Methane emissions among individual dairy cows during milking quantified by eructation peaks or ratio with carbon dioxide

M. J. Bell, N. Saunders, R. H. Wilcox, E. M. Homer, J. R. Goodman, J. Craigon, and P. C. Garnsworthy¹

The University of Nottingham, School of Biosciences, Sutton Bonington Campus, Loughborough LE12 5RD, UK

ABSTRACT

The aims of this study were to compare methods for examining measurements of CH₄ and CO₂ emissions of dairy cows during milking and to assess repeatability and variation of CH₄ emissions among individual dairy cows. Measurements of CH₄ and CO₂ emissions from 36 cows were collected in 3 consecutive feeding periods. In the first period, cows were fed a commercial partial mixed ration (PMR) containing 69% forage. In the second and third periods, the same 36 cows were fed a high-forage PMR ration containing 75% forage, with either a high grass silage or high maize silage content. Emissions of CH₄ during each milking were examined using 2 methods. First, peaks in CH₄ concentration due to eructations during milking were quantified. Second, ratios of CH₄ and CO₂ average concentrations during milking were calculated. A linear mixed model was used to assess differences between PMR. Variation in CH₄ emissions was observed among cows after adjusting for effects of lactation number, week of lactation, diet, individual cow, and feeding period, with coefficients of variation estimated from variance components ranging from 11 to 14% across diets and methods of quantifying emissions. No significant difference was detected between the 3 PMR in CH₄ emissions estimated by either method. Emissions of CH₄ calculated from eructation peaks or as CH₄ to CO₂ ratio were positively associated with forage dry matter intake. Ranking of cows according to CH₄ emissions on different diets was correlated for both methods, although rank correlations and repeatability were greater for CH₄ concentration from eructation peaks than for CH₄-to-CO₂ ratio. We conclude that quantifying enteric CH₄ emissions either using eructation peaks in concentration or as CH₄-to-CO₂ ratio can provide highly repeatable phenotypes for ranking cows on CH₄ output.

Key words: dairy cow, methane, carbon dioxide, phenotype, repeatability

INTRODUCTION

Enteric methane (CH₄) emissions from ruminants have gained research interest due to the association between greenhouse gas concentrations in the atmosphere and global climate change. A large proportion of the variation in enteric CH₄ emissions from animals can be explained by diet composition and feed intake (Bell and Eckard, 2012). In addition to the variation in CH₄ explained by diet, considerable variation exists among individual dairy cows (de Haas et al., 2011; Garnsworthy et al., 2012a; Huhtanen et al., 2013), suggesting a scope for selective breeding. Compared with diet manipulation, outcomes of selective breeding are permanent and cumulative. A repeatable and accurate phenotype is required, however, to allow selection of animals for reduced emissions.

Use of respiration chambers is impractical for large-scale estimation of CH₄ emissions by individual cows on commercial dairy farms. Quantifying enteric CH₄ emissions during milking by using low-cost and mobile technologies has been demonstrated to provide repeatable phenotypic estimates of CH₄ emissions under commercial conditions (Garnsworthy et al., 2012a,b; Lassen et al., 2012). In the study of Garnsworthy et al. (2012a), estimates of CH₄ made during milking were correlated with total daily CH₄ emissions by the same cows when housed subsequently in respiration chambers.

The studies of Garnsworthy et al. (2012a) and Lassen et al. (2012) used similar technologies for measuring CH₄; both sampled gas from the feed bin of automatic (robotic) milking stations while cows were being milked, and measured CH₄ concentrations with portable gas analyzers. Subsequent handling and analysis of data, however, differed between studies; Garnsworthy et al. (2012a) analyzed CH₄ only released by eructation, whereas Lassen et al. (2012) calculated ratios of average CH₄ to average CO₂ concentrations of cows throughout milking, as proposed by Madsen et al. (2010). The equivalence of these 2 approaches is unknown, but is fundamentally important for comparison of findings from these and subsequent studies.

The objective of the current study was to assess repeatability and variation in CH₄ and CO₂ emissions

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¹Corresponding author: Phil.Garnsworthy@nottingham.ac.uk

from eructation peaks, average concentrations during milking, and their ratio, by dairy cows fed on diets differing in forage composition.

