



Accuracy of noninvasive breath methane measurements using Fourier transform infrared methods on individual cows

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ABSTRACT

Individual methane (CH₄) production was recorded repeatedly on 93 dairy cows during milking in an automatic milking system (AMS), with the aim of estimating individual cow differences in CH₄ production. Methane and CO₂ were measured with a portable air sampler and analyzer unit based on Fourier transform infrared (FTIR) detection. The cows were 50 Holsteins and 43 Jerseys from mixed parities and at all stages of lactation (mean = 156 d in milk). Breath was captured by the FTIR unit inlet nozzle, which was placed in front of the cow's head in each of the 2 AMS as an admixture to normal barn air. The FTIR unit was running continuously for 3 d in each of 2 AMS units, 1 with Holstein and another with Jersey cows. Air was analyzed every 20 s. From each visit of a cow to the AMS, CH₄ and CO₂ records were summarized into the mean, median, 75, and 90% quantiles. Furthermore, the ratio between CH₄ and CO₂ was used as a derived measure with the idea of using CO₂ in breath as a tracer gas to quantify the production of methane. Methane production records were analyzed with a mixed model, containing cow as random effect. Fixed effects of milk yield and daily intake of the total mixed ration and concentrates were also estimated. The repeatability of the CH₄-to-CO₂ ratio was 0.39 for Holsteins and 0.34 for Jerseys. Both concentrate intake and total mixed ration intake were positively related to CH₄ production, whereas milk production level was not correlated with CH₄ production. In conclusion, the results from this study suggest that the CH₄-to-CO₂ ratio measured using the noninvasive method is an asset of the individual cow and may be useful in both management and genetic evaluations.

Key words: methane, dairy cattle, repeatability

INTRODUCTION

Emission of greenhouse gasses is a great concern in society today. Dairy cattle are contributing greenhouse gasses such as methane (CH₄) from rumination, and CH₄ is one of the most powerful greenhouse gases. However, modern dairy production, characterized by high milk production per cow, wastes relatively less of the energy in feed as emitted CH₄ from the rumination process, and is thereby also more efficient in converting feed energy to human-edible food, such as milk and meat (Capper et al., 2009). Comparing production circumstances from 1944 to 2007 the proportion of energy wasted as CH₄ per kilogram of milk produced from dairy production has been more than halved (Capper et al., 2009). On a global scale, livestock contributes about 15% of the total greenhouse gas production (Steinfeld et al., 2006) and a major contributor is the CH₄ produced in the rumen of cattle. The global warming potential of CH₄ is about 22 times that of CO₂, and therefore, just a small decrease in CH₄ production will be beneficial for the environment (Hegarty and McEwan, 2010). With a half-life of 7 yr, CH₄ lasts around 10 yr in our atmosphere (Steinfeld et al., 2006). This means that, on the one hand, the total amount of CH₄ on a world scale is relatively stable, but on the other hand, it also means that this is a greenhouse gas for which a potential exists of really decreasing the amount emitted. No doubt exists that feeding plays a role in CH₄ production from dairy cattle, as CH₄ comes from the digestion of high-fiber diets. There is also reason to expect a genetic component affecting CH₄ production from cattle. However, genetic selection for decreased CH₄ production is hampered by lack of methods for accurate individual CH₄ measurements from large numbers of dairy cattle.

In precision studies where CH₄ exhalation has been investigated, a whole-animal respiration chamber has been used (Ellis et al., 2007). In such a system, one has full control of gas entering and leaving the chamber, but only 1 cow can be studied at a time and each test occupies the respiration chamber for several hours, thus restricting testing capacity. However, Robinson et al. (2010) successfully used simpler chambers for 1-h CH₄

Received May 18, 2011.

Accepted October 20, 2011.

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production records on 708 sheep. Attempts to develop noninvasive methods potentially applicable under commercial dairy herd conditions have used hand-held laser-reflectance equipment (Chagunda et al., 2009). Methane production has also been estimated using the SF₆-tracer gas (e.g., Grainger et al., 2007), but this method is invasive in the way that the cow needs to ingest a bolus containing the tracer, and that the SF₆ tracer is itself an extremely potent greenhouse gas. A noninvasive approach was taken by Madsen et al. (2010) using a portable unit combining air sampling and gas detection. Their method relies on analyzing air samples for CH₄ and CO₂ simultaneously with a gas analyzer that is based on Fourier transform infrared (FTIR) detection, and uses CO₂ from the breath of cows as the tracer gas. Their initial findings showed good agreement with expected values and the method has potential for large-scale use.

