated time points for Lee et al. (2015) and Warner et al. (2017), often no measurement at the designated time was found (e.g., if the designated time was 1400 h) measurement was conducted at 1352 and 1406 h. In this case, we selected the measurement that was nearest to the designated time. Because Judy et al. (2018) measured gaseous exchanges for only 1 d for individual cows in each period, spot sampling data points were collected within the one day [i.e., FQ12, 12 time points over 24 h (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 h after morning feeding)]; FQ8, 8 time points over 24 h (0, 3, 6, 9, 12, 15, 18, and 21 h after feeding); FQ6, 6 time points over 24 h (0, 4, 8, 12, 16, and 20 h after feeding); FQ4, 4 time points over 24 h (0, 6, 12, and 18 h after feeding). The data points extracted for each FQ (FQ12, FQ8, FQ6, or FQ4) were averaged by animal (Warner et al., 2017) or animal within period (Lee et al., 2015; Judy et al., 2018) and used to calculate daily O_2 consumption, CO_2 and CH_4 production, and HP. Therefore, a total of 32, 56, and 24 observations were obtained for each FQ in the study by Lee et al. (2015), Warner et al. (2017), and Judy et al. (2018), respectively.

Statistical Analyses

The daily gaseous exchanges and HP obtained from each FQ were analyzed within study using the MIXED procedure of SAS (version 9.4; SAS Institute Inc.). The models used in the original studies (Lee et al., 2015; Warner et al., 2017; Judy et al., 2018) were used with modifications where FQ and an interaction of dietary treatment by FQ were included in the model as fixed

Table 1. Descriptive statistic summary of gaseous exchanges and heat production (HP) in Lee et al. (2015), Warner et al. (2017), and Judy et al. (2018)

Study	Variable[1]	N[2]	Mean	SD	Minimum	Maximum
Lee et al. (2015)						
	O_2, L/d	32	2,362	214.0	1,881	2,721
	CO_2, L/d	32	3,463	362.4	2,879	4,398
	CH_4, L/d	32	233	32.4	170	293
	HP, Mcal/d	32	12.9	0.86	11.1	14.4
Warner et al. (2017)[3]						
	O_2, L/d	55	4,988	472.3	3,905	6,023
	CO_2, L/d	55	5,593	489.5	4,444	6,573
	CH_4, L/d	55	483	56.4	366	622
	HP, Mcal/d	55	25.8	2.38	20.2	30.9
Judy et al. (2018)[4]						
	O_2, L/d	22	4,124	687.0	2,929	5,314
	CO_2, L/d	22	4,160	620.2	3,208	5,115
	CH_4, L/d	22	361	51.3	251	456
	HP, Mcal/d	22	20.4	3.29	15.4	25.9

[1]Descriptive statistics of variables measured from respiratory chambers over 3 d for Lee et al. (2015; beef heifers) and Warner et al. (2017; lactating Holstein-Friesian dairy cows) or over 1 d for Judy et al. (2018; lactating dairy Jersey cows).

[2]N = the number of experimental units.

[3]One cow was removed during the experiment and the data were excluded from the analysis.

[4]One cow was removed during the experiment and the data were excluded from the analysis.

effects. Within study, animal-to-animal and day-to-day variation were calculated at each FQ. Gaseous exchanges and HP obtained from each FQ were averaged by animal or day and SD and CV (SD/average × 100) were calculated. Because Judy et al. (2018) measured gaseous exchanges and HP for 1 d, there is no day-to-day variation for this study. A regression analysis was also conducted to examine estimation accuracy of gaseous exchanges and HP obtained from FQ12, FQ8, FQ6, and FQ4 by comparing with FQALL within study using the REG procedure of SAS. Gaseous exchanges and HP from FQ12, FQ8, FQ6, or FQ4 were plotted on the observed values (i.e., FQALL) and intercepts and slopes were compared with 0 and 1, respectively. Root mean square prediction error (**RMSPE**; root of the sum of the squared residual errors divided by the number of observations) and concordance correlation coefficient (**CCC**; Lin, 1989) were calculated and used to determine estimation accuracy.

Data of gaseous exchanges from the 3 experiments were combined and a regression analysis was conducted to examine estimation accuracy of FQ12, FQ8, FQ6, and FQ4 using the following model in the MIXED procedure of SAS (version 9.4):

$$Y_{ij} = B_0 + S_i + B_1X_{ij} + s_iX_{ij} + e_{ij},$$

where Y_{ij} is the observed variable (FQALL), X_{ij} represents the estimator variables (FQ12 to FQ4), B_0 is the overall intercept (fixed), S_i is the study effect (random), B_1 is the overall regression slope (fixed), s_i is the slope associated with study (random), and e_{ij} is the residual error. Initially, the model included interactions of study by X_{ij}, but their effects were almost equal to 0 ($P <$ 0.05) and thus were removed from the model. Estimated gaseous exchanges (FQ12 to 4) were plotted on the corresponding observed values (FQALL) where observed values were converted to adjusted values (St-Pierre, 2001) for graphical presentation, and the adjusted values were used for calculation of RMSPE and CCC. The results from the MIXED procedure were restored in the PLM procedure of SAS (version 9.4) and the slope was compared with 1. The residuals from the regression analysis within study and from all studies were plotted and shown in Figure 3.

RESULTS

Descriptive statistics of gaseous exchanges and HP are presented in Table 1. Gaseous exchanges measured for individual animals in Lee et al. (2015), Warner et al. (2017), and Judy et al. (2018) were averaged by time point within study and are shown in Figure 1. Oxygen consumption and production of CO_2 and CH_4 within study had clear and similar diurnal variations, where the diurnal variation depended on feeding frequency. Therefore, within a 24-h feeding cycle there was 1 clear peak of gaseous exchanges in Lee et al. (2015; once per d feeding) and 2 clear peaks in Warner et al. (2017; twice per d feeding). Because the experiment by Judy et al. (2018) had feeding frequency as a treatment (once vs. twice daily), peaks associated with feeding frequency are visually not as clear when the data are averaged across animals and treatment.

