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Individual variation and repeatability of methane production from dairy cows estimated by the CO2 method in automatic milking system

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The objectives of this study were to investigate the individual variation, repeatability and correlation of methane (CH4) production from dairy cows measured during 2 different years. A total of 21 dairy cows with an average BW of 619 ± 14.2 kg and average milk production of 29.1 ± 6.5 kg/day (mean ± s.d.) were used in the 1st year. During the 2nd year, the same cows were used with an average BW of 640 ± 8.0 kg and average milk production of 33.4 ± 6.0 kg/day (mean ± s.d.). The cows were housed in a loose housing system fitted with an automatic milking system (AMS). A total mixed ration was fed to the cows ad libitum in both years. In addition, they were offered concentrate in the AMS based on their daily milk yield. The CH4 and CO2 production levels of the cows were analysed using a Gasmet DX-4030. The estimated dry matter intake (EDMI) was 19.8 ± 0.96 and 23.1 ± 0.78 (mean ± s.d.), and the energy-corrected milk (ECM) production was 30.8 ± 8.03 and 33.7 ± 5.25 kg/day (mean ± s.d.) during the 1st and 2nd year, respectively. The EDMI and ECM had a significant influence ($P < 0.001$) on the CH4 (l/day) yield during both years. The daily CH4 (l/day) production was significantly higher ($P < 0.05$) during the 2nd year compared with the 1st year. The EDMI (described by the ECM) appeared to be the key factor in the variation of CH4 release. A correlation ($r = 0.54$) of CH4 production was observed between the years. The CH4 (l/day) production was strongly correlated ($r = 0.70$) between the 2 years with an adjusted ECM production (30 kg/day). The diurnal variation of CH4 (l/h) production showed significantly lower ($P < 0.05$) emission during the night (0000 to 0800 h). The between-cows variation of CH4 (l/day, l/kg EDMI and l/kg ECM) was lower compared with the within-cow variation for the 1st and 2nd years. The repeatability of CH4 production (l/day) was 0.51 between 2 years. In conclusion, a higher EDMI (kg/day) followed by a higher ECM (kg/day) showed a higher CH4 production (l/day) in the 2nd year. The variations of CH4 (l/day) among the cows were lower than the within-cow variations. The CH4 (l/day) production was highly repeatable and, with an adjusted ECM production, was correlated between the years.

Keywords: breath, diurnal variation, methane, phenotypic correlation, dairy cows

Implications

Daily methane (CH4) production is different between cows. CH4 production mainly depends on the feed intake, which is related to the milk production. The variation of CH4 production remained even after the standardization of the feed intake and milk yield. This animal variation can most likely be used to select cows with low CH4 production as a long-term mitigation approach. For the selection of the correct low CH4 emitting cows, it is important that the measured low emission can be repeated. This experiment shows that the ranking of the cows can be repeated over different years.