Before CH₄ measurements can be applied in large-scale studies, some issues must be resolved. The first issue is the definition of the focus trait and the variables that need to be measured to have it calculated. Another issue is to establish a protocol for measurements under the given circumstances. As to the first issue with a view to mitigate greenhouse gas effects of dairy production, the production of CH₄ per kilogram of produced milk over the lifetime of a cow would be the ideal trait. As that would be very difficult to obtain, some variables that are closely related could be useful indicator traits (e.g., production of CH₄ per kilogram of produced milk on a lactation basis, or on a daily basis; IDF, 2010). Alternatively, production of CH₄ per unit of feed intake could be a useful trait. Another issue is how the raw measurements are used to calculate the variables of interest. That includes designing a testing protocol with details of how much time is needed to get a reliable estimate and how many times a testing sequence should be repeated to obtain a reliable day-average estimate. During validation of a suggested protocol, influences from time of day and similar disturbances also need to be assessed to evaluate robustness and needs for adjustments.

The aim of this study was to obtain and analyze measurements of CH₄ production from individual dairy cattle using the FTIR analysis and to estimate variance components and short-term repeatability for the trait as a first step toward a genetic evaluation.

MATERIALS AND METHODS

Design, Animals, and Feeding

Data was obtained at the experimental herd at the Danish Cattle Research Centre (DCRC, Foulum, Den-

Table 1. Means of data analyzed for Holstein and Jersey cows¹

Item	Holstein	Jersey
Milk (kg/d)	30.8	20.8
CH ₄ :CO ₂	0.065	0.050
Roughage (kg/d)	50.5	31.98
Concentrate (kg/d)	2.50	2.45
DIM	156	165

¹For milk, roughage, and concentrate, the mean is the average over a 3-wk period starting 1 wk before breath measurements and ending 1 wk after breath measurements were done. For the ratio between CH₄ and CO₂, it is the mean of all measurements done in the data-collection period when cows were in the automatic milking system (AMS). For DIM, it is the average number of DIM at the last day of measuring in each AMS.

mark). A total of 93 cows were in the study. These included 50 Holstein and 43 Jersey cows, kept in 2 separate groups, each allocated to an automatic milking system (AMS; voluntary milking system, VMS; DeLaval International AB, Tumba, Sweden). Measurements were conducted over 3 consecutive days in each group. There were between 2 and 12 AMS visits with CH₄ measurements per cow during the 3-d period (Table 1).

Data on feed intake, weight, and milk production are recorded automatically at the DCRC. In this study, the phenotypes used for these 3 traits were the mean of the daily records for each trait over a 3-wk period, starting 1 wk before the week of CH₄ recording and ending 1 wk after CH₄ recording (Table 1). The feed intake was recorded separately for the TMR and for concentrates. The TMR consisted of corn silage, grass silage, rapeseed meal, and soybean meal. The concentrate feed in the AMS was used to attract the cows into the AMS.

Breath Sampling and Analysis

Breath was sampled and analyzed directly for CH₄ and CO₂ using a portable FTIR gas analyzer (GASMET 4030; Gasmeter Technologies Oy, Helsinki, Finland). The instrument air inlet was placed in front of the cow's head in each of the 2 AMS and measurements were performed continuously every 20 s for 3 d in each AMS. Between 2 and 12 visits with measurements per cow occurred during that period.

The FTIR measurement is an effective and accurate way to measure CH₄ from dairy cattle in low concentrations of air. The FTIR technique uses an infrared transmission spectrum of an air sample. The FTIR technology is incorporated into the GASMET DX-4000 equipment, which can be calibrated to measure several gasses at the same time (e.g., CO₂ and CH₄; Teye et al., 2009). During each visit, the cow's breath contains CH₄ and CO₂ in concentrations clearly above the baseline in the surrounding air.

Methane Production Variables

In this study, we were specifically interested in measuring CH₄ and CO₂ concentrations and to calculate the ratio between CH₄ and CO₂ (CH₄:CO₂) in the breath of the cow (Madsen et al., 2010). The CH₄:CO₂ ratio was used for the evaluation and no calculations were made to quantify CH₄ production. Moreover, as this was a preliminary evaluation of the potential for differentiation between cows, and not for quantification of CH₄ production, no correction was made for background CH₄ and CO₂ concentrations. Given that the CH₄:CO₂ ratio is concentration independent, this ratio describes the CH₄ production of each cow. The direct measurements of CH₄ and CO₂ were also analyzed. For each of the 3 traits (CH₄, CO₂, and CH₄:CO₂ ratio) 4 methods of summarizing the measurements per visit were analyzed: median, mean, the 75% quantile, and the 90% quantile of all recordings from each visit. The best summarizing method would give the highest repeatability.

With further information on BW, milk production, and feed intake, total daily production of CH₄ can also be calculated (Madsen et al., 2010). This estimate essentially quantifies the heat production of the cow. It is possible to estimate the quantitative CO₂ production from cows in different ways (Pedersen et al., 2008; Madsen et al., 2010) and by using this estimate together with the measured CH₄-to-CO₂ ratio, it is possible to quantify CH₄ production (Madsen et al., 2010). In this study, the main purpose was to generate a phenotype, which can be recorded in a precise and repeatable way.

