Short communication: Use of a portable, automated, open-circuit gas quantification system and the sulfur hexafluoride tracer technique for measuring enteric methane emissions in Holstein cows fed ad libitum or restricted

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ABSTRACT

The objective of this study was to measure enteric CH₄ emissions using a new portable automated open-circuit gas quantification system (GQS) and the sulfur hexafluoride tracer technique (SF₆) in midlactation Holstein cows housed in a tiestall barn. Sixteen cows averaging 176 ± 34 d in milk, 40.7 ± 6.1 kg of milk yield, and 685 ± 49 kg of body weight were randomly assigned to 1 out of 2 treatments according to a cross-over design. Treatments were (1) ad libitum (adjusted daily to yield 10% orts) and (2) restricted feed intake [set to restrict feed by 10% of baseline dry matter intake (DMI)]. Each experimental period lasted 22 d, with 14 d for treatment adaptation and 8 d for data and sample collection. A common diet was fed to the cows as a total mixed ration and contained 40.4% corn silage, 11.2% grass-legume haylage, and 48.4% concentrate on a dry matter basis. Spot 5-min measurements using the GQS were taken twice daily with a 12-h interval between sampling and sampling times advanced 2 h daily to account for diurnal variation in CH₄ emissions. Canisters for the SF₆ method were sampled twice daily before milking with 4 local background gas canisters inside the barn analyzed for background gas concentrations. Enteric CH₄ emissions were not affected by treatments and averaged 472 and 458 g/d (standard error of the mean = 18 g/d) for ad libitum and restricted intake treatments, respectively (data not shown). The GQS appears to be a reliable method because of the relatively low coefficients of variation (ranging from 14.1 to 22.4%) for CH₄ emissions and a moderate relationship (coefficient of determination = 0.42) between CH₄ emissions and DMI. The SF₆ resulted in large

coefficients of variation (ranging from 16.0 to 111%) for CH₄ emissions and a poor relationship (coefficient of determination = 0.17) between CH₄ emissions and DMI, likely because of limited barn ventilation and high background gas concentration. Research with improved barn ventilation systems or outdoors is warranted to further assess the GQS and SF₆ methodologies.

Key words: dairy cow, gas quantification system, methane, sulfur hexafluoride tracer technique

Short Communication

Several methodologies have been used to measure enteric CH₄ emissions from dairy cattle, including calorimetric chambers (Blaxter and Clapperton, 1965; Hellwing et al., 2012), the sulfur hexafluoride tracer technique (SF₆; Johnson et al., 1994; Williams et al., 2011), open-path laser measurements (Laubach and Kelliher, 2005), continuous gas analyzer systems (Teye et al., 2009), head chambers (Kelly et al., 1994; Garnsworthy et al., 2012; Hegarty, 2013), and CO₂ as a tracer (Madsen et al., 2010). All of these methods have some limitations and potential errors, which have been discussed in depth in the literature (Storm et al., 2012). An alternative method, a portable automated open-circuit gas quantification system (GQS; GreenFeed; C-Lock Inc., Rapid City, SD), was recently developed and it has now been used by researchers globally (Scott Zimmerman, C-Lock Inc., personal communication). The GQS allows for spot, automated measurements of CH₄ and CO₂ in real time with minimal disturbance of cow's natural behavior (Hegarty, 2013). The objective of the current study was to evaluate the enteric CH₄ measurements obtained by the GQS and the SF₆ tracer technique within a tiestall dairy barn. We hypothesized that short-term emissions using the GQS could be a precise (i.e., low CV) technique for capturing differences in CH₄ emissions between treatments, whereas the SF₆ tracer technique would lack accuracy under

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high background gas concentrations as a result of poor barn ventilation.