Introduction

The livestock sector represents a significant source of greenhouse gas (GHG) emissions worldwide, generating carbon dioxide (CO2), methane (CH4) and nitrous oxide throughout the production process. This sector is often the focus of study because of its large impact on the environment. A recent report by Gerber et al. (2013) described that the majority of CH4 emissions occurred from the livestock sector as a result of enteric fermentation.
and feed production. In the livestock sector, cattle are the highest contributors of GHG emissions; the GHG emissions from cattle account for 65% of the GHG emissions from the livestock sector (4.6 Gt CO$_2$ eq). Of the total emissions, cattle emit the most enteric CH$_4$, that is, ~77%, followed by the other domesticated species (Gerber et al., 2013). Another consideration in addition to environmental pollution is that between 2% and 12% of the ingested gross energy is lost through CH$_4$ emission (Johnson and Johnson, 1995); this loss of energy could potentially be used by the animals. The CH$_4$ emissions from the animals vary according to the level of feed intake, type of carbohydrate, type of feed processing, addition of lipids, alteration of rumenal microflora (Johnson and Johnson, 1995) and measurement techniques (Vlaming et al., 2008). In addition, it can also vary as a result of the genetic variation of the animals (Pinares-Patiño et al., 2013). One of the earlier studies using a standard respiration chamber reported a CV of 7% for within-animal variation for CH$_4$ production and of 7% to 8% for between-animal variation (Blaxter and Clapperton, 1965). More recently, several authors reported a CV of 4.3% for within-animal variation and 17.8% for between-animal variation using open-circuit calorimetry (Grainger et al., 2007). Using the SF$_6$ technique, Vlaming et al. (2008) mentioned a wider range of variation in CH$_4$ emissions for two different diets (6.91% to 10.09% for within cow and 6.23% to 27.79% for between cow). Moreover, under grazing conditions, Lassey et al. (1997), Boadi et al. (2002) and McNaughton et al. (2005) reported between-animal variations of 11.5%, 15.5% and 25% CV, respectively, using the SF$_6$ technique. In a comparative study using two different techniques, Grainger et al. (2007) mentioned a higher within-cow variation (CV = 19.6%) for SF$_6$ techniques compared with the chamber technique (CV = 17.8%). To date, most studies have estimated the animal variation in CH$_4$ production, either by using the traditional chamber technique or SF$_6$ techniques, where handling and confinement of the animals is required. A drawback of these methods is that they might have an influence on the normal metabolism of the animals. In this study, we assume that the animal should be free from any influential factors to understand individual variability in CH$_4$ production. We hypothesize that CH$_4$ production resulting from animal variation would be lower if the measurements are taken from their natural environment. In the dairy industry, automatic milking systems (AMS) reduce human involvement and interactions with cows, thus allowing the cows to have free movement. Therefore, under this condition, normal feeding and milking behaviour as well as rumen metabolism and gas production can be expected. The ‘CO$_2$ method’, a newly developed technique for CH$_4$ estimation, was used in this study. This method is non-invasive and measures the CH$_4$ production from cows by keeping them in their natural environment. The objectives of this study were (i) to investigate individual variation and CH$_4$ production repeatability measured in an AMS and (ii) to investigate the correlation of CH$_4$ production of individual cows during 2 different years.

### Material and methods

#### Animals, experimental design and feeding

A total of 21 dairy cows with an average BW of 619 ± 14.2 kg and average milk production of 29.1 ± 6.5 kg/day (mean ± s.d.) were used in the 1st year. Among the total number of cows, 14 were primiparous and seven were multiparous in the 1st year. The cows were in the same lactation stage, with an approximate calving interval of 12 months. During the 2nd year, the same cows were used, with an average BW of 640 ± 8.0 kg and average milk production of 33.4 ± 6.0 kg/day (mean ± s.d.). The cows were housed in a loose housing system that had adequate ventilation and was fitted with an AMS. The study was conducted without interfering with the feeding and management planned by the farm. During both years, the measurements were taken from the same cows in the same AMS. The experimental period was 7 days in the 2nd week of May each year. The cows were offered a total mixed ration (TMR) ad libitum (Table 1) in both years. In addition to the TMR, they were offered concentrate in the AMS based on their daily average milk production. The TMR was allocated in the morning at ~0700 h, and at ~1500 h, the remaining feed residuals were mixed and moved closer to the cow. A total of 57 cows were milked in the AMS; of these 57, 23 cows were common in both years. Among the common cows, two cows showed abnormal milking behaviour. One cow had just calved and only visited the AMS for 3 of the 7 days of measurements. The other cow visited the AMS once per day and was treated for lameness. These two cows were therefore excluded from the analysis; thus, 21 cows were studied.

#### Gas measurement

The CH$_4$ and CO$_2$ production levels of the cows was analysed using a continuous gas analyser, the ‘Gasmert DX-4030’ (Gasmert Technologies Oy, Helsinki, Finland), based on Fourier transformed IR. The inlet filter of the Gasmert was fitted on the feeding pen of the AMS to obtain concentrated breath samples from individual cows. The breath samples pass through the inlet filter and then through the Gasmert to determine the concentration of CH$_4$ and CO$_2$. The measurements were performed every 15 s over 24 h for 7 consecutive days during milking in the AMS. Each individual cow visited the AMS at least two times per day (ranging from 1 to 4, average 2.54). Before the first measurement, the Gasmert was calibrated with standard gases to check the accuracy of the measurements. The Gasmert was disconnected for 10 min randomly during each measurement day to obtain the blank concentration of CH$_4$ and CO$_2$. The measurement of this concentration was used as a correction factor for the entire experimental period to obtain the actual breath concentration of CH$_4$ and CO$_2$. The measurements were remotely monitored via the internet using TeamViewer.