MATERIALS AND METHODS

Animal work was conducted under the authority of the United Kingdom Animal (Scientific Procedures) Act 1986, and approval was obtained from the University of Nottingham animal ethics committee (Sutton Bonington, UK) before commencement of the study.

Data

Concentrations of CH₄ and CO₂ from Holstein-Friesian dairy cows were measured during milking at Nottingham University Dairy Centre (Sutton Bonington, UK). Cows were group housed in a freestall barn and milked individually at an automatic (robotic) milking station (AMS). Gas concentrations in air sampled from the feed bin of the AMS were measured continuously by infrared analyzers (Guardian Plus; Edinburgh Instruments Ltd., Livingston, UK) throughout the sampling period of 35 d. For a full description of the technique, see Garnsworthy et al. (2012a). The technique is briefly described below.

The CH₄ and CO₂ concentrations were logged at 1-s intervals on data loggers (Simex SRD-99; Simex Sp. z o.o., Gdańsk, Poland) and visualized using logging software (Loggy Soft; Simex Sp. z o.o.). Analyzers were calibrated using standard mixtures of gases in nitrogen (0.0 and 1.0% CH₄, and 5% CO₂; Thames Restek UK Ltd., Saunderton, UK).

Concentrations of CH₄ and CO₂ measured in parts per million (vol/vol) were converted to milligrams per liter by assuming the density of CH₄ to be 655.7 mg/L and CO₂ to be 1798.9 mg/L at 25°C, 1 atmosphere (101.3 kPa) pressure, with the analyzer sampling air at 1 L/min. Concentrations of CH₄ and CO₂ emitted during each milking were calculated from (1) area under the curve of eructation peaks [integral of concentrations minus concentration at the start of each peak; (mg/L)·min], multiplied by frequency of eructation peaks (peaks per minute) or (2) average concentration during the milking period minus the minimum (baseline or background) concentration at the start of the milking (Figure 1). Method 1 used a custom-designed program to identify and quantify eructation peaks of CH₄ concentration during milking (eructation CH₄) from raw logger data (Garnsworthy et al., 2012a). In method 1, milkings with less than 3 eructation peaks for CH₄ concentration were excluded from the analysis. Peaks of CO₂ concentration were not calculated using

this method due to lack of distinct peaks originating from eructation (Figure 1). Method 2 used the average of all recorded CH₄ (average CH₄) and CO₂ (average CO₂) concentrations (mg/L) during each milking to derive the ratio of CH₄ to CO₂ concentrations (expressed as g/kg).

Emissions were measured during 3 consecutive feeding periods, in which cows were fed partial mixed rations (PMR; Table 1) ad libitum plus concentrates during milking (AMS concentrates). In the first period, 36 cows were fed for 7 d a commercial PMR containing 69% forage (Table 1). In the second and third periods, the same 36 cows were fed, in a 14-d crossover design, PMR containing 75% forage with high proportions of either grass silage or maize silage (Table 1). Feeding periods followed immediately with no adjustment period between diets. The daily AMS concentrate allowance fed during milking was 1.5 kg plus 0.16 kg per liter of milk yield above 23 L/d. The AMS concentrates were dispensed into the feed bin at 360 g/min in 6 portions per minute throughout the milking period, which helped to keep the cow's head within suitable proximity of the gas sampling tube. The AMS concentrate dispensers were calibrated monthly by weighing quantities dispensed. The AMS concentrate manufacturer's declared specification per kilogram as fed was as follows: ME: 12.2 MJ, CP: 16%, NDF: 24%, starch: 21%, and fat: 6.2%. Milk yield, BW, and AMS concentrate intake were recorded automatically at each milking. Dry matter intake of PMR was recorded automatically by electronic feeders. Total daily DMI of concentrates was calculated from AMS concentrate intake plus intake of concentrates in the PMR.

For comparison with other studies, the method of Madsen et al. (2010) was used to estimate daily heat produced by each cow in megajoules per day ($5.6 \times \text{kg of BW}^{0.75} + 22 \times \text{kg of milk yield per day} + 0.000016 \times \text{days pregnant}^3 \times 0.0864$), which was then converted to estimated CO₂ emissions in grams per day.