Statistical Models

Data was analyzed with linear mixed models (Equations 1 and 2) using the MIXED procedure in SAS (SAS Institute, 2008):

$$y_{ij} = \mu + \beta_1 \times dm + lact_j + a_i + e_i; \quad [1]$$

$$y_{ij} = \mu + \beta_1 \times dm + \beta_2 \times conc + \beta_3 \times milk + \beta_4 \times roug + lact_j + \sum_{j=1}^1 (\cos j\theta 2\pi + \sin j\theta 2\pi) + a_i + e_i, \quad [2]$$

where y_{ij} is the dependent phenotype, which is either CH₄, CO₂, or the CH₄:CO₂ ratio of each AMS visit; μ is the overall intercept; β_1 to β_4 are fixed regression coefficients; dm is the DIM at recording; $lact_j$ is the lactation number at recording; a_i are the random animal effects; e_i is the random residual effect; $roug$ is the mean TMR intake in kilograms over a 3-wk period; $conc$ is the mean concentrate intake over a 3-wk period; and

$milk$ is the mean daily milk production over a 3-wk period. Equation 2 is an extension of Equation 1 with systematic effects of feeding level and milk production. Diurnal variation was modeled using a Fourier series approach previously used by Løvendahl and Bjerring (2006), where θ is the decimal fraction of the 24-h diurnal cycle when the breath recording was initiated (i.e., $\theta = h/24$). The models allow estimation of repeatability coefficients between visits. The repeatability (rep) is

defined as $rep = \frac{\sigma_{animal}^2}{\sigma_{animal}^2 + \sigma_{residual}^2}$, where σ^2 is the variance. The repeatability is used to infer which phenotype is most suitable for genetic evaluations.

RESULTS AND DISCUSSION

Overall Results for Raw and Calculated Variables

A sample of data for CH₄ concentrations is shown in Figure 1. When a cow enters the AMS, the CH₄ level increases and, during the visit, the concentration fluctuates and immediately falls back to baseline at the end of the visit. From Figure 1 it can be seen that the first 2 to 3 records after a cow enters the AMS have lower values than the rest of the period.

Air sampling is a major issue when doing breath analysis. If one only looks at the pure concentrations of CH₄ and CO₂ in the breath, these numbers are highly influenced by the distance from the cow's head to the sampling unit. However, by using the ratio between CH₄ and CO₂ as a measurement, the phenotype is more stable, although bias will still exist because the ratio between the surrounding air and the actual air from the cow's breath will be influenced by the distance from the cow's head to the sampling unit. This bias is very hard to quantify. One possible way to decrease this bias is to increase the number of recordings and filter data. Like for many other phenotypes, it is a tradeoff between the time it will take to generate a record and measurement noise on records. In this study, we recorded over a 3-d period, which was the time available in the research farm. We believe that this is an appropriate number of days to collect records from but we have not investigated data from more than 3 d of recordings. For large-scale recording, more than 3 d of recording would make phenotyping both expensive and impractical.

The recordings done in this study are snapshots of the daily life of the cows. Given the way the recordings were done, we were only able to perform recordings when the cows were milked. This could bias the phenotype, as the cow might be motivated to get milked at certain time points each day. However, the cows were fed ad libitum with TMR and had free access to the

