Technical note: Interchangeability and comparison of methane measurements in dairy cows with 2 noninvasive infrared systems

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ABSTRACT

This study aimed to compare measurements of methane (CH₄) and carbon dioxide (CO₂) concentrations in the breath of dairy cows kept in commercial conditions using the Fourier-transform infrared spectroscopy (FTIR) and nondispersive infrared spectroscopy (NDIR) methods. The measurement systems were installed in an automated milking system. Measurements were carried out for 5 d using both systems during milkings. The measurements were averaged per milking, giving 467 observations of CH₄ and CO₂ concentrations of 44 Holstein Friesian cows. The Pearson correlation between observations from the 2 systems was 0.86 for CH₄, 0.84 for CO₂, and 0.88 for their ratio. The repeatability of FTIR (0.53 for CH₄, 0.57 for CO₂, and 0.28 for their ratio) was somewhat higher than that of NDIR (0.57 for CH₄, 0.47 for CO₂, and 0.25 for their ratio). The coefficient of individual agreement was 0.98 for CH₄, 0.89 for CO₂, and 0.89 for their ratio; the concordance correlation coefficient was 0.48 for both gases and 0.24 for their ratio. We showed that FTIR and NDIR give similar results in commercial farm conditions. They can therefore be used interchangeably to generate a larger data set, which could then be further used for genetic evaluation.

Key words: methane emission, nondispersive infrared, Fourier-transform infrared spectroscopy, method comparison

Technical Note

Global livestock methane (CH₄) emissions have been estimated to be 119.1 ± 18.2 Tg (Wolf et al., 2017). Methane emitted by animals can be measured in multiple ways (Hill et al., 2016). Although respiration chambers are considered the gold standard for this task, they are expensive, labor intensive, and time

consuming, which has led to a search for alternatives. So-called sniffers are an example of devices with the potential to generate large amounts of data under farm conditions (Garnsworthy et al., 2012; Lassen et al., 2012; Huhtanen et al., 2015). These devices use 2 infrared light absorption methods that differ in light wavelength to determine the CH₄ and carbon dioxide (CO₂) concentrations of the air sample. The first method, nondispersive infrared spectroscopy (NDIR), uses short wavelengths and has been used in several studies (Garnsworthy et al., 2012; Bell et al., 2014; Huhtanen et al., 2015). The second method, Fourier-transform infrared spectroscopy (FTIR), uses longer wavelengths and is capable of measuring multiple gases at the same time (Lassen et al., 2012; Pszczola et al., 2017). Several studies have compared FTIR systems with NDIR systems. Difford et al. (2016) compared the infrared systems usually used in the sniffer setup, whereas Sorg et al. (2018) compared FTIR and NDIR with the laser CH₄ detector. To the best of our knowledge, this is the first study to directly compare the NDIR system with the FTIR system used in the sniffer setup. The aim of this study was thus to compare measurements of the FTIR system by comparing the CH₄ and CO₂ concentrations, and their ratio, measured during milking under commercial conditions with those of the NDIR system.

The CH₄ and CO₂ concentrations were measured during milking with an automated milking system (AMS) at a commercial farm located in western Poland. Forty-four Holstein Friesian cows were monitored for 5 d, giving a total of 467 observations from milking. The cows had ad libitum group access to partial mixed rations, and additional concentrates were given to cows during milking. The feeding routine was as follows: morning feeding at 0600 h, pushing the feed from the previous feeding such that cows could reach it at 0730 h, feed pushing and feeding at 1130 h, and final feed pushing at 1800 h. Sampling inlets of both systems were placed in the middle part of the feeding bin 5 cm from each other. Measurements were performed simultaneously and independently by each system.

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The FTIR system measured the CH₄ and CO₂ concentrations using a Gasmeter 4030 system (Gasmeter Technologies, Helsinki, Finland). Zero-point calibration was performed every day using nitrogen gas. Measurements were taken during milking for 24 h with a 5-s measuring interval. The clocks of the systems and of the milking machine were synchronized with the same time server to assign particular milkings to gas measurements and to specific cows. Air samples were acquired by sucking in air exhaled by the cow. The airflow rate was 4 L/min. Next, the air passed through polytetrafluoroethylene hoses warmed to 60°C before passing a check valve. It was then heated to 180°C in another filter and passed through a pump. Finally, the gas entered the system. The FTIR system was equipped with a 0.4-L cuvette with a 5-m optical track length.