Statistical Analysis

Data were analyzed using a linear mixed model in GenStat software (version 15.1; VSN International Ltd., Hemel Hempstead, UK; Lawes Agricultural Trust, 2012). Equation 1 was used to assess the effect of diet on average DMI, milk yield (both kg/d), average number of milkings per day, average duration of milking (s), BW (kg), eructation CH₄, average CH₄, average CO₂, and CH₄-to-CO₂ ratio per individual cow:

$$y_{ijk} = \mu + L_i + W_j + D_i + C_j + C_j P_k + E_{jk}, \quad [1]$$

Figure 1. Concentration in parts per million of (a) CH₄ and (b) CO₂ during a single milking visualized by the data logging software (Loggy Soft; Simex Sp. z o.o., Gdańsk, Poland).

where y_{ijk} is the dependent variable, μ = overall mean, L_i = fixed effect of lactation number (1, 2, or 3+), W_j = fixed effect of week of lactation (1, 2, 3, . . .), D_i = fixed effect of diet, C_j = random effect of individual cow, P_k

= random effect of sampling period within cow, and E_{jk} = random error term (df = 53).

The residual coefficient of variation was calculated from variance components as root mean square error

Table 1. Composition and analysis of commercial, high-grass-silage, and high-maize-silage partial mixed rations (PMR)

Composition (g/kg of DM)	PMR		
	Commercial	Grass silage	Maize silage
Grass silage	226	360	193
Maize silage	253	210	361
Whole-crop wheat silage	215	178	184
Soybean meal	80	66	68
Rapeseed meal	80	66	68
DDGS ¹	24	20	20
Soybean hulls	24	20	20
Sugar beet pulp	24	20	20
Beet molasses	40	33	34
Fat supplement ²	13	11	11
Minerals and vitamins ³	22	18	19
Analysis ⁴			
DM (g/kg)	463	425	453
ME (MJ/kg of DM)	12.0	12.1	11.9
CP (g/kg of DM)	175	171	162
NDF (g/kg of DM)	367	374	379
Starch (g/kg of DM)	163	135	200
Sugars (g/kg of DM)	67	60	58
Crude fat (g/kg of DM)	37	37	36
Forage DM (% of total DM)	69	75	75

¹Distillers dried grains with solubles (maize).

²Butterfat Extra (Trident Feeds, Peterborough, UK).

³Contained 18% Ca, 10% P, 5% Mg, 17% salt, 2,000 mg of Cu/kg, 5,000 mg of Mn/kg, 100 mg of Co/kg, 6,000 mg of Zn/kg, 500 mg of I/kg, 25 mg of Se/kg, 400,000 IU of vitamin A/kg, 80,000 IU of vitamin D₃/kg, and 1,000 mg of vitamin E/kg.

⁴All ingredients were analyzed by a commercial analytical laboratory (Sciencetec Analytical Services, Cawood, UK).

divided by estimated mean. Repeatability of production and gas emission variables were assessed by σ^2 animal/ $(\sigma^2$ animal + σ^2 residual), where σ^2 is the variance. Spearman's rank correlation was used to assess persistency of ranking of individual cow emissions on the commercial diet and high-forage diets. Pearson correlation coefficient was used to assess the association between total DMI, forage DMI, concentrate DMI, milk yield, BW, and eructation CH₄, and CH₄-to-CO₂ ratio across all individual cow records.

RESULTS

Cow Performance

No effect of diet on BW was detected, but DMI was lower when cows were fed the commercial diet than when they were fed the high-forage diets (Table 2). The milk yield of cows on the commercial diet was lower than when they were fed the high-grass-silage diet. No difference existed between the high-forage diets, however, in DMI, milk yield, or BW. Cows presented themselves for milking fewer times per day when they were fed the commercial diet than when they were fed the high-forage diets, but no effect of diet on duration of milking visits to the AMS was detected (Table 2).

CH₄ and CO₂ Concentrations

No effect of diet on frequency of eructations during milking was detected, as indicated by peaks in CH₄ concentration (mean 1.0 ± 0.1 eructations per minute). Diurnal variation was observed in eructation CH₄ concentration and in CH₄-to-CO₂ ratio; both were at their lowest during early morning and generally highest in the afternoon (Figure 2).