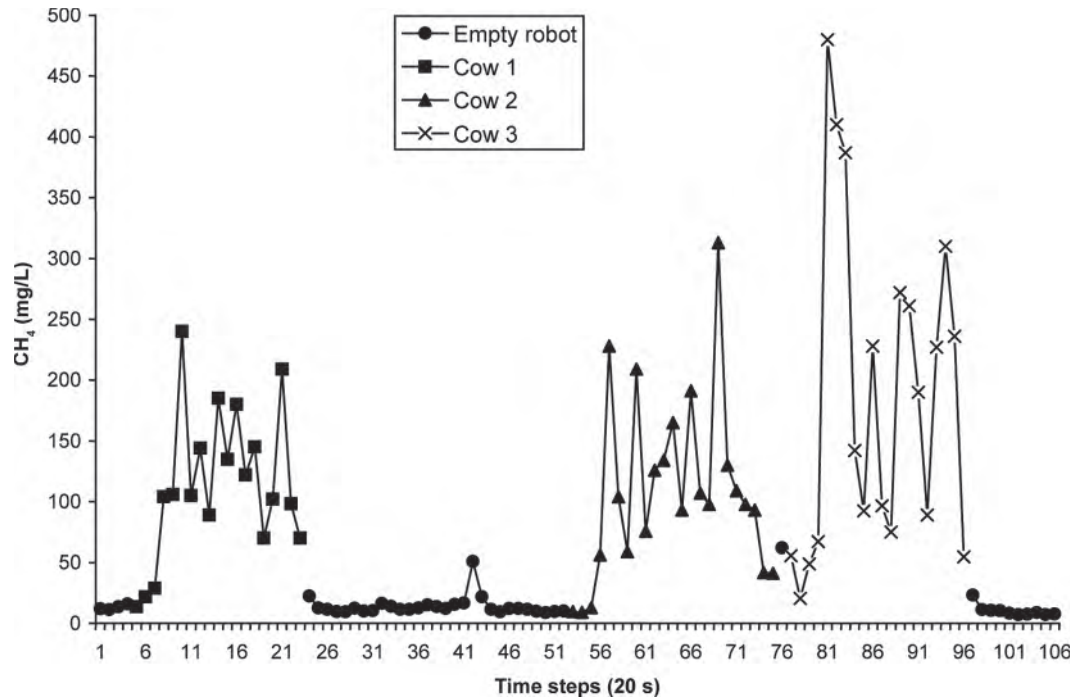


Figure 1. Data points of measured CH_4 in milligrams per liter from a time sample when 3 Jersey cows were milked in the automatic milking system. Each time step covers a 20-s period.

AMS; thus, further studies would be required to obtain insights into complex relations between CH_4 records and feeding or milking events.

Repeatability

The fluctuating levels were handled by summarizing measurements from each visit so that they were condensed into the mean, median, and upper quantiles of 75 and 90%. When using the median of each visit as a phenotype, we obtained the largest repeatability and thus, the most stable measure. The estimated repeatability of CH_4 production in terms of the $\text{CH}_4:\text{CO}_2$ ratio was 0.37 for Holsteins (Table 2) and 0.33 for Jerseys for the median of each visit. For the mean of each visit, the repeatability was 0.38 for Holsteins and 0.31 for Jerseys. For the 75% quantile and for the 90% quantile, the repeatability was somewhat lower for both Holsteins and Jerseys. The simple mean of all recordings during a visit seem to be as good a measure for CH_4 production as the median for all recordings during a visit. When looking at the recording (Figure 1) during a visit, some fluctuation exists from time stamp to time stamp. Some extremes will occur and we would have expected that the median of all records during a visit would have been at least as good a summarized phenotype as the simple mean of each visit. That was not the case in this study. Repeatability estimates of similar

magnitude were found for pure CH_4 measurements as well as CO_2 level (Table 2). Data were analyzed first without systematic effects using Equation 1 and with the systematic effects included using Equation 2. However, the inclusion of systematic effects had little effect on estimates of repeatability, as their effects on animal and residual variance components leveled each other out (data not shown).

The repeatability estimates from this study are somewhat higher than the previous results by McCourt et al. (2005) and Grainger et al. (2007), who found a repeatability of 0.17 and 0.18 for CH_4 production, respectively. Their results came from an SF_6 tracer study and a whole-animal respiration chamber study. In the study by Grainger et al. (2007), only 16 animals were measured and in the study by McCourt et al. (2005), the CH_4 production was measured on beef cattle steers. So, measures of quantified CH_4 and CO_2 production were repeatable themselves but more so were the ratios between them. Using a measuring approach as in this study, controlling the direction of the cow's breath is not possible, because the cow is moving around during milking. Therefore, the $\text{CH}_4:\text{CO}_2$ ratio will correct for the distance from the cow's head to the measuring device compared with the raw measures of CH_4 and CO_2 production. Therefore, the $\text{CH}_4:\text{CO}_2$ ratio as phenotype will be a better measure of the CH_4 production than the raw measure of CH_4 .

Table 2. Estimates of repeatability (t) in CH_4 production from Holstein and Jersey cows during visits to the automatic milking system (AMS), recorded over 3 consecutive days¹

Measure	Statistic	Holstein ($t \pm \text{SE}$)	Jersey ($t \pm \text{SE}$)
CH_4	Mean	0.34 ± 0.006	0.33 ± 0.005
	Median	0.33 ± 0.006	0.37 ± 0.005
	Quantile 75%	0.39 ± 0.004	0.22 ± 0.005
	Quantile 90%	0.26 ± 0.005	0.30 ± 0.006
CO_2	Mean	0.46 ± 0.004	0.40 ± 0.005
	Median	0.45 ± 0.005	0.38 ± 0.007
	Quantile 75%	0.46 ± 0.008	0.29 ± 0.006
	Quantile 90%	0.33 ± 0.004	0.31 ± 0.004
$\text{CH}_4:\text{CO}_2$	Mean	0.37 ± 0.006	0.33 ± 0.003
	Median	0.39 ± 0.004	0.34 ± 0.005
	Quantile 75%	0.38 ± 0.005	0.28 ± 0.005
	Quantile 90%	0.29 ± 0.003	0.32 ± 0.004

¹All t are significantly different from 0 ($P < 0.001$).