#### Calculations

Identification numbers and the entrance and exit times of each individual cow were recorded in a computer connected to the AMS. These data were matched with the breath analysis data from the Gasmert. All of the calculations regarding
Individual variation of \( \text{CH}_4 \) production in dairy cows

(described in step I) multiplied by the total CO\(_2\) production per day (described in step II) and results in the amount of CH\(_4\) produced.

The concentrate intake in the AMS was measured individually on a daily basis while the TMR intake was considered to be a herd average. The total estimated dry matter intake (EDMI, kg/day) was calculated by adding the individually recorded concentrate dry matter intake (DMI) (kg/day) to the corrected TMR dry matter intake (kg/day) using equation (4) according to Kristensen and Ingvartsen (2003). In this case, a supplementation rate of 0.5 was considered for the concentrate intake. The actual energy-corrected milk (ECM, kg/day) was calculated using equation (5), according to Sjaunja et al. (1991). Standardized CH\(_4\) production and CH\(_4\) : CO\(_2\) ratios were calculated at the adjusted 30 (kg/day) ECM level according to equations (6) and (7).

\[
\text{HP (watt)} = 5.6 \times \text{BW}^{0.75} + [(Y \times 22) + (1.6 \times 10^{-5} \times \text{P}^3)]
\]

(1)

\[
\text{CO}_2 (L) = \text{HP} \times 180 \times 24
\]

(2)

\[
\text{CH}_4 (L) = \text{CO}_2 \times \frac{\text{CH}_4}{\text{CO}_2}
\]

(3)

\[
\text{TMRDMI (kg/day)} = a + 0.5(b - c) + d
\]

(4)

\[
\text{ECM (kg)} = Y \times [0.383 \times \text{milkfat} + 0.242 \times \text{milkprotein} + 0.7832] / 3.14
\]

(5)

\[
\text{Standardized CH}_4/L = \text{CH}_4 + (30 - \text{ECM}) \times q
\]

(6)

\[
\text{Standardized CH}_4/\text{CO}_2 \text{ ratio} = \frac{\text{CH}_4}{\text{CO}_2} + (30 - \text{ECM}) \times s
\]

(7)

where \( a \) is the average TMR intake; \( b \) the average concentrate intake; \( c \) the concentrate intake of the individual cows during the experimental periods; \( d \) the correction factor for the lactation number; \( d = -1.61 \) was used for first lactation and \( d = 0.39 \) was used for the second and subsequent lactations; HP the heat production of the animals; BW\(^{0.75}\) the metabolic BW of the animals; \( Y \) the milk yield of the cows; \( P \) the number of days the cows were pregnant; \( s \) the slope of the regression of CH\(_4\) : CO\(_2\) ratio as a function of ECM in each year separately; \( q \) the slope of the regression of CH\(_4\) as a function of ECM in each year separately; \( q \) the slope of the regression of CH\(_4\) as a function of ECM in each year separately.

**Statistical analyses**

Data were analysed with linear mixed models using the lmer function fitted by the restricted maximum likelihood from the package ‘lme4’ (Bates and Sarkar, 2009) using R software (R Development Core Team, 2013). An extension package ‘ImerTest’ was used to obtain the \( P \) value directly from the Imer function (Kuznetsova et al., 2012). Individual 24-h mean emissions were considered for the interpretation of the results. The analyses focused on making inferences on the

### Table 1 Feed allocation and nutrient composition of diet over the 2 years

| Ingredients                          | 1st year (DM, kg/day) | 2nd year (DM, kg/day) |
|--------------------------------------|-----------------------|-----------------------|
| Total mixed ration                   |                       |                       |
| Rapeseed cake                        | 1.6                   | 1.3                   |
| Soybean decorticated                 | 1.4                   | 1.0                   |
| Clover grass silage                  | 3.4                   | 2.9                   |
| Maize silage                         | 9.0                   | 10.7                  |
| Ryegrass straw                       | 0.6                   | 1.4                   |
| Urea                                 | –                     | 0.1                   |
| Beet pulp                            | –                     | 0.9                   |
| Vitamin mineral premix               | 0.2                   | 0.2                   |
| Concentrate supplied in AMS          |                       |                       |
| Concentrate                          | 4.0                   | 4.2                   |

**AMS** = automatic milking system; **DM** = dry matter; **MEI** = metabolizable energy intake; **AAT** = amino acids absorbed in the small intestine; **PBV** = protein balance in the rumen.