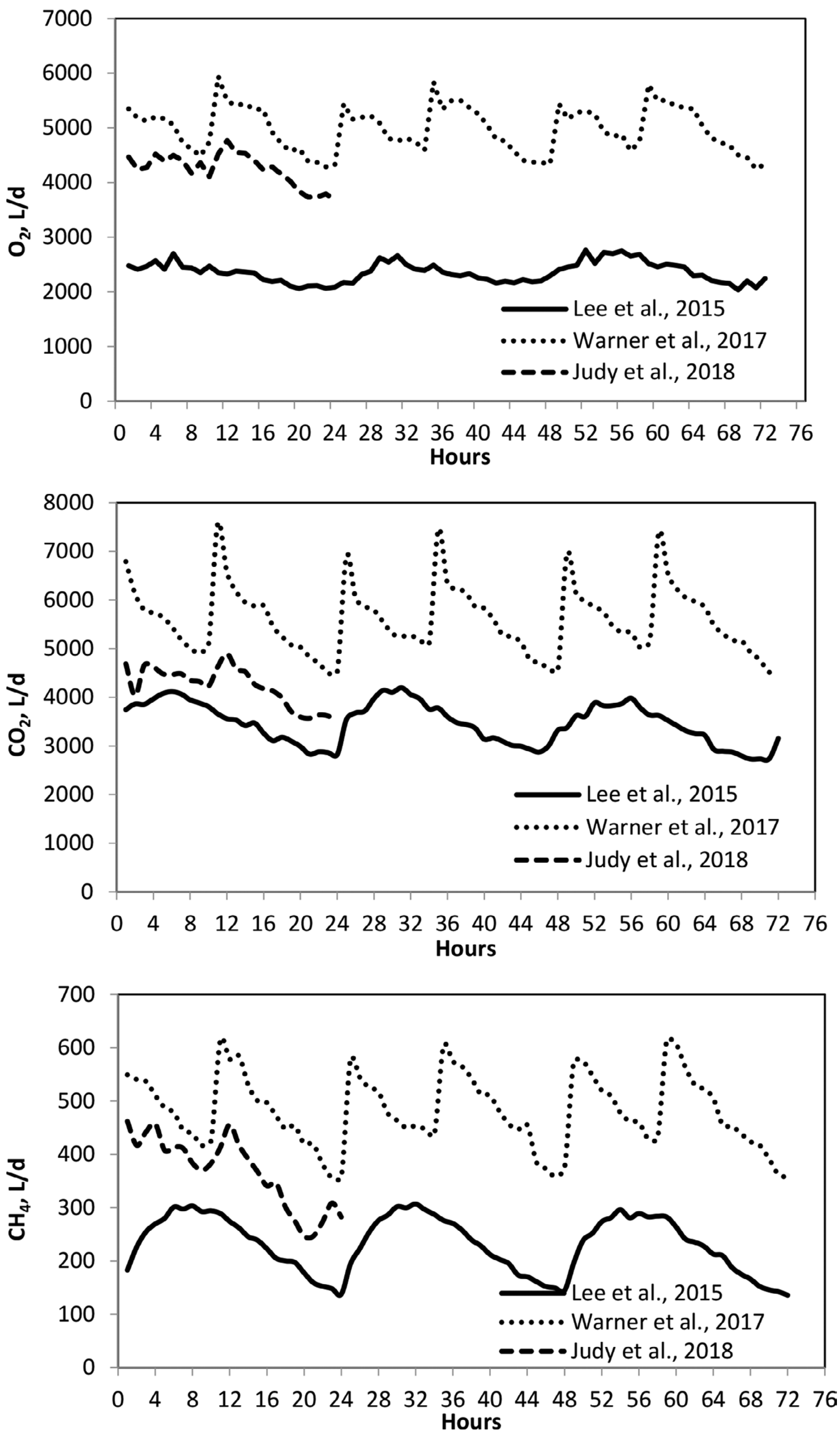

Figure 1. Diurnal variation of O_2 consumption and CO_2 and CH_4 production observed in studies by Lee et al. (2015), Warner et al. (2017), and Judy et al. (2018). Feeds were delivered at 0, 24, and 48 h in Lee et al. (2015) and at 0, 10, 24, 34, 48, and 58 h in Warner et al. (2017). In Judy et al. (2018), half of the cows were fed a diet once a day at 0 h and another half twice a day at 0 and 10 h.

Table 2. Oxygen consumption, CO_2 and CH_4 production, and heat production (HP) estimated from various spot gas sampling frequencies over 3 d and their variations in beef heifers (Lee et al., 2015)

Item	FQ[1]					SEM	*P*-value[2]		
	All	12	8	6	4		FQ	Diet	FQ × D
Gas production									
O_2, L/d	2,362	2,339	2,369	2,332	2,421	65.6	0.57	0.98	0.98
CO_2, L/d	3,463	3,509	3,517	3,537	3,606	89.6	0.36	0.18	0.99
CH_4, L/d	233	233	230	226	228	7.0	0.23	<0.01	0.08
CH_4, L/kg DMI	27.1	27.0	26.7	26.3	26.4	1.03	0.25	<0.01	0.13
HP, Mcal/d	12.9	12.9	13.0	12.9	13.3	0.30	0.39	0.93	0.98
HP, % of GEI[3]	28.1	28.0	28.2	28.0	28.9	0.80	0.31	0.56	0.94
Variation									
O_2, L/d									
Animal[4]	214.0	248.8	268.4	322.0	357.7				
SD									
CV	9.1	10.6	11.3	13.8	14.8				
Day[5]	62.6	3.1	159.4	110.2	126.2				
SD									
CV	2.7	0.1	6.7	4.7	5.1				
CO_2, L/d									
Animal	404.4	466.8	462.8	514.1	704.7				
SD									
CV	11.7	13.3	13.1	14.5	19.3				
Day	90.3	144.3	84.7	150.0	432.9				
SD									
CV	2.6	4.1	2.4	4.2	11.8				
CH_4, L/d									
Animal	32.9	37.8	43.7	47.7	72.5				
SD									
CV	14.1	16.2	18.8	21.0	30.1				
Day	3.2	2.1	12.9	12.3	54.2				
SD									
CV	1.4	0.9	5.5	5.4	22.5				

[1]FQ = various sampling frequencies. All, gas emissions were measured every 30 min for each chamber over 3 d. FQ12, 12 time points after feeding over 3 d: 0, 6, 12, and 18 h on d 1; 2, 8, 14, and 20 h on d 2; 4, 10, 16, and 22 h on d 3. FQ8, 8 time points after feeding over 3 d: 0, 9, and 18 h on d 1; 3, 12, and 21 h on d 2; 6 and 15 h on d 3. FQ6, 6 time points after feeding over 3 d: 0 and 12 h on d 1; 4 and 16 h on d 2; 8 and 20 h on d 3. FQ4, 4 time points after feeding over 3 d: 0 and 18 h on d 1; 6 h on d 2; 12 h on d 3.

[2]FQ = sampling frequency effect; diet = dietary treatment effect; FQ × D = interaction of frequency by diet.

[3]GEI = gross energy intake.

[4]Animal-to-animal variation; CV, %.

[5]Day-to-day variation; CV, %.

In the experiment by Lee et al. (2015), no interaction of diet by FQ was found for O_2 consumption, CO_2 production, and HP (Table 2). Also, no dietary and FQ effects were found for these variables. However, a tendency for an interaction between FQ and diet occurred for CH_4 production (L/d; $P = 0.08$) but no interaction was found for CH_4 yield (L/kg DMI). Methane emission expressed as L/d and L/kg DMI was affected by diet ($P < 0.01$) but not FQ. Animal-to-animal and day-to-day variation of gaseous exchanges generally increased as FQ decreased. Day-to-day variation was considerably larger for FQ4 compared with other FQ for CO_2 and CH_4 production.

Results from regression analyses for data of Lee et al. (2015) are shown in Table 3. When O_2 consumption, CO_2 and CH_4 production, and HP were estimated from FQ12, FQ8, FQ6, and FQ4, the intercepts and slopes became farther from 0 and 1, respectively, as FQ decreased. Intercepts and slopes were different ($P < 0.05$) from 0 and 1, respectively, except that the intercept and slope for CH_4 production from FQ12 and FQ8 were not different from 0 and 1, respectively. In general, RMSPE increased and CCC decreased as FQ decreased for gaseous exchanges and HP. Although a general trend of increasing RMSPE and decreasing CCC was observed as FQ decreased for CH_4 production, RMSPE and CCC were numerically similar between FQ6 and FQ4.

In the experiment by Warner et al. (2017), no interaction between FQ and diet by FQ was observed for all variables (Table 4). Whereas O_2 consumption, CO_2 and CH_4 production, and HP were affected ($P < 0.01$) by diet, CO_2 production was affected ($P < 0.01$) by FQ, where the estimated CO_2 production from FQ4 was lower compared with FQALL, FQ12, and FQ8. The production of CH_4 tended to be affected ($P = 0.09$) by FQ, where production of CH_4 from FQ4 was nu-

Table 3. Accuracy of estimated O_2 consumption, CO_2 and CH_4 production, and heat production (HP) with various spot gas sampling frequencies over 3 d in beef heifers (Lee et al., 2015)

Item	FQ[1]	Intercept (SE)	Slope (SE)	RMSPE[2]	CCC[3]
O_2, L/d	12	698* (207)	0.71* (0.09)	140 (5.9)	0.81
	8	930* (226)	0.60* (0.09)	179 (7.3)	0.74
	6	1,119* (171)	0.53* (0.07)	197 (8.3)	0.73
	4	1,488* (213)	0.36* (0.09)	287 (12.1)	0.52
CO_2, L/d	12	566* (141)	0.83* (0.04)	123 (3.5)	0.95
	8	531* (179)	0.83* (0.05)	138 (4.0)	0.93
	6	957* (203)	0.71* (0.06)	205 (5.9)	0.87
	4	914* (206)	0.71* (0.06)	239 (6.9)	0.83
CH_4, L/d	12	1.8 (12)	0.99 (0.05)	9 (3.8)	0.96
	8	29 (16)	0.88 (0.07)	13 (5.7)	0.92
	6	72* (17)	0.71* (0.07)	20 (8.8)	0.84
	4	58* (18)	0.77* (0.08)	19 (8.0)	0.85
HP, Mcal/d	12	4.2* (1.1)	0.67* (0.08)	0.6 (4.5)	0.81
	8	5.1* (1.3)	0.60* (0.10)	0.7 (5.5)	0.73
	6	6.4* (0.9)	0.51* (0.07)	0.8 (6.4)	0.72
	4	8.2* (1.1)	0.35* (0.08)	1.3 (9.7)	0.50

[1]Various sampling frequencies (FQ) were compared with frequency "all" (gas emissions measured every 30 min over 3 d). FQ12, 12 time points after feeding over 3 d: 0, 6, 12, and 18 h on d 1; 2, 8, 14, and 20 h on d 2; 4, 10, 16, and 22 h on d 3. FQ8, 8 time points after feeding over 3 d: 0, 9, and 18 h on d 1; 3, 12, and 21 h on d 2; 6 and 15 h on d 3. FQ6, 6 time points after feeding over 3 d: 0 and 12 h on d 1; 16 h on d 2; 8 and 20 h on d 3. FQ4, 4 time points after feeding over 3 d: 0 and 18 h on d 1; 6 h on d 2; 12 h on d 3.