Systematic Effects

Systematic effects of TMR and concentrate feeding level and milk yield were obtained as regression coefficients in model 2 (Equation 2). For both Holstein and Jersey breeds, significant effects of TMR and concentrate intake were found. This pattern is also illustrated

in Figures 2 and 3. On the other hand, no significant effect of production level, DIM, or lactation number on the $\text{CH}_4:\text{CO}_2$ ratio was observed for Jerseys or for Holsteins. However, there seemed to be a tendency that higher milk production also meant higher CH_4 production (Figure 4) and for Holsteins, a tendency that more DIM meant higher CH_4 production (Figure 5),

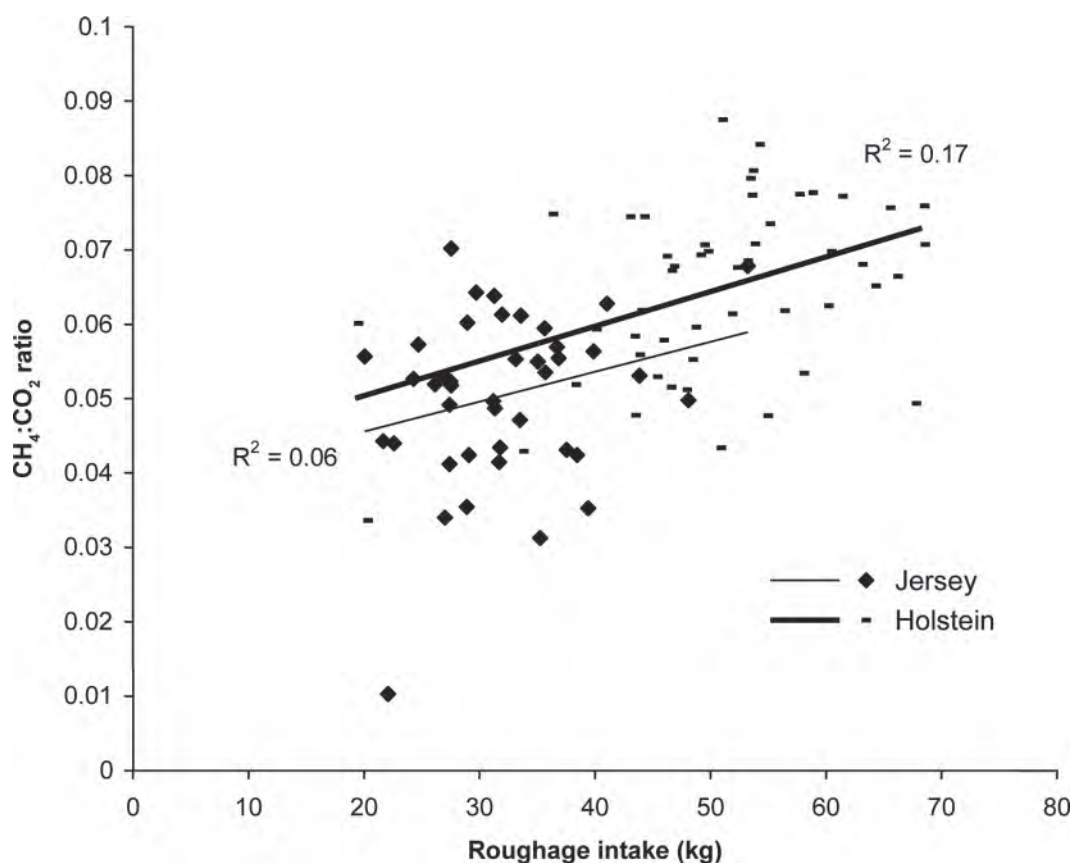


Figure 2. Plots of mean of medians for all automatic milking system visits/cow for $\text{CH}_4:\text{CO}_2$ ratio against kilograms of roughage intake for Jersey (\blacklozenge) and Holstein (\blacksquare) cows.