1. Nutrient and energy values were calculated using the Danish feed stuffs table (Møller et al., 2000).

2. Net energy for feed utilization (Nørgaard et al., 2011).

the CH\(_4\) estimation were performed according to the CO\(_2\) method (Madsen et al., 2010). The protocol of the method is described in the following three steps.

**Step I: Calculation of the CH\(_4\) : CO\(_2\) ratio.** The CO\(_2\) method uses the measured CH\(_4\) : CO\(_2\) ratio from the breath sample analysis of the individual cows. The average barn concentrations of CH\(_4\) (23.2 and 25.8 ppm) and CO\(_2\) (495.8 and 625.5 ppm) were obtained during measurements in the 1\(^{st}\) and 2\(^{nd}\) year, respectively. These concentrations were subtracted from the exhaled concentrations to get the corrected CH\(_4\) and CO\(_2\) (ppm) of the individual cows. The data that were below 400 ppm for the corrected CO\(_2\) were removed to avoid the influence of samples that contained a very low concentration of CH\(_4\) and CO\(_2\) (ppm). The ratio between CH\(_4\) and CO\(_2\) (CH\(_4\) : CO\(_2\)) was thereafter calculated.

**Step II: Calculation of the total CO\(_2\) production per day.** To calculate the total CO\(_2\) production from the individual cows, it is necessary to first calculate the total heat production (HP). The HP of the cows was calculated according to equation (1) using the cows’ body mass, milk production and number of days pregnant as described by CIGR (2002). Thereafter, the total CO\(_2\) production per day was calculated according to Pedersen et al. (2008), as shown in equation (2).

**Step III: CH\(_4\) estimation.** The amount of CH\(_4\) was calculated according to equation (3). This uses the CH\(_4\) : CO\(_2\) ratio
individual variation and repeatability of CH₄ production (l/day, l/kg EDMI and l/kg ECM). The models were fitted on the yearly data subset. The BW, EDMI, ECM, parity and days of pregnancy were included as fixed effects in the primary model that was fitted with the maximum likelihood method. Cows and the number of visits to the AMS were included as random effects. The final model (equation (6)) was confirmed by the stepwise elimination of non-significant variables. The significance of the fixed effects was assessed by $F$ ratio tests, and the significance of the random effects was assessed by likelihood-ratio tests. Model validations were performed with ANOVA based on the Akaike Information Criterion. The model residuals were checked for normality by visual inspection of qqplots. The final model is:

$$y_j = \mu + X_{ij} + X_{ij} + \delta_j + C_j + \epsilon_j$$ (8)

where \(y_j\) is the response variable \(y\) (CH₄ (l/day), CH₄ (l/kg EDMI), CH₄ (l/kg ECM) and CH₂ (l/kg ECM)) of cow \(j\) and \(\mu\) the overall mean. The fixed effects are the \(X_{ij}\) (EDMI (kg/day)) of cow \(j\); \(X_{ij}\) (ECM (kg/day)) of cow \(j\); \(X_{ij}\) (parity of cow \(j\)); and \(X_{ij}\) (random effect of cow \(j\)). \(\delta_j\) is the parity of cow \(j\); \(C_j\) the random effect of cow \(j\) and \(\epsilon_j\) are the residual errors. Model estimates were extracted using the glht function from the ‘multcomp’ package (Hothorn et al., 2008). The CVs of CH₄ production between cows (CVbc) and within cow (CVwc) were calculated from the variance components of the model (equation (8)) using equations (9) and (10). The variance components were defined as the ratio of the individual random effect (\(\sigma^2\)) and the variance of the random error (\(\sigma^2\)) to the estimated mean (\(\bar{X}\)).

$$CV_{bc} = \frac{\sigma^2_D}{\bar{X}} \times 100$$ (9)

$$CV_{wc} = \frac{\sigma^2_e}{\bar{X}} \times 100$$ (10)

The variance components from the same model (equation (8)) were used to obtain the repeatability (\(R\)) within a given year, calculated as the proportion of between-animal variation with respect to the total variance as:

$$R = \frac{\sigma^2_D}{\sigma^2_D + \sigma^2_e}$$ (11)

The differences of CH₄ production between the 2 years were assessed by the following model:

$$y_{ij} = \mu + \lambda_i + X\beta_j + X_1\gamma_{ij} + \delta_j + C_j + \epsilon_{ij}$$ (12)

where \(\lambda_i\) is the year of measurement with \(i = 1:2\) years; \(X\beta_j\) the EDMI (kg/day) of year \(i\) and cow \(j\); \(X_1\gamma_{ij}\) the ECM (kg/day) of year \(i\) and cow \(j\); \(\delta_j\) the parity of cow \(j\); \(\gamma_{ij}\) the random effect of cow and \(\epsilon_{ij}\) are the residual errors. The between-year repeatability (\(R_y\)) of CH₄ production was calculated using the variance components of the model fitted with EDMI (kg/day), ECM (kg/day) and parity as fixed effects and the year of the measurements as the random effect.