[2]RMSPE = root mean square prediction error; prediction error as a % of the observed mean value shown in parentheses.

[3]CCC = concordance correlation coefficient.

*Asterisk for intercepts and slopes indicates a significant difference from 0 and 1, respectively.

merically lower than the other FQ. Animal-to-animal and day-to-day variation generally increased as FQ decreased. Results from the regression analyses for data of Warner et al. (2017) are shown in Table 5. In general, the intercepts and slopes generally became farther from 0 and 1, respectively, for all gaseous exchanges and HP as FQ decreased. The intercepts and slopes were different ($P < 0.05$) from 0 and 1, respectively, except that the slope of FQ12 for CH_4 production was not different from 1. In general, RMSPE increased and CCC decreased for all gaseous exchanges and HP as FQ decreased.

In the study by Judy et al. (2018), no interaction between FQ and diet was observed for all variables (Table 6). Diet affected CH_4 yield (L/kg DMI; $P < 0.01$) but did not affect any other variables. Gaseous exchanges and HP were not affected by FQ. Animal-to-animal variation generally increased as FQ decreased, but the increasing trend was not as clear as that observed in the studies by Lee et al. (2015) and Warner et al. (2017). Results from the regression analysis for data of Judy et al. (2018) are shown in Table 7. For O_2 consumption, the intercepts and slopes differed ($P < 0.05$) from 0 and 1, respectively, for FQ12 and FQ6, but did not differ from 0 and 1 for FQ8 and FQ4 (Table 7). The RMSPE increased from 5.9 to 13.0% of observed mean and CCC decreased from 0.95 to 0.78 when FQ decreased from FQ12 to FQ6. However, RMSPE was lower and CCC was numerically greater for FQ4 compared with FQ6 and were similar between FQ4 and FQ8. For CO_2 production, the intercepts and slopes were not different from 0 and 1 ($P < 0.05$), respectively, for FQ12 and FQ8 but did differ from 0 and 1 for FQ6 and FQ4. The RMSPE increased and CCC decreased for CO_2 as FQ decreased. For CH_4 production, the intercepts and slopes of FQ12, FQ6, and FQ4, but not of FQ8, differed from 0 and 1, respectively. When CH_4 production was estimated from FQ, RMSPE increased and CCC decreased for CO_2 production as FQ decreased. For HP, the intercepts and slopes for FQ12 and FQ6 were different ($P < 0.05$) from 0 and 1, respectively, but not different for FQ8. For FQ4, the intercept was not different from 0 but the slope was different from 1. As FQ decreased from FQ12 to FQ6, RMSPE increased from 4.8 to 10.2% of observed mean and CCC decreased from 0.96 to 0.84. However, FQ4 had lower RMSPE and greater CCC compared with FQ6.

Regression analyses of combined data from the 3 experiments are shown in Figure 2 and their residual plots are presented in Figure 3. When O_2 consumption was estimated, RMSPE increased from 6.3 to 11.1% and CCC decreased from 0.98 to 0.91 as FQ decreased. For CO_2 production, RMSPE increased from 4.3 to 7.9% and CCC decreased from 0.98 to 0.92 as FQ decreased. A similar pattern of RMSPE (6.9 to 16.9%) and CCC (0.97 to 0.80) for CH_4 production was observed as FQ decreased. For all variables, intercepts and slopes differed ($P < 0.05$) or tended to differ ($P < 0.10$) from

Table 4. Oxygen consumption, CO_2 and CH_4 production, and heat production (HP) estimated using various spot gas sampling frequencies over 3 d and their variations in lactating Holstein-Friesian dairy cows (Warner et al., 2017)

	FQ[1]						*P*-value[2]		
Item	All	12	8	6	4	SEM	FQ	Diet	FQ × D
Gas production									
O_2, L/d	4,998	4,920	4,886	4,970	4,893	120.2	0.22	<0.01	0.99
CO_2, L/d	5,605[a]	5,558[a]	5,525[a]	5,521[ab]	5,411[b]	130.0	<0.01	<0.01	0.85
CH_4, L/d	485	474	473	482	468	14.1	0.09	<0.01	0.95
CH_4, L/kg DMI	30.3	29.7	29.6	30.2	29.2	0.48	0.11	<0.01	0.98
HP, Mcal/d	25.8	25.3	25.2	25.6	25.2	0.61	0.11	<0.01	0.98
HP, % of GEI[3]	35.5	34.8	34.6	35.2	34.6	0.48	0.21	<0.01	0.99
Variation									
O_2, L/d									
Animal[4]									
SD	476.4	510.5	505.0	510.6	592.8				
CV	9.6	10.4	10.4	10.3	12.1				
Day[5]									
SD	22.6	52.4	145.2	292.2	348.9				
CV	0.5	1.1	3.0	5.9	7.0				
CO_2, L/d									
Animal									
SD	489.2	514.7	525.9	525.6	633.9				
CV	8.8	9.4	9.7	9.5	11.7				
Day									
SD	30.2	85.5	304.0	304.9	706.3				
CV	0.5	1.6	5.6	5.5	12.7				
CH_4, L/d									
Animal									
SD	55.3	57.1	64.0	60.9	74.5				
CV	11.5	12.1	13.6	12.7	16.0				
Day									
SD	4.6	11.6	37.8	24.8	82.6				
CV	1.0	2.5	8.1	5.2	17.1				

[a,b]Within a row, means without a common superscript letter differ ($P < 0.05$).

[1]FQ = various sampling frequencies; All, gas emissions were measured every 12 min for each chamber over 3 d. FQ12, 12 time points after feeding over 3 d: 0, 6, 12, and 18 h on d 1; 2, 8, 14, and 20 h on d 2; 4, 10, 16, and 22 h on d 3. FQ8, 8 time points after feeding over 3 d: 0, 9, and 18 h on d 1; 3, 12, and 21 h on d 2; 6 and 15 h on d 3. FQ6, 6 time points after feeding over 3 d: 0 and 12 h on d 1; 4 and 16 h on d 2; 8 and 20 h on d 3. FQ4, 4 time points after feeding over 3 d: 0 and 18 h on d 1; 6 h on d 2; 12 h on d 3.

[2]FQ, sampling frequency effect; diet, dietary treatment effect; FQ × D, interaction of frequency by diet.

[3]GEI = gross energy intake.

[4]Animal-to-animal variation; CV, %.

[5]Day-to-day variation; CV, %.

0 and 1, respectively, except for the intercept for O_2 consumption at FQ8 and for CH_4 production at FQ12.

DISCUSSION

Accurate measurements of daily O_2 consumption and CO_2 and CH_4 production are essential to examine effects of dietary manipulation on energy metabolism and environmental effects (i.e., enteric CH_4 emission) of ruminant animals. The purpose of this study was to evaluate the potential use of spot sampling and to optimize spot sampling frequency to estimate daily gaseous exchanges and HP of beef and dairy cattle. If spot sampling frequency can be quantitatively optimized, techniques that collect spot breath samples such as the GreenFeed and face mask systems may be more reliably used to estimate daily gaseous exchanges and HP, allowing a larger number of animals to be used in such studies compared with the use of respiratory chambers. Spot sampling to estimate gaseous exchanges is used by the GreenFeed system (e.g., Hristov et al., 2015a; Hammond et al., 2016). This technique has been mostly used for CH_4 production and to a somewhat smaller extent CO_2 and H_2 production, but the sampling frequency has not yet been fully optimized. In studies where the GreenFeed system is used with animals in tie stalls, Hristov et al. (2015a,b) suggested sampling at 8 time points after feeding over 3 d to represent measurements every 3 h within a 24-h cycle. Manafiazar et al. (2017) suggested that for beef steers housed in pens, a minimum of 20 spot samples over 7 to 14 d is required to produce repeatable and reliable averaged CH_4 and CO_2 emissions using the GreenFeed system.