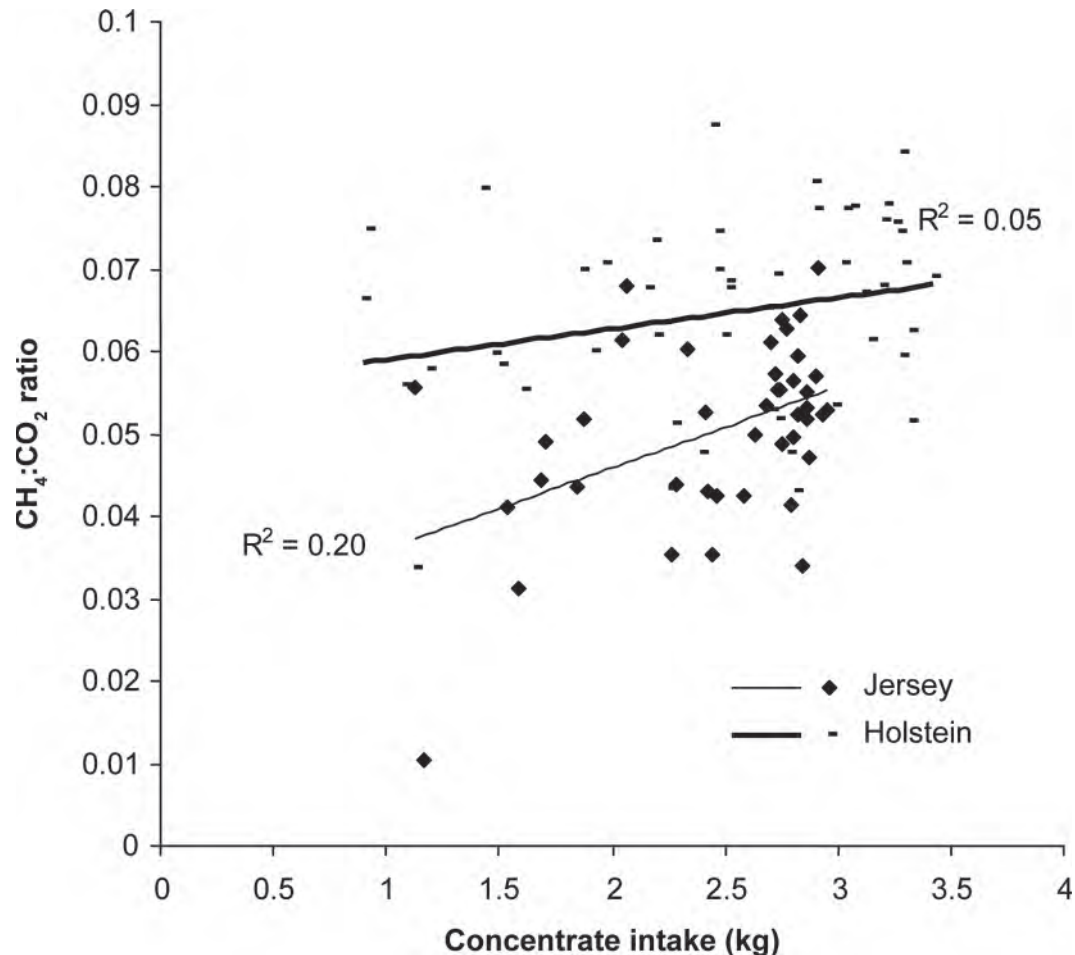


Figure 3. Plots of mean of medians for all automatic milking system visits/cow for $\text{CH}_4:\text{CO}_2$ ratio against kilograms of concentrate intake for Jersey (\blacklozenge) and Holstein (\square) cows.

although none of these effects was significant. Several reasons could exist why no significant effect of milk production level on CH_4 production was observed. One could be that the majority of the animals we measured were not in the critical period of lactation with extreme milk production and usage of body reserves. Another reason could be a confounding between DIM and milk production, which would be hard to separate when the number of records is low. Madsen et al. (2010) found a slight positive correlation between milk production and the $\text{CH}_4:\text{CO}_2$ ratio when cows were fed ad libitum on a basal TMR diet and supplemented with a graded amount of concentrate according to their milk yield.

A higher proportion of TMR will increase the $\text{CH}_4:\text{CO}_2$ ratio, as relatively more CH_4 is produced and the production of CO_2 is unchanged when acetic acid or butyric acid fermentation and hydrogen production in the rumen is high (Aguerre et al., 2010). This will be even higher if the TMR ration is rich in fiber, low in starch, and rich in fat (Aguerre et al., 2010; Johannes

et al., 2010). Also, a high feed intake will increase the $\text{CH}_4:\text{CO}_2$ ratio as the extra feed is fermented and produces CH_4 , but the cow does not necessarily produce proportionally more heat or CO_2 ; that is, if the energy is deposited and only about 50% of the extra energy intake is metabolized. Thus, some extra CO_2 is produced or energy is delivered in the milk and only about 30% of the extra energy intake is metabolized to CO_2 .

In addition to these factors, variation exists over the day in relation to the feed intake and the activity of the cows. Methane production is high when the fermentation of the feeds in the rumen is high, and a maximum is expected a few hours after feeding (Figure 6). So, the CH_4 production is highest in the daytime and lowest during the night. On the other hand, CO_2 production is high when the activity of the cows is high. These factors make the $\text{CH}_4:\text{CO}_2$ ratio change slightly over the day as was modeled using sinusoid functions in Equation 2.

Some factors will decrease the $\text{CH}_4:\text{CO}_2$ ratio. A high proportion of concentrates, lipids, or other components