The CH₄ and CO₂ concentrations were measured using 2 separate NDIR systems (1 system per gas) operating in the near-infrared spectrum (detector 1210 Gfx, Servomex 4100; Servomex, Crowborough, UK). Measurements were taken for 24 h at 2-s measuring intervals. Two measuring channels were used: CO₂ concentration in the 0 to 2.5% (0–48,450 mg/m³) range and CH₄ concentration in the 0 to 1,000 ppm (0–706 mg/m³) range. The sample was collected and then ducted to the system via a polyethylene tube with an 8-mm diameter. Before entering the analyzer, air samples were dried using a gas cooler (TC-standard with polyvinylidene fluoride exchanger; Bühler Technologies, Ratingen, Germany). The sampling rate was 0.6 L/min. Before beginning, the experimental systems were calibrated using calibration gases: nitrogen 5.0 (99.999 vol. % purity) and 1,210 ppm of CH₄ in nitrogen. The system was equipped with a 0.17-L cuvette with 540-mm optical track length for CH₄ and a 0.012-L cuvette with 154-mm optical track length for CO₂.

The variances within and between the cows and the correlation between the average gas observations from the 2 systems were assessed using a linear mixed-effects model with Kronecker product covariance structure in a doubly multivariate setup (Hamlett et al., 2004; Roy, 2009) using the MIXED procedure in SAS University edition (version 9.3; SAS Institute Inc., Cary, NC). The number of replicated observations per cow per day varied and was not equal between cows. The number of replicated observations with each method for each cow is thus p_i , and the number of observations of cow i (n_i) equals $2 \times p_i$. The linear mixed-effects model for cow i was

$$y_i = \beta_0 + \beta_1 m_{i1} + \beta_2 m_{i2} + \beta_3 day_k + \sum_{n=1}^5 (f_{sm} \sin \varnothing + f_{cm} \cos \varnothing) + b_{1i} z_{i1} + b_{2i} z_{i2} + \varepsilon_i,$$

where y_i is the response [any combination of method and observation (CH₄ or CO₂) replication] of cow i ; β_0 is the intercept; β_1 and β_2 are fixed effect parameters corresponding to the 2 systems (1 for FTIR and 2 for NDIR); m_{i1} and m_{i2} indicate the method to which the response y_i belongs and take the value 1 or 0 (thus, if the response comes from the first method, m_{i1} will equal 1 and m_{i2} will equal 0); β_3 is the fixed effect of the date of milking; the variable day_k describes the calendar day of milking; f_{sm} and f_{cm} are fixed regression coefficients of the Fourier series linear covariates of the time of measurement; and $\varnothing$ is an angle in radians equal to $2\pi s/86,400$, where s is the second of the day at which the measurement was taken. The terms b_{1i} and b_{2i} are random effects parameters corresponding to the 2 systems; z_{i1} and z_{i2} relate the responses y_i to the 2 systems and take the values 0 or 1 in the same way as m_{i1} and m_{i2} ; and ε_i is the error in the i th observation. The Kenward–Roger correction was used in computing the correct denominator degrees of freedom of the fixed effects in the presence of repeated measures (Kenward and Roger, 1997). This model is equivalent to a bi-variate model in which the observations from FTIR are treated as one trait and those from NDIR as a second trait. The agreement between the observations from the 2 systems was determined by computing the repeatability correlation (i.e., the correlation between random cow solutions for the 2 systems), the concordance correlation coefficient (CCC), and the coefficient of individual agreement (CIA) without a reference method (Barnhart et al., 2007a).

Comparison of measurements from a single, randomly chosen milking (Figure 1 for CH₄, Figure 2 for CO₂, and Figure 3 for the CH₄/CO₂ ratio) showed similarities in the general shape of the curve between the systems. The selected milking lasted for 275 s; due to differences in the sampling rates of the systems, the number of measurements obtained for this milking was 56 in the case of FTIR and 138 in the case of NDIR. The NDIR method yielded more extreme values of gas concentration, and the slopes were steeper compared with FTIR. Moreover, NDIR detected gases more rapidly (earlier retention time and earlier end time of peaks); therefore, peaks obtained from NDIR had sharp edges. These effects were due to technical differences between the systems. Peaks in the NDIR readings for CH₄ were steep and occurred earlier due to the smaller cuvette (0.17 vs. 0.4 L in FTIR), shorter optical track (540 mm vs. 5 m in FTIR), and differences in sampling rates. The NDIR CO₂ measurements were performed in a separate and even smaller cuvette (0.012 L with a 154-mm optical track length), whereas the FTIR used the same cuvette for both gases. Figure 3 demonstrates these differences,

Figure 1. Sample methane (CH_4) measurements of a randomly chosen milking using Fourier-transform infrared spectroscopy (solid line) and nondispersive infrared (dashed line) methods.

Figure 2. Sample carbon dioxide (CO_2) measurements of a randomly chosen milking using Fourier-transform infrared spectroscopy (solid line) and nondispersive infrared (dashed line) methods.

showing that the NDIR is capable of capturing more CH_4 than CO_2 compared with the FTIR system.

