INTERPRETIVE SUMMARY

Methane emissions among individual dairy cows during milking quantified by eructation peaks or ratio with carbon dioxide. By Bell et al., page 0000. Methane (CH_4) and carbon dioxide (CO_2) emissions of dairy cows were measured during milking within an automatic milking station. Cows were fed a commercial partial mixed ration followed by 2 high forage rations during 3 feeding periods. Emissions of CH_4 during milking were examined using 2 methods: CH_4 released in eructation peaks; and ratio of CH_4 and CO_2 average concentrations. Both methods can provide highly repeatable phenotypes for ranking cows by CH_4 output on different diets.

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

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ABSTRACT

The aims of this study were to compare methods for examining measurements of methane (CH_4) and carbon dioxide (CO_2) emissions of dairy cows during milking and to assess repeatability and variation of CH_4 emissions among individual dairy cows. Measurements of CH_4 and CO_2 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 CH_4 during each milking were examined using 2 methods. Firstly, peaks in CH_4 concentration due to eructations during milking were quantified. Secondly, ratios of CH_4 and CO_2 average concentrations during milking were calculated. A linear mixed model was used to assess differences between PMRs. Variation in CH_4 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. There was no significant difference between the 3 PMR in CH_4 emissions estimated by either method. Emissions of CH_4 calculated from eructation peaks or as CH_4 to CO_2 ratio were positively associated with forage DM intake. Ranking of cows according to CH_4 emissions on different diets was correlated for both methods, although rank correlations and repeatability were greater for CH_4 concentration from eructation peaks than for CH_4 to CO_2 ratio. It is concluded that quantifying enteric CH_4 emissions either using eructation peaks in concentration or as CH_4 to CO_2 ratio can provide highly repeatable phenotypes for ranking cows on CH_4 output.

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

INTRODUCTION

Enteric methane (CH_4) 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 CH_4 emissions from animals can be explained by diet composition and feed intake (Bell and Eckard, 2012). In addition to the variation in CH_4 explained by diet, there is considerable variation among individual dairy cows (de Haas et al., 2011; Garnsworthy et al., 2012a; Huhtanen et al., 2013), suggesting scope for selective breeding. Compared to 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 CH_4 emissions by individual cows on commercial dairy farms. Quantifying enteric CH_4 emissions during milking by using low cost and mobile technologies has been demonstrated to provide repeatable phenotypic estimates of CH_4 emissions under commercial conditions (Garnsworthy et al., 2012a,b; Lassen et al., 2012). In the study of Garnsworthy et al. (2012a), estimates of CH_4 made during milking were correlated with total daily CH_4 emissions by the same cows when housed subsequently in respiration chambers.

The studies of Garnsworthy et al. (2012a) and Lassen et al. (2012) employed similar technologies for measuring CH_4; both sampled gas from the feed bin of automatic (robotic) milking stations whilst cows were being milked, and measured CH_4 concentrations with portable gas analyzers. Subsequent handling and analysis of data, however, differed between studies; Garnsworthy et al. (2012a) analyzed CH_4 only released by eructation, whereas Lassen et al. (2012) calculated ratios of average CH_4 to average CO_2 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 CH_4 and CO_2 emissions 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 authority of the UK Animal (Scientific Procedures) Act 1986, and approval was obtained from the University of Nottingham animal ethics committee before commencement of the study.

Data

Concentrations of CH_4 and CO_2 from Holstein Friesian dairy cows were measured during milking at Nottingham University Dairy Centre (Sutton Bonington, Leicestershire, UK). Cows were grouped 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 days. For a full description of the technique see Garnsworthy et al. (2012a). The technique is briefly described below.

The CH_4 and CO_2 concentrations were logged at 1 second 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, 1.0% CH_4, and 5% CO_2, Thames Restek UK Ltd., Saunderton, UK).

Concentrations of CH_4 and CO_2 measured in parts per million (v/v) were converted to mg/L by assuming the density of CH_4 to be 655.7 mg/L and CO_2 to be 1798.9 mg/L at 25°C, 1 atm, with the analyzer sampling air at 1 L/min. Concentrations of CH_4 and CO_2 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), 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 CH_4 concentration during milking (eructation CH_4) from raw logger data (Garnsworthy et al., 2012a). In Method 1, milkings with less than 3 eructation peaks for CH_4 concentration were excluded from the analysis. Peaks of CO_2 concentration were not calculated using this method due to lack of distinct peaks originating from eructation (Figure 1). Method 2 used average of all recorded CH_4 (average CH_4) and CO_2 (average CO_2) concentrations (mg/L) during each milking to derive the ratio of CH_4 to CO_2 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 days on 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, PMRs containing 75% forage with high proportions of either grass silage or maize silage (Table 1). Feeding periods followed on immediately with no adjustment period between diets. Daily AMS concentrate allowance fed during milking was 1.5 kg plus 0.16 kg per liter of milk yield above 23 L/d. AMS concentrates were dispensed into the feed bin at 360 g/min in 6 portions per minute throughout the milking period, which helps to keep the cow's head within suitable proximity of the gas sampling tube. AMS concentrate dispensers were calibrated monthly by weighing quantities dispensed. AMS concentrate manufacturer's declared specification per kilogram as fed was: ME, 12.2 MJ; CP, 16%; NDF, 24%; starch, 21%; and fat, 6.2%. Milk yield, live weight, and AMS concentrate intake were recorded automatically at each milking. Dry matter intake of PMR was recorded automatically by electronic feeders. Total daily DM intake 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 MJ per day ($5.6 \times$ kg live weight$^{0.75}$ $+ 22 \times$ kg milk yield per day $+ 0.000016 \times$ days pregnant^3 $\times 0.0864$), which was then converted to estimated CO_2 emissions in grams per day.