Eructation CH₄ concentration was lower than average CH₄ concentration (Table 2). For both indicators of CH₄ emissions, means were not significantly different when cows were fed the commercial diet compared with when cows were fed the high-forage diets. Average CO₂ concentration and CH₄-to-CO₂ ratio were not affected by diet.

Daily DMI was positively associated with average CH₄ concentration ($r = 0.22$; $P < 0.05$). Forage DMI was positively associated with eructation CH₄ concentration ($r = 0.19$; $P < 0.05$), average CH₄ concentration ($r = 0.29$; $P < 0.001$), and CH₄-to-CO₂ ratio ($r = 0.24$; $P < 0.05$). Daily milk yield was negatively correlated ($r = -0.21$; $P < 0.05$) with eructation CH₄ concentration. No association existed between BW and CH₄ or CO₂ concentrations.

Table 2. Least squares means, variability, repeatability, and rank correlation (*r*) of production, CH₄, and CO₂ phenotypes for cows fed commercial, high-grass-silage, and high-maize-silage partial mixed rations

Phenotype	Units	Partial mixed ration ¹				Residual CV (%)	Repeatability	Rank correlation ³	
		Commercial	High grass silage	High maize silage	SED ²			<i>r</i>	<i>P</i> -value
DMI	kg/d	17.8 ^a	19.8 ^b	19.4 ^b	0.7	<0.05	0.42	0.63	<0.001
Milk yield	kg/d	29.7 ^a	33.3 ^b	31.5 ^{ab}	1.2	<0.05	0.82	0.92	<0.001
BW	kg/d	662	664	661	2.8	0.29	0.98	0.97	<0.001
Milkings per day	No.	2.6 ^a	3.2 ^b	3.1 ^b	0.2	<0.05	0.26	0.75	<0.001
Milking duration	s	389	387	386	9.6	0.97	0.92	0.96	<0.001
Eructation peaks									
CH ₄	mg/L	0.12	0.11	0.11	0.01	0.75	0.75	0.80	<0.001
Average concentration									
CH ₄	mg/L	0.25	0.24	0.24	0.01	0.54	0.74	0.72	<0.001
CO ₂	mg/L	8.4	8.6	8.7	0.2	0.29	0.86	0.82	<0.001
CH ₄ :CO ₂ ratio	g/kg	29.8	30.7	29.7	1.1	0.59	0.54	0.59	<0.001

^{a,b}Means within a row with different superscript letters differ ($P < 0.05$).

¹In consecutive feeding periods, 36 cows were fed a commercial ration (period 1) followed by 2 diets containing higher proportions of grass silage or maize silage in a crossover design (periods 2 and 3).

²Standard error of the difference.

³Values for 36 cows fed on a commercial diet (period 1) were compared with average values for the same 36 individual cows in periods 2 and 3.

Variation Among Cows and Repeatability of Phenotypes

Residual coefficient of variation was slightly greater for eructation CH₄ concentration than for average CH₄ concentration, average CO₂ concentration, and CH₄-to-CO₂ ratio (Table 2). Residual coefficients of variation in DMI and milk yield were of similar magnitude to that of CH₄-to-CO₂ ratio. Repeatability was similar for eructation CH₄ concentration, average CH₄ concentration, average CO₂ concentration, daily milk yield, milking duration, and BW, but repeatability values for DMI, milkings per day, and CH₄-to-CO₂ ratio were lower than for other phenotypes (Table 2).

When cows were fed the commercial diet, rank correlations were 0.62 ($P < 0.001$) between ranking on eructation CH₄ concentration and ranking on average CH₄ concentration (Figure 3a), and 0.35 ($P < 0.05$) between ranking on eructation CH₄ concentration and ranking on CH₄-to-CO₂ ratio (Figure 3b). When fed on the high-forage diets, rank correlations were 0.86 ($P < 0.001$) between ranking on eructation CH₄ concentration and ranking on average CH₄ concentration (Figure 3a), and 0.53 ($P < 0.05$) between ranking on eructation CH₄ concentration and ranking on CH₄-to-CO₂ ratio (Figure 3b).

Rank correlation coefficients obtained by comparing ranking of cows when fed the commercial PMR and when fed the high-forage diets were high and positive for all production and emission phenotypes (Table 2). The rank correlation coefficient was higher, however, for eructation CH₄ concentration than for CH₄-to-CO₂ ratio (Table 2; Figure 4).