Use and care of animals was approved by the University of New Hampshire Institutional Animal Care Use Committee (no. 121203). The experiment was conducted at the University of New Hampshire Fairchild Dairy Teaching and Research Center located in Durham (43.14°N, 70.95°W). A naturally ventilated tiestall barn with individual stalls (1.8 × 1.5 m) covering both sides of the barn (81 m) equipped with a daily flushed manure gutter system was used. Ventilation was provided by windows that automatically opened to varying degrees depending on outside temperature. Windows were set to remain closed when the temperature was below 7.2°C. The outside air temperature averaged −1.3 and 6.1°C during periods 1 and 2, respectively, and was determined by hourly data from the National Climate Data Center weather station at the University of New Hampshire Kingman Farm, Durham (43.13°N, 70.98°W; 6 km away). Sixteen Holstein cows (12 multiparous and 4 primiparous) averaging 176 ± 34 DIM, 40.7 ± 6.1 kg of milk yield, and 685 ± 49 kg of BW at the start of the study were used. Baseline DMI (mean = 24.5 ± 2.56 kg/d) was determined for 7 consecutive days before the start of the experiment. Cows were then blocked by DIM, parity, and DMI as a percentage of BW (mean = $3.94 \pm 0.35\%$) and, within each block, randomly assigned to 1 out of 2 treatments according to a crossover design. The treatments were (1) ad libitum (**AL**) adjusted daily to yield 10% Orts and (2) restricted feed intake (**RFI**) set to restrict feed by 10% of baseline DMI. Specifically, cows ($n = 16$) in period 1 were split equally across treatments ($n = 8/\text{treatment}$) and then switched to a new treatment (AL or RFI) in period 2. Each period lasted 22 d with 14 d for diet adaptation and 8 d for data and sample collection with both methods (i.e., GQS and SF₆) used simultaneously to measure CH₄ emissions from AL and RFI treatments. Cows were milked twice daily (0430 and 1600 h) with milk yield recorded throughout the duration of the study.

A TMR was fed to all cows and consisted (DM basis) of 40.4% corn silage, 11.2% grass-legume haylage, and 48.4% of a concentrate mix [3.61% steam-flaked corn, 9.86% ground dry corn, 11.4% soybean meal, 7.33% citrus pulp, 0.71% molasses, 1.07% dry distillers grains with solubles, 3.50% canola meal, 2.93% soybean hulls, 0.18% urea, 3.30% minerals and vitamins, 1.59% ProAAL Elite (AA supplement; Perdue Agribusiness Inc., Salisbury, MD), and 0.81% fat (BergaFat; Berg+Schmidt GmbH & Co., Hamburg, Germany)]. Alfalfa pellets (Poulin Grain, Newport, VT; 16.9% CP, 56.0% NDF, and 35.0% ADF) used in the GQS comprised 2.11% (DM basis) of the TMR. The resulting

TMR averaged (DM basis): 16.8% CP, 34.7% NDF, 24.3% ADF, and 19.2 MJ/kg of gross energy (Dairy One Laboratory, Ithaca, NY). Animals were fed twice a day (0500 and 1400 h) with 40% of the total daily ration fed in the morning and the remaining 60% in the afternoon to account for the difference in feeding time intervals. Orts, if present, were removed and weighed daily before the afternoon feeding. Samples of TMR were collected daily, pooled by week, and stored at −20°C until determination of DM (55°C for 48 h) in a forced-air oven (VWR Scientific, Radnor, PA).

The GQS operated by releasing 50 g of alfalfa pellets up to 5 times per feeding with a minimum of 45 s apart triggered by a radio frequency ear tag and the cow's head located inside the manger. This allowed 250 g of delivered alfalfa pellets per sampling and encouraged maintenance of the animals head inside the manger for feeding bouts lasting approximately 5 min, which was needed for accurate breath sampling and near real-time analysis of CH₄. Sampling times started at 0700 and 1900 h on d 1 of the sampling period with the GQS moved sequentially from cow to cow with at least a 1-min interval between samplings for determination of background gas concentration. Sampling was advanced 2 h daily to account for diurnal variations in CH₄ emissions, thus allowing for 14 measurements per cow per period. Cows were trained with the GQS during the 10 d before the beginning of the study. The CH₄ sensor was calibrated weekly by purging sensors of any gas using purified N followed by a 1,000 mg/kg standard of CH₄. At the beginning of each experimental period, the air flux sensor was calibrated using a 90-g prefilled CO₂ cylinder (KEE Action Sports LLC, Sewell, NJ). Further details about sensors calibration, GQS operation, CH₄ calculations, and data upload can be found on the manufacturer website (www.c-lockinc.com) and Hegarty (2013).