Statistical analysis

Data were analyzed using a linear mixed model in Genstat Version 15.1 (Lawes Agricultural Trust, 2012). Equation 1 was used to assess the effect of diet on average DM intake, milk yield (both kg/d), average number of milkings per day, average duration of milking (s), live weight (kg), eructation CH_4, average CH_4, average CO_2, and CH_4 to CO_2 ratio per individual cow:

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

where y_{ijk} is the dependent variable; μ = overall mean; L_i = fixed effect of lactation number (1, 2 and 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; E_{jk} = random error term (df = 53).

The residual coefficient of variation was calculated from variance components as root mean square error divided by estimated mean. Repeatability of production and gas emission variables were assessed by σ^2 animal / (σ^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 DM intake, forage DM intake, concentrate DM intake, milk yield, live weight and eructation CH_4, and CH_4 to CO_2 ratio across all individual cow records.

RESULTS

Cow performance

There was no effect of diet on live weight, but DM intake was lower when cows were fed on the commercial diet than when they were fed on the high forage diets (Table 2). Milk yield of cows on the commercial diet was lower than when they were fed on the high grass silage diet. There was no difference between the high forage diets, however, in DM intake, milk yield or live weight. Cows presented themselves for milking fewer times per day when they were fed on the commercial diet than when they were fed the high forage diets, but there was no effect of diet on duration of milking visits to the AMS (Table 2).

Methane and CO_2 concentrations

There was no effect of diet on frequency of eructations during milking, as indicated by peaks in CH_4 concentration (mean 1.0 ± 0.1 eructations per minute). Diurnal variation was observed in eructation CH_4, and in CH_4 to CO_2 ratio; both were at their lowest during early morning and generally highest in the afternoon (Figure 2).

Eructation CH_4 was lower than average CH_4 (Table 2). For both indicators of methane emissions, means were not significantly different when cows were fed on the commercial diet than when cows were fed on the high forage diets. Average CO_2, and CH_4 to CO_2 ratio were not affected by diet.

Daily DM intake was positively associated with average CH_4 (r = 0.22, $P < 0.05$). Forage DM intake was positively associated with eructation CH_4 (r = 0.19, $P < 0.05$), average CH_4 (r = 0.29, $P < 0.001$), and CH_4 to CO_2 ratio (r = 0.24, $P < 0.05$). Daily milk yield was negatively correlated (r = -0.21, $P < 0.05$) with eructation CH_4. There was no association between live weight and CH_4 or CO_2 concentrations.

Variation among cows and repeatability of phenotypes

Residual coefficient of variation was slightly greater for eructation CH_4 than for average CH_4, average CO_2, and CH_4 to CO_2 ratio (Table 2). Residual coefficients of variation in DM intake and milk yield were of similar magnitude to that of CH_4 to CO_2 ratio. Repeatability was similar for eructation CH_4, average CH_4, average CO_2, daily milk yield, milking duration and live weight, but repeatability values for DM intake, milkings per day and CH_4 to CO_2 ratio were lower than for other phenotypes (Table 2).

When cows were fed on the commercial diet, rank correlations were 0.62 ($P < 0.001$) between ranking on eructation CH_4 and ranking on average CH_4 (Figure 3a), and 0.35 ($P<0.05$) between ranking on eructation CH_4 and ranking on CH_4 to CO_2 ratio (Figure 3b).

When fed on the high forage diets rank correlations were 0.86 (P < 0.001) between ranking on eructation CH_4 and ranking on average CH_4 (Figure 3a), and 0.53 (P < 0.05) between ranking on eructation CH_4 and ranking on CH_4 to CO_2 ratio (Figure 3b).

Rank correlation coefficients obtained by comparing ranking of cows when fed on the commercial PMR and when fed on 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 CH_4 than for CH_4 to CO_2 ratio (Table 3; Figure 4).

Average heat production estimated by the equation (5.6 × kg live weight$^{0.75}$ + 22 × kg milk yield per day + 0.000016 × days pregnant^3 × 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 CO_2 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. There was no relationship between observed CO_2 concentrations during milking and daily CO_2 emissions estimated from heat production (Figure 5). Observed average CO_2 concentration was more variable (CV 18.7%) than estimated daily CO_2 emission (CV 13.4%).

DISCUSSION

This study is the first to compare online methods for estimating enteric CH_4 emissions from dairy cows during milking in the same individual cows. Because measurements of CH_4 and CO_2 were made concurrently, 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. Furthermore, the design of the study permits separation of within-cow, between-cow, diet and temporal effects on methane emissions in order to examine variation and repeatability of estimates. Quantifying variation and repeatability of phenotypes is an essential pre-requisite for combining datasets derived by different methods in international collaborations.

Individual cow eructation CH_4 was a highly repeatable phenotype, confirming our previous studies (Garnsworthy et al., 2012a,b). Average CH_4 and average CO_2 showed a level of repeatability similar to that of eructation CH_4, but CH_4 to CO_2 ratio was less repeatable. Repeatability of CH_4 to CO_2 ratio (0.54) is consistent with 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 experiment 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, repeatability was 0.78 between diets (Garnsworthy et al., 2012a).