Table 5. Accuracy of estimated O_2 consumption, CO_2 and CH_4 production, and heat production (HP) with various spot gas sampling frequencies over 3 d in lactating Holstein-Friesian dairy cows (Warner et al., 2017)

Item	FQ	Intercept (SE)	Slope (SE)	RMSPE[2]	CCC[3]
O_2, L/d	12	686* (200)	0.88* (0.04)	177 (3.5)	0.94
	8	802* (248)	0.86* (0.05)	227 (4.5)	0.89
	6	806* (261)	0.84* (0.05)	210 (4.2)	0.91
	4	1,582* (260)	0.70* (0.05)	306 (6.1)	0.84
CO_2, L/d	12	794* (269)	0.88* (0.05)	248 (4.4)	0.89
	8	1,067* (305)	0.84* (0.06)	279 (5.0)	0.86
	6	1,118* (346)	0.81* (0.06)	270 (4.8)	0.86
	4	1,914* (272)	0.68* (0.05)	360 (6.4)	0.81
CH_4, L/d	12	65* (29)	0.89 (0.06)	24 (5.0)	0.91
	8	133* (31)	0.74* (0.07)	35 (7.2)	0.84
	6	96* (30)	0.81* (0.06)	30 (6.2)	0.87
	4	196* (28)	0.62* (0.06)	46 (9.6)	0.76
HP, Mcal/d	12	3.6* (1.1)	0.88* (0.04)	0.9 (3.7)	0.93
	8	4.2* (1.3)	0.86* (0.05)	1.2 (4.6)	0.89
	6	4.3* (1.4)	0.84* (0.05)	1.1 (4.3)	0.90
	4	8.3* (1.3)	0.70* (0.05)	1.6 (6.1)	0.83

[1]Various sampling frequencies (FQ) were compared with "All" (gas emissions measured every 12 min for each chamber over 3 d). FQ12, 12 time points after feeding over 3 d: 0, 6, 12, and 18 h on d 1; 2, 8, 14, and 20 h on d 2; 4, 10, 16, and 22 h on d 3. FQ8, 8 time points over 3 d: 0, 9, and 18 h on d 1; 3, 12, and 21 h on d 2; 6 and 15 h after feeding on d 3. FQ6, 6 time points after feeding over 3 d: 0 and 12 h on d 1; 4 and 16 h on d 2; 8 and 20 h on d 3. FQ4, 4 time points after feeding over 3 d: 0 and 18 h on d 1; 6 h on d 2; 12 h on d 3.

[2]RMSPE = root mean square prediction error; prediction error as a % of the observed mean value shown in parentheses.

[3]CCC = concordance correlation coefficient.

*Asterisk for intercepts and slopes indicates a significant difference from 0 and 1, respectively.

Table 6. Oxygen consumption, CO_2 and CH_4 production, and heat production (HP) estimated using various spot gas sampling frequencies over 24 h and their variations in lactating Jersey cows (Judy et al., 2018)

	FQ[1]						*P*-value[2]		
Item	All	12	8	6	4	SEM	FQ	Diet	FQ × D
Gas production									
O_2, L/d	4,124	4,036	4,192	4,029	4,222	211.8	0.38	0.33	0.76
CO_2, L/d	4,160	4,128	4,156	4,147	4,158	198.5	0.93	0.24	0.73
CH_4, L/d	361	357	356	355	357	17.3	0.99	0.19	0.83
CH_4, L/kg DMI	21.8	21.2	21.3	21.0	21.1	1.04	0.85	<0.01	0.89
HP, Mcal/d	20.4	20.1	20.7	20.1	20.8	1.02	0.39	0.27	0.75
HP, % of GEI[3]	27.4	26.8	27.7	26.7	27.8	1.30	0.48	0.37	0.73
Variation									
O_2, L/d									
Animal[4]									
SD	687.0	841.2	645.1	943.2	678.1				
CV	16.7	20.8	15.4	23.4	16.1				
CO_2, L/d									
Animal									
SD	620.2	660.6	650.8	682.8	705.0				
CV	14.9	16.0	15.7	16.5	17.0				
CH_4, L/d									
Animal									
SD	51.3	62.4	52.0	69.7	76.4				
CV	14.2	17.5	14.6	19.7	21.4				

[1]FQ = various sampling frequencies; All, proportion of gases exiting the chambers was continuously collected every hour over 24 h (i.e., 24 time points). FQ12, 12 time points over 24 h: 0, 2, 4, 6, 8, 12, 14, 16, 18, 20, and 22 h after feeding. FQ8, 8 time points over 24 h: 0, 3, 6, 9, 12, 15, 18, and 21 h after feeding. FQ6, 6 time points over 24 h: 0, 4, 8, 12, 16, and 20 h after feeding. FQ4, 4 time points over 24 h: 0, 6, 12, and 18 h after feeding.

[2]FQ = sampling frequency effect; diet = dietary treatment effect; FQ × D = interaction of frequency by diet.

[3]GEI = gross energy intake.

[4]Animal-to-animal variation; CV, %.

Table 7. Accuracy of estimated O_2 consumption, CO_2 and CH_4 production, and heat production (HP) with various spot gas sampling frequencies over 24 h in lactating Jersey cows (Judy et al., 2018)

Item	FQ[1]	Intercept (SE)	Slope (SE)	RMSPE[2]	CCC[3]
O_2, L/d	12	915* (171)	0.80* (0.04)	244 (5.9)	0.95
	8	138 (455)	0.95 (0.11)	311 (7.6)	0.89
	6	1,697* (378)	0.60* (0.09)	534 (13.0)	0.78
	4	319 (442)	0.90 (0.10)	328 (8.0)	0.88
CO_2, L/d	12	362 (175)	0.92 (0.04)	135 (3.3)	0.98
	8	330 (228)	0.92 (0.05)	162 (3.9)	0.97
	6	812* (213)	0.88* (0.05)	172 (4.1)	0.96
	4	733* (290)	0.82* (0.07)	244 (5.9)	0.93
CH_4, L/d	12	93* (27)	0.75* (0.08)	26 (7.1)	0.89
	8	86 (49)	0.77 (0.14)	34 (9.3)	0.78
	6	138* (31)	0.63* (0.09)	37 (10.2)	0.81
	4	193* (39)	0.47* (0.11)	53 (14.8)	0.65
HP, Mcal/d	12	3.8* (0.77)	0.83* (0.04)	0.99 (4.8)	0.96
	8	0.8 (2.0)	0.95 (0.09)	1.33 (6.5)	0.91
	6	6.8* (1.7)	0.68* (0.08)	2.08 (10.2)	0.84
	4	2.1 (2.0)	0.88* (0.09)	1.47 (7.2)	0.90

[1]Various sampling frequencies (FQ) were compared with "All" (proportion of gases exiting the chambers was continuously collected every hour over 24 h). FQ12, 12 time points over 24 h: 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 h after feeding. FQ8, 8 time points over 24 h: 0, 3, 6, 9, 12, 15, 18, and 21 h after feeding. FQ6, 6 time points over 24 h: 0, 4, 8, 12, 16, and 20 h after feeding. FQ4, 4 time points over 24 h: 0, 6, 12, and 18 h after feeding.

[2]RMSPE = root mean square prediction error; prediction error as a % of the observed mean value shown in parentheses.

[3]CCC = concordance correlation coefficient.

*Asterisk for intercepts and slopes indicates a significant difference from 0 and 1, respectively.

Overall, the 3 experiments had a good agreement that spot sampling can be used to estimate daily CH_4 production. In the experiment by Lee et al. (2015), the tendency for a significant interaction between diet and FQ occurred due to lower estimates of CH_4 production for FQ6 and FQ4 depending on dietary treatments. This interaction suggests that FQ12 and FQ8 were the appropriate sampling FQ to estimate daily CH_4 production and detect the effect of dietary treatments. Similar interactions, however, were not observed in the experiments by Warner et al. (2017) and Judy et al. (2018). In the experiment of Warner et al. (2017), FQ tended to affect CH_4 production (L/d), with FQ4 tending to result in lower CH_4 production compared with FQALL. Increasing RMSPE and decreasing CCC as FQ decreased in all the 3 experiments was expected and agrees with our hypothesis that decreasing FQ increases estimation errors. Overall, our results suggest that spot sampling with a minimum of 8 time points (i.e., FQ8 or FQ12) that represents every 3 or 2 h sampling, respectively, in a 24-h cycle is necessary to estimate daily CH_4 production and detect dietary effects.