Despite noticeable differences in the standard deviations of the systems, the differences between measurements during milking were statistically insignificant, with both systems yielding similar measurements averaged across the whole milking, as shown in Table 1. This indicates that even though FTIR tended to smooth measurements within a milking, it was capable of capturing an average gas concentration that was similar to that of the more responsive NDIR over the whole period of a single milking.

The observations were averaged within hour of the day across all days, and all cows were similar for both systems, as shown in Figures 4, 5, and 6. Only small differences between systems were seen in terms of the CH_4 hourly observations (Figure 4; FTIR = 531.09 ± 55.58 ; NDIR = 530.101 ± 52.098 ; $\pm$ SD). These differences were not significant ($P = 0.9384$). The CO_2 observations (Figure 5) were more divergent (FTIR = $6,646.55 \pm 1,875.34$; NDIR = $6,318.72 \pm 1,644.24$; $\pm$ SD) and were significantly different from each other ($P = 0.02274$). The shape of the curves for both gases (Figures 4 and 5) was similar between systems. However, CO_2 observations (Figure 5) were less consistent than those of CH_4 (Figure 4). Observations for the ratio of CH_4/CO_2 (Figure 6) were significantly different ($P < 0.001$). However, the shape of the curves was very similar, which is to be expected because they represent the combination of the 2 gases. The variability of daily averages did not differ significantly between the systems ($P = 0.82$ for CH_4 ; $P = 0.092$ for CO_2). The FTIR had higher variability than the NDIR in the case of CH_4 . In the case of CO_2 , the NDIR had higher variability than the FTIR. The overall mean of CH_4 observations (i.e., averaged within the whole period), as shown in Table 1, was comparable between the 2 systems (FTIR = 548.13

± 49.018 ; NDIR = 541.96 ± 28.81 ; $\pm$ SD) and the differences were not significant ($P = 0.82$). Overall CO_2 observations differed from each other (FTIR = $6,646.55 \pm 1,875.34$; NDIR = $6,430.61 \pm 1,644.24$; $\pm$ SD), with a P -value of 0.092. The average values reported here differ from those found by Negussie et al. (2017), where CH_4 concentrations were about 10% higher and CO_2 concentrations were almost 2 times lower. A possible explanation for these differences could be the different technique used in that study (photoacoustic spectroscopy with CO_2 as the tracer gas) and the differences between the analyzed populations. Similarly, Difford et al. (2016) reported considerably higher average CH_4 and lower CO_2 concentrations than those in our study. The differences in the average level of gas concentrations may be due to the different systems used in the measurements, the type of diet, and differences in animals used (in terms of the cows' productivity, body size, and genetics). The deviations may also be due to differences in AMS feed bin geometry, as the 3 studies

Figure 3. Sample methane (CH_4)/carbon dioxide (CO_2) ratio of a randomly chosen milking using Fourier-transform infrared spectroscopy (solid line) and nondispersive infrared (dashed line) methods.

Table 1. Mean methane (CH₄) and carbon dioxide (CO₂) concentrations (ppm) over the entire measuring period using Fourier-transform infrared spectroscopy (FTIR) and nondispersive infrared spectroscopy (NDIR) systems

Item	CH ₄		CO ₂		CH ₄ /CO ₂ ratio	
	FTIR	NDIR	FTIR	NDIR	FTIR	NDIR
Mean	548.13 ¹	541.96 ¹	6,880.54 ²	6,430.61 ²	0.079 ³	0.084 ³
SD	49.02	28.81	317.14	418.17	0.0064	0.0065
Maximum	603.90	570.61	7,208.76	7,090.81	0.093	0.097
Minimum	478.56	495.4	6,461.11	5,978.72	0.063	0.067

¹*P* = 0.82 for differences between estimates.²*P* = 0.092 for differences between estimates.³*P* < 0.001 for differences between estimates.

used different AMS systems. Measurements with the sniffer method can be affected by the variable air-mixing conditions created by the geometry of the feed bin, sampling inlet movement, and its position (Huhtanen et al., 2015). In genetic studies, these differences can be adjusted during modeling and should not affect the genetic evaluation when all individuals are measured under similar conditions and when measurements are repeated multiple times per individual. The estimated repeatability was similar for both systems and gases (see Table 2). The repeatability for FTIR was 0.53 for CH₄ and 0.57 for CO₂; for NDIR, the values were 0.45 for CH₄ and 0.47 for CO₂. Our repeatabilities were similar to those of Negussie et al. (2017), who used photoacoustic spectroscopy but with measurements averaged over the entire day; this had the effect of decreasing residual error variance and leading to an increase in the estimated repeatability. The repeatability we obtained for the ratio measurement (0.28 for FTIR and 0.25 for NDIR) was lower than the repeatability obtained by Lassen et al. (2012), who used FTIR measurements

per milking. The lower values reported in our study may result from the smaller number of animals, shorter measurement period, or differences in the statistical model.