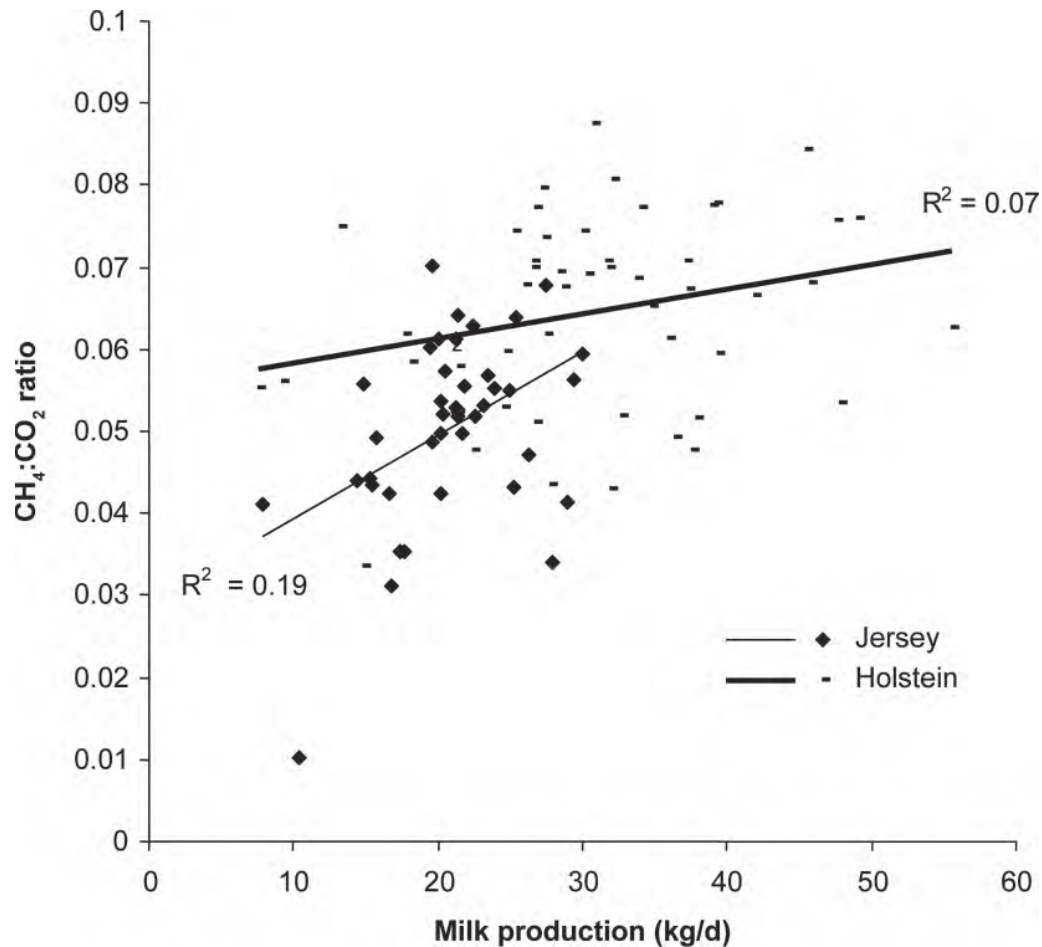


Figure 4. Plots of mean of medians for all automatic milking system visits for $\text{CH}_4\text{:CO}_2$ ratio/cow against kilograms of milk produced per day for Jersey (\blacklozenge) and Holstein ($-$) cows.

of limited physical structure in the diet will decrease acetic acid or butyric acid production. Also, high milk production without a proportionally higher feed intake, as during mobilization of energy from body tissues, may decrease the $\text{CH}_4\text{:CO}_2$ ratio. The listed effects of feed intake and milk yield may counterbalance each other, showing only a weak correlation between milk production and the $\text{CH}_4\text{:CO}_2$ ratio.

In this study, all cows were fed the exact same TMR ration and, therefore, it was not possible to draw inference on how different feedstuffs influence CH_4 production. Additionally, no analysis was done on diurnal or day-to-day changes in the TMR ration that the cows were fed. As a consequence, any potential dietary variation was deliberately ignored in the analysis.

The observation period was restricted to 3 d so that the estimated repeatabilities are strictly short term. To evaluate the stability of measures in more depth, further records covering longer time intervals (months)

are needed. However, the magnitude of the short-term repeatability indicates that repeated records are needed within each measurement session to obtain reliable records.

Toward Genetic Evaluation

Even using a model where we account for feed intake, production level, DIM, and lactation number we were able to show clear individual variation. A part of this variation is likely to be under genetic control and thereby, the trait is heritable. The repeatability is assumed to be the upper boundary for the heritability of the trait. With only 50 and 43 cows it is not possible to estimate reliable genetic parameters for any trait. It would, therefore, be interesting to make more recordings for the trait to estimate heritability and correlations to other traits such as milk production. With repeatability estimates of 0.35 and 0.37 for