The efficacy of spot sampling for estimating daily CH_4 production has been previously studied using the GreenFeed system. Hammond et al. (2015) collected spot breath samples using the GreenFeed and compared the estimates of CH_4 production with measures from a respiratory chamber. Animals voluntarily visited the GreenFeed units at an average of 2 visits/animal per day over a week, where the timing of the measurements (visits) within a day differed between animals (i.e., various time points after feeding). In that study, although the daily CH_4 production was similar between the GreenFeed system and the respiratory chamber, the CCC (0.10) indicated no agreement between the 2 methods. In addition, the GreenFeed was not able to detect treatment differences in CH_4 production that were detected by the respiratory chamber. The authors attributed the poor CCC and lack of detection of dietary effects to the limited number of measurements per animal during the 7-d measurement period and the timing of measurements obtained using the GreenFeed system. The estimation accuracy from this type of spot sampling with the GreenFeed in situations of voluntary visits to GreenFeed units can be improved by having a sufficient length of measurement period (7–14 d) and number of samples (20 or more per animal) so that all animals receive sufficient measurements within a 24 h cycle to account for diurnal variation of CH_4 production (Manafiazar et al., 2017). As shown in Figure 1, the timing of measurements within a feeding cycle is critical due to the large diurnal variation in gaseous exchange related to the feeding patterns of cattle.

In cattle, the relationship between DMI and CH_4 production is well established (Niu et al., 2018). However, the lack of strong relationship between DMI and CH_4 observed in some studies (Hristov et al., 2018) raises concern that sampling FQ and timing may pro-

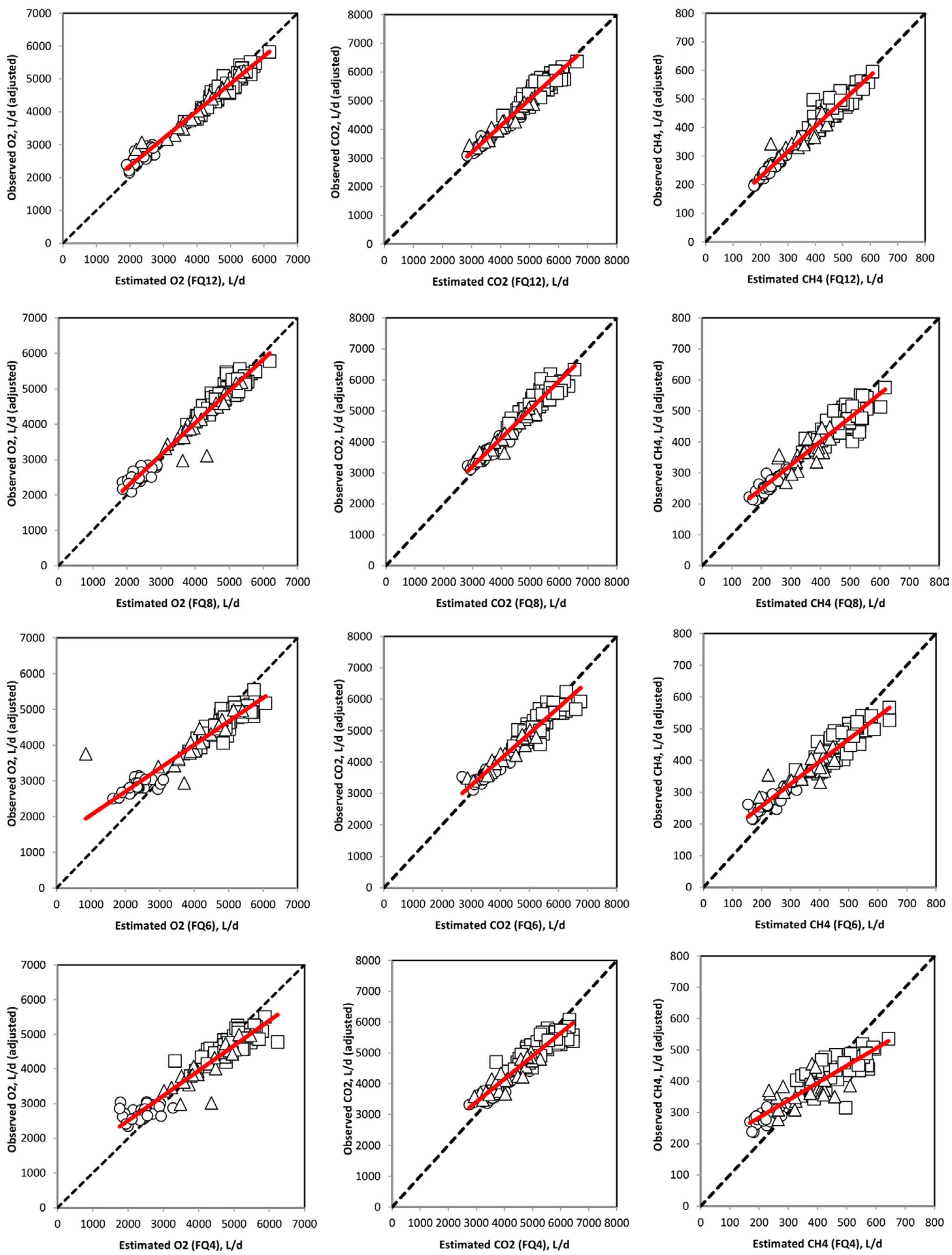

Figure 2. Estimation of O_2 consumption, CO_2 production, and CH_4 production using various spot gas sampling frequencies in studies by Lee et al. (2015; circle), Warner et al. (2017; square), and Judy et al. (2018; triangle). Observed values were obtained from a mixed model including experiment as discrete class variable with experiment effect not shown. Estimation of O_2 consumption (L/d): FQ12, 12 time points [intercept = 687, slope = 0.83; root mean square prediction error (RMSPE) = 251 (6.3%), concordance correlation coefficient (CCC) = 0.98]; FQ8, 8 time points [intercept = 441, slope = 0.88; RMSPE = 255 (6.3%), CCC = 0.97]; FQ6, 6 time points [intercept = 1,180, slope = 0.70; RMSPE = 512 (12.8%), CCC = 0.89]; FQ4, 4 time points [intercept = 1,027, slope = 0.73; RMSPE = 439 (11.1%), CCC = 0.91]. The intercept for FQ8 was not different from 0 ($P > 0.10$) and intercepts for FQ12, FQ6, and FQ4 tended to be different from 0 ($P < 0.10$). All the slopes were different from 1 ($P < 0.05$). Estimation of CO_2 production (L/d): FQ12, 12 time points [intercept = 520, slope = 0.89; RMSPE = 202 (4.3%), CCC = 0.98]; FQ8, 8 time points [intercept = 608, slope = 0.87; RMSPE = 214 (4.6%), CCC = 0.97]; FQ6, 6 time points [intercept = 790, slope = 0.82; RMSPE = 290 (6.3%), CCC = 0.95]; FQ4, 4 time points [intercept = 1,206, slope = 0.73; RMSPE = 366 (7.9%), CCC = 0.92]. All the intercepts tended to be different from 0 ($P < 0.10$) and all the slopes were different from 1 ($P < 0.05$). Estimation of CH_4 production (L/d): FQ12, 12 time points [intercept = 49, slope = 0.88; RMSPE = 27 (6.9%), CCC = 0.97]; FQ8, 8 time points [intercept = 84, slope = 0.78; RMSPE = 40 (10.4%), CCC = 0.93]; FQ6, 6 time points [intercept = 91, slope = 0.76; RMSPE = 45 (11.8%), CCC = 0.91]. FQ4, 4 time points [intercept = 149, slope = 0.60; RMSPE = 64 (16.9%), CCC = 0.80]. The intercept for FQ12 was not different from 0 and intercepts for FQ8, FQ6, and FQ4 tended to be different from 0 ($P < 0.10$). All the slopes were different from 1 ($P < 0.05$). The solid red line represents the linear regression.