Mean average CH_4 was approximately double mean eructation CH_4, as expected from the methods of calculation. Average CH_4 was calculated across each milking, subtracting the lowest concentration at the start of the milking; eructation CH_4 was calculated across each eructation peak, subtracting the lowest concentration at the start of the peak. Average CH_4, therefore, adjusts for changes in ambient CH_4 at different milkings, whereas eructation CH_4 adjusts not only for ambient CH_4, but also for build-up of CH_4 during milking, and considers only CH_4 released by eructation rather than in breath.

The coefficient of variation in CH_4 emissions ranged from 11% for CH_4 to CO_2 ratio to 14% for eructation CH_4. The greater variation in eructation CH_4, average CH_4, CH_4 to CO_2 ratio compared with average CO_2, may be explained partly by differences in the way that CH_4 and CO_2 are emitted by cows. Methane emissions arise from enteric fermentation, whereas CO_2 emissions arise from both enteric fermentation and metabolic CO_2 excreted via the lungs. For CH_4, 83% of daily production by sheep was released by eructation irrespective of feeding level (Blaxter and Joyce, 1963; Murray et al., 1976), whereas for CO_2, the proportion of CO_2 released by eructation varied with CH_4 production and level of feeding, so that in eructed gas CO_2 concentration was 30% of CH_4 concentration when CH_4 production was 1 L/hr and 140% of CH_4 concentration when CH_4 production was 2.5 L/hr (Blaxter and Joyce, 1963). This effect would dampen variation in CO_2 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 CH_4 rather than more slowly emitted CO_2 emissions in breath where peaks in concentration are less defined (Figure 1). Furthermore, Blaxter and Joyce (1963) reported that during feeding the loss of CO_2 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 coefficients 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 (Grainger et al., 2007; Ellis 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 CH_4 emissions as a ratio to CO_2 emissions, variation among cows and repeatability of the phenotype were similar to variation and repeatability of DM intake, which was also found by Huhtanen et al. (2013).

All CH_4 emission phenotypes studied were positively (r = 0.19 to 0.24) correlated with forage DM intake, although only average CH_4 concentration was positively (r = 0.22) associated with total DM intake. Positive correlations with forage DM intake are expected because CH_4 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 DM intake and eructation CH_4, however, does not agree with chamber studies (e.g. Grainger et al., 2007; Ellis et al., 2007; Yan et al., 2010), in which strong positive relationships were observed. The explanation for this apparent discrepancy lies in the relative effects on CH_4 of

DM intake and diet composition. Although increased intake of most diets leads to greater CH_4 production, increasing the proportion of concentrates, fat or starch in a diet will reduce CH_4 production (Beauchemin et al., 2009; Bell and Eckard, 2012). In our previous study (Garnsworthy et al., 2012a), CH_4 emission rate during milking was positively related to both total DM and forage DM intakes, but negatively related to concentrate DM intake. 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 CH_4 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 DM intake.

Although DM intake and forage intake were greater when cows were fed on the high forage PMR rather than the commercial PMR, none of the estimates of CH_4 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 DM intake. ,

A previous study on the same research herd demonstrated that measuring CH_4 emissions in eructation peaks provides a method that is correlated with daily CH_4 emissions by the same cows when housed in respiration chambers (Garnsworthy et al., 2012a). Since the CH_4 analyzer in this study processes one liter of air per minute, the average concentration of 0.11 mg/L for cows fed a high forage PMR (Table 2) would equate to 422 g CH_4/d based on the equation of Garnsworthy et al. (2012a) derived from 24-hour chamber measurements (CH_4 g/d = 252 + 57.2 × [0.11 mg/min / 0.037], with the analyzer sampling 3.7% of eructed gas). This value is within the range of 278 to 456 g CH_4/d (mean of 369 g CH_4/d) found in a study by Garnsworthy et al. (2012a) on the same herd, and similar to the average value of 430 g CH_4/d for dairy cows at peak milk yield reported by Cottle et al. (2011).

Using the method of Madsen et al. (2010) to estimate CO_2 emissions from theoretical heat emitted by each cow in MJ per day, the average daily CH_4 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 CO_2 emitted per day derived using the method of Madsen et al. (2010) were not consistent with measured average CO_2 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, CO_2 concentration in breath varies with breathing rate, tidal volume, eructation rate, and rumen CO_2 production; and large amounts of CO_2 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 CO_2 emissions (Madsen et al., 2010; Lassen et al., 2012). In our previous study involving daily measurement of 215 cows over 5 months, CH_4 emissions increased over the first 10 weeks of lactation, and then declined in parallel with likely changes in DM intake (Garnsworthy et al., 2012b). Further assessment of temporal variation in CH_4 to CO_2 ratio is required, but the current study showed diurnal variation exists, with the ratio being at its lowest in the morning prior to feeding (Figure 2), which is consistent with other studies (Kinsman et al., 1995; Lassen et al., 2012). Diurnal variation in eructation CH_4 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 years, through genetic selection and nutrition, have reduced CH_4 emissions per unit product by about 1.3% per year. Reductions will continue, but at a slower rate per year based on current breeding objectives (Jones et al., 2008). Greater reductions in enteric CH_4 emissions are possible by selecting animals on traits associated with enteric CH_4 such as feed intake or feed efficiency without compromising production (Hegarty et al., 2007), with a theoretical potential for enteric CH_4 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 CH_4 emissions per unit DM intake or kg 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 CH_4 phenotypes requires combining international datasets, 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 CH_4 emissions using eructation peaks in concentration or as a ratio to CO_2 emissions averaged over a milking can provide a highly repeatable phenotype for ranking cows on CH_4 output. There was no significant difference between CH_4 and CO_2 emissions from the same cows when fed on diets containing different percentages and types of forage. Considerable variation in enteric CH_4 emissions exists among cows. All CH_4 emission phenotypes studied were positively correlated with forage DM intake. Importantly, there were significant correlations in ranking cows on emissions of CH_4 calculated from eructation peaks or as CH_4 to CO_2 ratio, although calculation of CH_4 emissions from eructation peaks produced a more repeatable phenotype.