Average heat production estimated by the equation ($5.6 \times \text{kg BW}^{0.75} + 22 \times \text{kg of milk yield per day} + 0.000016 \times \text{days pregnant}^3 \times 0.0864$) of Madsen et al. (2010) was 124 MJ/d for the commercial diet, 127 MJ/d for the high-grass-silage diet, and 126 MJ/d for the high-maize-silage diet. Extrapolated estimates of daily CO₂ emissions were 11,161 g/d for the commercial diet, 11,454 g/d for the high-grass-silage diet, and 11,308 g/d for the high-maize-silage diet. No relationship existed between observed CO₂ concentrations during milking and daily CO₂ emissions estimated from heat production (Figure 5). Observed average CO₂ concentration was more variable (CV = 18.7%) than estimated daily CO₂ emission (CV = 13.4%).

DISCUSSION

This study is the first to compare online methods for estimating enteric CH₄ emissions from dairy cows during milking in the same individual cows. Because measurements of CH₄ and CO₂ were made concur-

Figure 2. Average CH_4 concentration (with SE bars) calculated from (a) eructation peaks and (b) the ratio of CH_4 to CO_2 concentrations for individual cows for each hour of the day from all records collected during the study period.

rently, using the same gas samples and instruments, any differences between methods can be ascribed to differences in kinetics of CH_4 and CO_2 release. Thus, comparisons are not confounded by differences between experimental conditions and research centers. Further-

more, the design of the study permits separation of within-cow, between-cow, diet, and temporal effects on CH_4 emissions to examine variation and repeatability of estimates. Quantifying variation and repeatability of phenotypes is an essential prerequisite for combining

Figure 3. Relationship between CH₄ concentration calculated from eructation peaks and (a) the average CH₄ concentration over each milking, and (b) the ratio of CH₄ to CO₂ concentrations for individual cows fed a commercial partial mixed ration (PMR; ■) or high-forage PMR (□). The rank correlation (r) is shown with the line of best fit for the commercial PMR (dashed line) and high-forage PMR (solid line).

data sets derived by different methods in international collaborations.

Individual cow eructation CH₄ was a highly repeatable phenotype, confirming our previous studies (Garn-

sworthy et al., 2012a,b). Average CH₄ and average CO₂ concentration showed a level of repeatability similar to that of eructation CH₄, but CH₄-to-CO₂ ratio was less repeatable. Repeatability of CH₄-to-CO₂ ratio (0.54)

Figure 4. Relationship between CH_4 concentration during milking from individual cows on a commercial partial mixed ration (PMR) and high-forage PMR calculated from (a) eructation peaks and (b) ratio of CH_4 to CO_2 concentrations averaged over each milking. The rank correlation (r) is shown with the line of best fit.

was higher than repeatability values of 0.37 in the study of Lassen et al. (2012), and 0.34 in experiment 1 of Huhtanen et al. (2013), although in a second experi-

ment, Huhtanen et al. (2013) found a repeatability of 0.9 for CH_4 -to- CO_2 ratio. In our previous studies, where CH_4 emissions were calculated from eructation peaks,

Figure 5. Relationship between average CO₂ concentration over each milking and average daily CO₂ emissions estimated using the method of Madsen et al. (2010) for individual cows fed commercial partial mixed ration (PMR; ■), high-grass-silage PMR (+), and high-maize-silage PMR (▲).

repeatability was 0.78 between diets (Garnsworthy et al., 2012a).

Mean average CH₄ concentration was approximately double the mean eructation CH₄ concentration, as expected from the methods of calculation. Average CH₄ concentration was calculated across each milking, subtracting the lowest concentration at the start of the milking; eructation CH₄ concentration was calculated from the integral of concentrations across each eructation peak, subtracting the lowest concentration at the start of the peak. Average CH₄ concentration, therefore, adjusts for changes in ambient CH₄ concentration at different milkings, whereas eructation CH₄ concentration adjusts not only for ambient CH₄ concentration, but also for build-up of CH₄ during milking, and considers only CH₄ released by eructation rather than in breath.