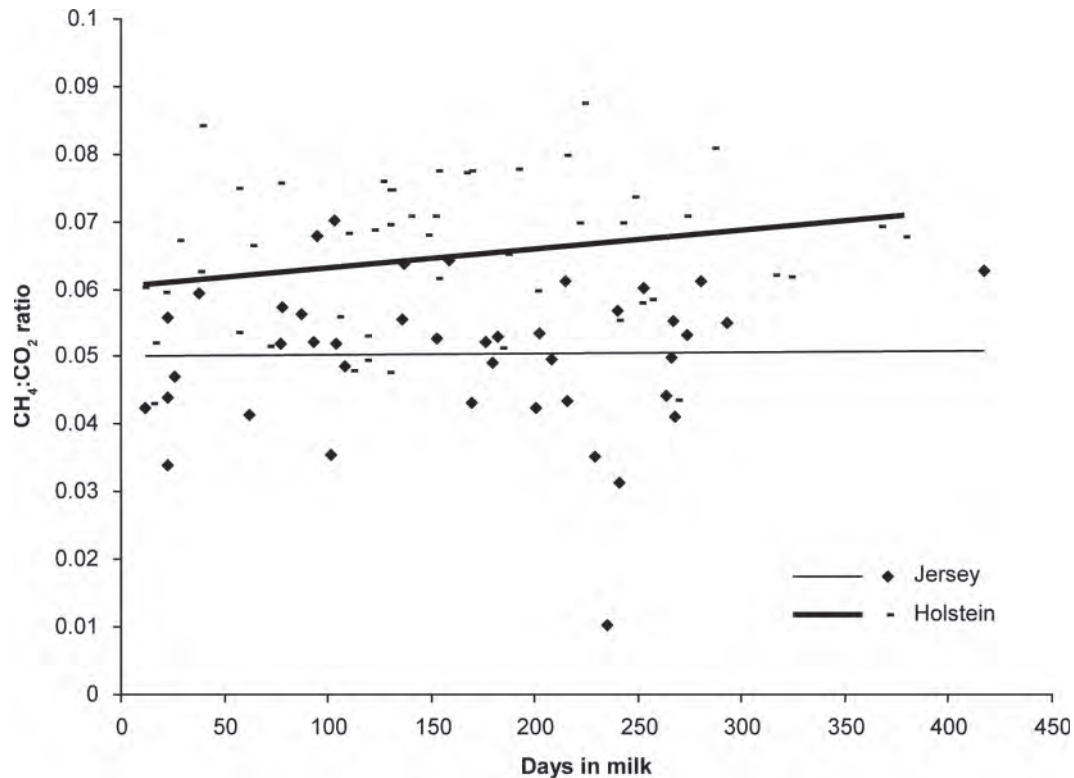


Figure 5. Plots of mean of medians for all automatic milking system visits for $\text{CH}_4:\text{CO}_2$ ratio against DIM at registration for Jersey (\blacklozenge) and Holstein (\diamond) cows.

the $\text{CH}_4:\text{CO}_2$ ratio we have reasons to believe that the FTIR instrument is useful in providing further insight into genetic variation in CH_4 production and possible correlations to production and health traits. So far, no genetic evaluation of CH_4 production from dairy cattle has been performed. In a study by Robinson et al. (2010), a repeatability of 0.32 and a heritability of 0.13 were presented for 1-h CH_4 production records on 708 sheep. With a heritability of that magnitude, it would be possible to select for the trait and thereby change the level of CH_4 production in dairy cattle.

The CH_4 measurements based on noninvasive FTIR methods could be introduced as a dairy herd management tool, as it can measure a range of other gasses from the cow's breath and thereby indicate aberrations in the cow's metabolic status.

CONCLUSIONS

This study has shown that individual differences between cows in their production of CH_4 are measurable using a portable FTIR measuring unit in an AMS. Repeatability estimates of 0.37 and 0.33 were found for Holsteins and Jerseys, respectively, for the median of the ratio between CH_4 and CO_2 . The FTIR instrument

combined with AMS may be useful to generate large-scale data for genetic evaluation of CH_4 production in dairy cattle.

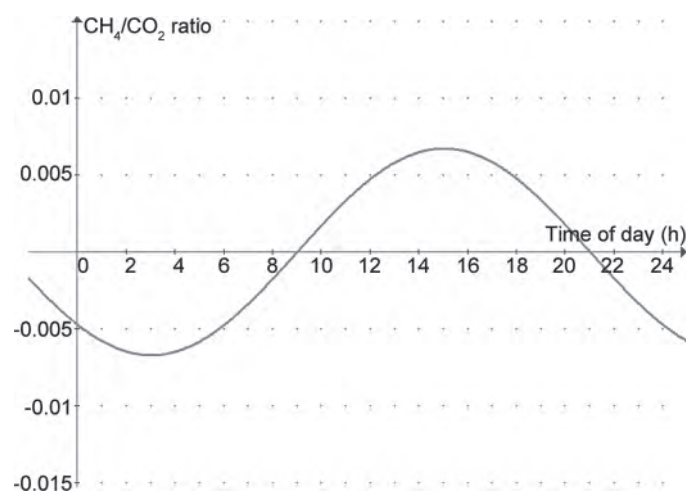


Figure 6. Diurnal changes in the median of the $\text{CH}_4:\text{CO}_2$ ratio fitted from solutions from Equation 2 using Holstein data.

ACKNOWLEDGMENTS

The authors acknowledge the Danish Cattle Research Centre (Foulum, Denmark) for providing data for this study and for providing the opportunity to take breath measurements of the cows in their herd. This study was partly financed by the Danish Council for Independent Research | Technology and Production Sciences. The project number is 11-105913.

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