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REFERENCES

Beauchemin, K. A., T. A. McAllister, and S. M. McGinn. 2009. Dietary mitigation of enteric methane from cattle. CAB Rev.: Perspec. Agric. Vet. Sci. Nutr. Nat. Resour. 4:1–18.

Bell, M. J., and R. J. Eckard. 2012. Reducing enteric methane losses from ruminant livestock – Its measurement, prediction and the influence of diet. In Livestock Production (ed. K Javed), pp.135-150. InTech Publishing, Croatia.

Cottle, D. J., J. V. Nolan, and S. G. Wiedemann. 2011. Ruminant enteric methane mitigation: a review. Anim. Prod. Sci. 51:491-514.

de Haas, Y., J. J. Windig, M. P. L. Calus, J. Dijkstra, M. de Haan, A. Bannink, and R. F. Veerkamp. 2011. Genetic parameters for predicted methane production and the potential for reducing enteric emissions through genomic selection. J. Dairy Sci. 94:6122-6134.

de Haas, Y., M. P. L. Calus, R. F. Veerkamp, E. Wall, M. P. Coffey, H. D. Daetwyler, B. J. Hayes, and J. E. Pryce. 2012. Improved accuracy of genomic prediction for dry matter intake of dairy cattle from combined European and Australian data sets. J. Dairy Sci. 95:6103-6112.

Ellis, J. L., E. Kebreab, N. E. Odongo, B. W. McBride, E. K. Okine, and J. France. 2007. Prediction of methane production from dairy and beef cattle. J. Dairy Sci. 90:3456-3467.

Garnsworthy, P. C., J. Craigon, J. H. Hernandez-Medrano, and N. Saunders. 2012a. On-farm methane measurements during milking correlate with total methane production by individual dairy cows. J. Dairy Sci. 95:3166-3180.

Garnsworthy, P. C., J. Craigon, J. H. Hernandez-Medrano, and N. Saunders. 2012b. Variation among individual dairy cows in methane measurements made on farm during milking. J. Dairy Sci. 95:3181-3189.

Grainger, C., T. Clarke, S.M. McGinn, M.J. Auldist, K. A. Beauchemin, M. C. Hannah, G. C. Waghorn, H. Clark, and R. J. Eckard. 2007. Methane emissions from dairy cows measured using the sulfur hexafluoride (SF_6) tracer and chamber techniques. J. Dairy Sci. 90:2755-2766.

Hayes, B. J., J. H. J. van der Werf, and J. E. Pryce. 2011. Economic benefit of genomic selection for residual feed intake (as a measure of feed conversion efficiency) in Australian dairy cattle. In: Recent advances in animal nutrition, 18, pp. 31-35.

Hegarty, R. S., J. P. Goopy, R. M. Herd, and B. McCorkell. 2007. Cattle selected for lower residual feed intake have reduced daily methane production. J. Anim. Sci. 85:1479-1486.

Huhtanen, P., S. J. Krizsan, M. Hetta, H. Gidlund, and E. H. Cabezas Garcia. 2013. Repeatability and between cow variability of enteric methane and total carbon dioxide emissions. In: Proceedings of the Greenhouse Gases in Animal Agriculture conference, Advances in Animal Biosciences, 23-27 June, Dublin, Ireland, 4:588.

Jones, H. E., C. C. Warkup, A. Williams, and E. Audsley. 2008. The effect of genetic improvement on emission from livestock systems. In: Proceedings of the European Association of Animal Production, 24-27 August, Vilnius, Lithuania, p. 28.

Kinsman, R., F. D. Sauer, H. A. Jackson, and M. S. Wolynez. 1995. Methane and carbon dioxide emissions from dairy cows in full lactation monitored over a six-month period. J. Dairy Sci. 78:2760-2766.

Lassen, J., P. Løvendahl, and J. Madsen. 2012. Accuracy of noninvasive breath methane measurements using Fourier transform infrared methods on individual cows. J. Dairy Sci. 95:890-898.

Lawes Agricultural Trust. 2012. Genstat 15, Version 15.1 Reference Manual. Clarendon Press, London, UK.

Madsen, J., B. S. Bjerg, T. Hvelplund, M. R. Weisbjerg, and P. Lund. 2010. Methane and carbon dioxide ratio in excreted air for quantification of the methane production from ruminants. Livest. Sci. 129:223-227.

Yan, T., C. S. Mayne, F. G. Gordon, M. G. Porter, R. E. Agnew, D.C. Patterson, C. P. Ferris, and D. J. Kilpatrick. 2010. Mitigation of enteric methane emissions through improving efficiency of energy utilization and productivity in lactating dairy cows. J. Dairy Sci. 93:2630-2638.

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

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

[1] Distillers dried grains with solubles (maize)

[2] Butterfat extra (Trident Feeds, Peterborough, UK)

[3] containing calcium, 18%; phosphorus, 10%; magnesium, 5%; salt, 17%; copper, 2,000 mg/kg; manganese, 5,000 mg/kg; cobalt, 100 mg/kg; zinc, 6,000 mg/kg; iodine, 500 mg/kg; selenium, 25 mg/kg; vitamin A, 400,000 IU/kg; vitamin D3, 80,000 IU/kg; and vitamin E, 1,000 mg/kg.