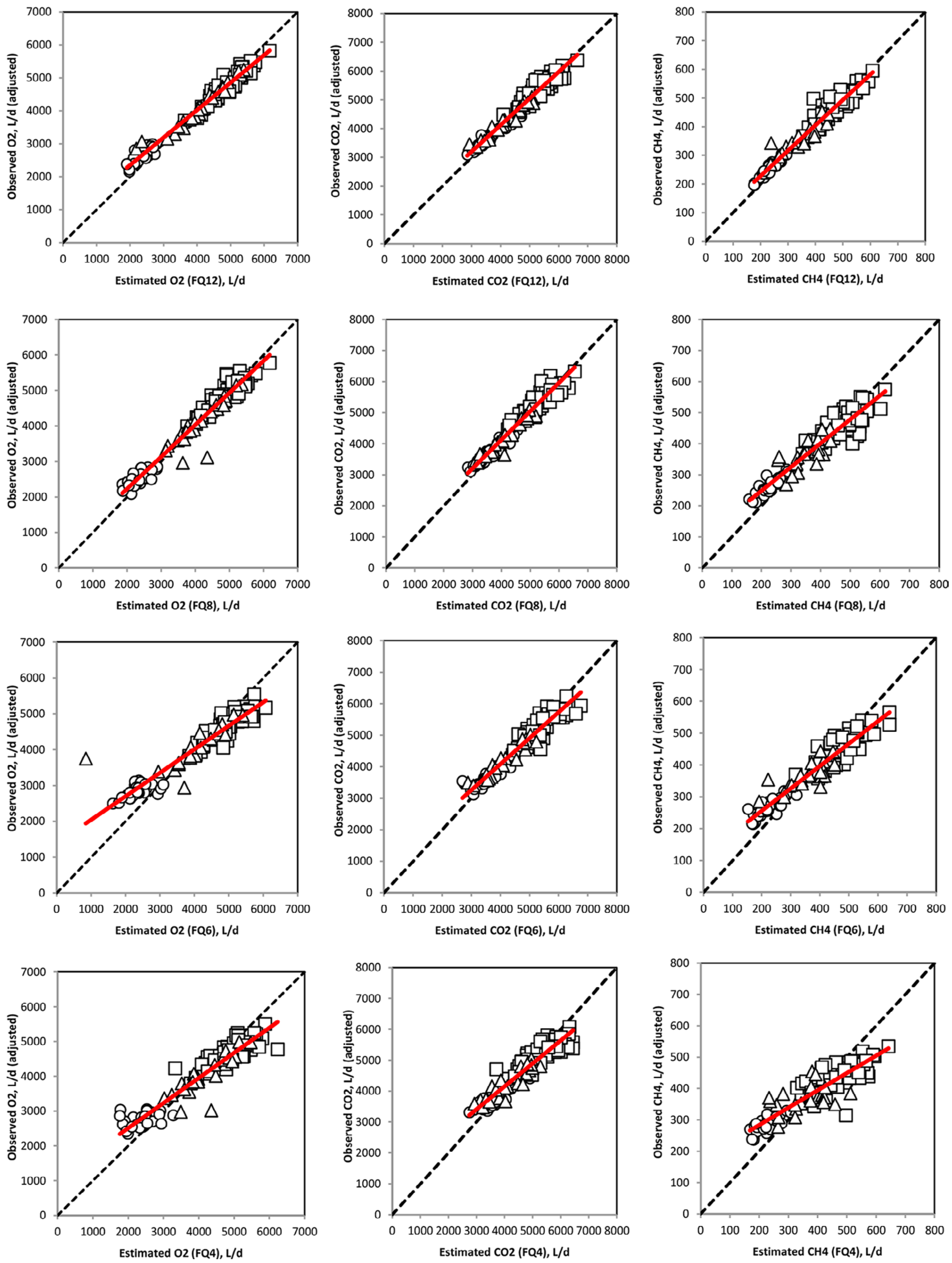

Figure 2 (Continued). Estimation of O_2 consumption, CO_2 production, and CH_4 production using various spot gas sampling frequencies in studies by Lee et al. (2015; circle), Warner et al. (2017; square), and Judy et al. (2018; triangle). Observed values were obtained from a mixed model including experiment as discrete class variable with experiment effect not shown. Estimation of O_2 consumption (L/d): FQ12, 12 time points [intercept = 687, slope = 0.83; root mean square prediction error (RMSPE) = 251 (6.3%), concordance correlation coefficient (CCC) = 0.98]; FQ8, 8 time points [intercept = 441, slope = 0.88; RMSPE = 255 (6.3%), CCC = 0.97]; FQ6, 6 time points [intercept = 1,180, slope = 0.70; RMSPE = 512 (12.8%), CCC = 0.89]; FQ4, 4 time points [intercept = 1,027, slope = 0.73; RMSPE = 439 (11.1%), CCC = 0.91]. The intercept for FQ8 was not different from 0 ($P > 0.10$) and intercepts for FQ12, FQ6, and FQ4 tended to be different from 0 ($P < 0.10$). All the slopes were different from 1 ($P < 0.05$). Estimation of CO_2 production (L/d): FQ12, 12 time points [intercept = 520, slope = 0.89; RMSPE = 202 (4.3%), CCC = 0.98]; FQ8, 8 time points [intercept = 608, slope = 0.87; RMSPE = 214 (4.6%), CCC = 0.97]; FQ6, 6 time points [intercept = 790, slope = 0.82; RMSPE = 290 (6.3%), CCC = 0.95]; FQ4, 4 time points [intercept = 1,206, slope = 0.73; RMSPE = 366 (7.9%), CCC = 0.92]. All the intercepts tended to be different from 0 ($P < 0.10$) and all the slopes were different from 1 ($P < 0.05$). Estimation of CH_4 production (L/d): FQ12, 12 time points [intercept = 49, slope = 0.88; RMSPE = 27 (6.9%), CCC = 0.97]; FQ8, 8 time points [intercept = 84, slope = 0.78; RMSPE = 40 (10.4%), CCC = 0.93]; FQ6, 6 time points [intercept = 91, slope = 0.76; RMSPE = 45 (11.8%), CCC = 0.91]. FQ4, 4 time points [intercept = 149, slope = 0.60; RMSPE = 64 (16.9%), CCC = 0.80]. The intercept for FQ12 was not different from 0 and intercepts for FQ8, FQ6, and FQ4 tended to be different from 0 ($P < 0.10$). All the slopes were different from 1 ($P < 0.05$). The solid red line represents the linear regression.