Pearson correlations between observations from the 2 systems were 0.85 for CH₄, 0.84 for CO₂, and 0.88 for the CH₄/CO₂ ratio. The CCC was equal for both gases at 0.48 and for the ratio was 0.24; the value of CIA equaled 0.98 for CH₄ and 0.89 for both CO₂ and the ratio. Difford et al. (2016) showed that NDIR outperformed FTIR on the basis of repeatability estimates. We showed that the FTIR and NDIR systems were similar. Moreover, the correlations observed in this study exceed those reported by Difford et al. (2016).

Pearson correlation coefficient was high; however, it does not account for the repeated measurements and is usually discouraged from use in method comparison studies (Bland and Altman, 1986; Carstensen, 2011). The CIA and CCC depend on the between-subjects and within-subject variances (cow variance in this case). The CCC decreases as the within-cow variance

Figure 4. Comparison of average hourly methane (CH₄) observations from Fourier-transform infrared spectroscopy (solid line) and nondispersive infrared (dashed line) methods. Shaded areas correspond to SE. **Figure 5.** Comparison of average hourly carbon dioxide (CO₂) observations from Fourier-transform infrared spectroscopy (solid line) and nondispersive infrared (dashed line) methods. Shaded areas correspond to SE.

increases and increases with the between-cows variance, thus making it partly population specific and less useful for the assessment of a more uniform population. In contrast, the CIA increases together with the within-cow variance and is thus more suitable for measurements on less-differentiated populations. Our data replicated the observations for subjects, and the number of replicates per subject differed. We thus suggest that the CIA is the most appropriate criterion for evaluating the interchangeability of the systems investigated here. However, when assessing the agreement in terms of averages of test groups of animals, the within-group error variance is ignored, and it is thus not possible to compute the CIA or the precision unless the group measurements are repeatedly recorded. In such instances, CCC is the only metric available (Barnhart et al., 2007b).

The high CIA value (Table 2) for CH₄ suggests strong similarities between the observations obtained using the 2 systems (particularly that the accuracy and precision of the systems were similar). It is therefore possible to use both systems interchangeably after rescaling the measurements to achieve similarity in the scales of the variances. The use of the sniffer method based on the FTIR and NDIR systems combined with an AMS on a larger scale could prove useful for generating large-scale data on CH₄ emission in dairy cattle.

This study validated the FTIR and NDIR sniffers under commercial conditions. We showed that, despite their technical differences, the FTIR and NDIR systems provide similar results when measuring CH₄ and CO₂. Thus, if installed in similar conditions, these 2 systems can be used interchangeably to measure CH₄ and CO₂ concentrations during milking, with the aim of generating a larger data set that can be further used for genetic evaluation.

Figure 6. Comparison of average hourly methane (CH₄)/carbon dioxide (CO₂) ratio obtained from Fourier-transform infrared spectroscopy (solid line) and nondispersive infrared (dashed line) methods. Shaded areas correspond to SE.

Table 2. Sources of agreement and disagreement for methane (CH₄) and carbon dioxide (CO₂) observations from the Fourier-transform infrared spectroscopy (FTIR) and nondispersive infrared spectroscopy (NDIR) systems¹

Item ²	CH ₄		CO ₂		CH ₄ /CO ₂ ratio	
	FTIR	NDIR	FTIR	NDIR	FTIR	NDIR
LSM	540 (19.10)	533 (16.03)	6,766 (196.13)	6,319 (158.33)	0.079 (0.10)	0.084 (0.10)
Between-cows variance	14,333 ^a	9,727 ^b	1,534,967 ^a	937,916 ^b	3.32 × 10 ^{-5a}	3.18 × 10 ^{-5b}
Within-cow variance	12,810 ^a	11,876 ^b	1,165,301 ^a	1,101,365 ^b	8.74 × 10 ^{-5a}	9.44 × 10 ^{-5b}
Total variance	27,143 ^a	21,603 ^b	2,700,268 ^a	2,058,464 ^b	1.21 ^a	1.26 ^b
Repeatability	0.53 (0.065)	0.45 (0.065)	0.57 (0.063)	0.47 (0.064)	0.28 (0.059)	0.25 (0.055)
Pearson correlation	0.85*		0.84*			0.88*
CCC	0.48		0.48			0.24
CIA	0.98		0.89			0.89

^{a,b}Estimates with different letters differ with $P < 0.001$. The significance of the differences between estimates for FTIR and NDIR was assessed for each gas separately.
¹Measurements were averaged per milking; SE in parentheses.
²CCC = concordance correlation coefficient; CIA = coefficient of individual agreement.
* $P < 0.001$ for statistically significant correlation.

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