The coefficient of variation in CH₄ emissions ranged from 11% for CH₄-to-CO₂ ratio to 14% for eructation CH₄ concentration. The greater variation in eructation CH₄ concentration, average CH₄ concentration, and CH₄-to-CO₂ ratio compared with average CO₂ concentration may be explained partly by differences in the way that CH₄ and CO₂ are emitted by cows. Methane emissions arise from enteric fermentation, whereas CO₂ emissions arise from both enteric fermentation and metabolic CO₂ excreted via the lungs. For CH₄, 83% of daily production by sheep was released by eructation, irrespective of feeding level (Blaxter and Joyce, 1963;

Murray et al., 1976), whereas for CO₂, the proportion of CO₂ released by eructation varied with CH₄ production and level of feeding, so that in eructed gas, CO₂ concentration was 30% of CH₄ concentration when CH₄ production was 1 L/h and 140% of CH₄ concentration when CH₄ production was 2.5 L/h (Blaxter and Joyce, 1963). This effect would dampen variation in CO₂ concentrations measured in eructed gas. When quantifying emissions from eructation peaks, it can be expected that this method would be more appropriate for identifying eructed CH₄ rather than more slowly emitted CO₂ emissions in breath where peaks in concentration are less defined (Figure 1). Furthermore, Blaxter and Joyce (1963) reported that during feeding, the loss of CO₂ is proportionally greater than it is between meals; an observation made also in our chamber studies (Garnsworthy et al., 2012a). This is an important consideration when analyzing gas samples produced during milking in an AMS, which involves concurrent feeding.

The range in coefficient of variation among cows is within the range of 3 to 34% in coefficient of variation found in studies using respiration chambers to measure emissions in research herds (Ellis et al., 2007; Grainger et al., 2007; Yan et al., 2010), and is lower than the value of 28.8% found using eructation peaks on farm in our previous study (Garnsworthy et al., 2012a). By expressing enteric CH₄ emissions as a ratio to CO₂ emissions, variation among cows and repeatability of

the phenotype were similar to variation and repeatability of DMI, which was also found by Huhtanen et al. (2013).

All CH₄ emission phenotypes studied were positively ($r = 0.19$ to 0.24) correlated with forage DMI, although only average CH₄ concentration was positively ($r = 0.22$) associated with total DMI. Positive correlations with forage DMI are expected because CH₄ arises primarily from hydrogen released during enteric fermentation of plant cell walls to produce acetate (Beauchemin et al., 2009). The lack of correlation between total DMI and eructation CH₄, however, does not agree with chamber studies (e.g., Ellis et al., 2007; Grainger et al., 2007; Yan et al., 2010), in which strong positive relationships were observed. A possible explanation for this apparent discrepancy lies in the relative effects on CH₄ of DMI and diet composition. Although increased DMI of most diets leads to greater CH₄ production, increasing the proportion of concentrates, fat, or starch in a diet will reduce CH₄ production (Beauchemin et al., 2009; Bell and Eckard, 2012). Reynolds et al. (2011) reported that increasing dietary proportions of cereal concentrates relative to forage, as indicated by ratio of starch to ADF, reduced CH₄ excretion per unit of DMI. In our previous study (Garnsworthy et al., 2012a), CH₄ emission rate during milking was positively related to both total DMI and forage DMI, but negatively related to concentrate DMI. As in the current study, higher intakes of DM were associated with higher intakes of concentrates. The negative correlation between daily milk yield and eructation CH₄ concentration can similarly be explained by changes in diet composition; cows with greater milk yields consumed greater proportions of high-fat concentrates fed in the AMS, which would offset increases in DMI. In another study (Bell et al., 2014), CH₄ emission rate during milking for 1,964 individual cows across 21 farms was positively related to daily milk yield overall, but the relationship was positive for farms feeding grass or grass silage plus low-fat concentrates, and negative for farms feeding corn silage and high-fat concentrates.

Although total DMI and forage DMI were greater when cows were fed the high-forage PMR rather than the commercial PMR, none of the estimates of CH₄ emissions differed between diets. It is possible that the lack of difference between diets is due to slightly increased concentrate consumption with the high-forage PMR; although concentrate percentage was lower than in the commercial PMR, as planned, the greater milk yield of cows resulted in a slightly greater ($+0.5$ kg/d; $P = 0.070$) concentrate DMI.