[4] All ingredients were analyzed by a commercial analytical laboratory (Sciantec analytical, Cawood, UK)

Table 2. Least square means, variability, repeatability and rank correlation (r) of production, methane (CH_4) and carbon dioxide (CO_2) phenotypes for cows fed on commercial, high grass silage and high maize silage partial mixed rations

Phenotype	Units	Partial mixed ration[1] Commercial	High grass silage	High maize silage	SED	*P* value	Residual CV (%)	Repeatability	Rank correlation[2] r	P value
		Mean								
DM intake	kg/d	17.8[a]	19.8[b]	19.4[b]	0.7	< 0.05	11.4	0.42	0.632	<0.001
Milk yield	kg/d	29.7[a]	33.3[b]	31.5[ab]	1.2	< 0.05	10.6	0.82	0.920	<0.001
Live weight	kg/d	662	664	661	2.8	0.294	1.0	0.98	0.967	<0.001
Milkings per day		2.6[a]	3.2[b]	3.1[b]	0.2	< 0.05	21.7	0.26	0.749	<0.001
Milking duration	s	389	387	386	9.6	0.972	6.6	0.92	0.956	<0.001
Eructation Peaks										
CH_4	mg/L	0.12	0.11	0.11	0.01	0.748	13.6	0.75	0.801	<0.001
Average Concentrations										
CH_4	mg/L	0.25	0.24	0.24	0.01	0.536	10.3	0.74	0.716	<0.001
CO_2	mg/L	8.4	8.6	8.7	0.2	0.293	6.6	0.86	0.821	<0.001
Ratio CH_4:CO_2	g/kg	29.8	30.7	29.7	1.1	0.592	11.0	0.54	0.587	<0.001

[a,b] Means within a row with different superscripts differ. SED = standard error of differences.

[1] In 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).

[2] Values for 36 cows fed on a commercial diet (Period 1) were compared to average values for the same 36 individual cows in Periods 2 and 3.

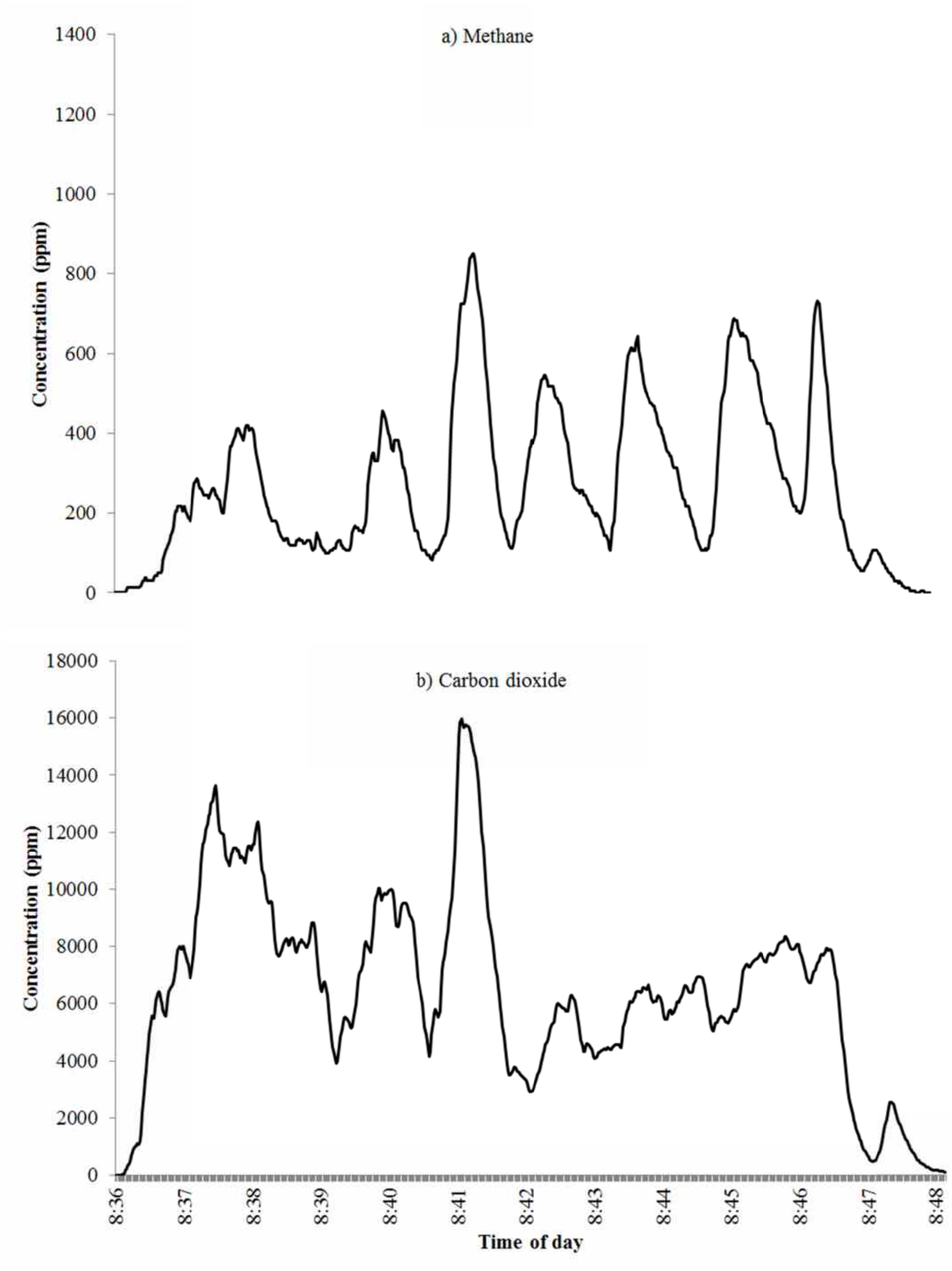

Figure 1. Concentration in parts per million of a) methane and b) carbon dioxide during a single milking visualized by the data logging software.