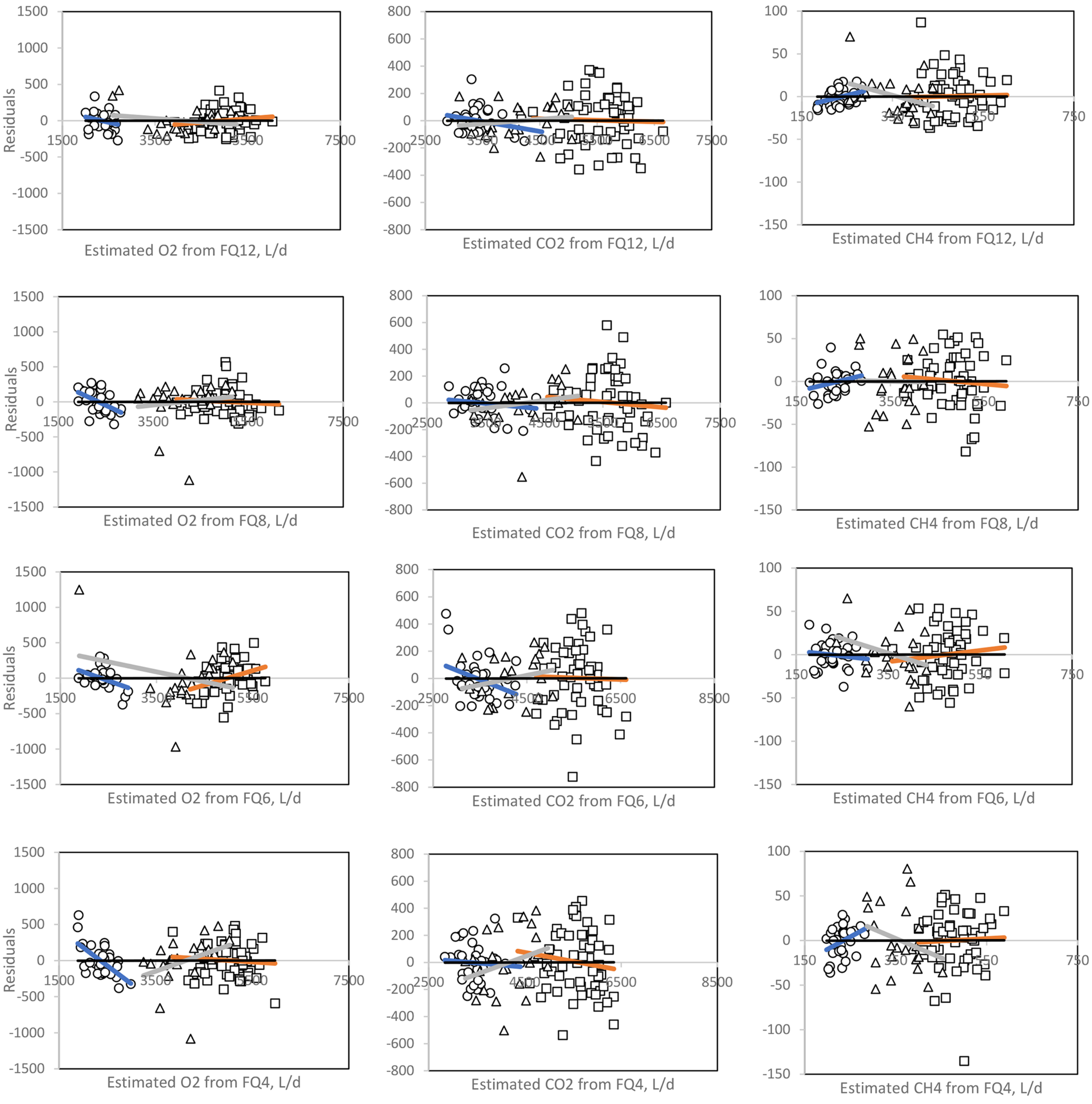

Figure 3. Residual plots for O_2 consumption and CO_2 and CH_4 production estimated from spot sampling with 12, 8, 6, and 4 time points in the studies by Lee et al. (2015; circle and blue line), Warner et al. (2017; square and brown line), and Judy et al. (2018; triangle and green line). Residuals were obtained from a mixed model including experiment as discrete class variable with experiment effect not shown. The solid black line represents the linear regression from all studies combined. FQ12, 12 time points over 24 h: 0, 2, 4, 6, 8, 12, 14, 16, 18, 20, and 22 h after feeding. FQ8, 8 time points over 24 h: 0, 3, 6, 9, 12, 15, 18, and 21 h after feeding. FQ6, 6 time points over 24 h: 0, 4, 8, 12, 16, and 20 h after feeding. FQ4, 4 time points over 24 h: 0, 6, 12, and 18 h after feeding.

vide inaccurate estimates of methane when using the GreenFeed system. The spot sampling procedure that we simulated in this study reflects an approach where all animals would be subjected to measurements of spot gaseous exchanges with equal frequency and at identical time points after feeding, as suggested by Hristov

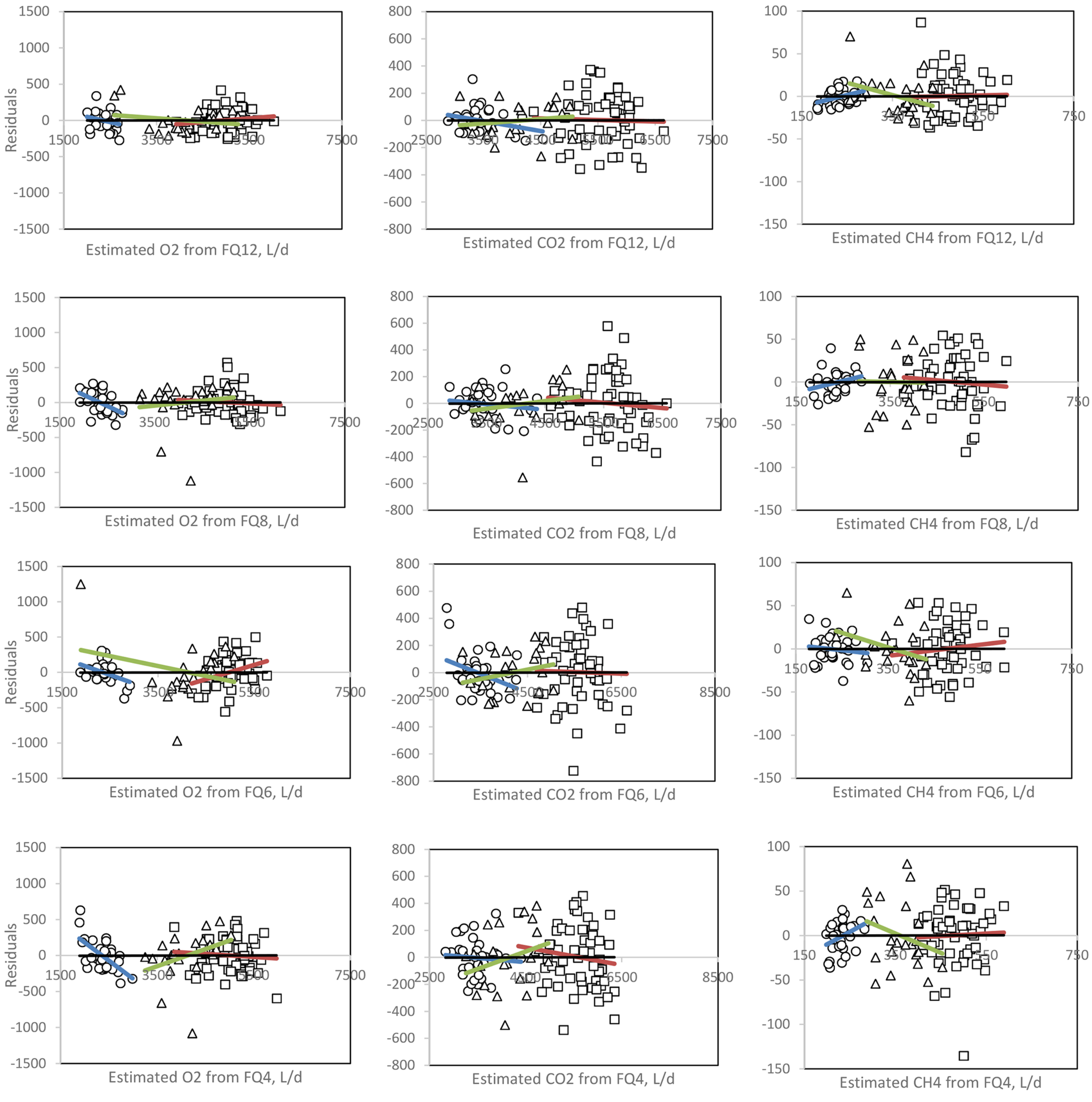

Figure 3 (Continued). Residual plots for O_2 consumption and CO_2 and CH_4 production estimated from spot sampling with 12, 8, 6, and 4 time points in the studies by Lee et al. (2015; circle and blue line), Warner et al. (2017; square and brown line), and Judy et al. (2018; triangle and green line). Residuals were obtained from a mixed model including experiment as discrete class variable with experiment effect not shown. The solid black line represents the linear regression from all studies combined. FQ12, 12 time points over 24 h: 0, 2, 4, 6, 8, 12, 14, 16, 18, 20, and 22 h after feeding. FQ8, 8 time points over 24 h: 0, 3, 6, 9, 12, 15, 18, and 21 h after feeding. FQ6, 6 time points over 24 h: 0, 4, 8, 12, 16, and 20 h after feeding. FQ4, 4 time points over 24 h: 0, 6, 12, and 18 h after feeding.

et al. (2015a). Our data suggest that, compared with daily CH_4 production measured in respiration chambers (whole-animal enclosure or headbox style), spot sampling has potential to estimate daily CH_4 production when sampling frequency and time points are consistent across animals and dietary treatments and when at least 8 time points of spot measurements are performed over multiple days to represent a 24-h feeding cycle.