A previous study on the same research herd demonstrated that measuring CH₄ emissions in eructation peaks provides a method that is correlated with daily

CH₄ emissions by the same cows when housed in respiration chambers (Garnsworthy et al., 2012a). As the CH₄ analyzer in the current study processes 1 L of air/min, the average concentration of 0.11 mg/L for cows fed a high-forage PMR (Table 2) would equate to 422 g of CH₄/d based on the equation of Garnsworthy et al. (2012a) derived from 24-h chamber measurements [g of CH₄/d = $252 + 57.2 \times (0.11 \text{ mg/min}/0.037)$, with the analyzer sampling 3.7% of eructed gas]. This value is within the range of 278 to 456 g of CH₄/d (mean of 369 g of CH₄/d) found in a study by Garnsworthy et al. (2012a) on the same herd, and similar to the average value of 430 g of CH₄/d for dairy cows at peak milk yield reported by Cottle et al. (2011).

Using the method of Madsen et al. (2010) to estimate CO₂ emissions from theoretical heat emitted by each cow in megajoules per day, the average daily CH₄ emissions would be higher for cows on the high-grass-silage PMR at about 346 g/d and lower for cows on the commercial PMR at 333 g/d. Estimates of average CO₂ emitted per day derived using the method of Madsen et al. (2010) were not consistent with measured average CO₂ concentration over milking (Figure 5). This is not surprising, as the equation of Madsen et al. (2010) is based on an average cow and assumes constant efficiency of energy utilization, whereas calorimeter studies show that these factors vary with animal, level of feeding, and diet composition (Yan et al., 2010). Furthermore, CO₂ concentration in breath measured over a short time period varies with ventilation rate, tidal volume, eructation rate, and rumen CO₂ production, and large amounts of CO₂ can be lost during feeding (Blaxter and Joyce, 1963).

During early lactation when metabolic activity is high, mobilizing body energy reserves for milk production can affect CO₂ emissions (Madsen et al., 2010; Lassen et al., 2012). In our previous study involving daily measurement of 215 cows over 5 mo, CH₄ emissions increased over the first 10 wk of lactation and then declined in parallel with likely changes in DMI (Garnsworthy et al., 2012b). Further assessment of temporal variation in CH₄-to-CO₂ ratio is required, but the current study showed that diurnal variation exists, with the ratio being at its lowest in the morning before feeding (Figure 2), which is consistent with other studies (Kinsman et al., 1995; Lassen et al., 2012). Diurnal variation in eructation CH₄ is similar to that observed in our previous study, where it was ascribed mainly to synchronized feeding behavior of the herd (Garnsworthy et al., 2012b).

Improvements in production efficiency of UK dairy systems over the last 20 yr, through genetic selection and nutrition, have reduced CH₄ emissions per unit product by about 1.3% per year. Reductions will con-

tinue, but at a slower rate per year, based on current breeding objectives (Jones et al., 2008). Greater reductions in enteric CH₄ emissions are possible by selecting animals on traits associated with enteric CH₄, such as feed intake or feed efficiency, without compromising production (Hegarty et al., 2007), with a theoretical potential for enteric CH₄ emissions from dairy cows to be reduced by up to 2.6% per cow per year by selecting on feed efficiency (de Haas et al., 2011). A breeding objective such as selecting cows for low CH₄ emissions per unit DMI or kilograms of milk may be a more cost-effective phenotype than feed intake and would include selection on energy utilization efficiency, which has not been possible in the past. To generate sufficient data for analyzing CH₄ phenotypes requires combining international data sets, derived using different methods. The current study provides initial evidence that such phenotypes are correlated, but that refinement is required before equivalence can be established.

CONCLUSIONS

This study showed that quantifying enteric CH₄ emissions using eructation peaks in concentration or as a ratio to CO₂ emissions averaged over a milking can provide a highly repeatable phenotype for ranking cows on CH₄ output. No significant difference existed between CH₄ and CO₂ emissions from the same cows when fed diets containing different percentages and types of forage. Considerable variation in enteric CH₄ emissions exists among cows. All CH₄ emission phenotypes studied were positively correlated with forage DMI. Importantly, significant correlations existed in ranking cows on emissions of CH₄ calculated from eructation peaks or as CH₄-to-CO₂ ratio, although calculation of CH₄ emissions from eructation peaks produced a more repeatable phenotype.

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