Yearly data subsets of the daily mean emissions during milking were considered for the visualization of the diurnal variation of CH₄ production following the model (equation (13)).

$$y_{ij} = \mu + \lambda_i + X\beta_j + X_1\gamma_{ij} + \delta_j + C_j + \epsilon_{ij}$$ (13)

where \(\mu\) is the overall mean; \(\lambda_i\) the hours of measurements in a day with \(i = 1:24\); \(X\beta_j\) the EDMI (kg/day) of cow \(j\); \(X_1\gamma_{ij}\) the ECM (kg/day) of cow \(j\); \(\delta_j\) the parity of cow \(j\); \(C_j\) the random effect of cow \(j\) and \(\epsilon_{ij}\) are the residual errors.

Results

Feed intake, milk and CH₄ production in 2 years

BW (kg), milk production (kg/day), ECM (kg/day) and EDMI (kg/day) were higher during the 2nd year compared with the 1st year (Table 2). The CH₄ production (l/day) was positively correlated with the ECM (kg/day) in both years (Figure 1a). A correlation was observed between CH₄ production (l/day) and EDMI (kg/day) during the 1st year (Figure 1b). However, CH₄ production (l/kg ECM) and EDMI (kg/day) were not correlated during the 2nd year (Figure 1b). The CH₄ production (l/kg ECM) revealed a negative correlation with the ECM (kg/day) in both years (Figure 1c). However, no correlation was found when the amount of CH₄ (l/kg EDMI) was plotted against the EDMI (kg/day) (Figure 1d).

Variation of CH₄ production in 2 years

CH₄ production, along with its variability and repeatability, were obtained from the fitted model (equation (6)) using the yearly data subsets (Table 3). The daily production of CH₄ (l/day) and l/kg ECM) was significantly lower \(P < 0.05\) in the 1st year compared with the 2nd year. However, CH₄ (l/kg EDMI) was similar in both years. The between-cow variation of CH₄ emissions (l/day, l/kg ECM and l/kg EDMI) was lower \((CV_{bc} = 8.8\%\) to 9.1\%) than the within-cow variation \((CV_{wc} = 15.7\) to 16.4\%) during the 1st year. The range of the variation during the 2nd year was narrower \((CV_{bc} = 5.9\) to 6.1 and CV_{wc} = 8.6 to 9.1\%) compared with that of the 1st year. Similarly, variations of the CH₄: CO₂ ratios were lower during the 2nd year \((CV_{bc} = 6.2\) and CV_{wc} = 8.8\%) compared with the variations during the 1st year \((CV_{bc} = 8.4\) and CV_{wc} = 15.9\%).

| Parameters       | 1st year        | 2nd year        |
|------------------|-----------------|-----------------|
| BW (kg)          | 619.9 ± 14.2    | 640.0 ± 8.0     |
| Milk yield (kg/day) | 29.1 ± 6.5      | 33.4 ± 6.0      |
| EDMI (kg/day)    | 30.8 ± 8.0      | 33.7 ± 5.3      |
| TMRI (DM, kg/day)| 15.8 ± 0.5      | 18.9 ± 0.5      |
| CI (DM, kg/day)  | 4.0 ± 1.0       | 4.2 ± 1.6       |
| EDMI (kg/day)    | 19.8 ± 1.0      | 23.1 ± 0.8      |

ECM = energy-corrected milk; TMRI = total mixed ration intake; DM = dry matter; CI = concentrate intake; EDMI = estimated dry matter intake.