Daily O_2 consumption and CO_2 production are the 2 major components required to calculate HP, and thus their accurate measurement is important to obtain accurate HP estimates. Gunter et al. (2018) estimated daily O_2 consumption of grazing cattle during a 77-d period where individual animals voluntarily visited a GreenFeed unit about 4 times a day. Although this study did not report details about the sampling procedure and did not report the O_2 consumption estimated from the spot sampling (only an abstract is available), the authors considered the calculated HP (12.2 Mcal/d of HP) reasonable for beef cattle (BW, 241 kg) fed mainly long-stemmed wheat hay. Guinguina et al. (2021) also used a GreenFeed system to measure O_2 consumption of dairy cows over 18 wk of lactation where each animal was allowed to receive measurement at a minimum of 5-h intervals (on average 395 measurements per cow over 18 wk). The estimated daily O_2 consumption in that study was 5,598 L/d and they reported repeatability estimates of O_2 to be higher (0.78) than for CO_2 (0.72) and CH_4 (0.58). In line with these repeatability estimates of Guinguina et al. (2021), in the 3 experiments examined in the current study we observed no difference in daily O_2 consumption among FQ and no interaction between diet and FQ. This suggests that all the FQ examined (i.e., FQ12 to FQ4) were able to estimate daily O_2 consumption with good agreement with that of FQALL. A good estimate of daily O_2 consumption with low frequency of spot sampling (e.g., FQ4) is likely because of its relatively small diurnal variation compared with other gaseous exchanges as shown in Figure 1. However, general trends of increasing RMSPE and decreasing CCC as FQ decreased in all 3 experiments suggests that estimation accuracy of O_2 consumption still becomes poor as FQ decreases, despite the similar daily O_2 consumption estimates compared with FQALL. Consumption of O_2 estimated from FQ4 in Lee et al. (2015) had poor CCC (0.52), suggesting at least FQ6 (CCC of 0.73) is needed to estimate O_2 consumption using spot sampling. We found no effect of FQ or interaction between diet and FQ for CO_2 production in the experiments of Lee et al. (2015) and Judy et al. (2018), suggesting that all simulated FQ estimated CO_2 production in good agreement with FQALL in these studies. However, lower CO_2 production for FQ4 compared with FQ8, FQ12, and FQALL in the experiment of Warner et al. (2017) indicated that FQ4 failed to accurately estimate daily CO_2 production in this study. The discrepancy between Warner et al. (2017) and the other 2 experiments probably occurred because of twice-a-day feeding for all animals in Warner et al. (2017) causing more dynamic diurnal variation (i.e., 2 peaks in a 24-h cycle) although some animals in Judy et al. (2018) were also fed twice daily. This suggests that optimal frequency of spot sampling to estimate gaseous exchanges can change depending on factors altering diurnal variation.

Estimating daily CO_2 production with spot sampling has been conducted in previous studies using the GreenFeed system (e.g., Lopes et al., 2016; Alemu et al., 2017; Melgar et al., 2021). As discussed earlier, the spot sampling procedure for CO_2 production was either the procedure similar to the simulation in the current study [i.e., procedure by Hristov et al. (2015a)], or voluntarily visiting GreenFeed (about 4 to 6 times per animal a day over several days). However, to our knowledge, spot sampling frequencies have not been previously validated for estimation accuracy of CO_2. In the current simulation, RMSPE and CCC from all 3 experiments indicated that the estimate of CO_2 production became more accurate as FQ increased, which is expected and in line with O_2 consumption and CH_4 production. Because RMSPE was less than 10% of observed mean and CCC was greater than 0.80 for all FQ in the 3 experiments, but the estimate for FQ4 was different from the estimate from FQALL in the experiment by Warner et al. (2017), we conclude that at least 6 time points of spot sampling within a 24-h cycle (every 4 h sampling in a feeding cycle) are required to estimate daily CO_2 production.

Heat production is calculated (Brouwer equation) using O_2 consumption and CO_2 production, with or without CH_4 production and N excretion. Based on the coefficient and mass of the components, changes in O_2 consumption have the largest effect on HP with a moderate effect of CO_2 changes. Effects by changes in CH_4 production and N excretion in urine are relatively small. This is probably the reason why the effect of FQ on HP was almost the same as that for O_2 consumption in all 3 experiments, although FQ, diet, or their interactions occasionally affected CO_2 (Warner et al., 2017) or CH_4 production (Lee et al., 2015 and Warner et al., 2017).

The present simulation analysis of 3 respiratory chamber experiments (Lee et al., 2015; Warner et al., 2017; Judy et al., 2018) indicates that spot sampling has potential to estimate daily gaseous exchanges and HP in ruminant animals. Spot sampling at a frequency of at least FQ8 was needed for all 3 experiments for accurate estimation of CH_4 production and detection of dietary treatment effects. For O_2 consumption and CO_2 production, spot sampling at a frequency of at least FQ6 was needed to be satisfactory for all 3 experiments. When gaseous exchanges are measured in an energy metabolism experiment, all the gaseous components (i.e., O_2, CO_2, CH_4) should be measured simultaneously at each time point. Therefore, we concluded that FQ8 is the minimum sampling frequency required to estimate daily gaseous exchanges and HP and detect effects of dietary treatments via breath spot sampling.

It is worth noting that the optimum frequency of spot sampling above was determined mainly based on FQ effects in comparison with FQALL and interactions between diet and FQ within experiment (Tables 2, 4, and 6). However, the intercepts and slopes (Tables 3, 5, and 7) can be also considered good indicators for accurate estimation. Although intercepts and slopes generally became farther from 0 and 1, respectively, as FQ decreased, most intercepts and slopes were significantly different from 0 and 1. In addition, when an intercept and slope did not differ from 0 and 1, respectively, for certain FQ, this pattern was not consistent across studies. Although FQ12 and FQ8 were considered optimum for gaseous exchanges, the intercepts and slopes for many of gaseous exchanges from FQ12 and FQ8 were different from 0 and 1 across the 3 experiments. This suggests that estimates of gaseous exchanges from FQ12 and FQ8 may be accurate only within the range of gaseous exchanges observed within the 3 experiments (Table 1) and not be appropriate for animals with extremely high or low daily production of gaseous exchanges. However, when CH_4 production becomes extremely depressed, diurnal variation of CH_4 production also decreases considerably. For example, Vyas et al. (2016) observed a decrease in CH_4 production by about 80% (162 to 25 L/d of CH_4) for beef cattle fed a finishing diet supplemented with 3-nitrooxypropanol and the diurnal variation of CH_4 production barely existed. Then, the spot sampling of FQ12 and FQ8 (even less FQ) would be able to estimate the extremely low CH_4 production accurately.

The results from the regression analysis of the combined data (Figure 2 and 3) support the results from analysis within study. The variation of estimating gaseous exchanges increased and CCC decreased as FQ decreased. Although the intercepts and slopes usually were different from 0 and 1, respectively, they came farther from 0 and 1 as FQ decreased. We expected to observe improvements on gaseous exchanges estimated from FQ in the combined data compared with estimation within study because of the larger number of observations, but the estimation accuracy was comparable according to RMSPE, CCC, intercept, and slope. Although the residual plots showed better symmetrical distribution when combined data were used compared with the plot within study, this lack of improvement of estimation probably indicates that sufficient collection of spot samples to account for diurnal variation of gaseous exchanges is more important than increasing the number of observations.

Researchers should consider 3 critical factors when spot sampling is used to accurately estimate daily gaseous exchanges and HP. First, the current study simulated spot sampling during a 1- or 3-d sampling period, but more days of measurement can be possible (i.e., 8 spot sample collection over days more than 3 d. As gaseous exchanges) especially CH_4 production, are directly affected by DMI (Nielsen et al., 2013; Knapp et al., 2014), it is important that DMI of the animals be similar each day. If one or more of the days has significantly lower or higher DMI during the measurement period it will increase estimation errors and decrease accuracy. Collecting spot samples over a period of less than 3 d can be done as the simulation from Judy et al. (2018), but more frequent measurements in each day is required and this may affect feeding behavior. Second, accurate and precise quantification of gaseous exchanges at each time point during spot sampling is essential to reduce estimation errors. To achieve this, appropriate system gas recovery tests need to be performed, to identify potential sources of experimental errors and reduce these errors (Gerrits et al., 2018; McGinn et al., 2021). Finally, in our analyses we assumed a consistent equally-spaced scheme of sampling within a day. Spot sampling biased toward specific periods before or after a large meal may result in biased estimates of gaseous exchange given diurnal variation in gas production and consumption. Thus, the sampling scheme used should reflect an appropriate balance of the variation in a full 24-h period.

CONCLUSIONS

The evaluation of data on gaseous exchange of 3 indirect calorimetry experiments showed that spot sampling with certain sampling frequency successfully estimated daily gaseous exchanges and HP from cattle. The current sampling procedure examined spot samples at 12, 8, 6, and 4 time point sampling FQ representing sampling every 2, 3, 4, and 6 h, respectively, in a 24-h cycle. Accuracy in estimating gaseous exchange and HP became poorer as sampling FQ decreased. Results from the 3 experiments indicated that spot sampling with FQ of at least 8 time points, over a single day or multiple days to represent sampling every 3 h within a 24-h cycle, provides accurate estimates of gaseous exchange and HP and could detect differences in response to dietary treatments. The minimal number of observations in spot sampling schemes suggested by the present analysis may be considered when using techniques that quantify gaseous exchanges from breath samples such as the GreenFeed or face mask systems in individual feeding studies (i.e., tiestalls). When used, the measuring units should be employed according to standard operating protocols to ensure adequate calibration and appropriate operation.

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