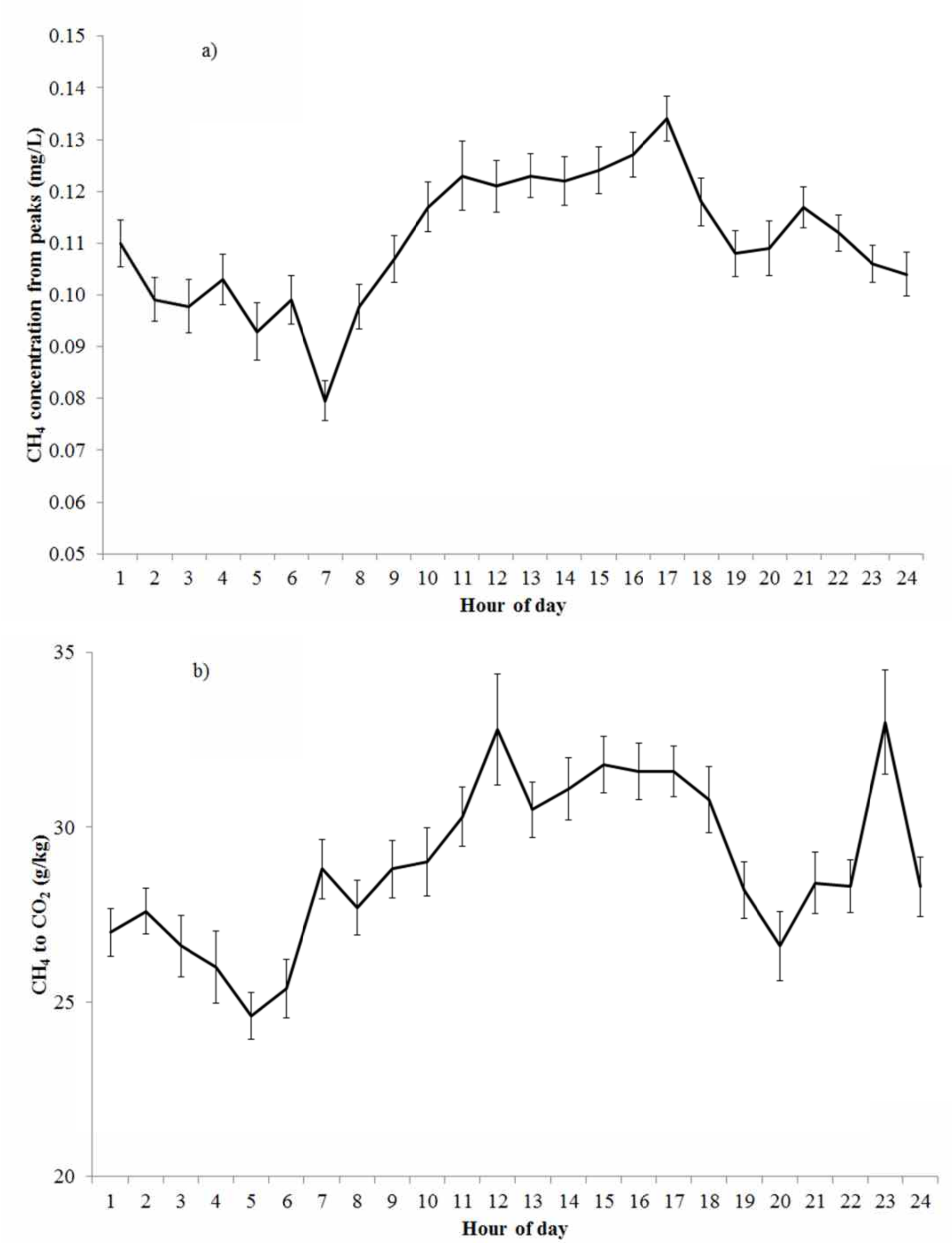

Figure 2. Average methane (CH_4) concentration (with SE bars) calculated from a) eructation peaks and b) the ratio of CH_4 to carbon dioxide (CO_2) concentrations for individual cows for each hour of the day from all records collected during the study period.