Values indicated arithmetic means and standard deviations (mean ± s.d.).
Correlation of CH4 production between 2 years

The individual mean emissions over 7 days were used to establish the correlation of CH4 emissions between years. A correlation (r = 0.54) was observed in the CH4 emission between the 2 years in the actual ECM (kg/day) production (Figure 2a). This correlation was increased (r = 0.70) when it was calculated with an adjusted ECM production (30 kg/day) (Figure 2b). The yearly difference of CH4 (l/day) in the actual ECM (kg/day) production was more (P = 0.008) compared with the difference in the adjusted ECM production (P = 0.01). However, the CH4 : CO2 ratio was significantly (P < 0.001) different between years in both the actual and adjusted ECM (kg/day) production. The correlation of the CH4 : CO2 ratio between years was slightly increased (r = 0.80) in the adjusted ECM compared with the value (r = 0.78) of the actual ECM production (Figure 2c and d).

Repeatability of CH4 production

The within-year repeatability (R) of CH4 production (l/day, l/kg EDMI and l/kg ECM) was lower (0.35 to 0.37) during the 1st year than in the 2nd year (0.40 to 0.41). The observed repeatability between years (R2) was 0.51 to 0.45 for the same parameters (Table 3). Likewise, the CH4 : CO2 ratio was more repeatable in the 2nd year (0.41) compared with the observed R during the 1st year (0.34), whereas the resultant R2 of the CH4 : CO2 ratio was 0.45 (Table 3).

Diurnal variation of CH4 production

The diurnal variations of CH4 (l/h) in 2 different years are shown in Figure 3. During the 2nd year, the diurnal variation indicated declining emissions between 0000 and 0800 h, with the lowest emission at 0800 h. The emissions reached a peak at ∼0900 h and continued
with the same magnitude up to 1600 h. The CH₄ production at this time ranged from 24 to 27 l/h. After 1600 h, the emissions declined. During the 1st year, a sudden drop in CH₄ (l/h) was observed at 1200 h. However, the rest of the hours followed a similar pattern, with more variable emissions over time.

When the CH₄ emissions (l/h) were aggregated into time intervals (0000 to 0600 h = night; 0601 to 1200 h = morning; 1201 to 1800 h = afternoon and 1801 to 2359 h = evening), a significant difference (data were not shown) was found over 6-h intervals (P = 0.01) during the 2nd year. However, during the 1st year, the CH₄ (l/h) emissions were not different, except for lower emissions at night (P = 0.02).

**Discussion**

The results of this study have implications for the selection of cows with low CH₄ production for breeding purposes. CH₄ production was quantified from 2 different years for the
same cows in a commercial dairy farm that were provided a similar diet in both years. Data from the same cows measured over 2 years were used to test different aspects of the variability in CH₄ production over time.

**Key source of variation for CH₄ production**

**Concentration of breath samples.** The estimation of CH₄ production using breath samples of cows indicates considerable variation. The concentration of the breaths collected by the inlet filter of the GASMET™ depends on the nose position of the cows. More importantly, the concentration of CH₄ depends on whether the breaths and/or the eructations come from the rumen. This study showed a higher CV of the individual breath concentration (Figure 4a). The same evidence was described by Haque et al. (2014a) in a previous study. The substantial variation among the individual breath concentrations are a reflection of normal biological rhythms. In this connection, Garnsworthy et al. (2012a) stated a certain variation in eructation frequency, and the CH₄ concentration in eructation is correlated with the differences in daily CH₄ emissions. Unlike the respiration chamber technique, the non-invasive methods for CH₄ estimation considered samples that had ambient exposure. Hence, some changes in the concentrations might occur. The average concentration of CO₂ in breath typically ranges from 30 000 to 50 000 ppm. To obtain a typical breath concentration through a sampling inlet is very sporadic and is mostly influenced by the physiology of the animals and the exposure of the breath samples to the ambient air. However, trapping 2% to 3% of breath samples through the sampling device was suggested to be sufficient for a reasonably precise CH₄ estimation from ruminants (Madsen et al., 2010). In terms of variation, the individual breath concentrations show very large fluctuations that often mislead CH₄ estimations. As shown in Figure 4, the CV gradually decreased when the visit-average (Figure 4b) or day-average (Figure 4c) data were considered. Moreover, a CV of 10.2% was found using period average data for 21 cows (Figure 4d). In this case, there is no repetition of the measurements for individual cows; hence, it is not possible to calculate within- and between-cow variations. However, these data can still be used to establish CH₄ production with 4.5% precision (s.e. = CV × √(1/n−1), i.e., 0.102 × 570/√(21−1) = 13) for the diet when measuring for 7 days on 21 cows. To be precise in the CH₄ estimation through breath sample analysis using the CO₂ method, it is important to consider the mean of several individual samples, such as the emission levels per visit or per day.