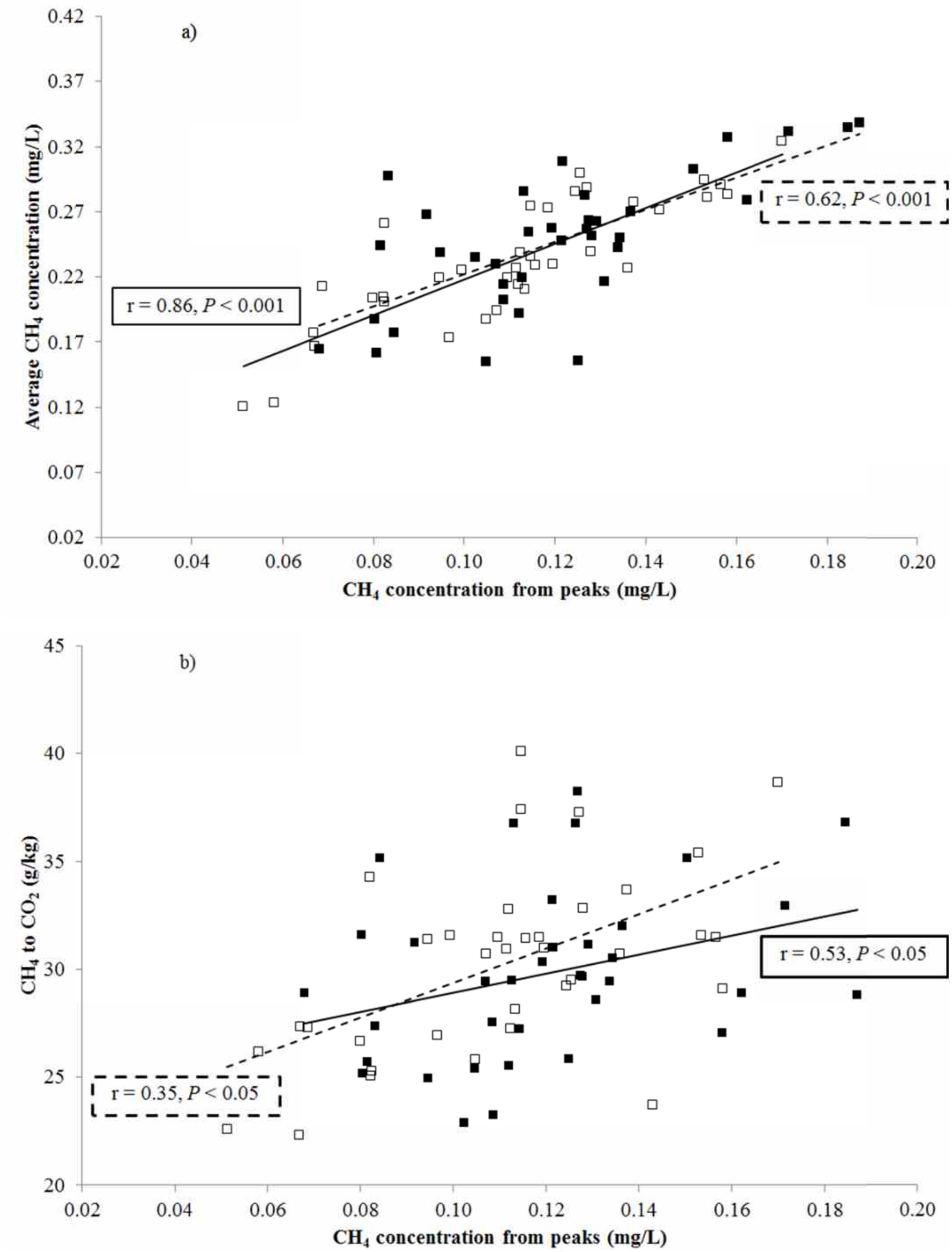

Figure 3. Relationship between methane (CH_4) concentration calculated from eructation peaks and a) the average CH_4 concentration over each milking, b) the ratio of CH_4 to carbon dioxide (CO_2) concentrations for individual cows fed a commercial PMR (■) or high forage

PMRs (□). The rank correlation (r) is shown with the line of best-fit for the commercial PMR (dashed line) and high forage PMRs (solid line).