**EDMI and ECM production.** Most of the studies agreed that DMI is a key factor in daily CH₄ emission (Blaxter and Clapperton, 1965; Johnson and Johnson, 1995; Grainger et al., 2007); a second key factor is determined by the digestibility of the diet (Blaxter and Clapperton, 1965; Johnson and Johnson, 1995) and the amount of concentrate or lipid supplement (Beauchemin, 2009). In this study, the EDMI and ECM had a significant influence on CH₄ yield during both years. The effect was most likely because the increased amount of EDMI was mediated by the increased body mass and ECM production. Therefore, in a commercial farming situation, where recording individual DMI is rare, the ECM can be used to explain the variation of CH₄ production. Higher ECM production and EDMI (kg/day) in the 2nd year resulted in significantly (P< 0.05) higher CH₄ (l/day). The CH₄ (l/kg EDMI) was similar in both years, which supports the fact that more CH₄ is produced at a higher EDMI. In this connection, Boadi and Wittenberg (2002) also mentioned that 64% of the variation in CH₄ production is explained by the DMI. The results of this study are also in line with several recent findings where diet effects on CH₄ emissions were investigated (Beauchemin, 2009; Doreau et al., 2011). In addition, Grainger et al. (2007) and Garnsworthy et al. (2012b) described similar results where DMI was mentioned as the primary determinant of CH₄ production. Moreover, the negative correlation between CH₄ (l/kg ECM) and the amount of ECM (kg/day) in this study revealed a reduced amount of CH₄ per unit of product in the same line as the results previously described by Tamminga et al. (2007).

**Levels of variation.** In a typical feed evaluation study using a respiration chamber, the animal variation of CH₄ production is minimized by a fixed amount of feed provided to the animals. Nevertheless, significant variation among the animals remained. A large scale CH₄ measurement study with 215 dairy cows (Garnsworthy et al., 2012b) indicated a between-cow variation of 23% (CV), whereas the within-cow variation was 6%. Based on the same data and using a mixed model, the reported variance components were 18.9% between cows and 11.5% within cows. Individual animal variations of 26.6% and 25.3% have been reported for dairy and beef heifers with *ad libitum* and restricted feeding, respectively (Boadi and Wittenberg, 2002). Blaxter and Clapperton (1965) analysed the results of 23 investigations in which sheep were offered the same amount of the same diet in contrast with another 30 investigations in which the intake was scaled according to the BW. In both analyses, the reported CV in CH₄ emission were 7% to 8% between animals and 5% to 7% within animals. The results from 16 calorimetric studies in dairy cows with *ad libitum* feeding showed a wider range of CV (3% to 34%) in CH₄ production (Ellis et al., 2010). This large variation in CH₄ emission was due to the wide range of DMI. Using a respiration chamber and SF₆ tracer technique to measure CH₄ production from lactating dairy cows that were fed *ad libitum*, Grainger et al. (2007) reported within- and between-cow variations of 6.1% and 19.6% for SF₆ techniques and of 4.3% and 17.8% for the chamber techniques, respectively. Furthermore, in a study using the SF₆ technique with four non-lactating dairy cows, Vlamings et al. (2008) indicated within- and between-cow variations of 6.91% to 10.09% and 6.23% to 27.79% in two diets, respectively. A wide range of individual cow variations of CH₄ emissions (22% to 67%) were reported in a recent study with 1964 cows from 21 commercial farms (Bell et al., 2014).
Figure 4 Levels of variation exist in different types of data: (a to c) are for one cow, and (d) is for 21 cows. (a) Individual observations of the concentration of corrected CH$_4$ (ppm), where the broken lines separate the visits to the AMS; (b) the mean CH$_4$ (l/day) (with s.e. bars) using visit-average data; and (c) the mean CH$_4$ (l/day) (with s.e. bars) using day-average data. The CVs shown on (a to c) are considering 21 cows using raw data, the visit-average data and the day-average data, respectively. (d) Mean CH$_4$ (l/day) (with s.e. bars) using the period average (7 days) data per cow, and the CV in this case is calculated as the s.d./expected mean. AMS = automatic milking system.
Repeatability and correlation of CH$_4$ production over 2 years. Repeatability expresses the total variation that is reproducible among repeated measures of the same subject (Nakagawa and Schielzeth, 2010). In this study, the repeatability of CH$_4$ (l/day) emissions was 0.36 and 0.41 during the 1st and 2nd years, respectively. The repeatability of CH$_4$ emissions in the 1st year was slightly lower presumably because of the higher within-cow variation. This result is similar to earlier findings in dairy cows and sheep (Vlaming et al., 2008; Pinares-Patiño et al., 2013). In agreement with the present study, the repeatability of the CH$_4$ : CO$_2$ ratio in Holstein cows was 0.37 (Lassen et al., 2012), which is considered to be an effective measure for the estimation of CH$_4$ production. Contrary to the present study, Pinares-Patiño et al. (2011) reported very low repeatability (0.16) in sheep where CH$_4$ was measured using a chamber technique to rank the animals according to their emission rate.