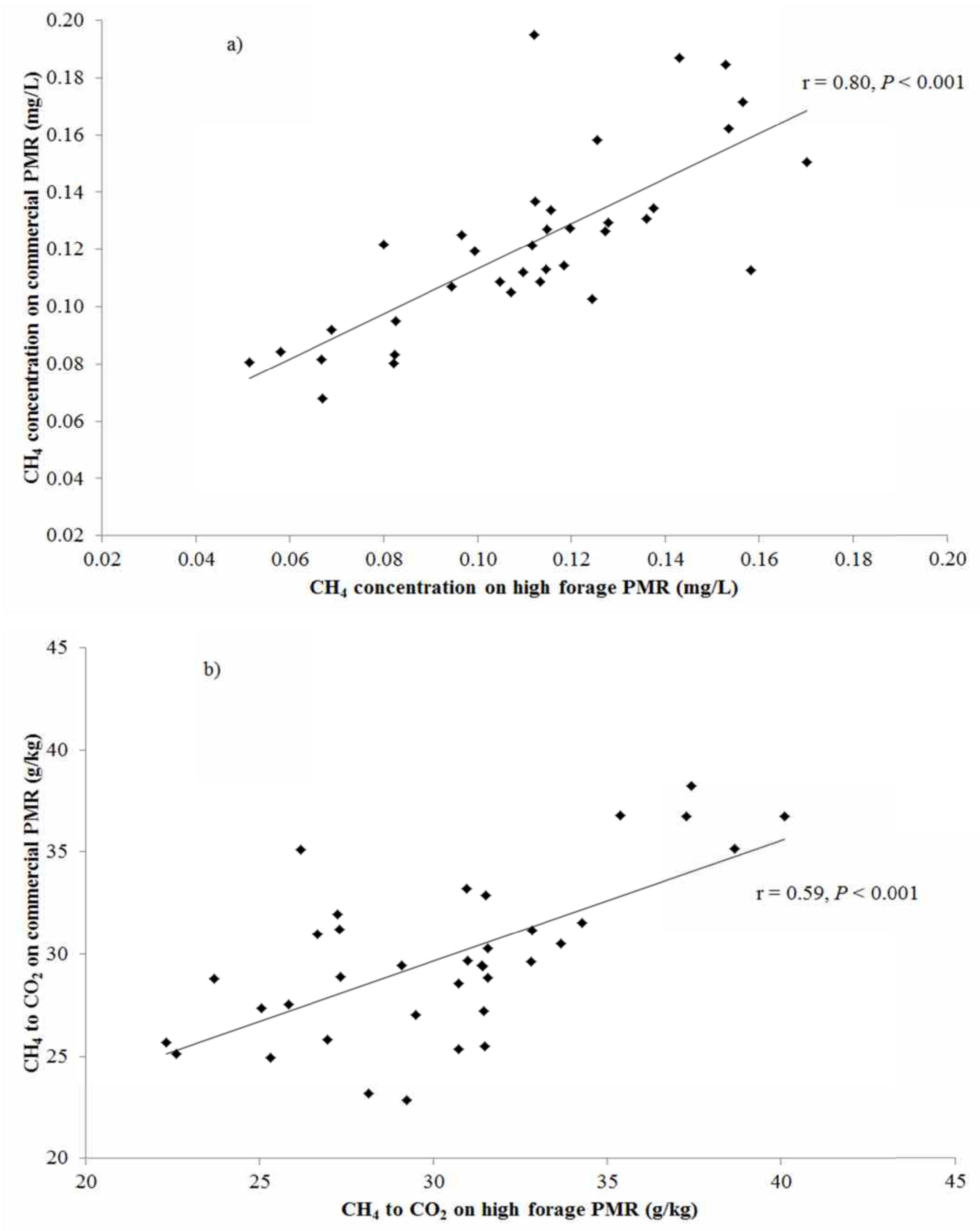

Figure 4. Relationship between methane (CH_4) concentration during milking from individual cows on a commercial PMR and high forage PMRs calculated from a) eructation peaks, and b) ratio of CH_4 to carbon dioxide (CO_2) concentrations averaged over each milking. The rank correlation (r) is shown with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

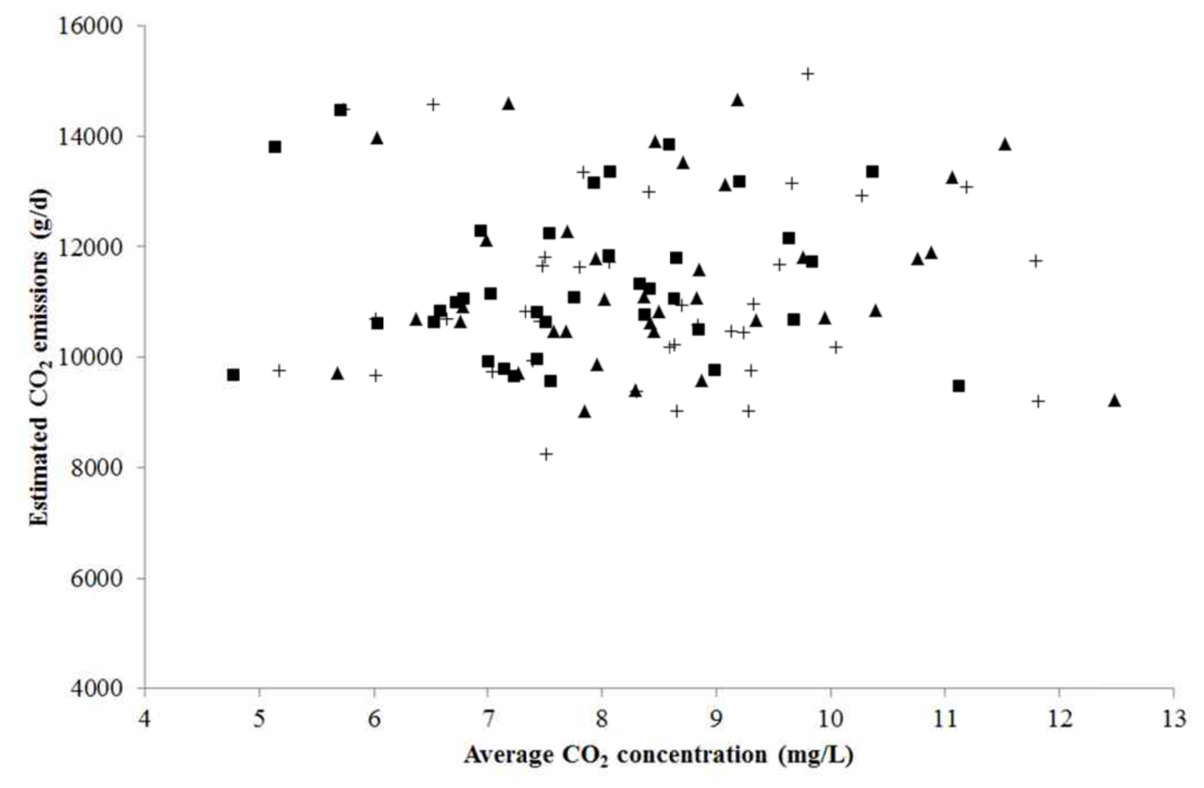

Figure 5. Relationship between average carbon dioxide (CO_2) concentration over each milking and average daily CO_2 emissions estimated using the method of Madsen et al. (2010) for individual cows fed commercial PMR (■), high grass silage PMR (+), and high maize silage PMR (▲).