A substantial variation in CH$_4$ (l/day) emissions was observed among individual cows during the 2 years. This variation was most likely caused by the differences in the EDMI and ECM between the 2 years. However, with the adjusted ECM production (30 kg/day), the CH$_4$ emissions were strongly correlated between the years. This correlation of CH$_4$ (l/day) is probably related to genetic variation, that is, the heritability of CH$_4$ production that was previously mentioned by Lassen et al. (2012) and Pinares-Patiño et al. (2013). The latter also stated that even after adjustment for feed intake or ECM, the trait will be repeatable. It is important to mention that cows normally show varying levels of production that ultimately results in a variable CH$_4$ production. Therefore, the estimation of CH$_4$ at a adjusted/standardized production is necessary in a herd, especially when ranking the cows based on CH$_4$ production over different time spans. The observed correlation of CH$_4$ production from individual cows in the current study could be used as an index in CH$_4$ mitigation strategies by selecting low-emitter cows for the breeding process. It is worth noting that when dealing with a large number of animals for CH$_4$ measurements, there will always be some individuals who are different from others because of oestrus, lameness or any other problems that affect normal feed intake, physiology, body activity or metabolism; consequently, these result in variations in CH$_4$ production. Therefore, these factors should be taken into consideration.

Diurnal variation. A sudden drop in CH$_4$ emissions (l/h) at the 1200 h during the 1st year is surprising and is therefore not comparable with other reports. This is most likely the result of a fewer number of cows that visited the AMS at that specific hour, consequently producing a lower number of observations. However, the diurnal pattern of CH$_4$ (l/h) in the 2nd year showed identical results to the results described by Garnsworthy et al. (2012b). Some other methods for CH$_4$ estimation, such as polytunnels grazing animals (Lockyer, 1997) and point source dispersion in grazing animals (McGinn et al., 2011), showed a comparable diurnal pattern. The diurnal variation is most likely linked with the animal’s behaviour, digestive physiology and ambient condition (Garnsworthy et al., 2012b), especially feeding behaviour. In the current study, feed was always available to the cows, the daily feed allocation was distributed at ~0700 h, and at ~1500 h, the remaining feed residuals were mixed and moved towards the cow. This might lead to synchronized feeding behaviour at a specific time. However, the milking time was widely different for every cow in the AMS, where milking was performed throughout a 24-h period. Therefore, the diurnal pattern might be more related to the feeding time rather than the milking time. The influence of the milking time could be considered for other methods where milking is performed, for example, twice a day at a fixed time.

Conclusions

On a herd average basis, daily CH$_4$ production was significantly higher in the 2nd year as a result of a higher EDMI (kg/day). The CH$_4$ emission per kg EDMI was similar throughout the 2 years. The study indicates that the key factor of variation in CH$_4$ production is EDMI; this key factor can also be described by ECM production. When measuring for a short period of time, for example, a visit in the AMS or in a single day, the variation in CH$_4$ (l/day) emission between cows was lower than within cows. The diurnal pattern of CH$_4$ (l/h) production was influenced by the feeding behaviour of the cows and was lowest from 0000 to 0800 h. The CH$_4$ production (l/day) was 51% repeatable over the 2 years. Individual cow variations over an average of 7 days show a
strong positive correlation, especially when CH₄ production is standardized using ECM in both years. This relation of CH₄ from individual cows between the 2 years shows a potential opportunity for the selection of low CH₄ emitter cows.

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