The SF₆ technique originally described by Johnson et al. (1994) was used with the following adaptations. The permeation tubes were built from small (11 mm o.d. × 32 mm) brass permeation rods bored in the center with a 25.4-mm deep × 4.80 mm i.d. wide hole and loaded with 800 to 900 mg of SF₆ by direct syringe injection. The permeation tubes were capped and tightened with a 0.38-mm-thick polytetrafluoroethylene Teflon disc (McMaster Carr, Elmhurst, IL), a 2-μm-pore stainless steel frit (Sigma-Aldrich, St. Louis, MO), and a 6.35-mm nut (Swagelok Inc., Solon, OH). Tubes were weighed immediately after filling and permeation release rates were determined by weighing tubes stored in a Daisy incubator (Ankom Technology, Macedon, NY; set to 39°C) twice weekly. Permeation tubes were weighed for a minimum of 6 wk to determine SF₆ permeation rates with rates varying from 1.13 to 4.97 mg/d (mean =

2.67 ± 1.09 mg/d; $R^2 > 0.99$). Permeation tubes were orally dosed to cows 2 wk before sample collection and were distributed evenly between the 2 treatments based on DMI as percentage of BW resulting in average permeation rates of 2.88 and 2.91 mg/d for AL and RFI treatments, respectively.

Canisters (10.2-cm diameter polyvinyl chloride, 17.8 cm length, 1 L) capped (10.2-cm polyvinyl chloride end caps) and adapted with a 1.10-cm hole fitted with a ball shutoff valve were connected to the sampling apparatus built into a halter as previously described (Makkar and Vercoe, 2007). Halters allowed for positioning of filters and capillary tubing (1.59 mm o.d. and 0.13 mm i.d.; Sigma-Aldrich) that sampled directly above the cows' nostrils and were designed to sample continuously and evenly for 12 h based on capillary tubing length and canister vacuum. Canisters were collected from cows twice daily immediately before milking times (0430 and 1600 h) and replaced with a new flushed and evacuated canister after cows returned from the milking parlor approximately 1 h later. Canisters were diluted (Makkar and Vercoe, 2007) with an ultra-high purity N gas (Specair; Maine Oxy, Southborough, MA) for sample collection and analysis. Gas samples were removed from the canisters and injected into pre-evacuated 10-mL vials with septa (Labco, Houston, TX) and stored until analysis using a Shimadzu GC-8A (Shimadzu, Kyoto, Japan) equipped with flame ionization detection and electron capture detection for CH₄ and SF₆, respectively.

Four local background gas (LBG) canisters were placed at 4 different locations among the cows to minimize spatial effects observed in tracer studies (Williams et al., 2011; Lassey, 2013). Based on locations of the cows and their respective permeation rates, 2 LBG canisters were closer to cows orally dosed with higher SF₆ permeation rates and 2 canisters were closer to the cows with lower SF₆ permeation rates. Due to the large and variable background concentrations measured within the barn, a data filter was applied to remove emission values that were unrealistic. Methane emissions were filtered within a range of 150 to 800 g/d, which was established based on values reported in the literature (Grainger et al., 2007; Hollmann et al., 2012; Muñoz et al., 2012). The background gas concentration used for the calculation of emissions from each cow was based on a selection procedure that consisted of (1) using the background gas concentrations from the closest LBG to the cow, (2) using the LBG average concentrations for SF₆ and CH₄ for the respective collection period of the cow sample, or (3) if the SF₆ sample had a higher SF₆ concentration than the LBG average concentration used in step 2, individual LBG canisters were used. Our

rationale for using this step-based protocol was that LBG concentrations need to be sampled near the cow to accurately calculate CH₄ emissions (Lassey, 2013). If an emission could not be calculated based on the closest LBG, it is conceivable that this specific LBG poorly represented the background gas concentration for that cow, or that there was a sampling issue. Sampling apparatus broken by cows or blockage of tubing from feed prevented sampling from all canisters resulting in 82 (period 1) and 88% (period 2) of canisters being sampled, which is in line with previous research (Grainger et al., 2007). Calculations of enteric CH₄ emissions were done according to Johnson et al. (1994).

Data were analyzed using the MIXED procedure of SAS (version 9.3; SAS Institute Inc., Cary, NC) for a crossover design with repeated measures over time using a statistical model similar to that reported by Brito et al. (2014). Neither day nor day $\times$ treatment interaction was significant and both were removed from the final model. Results are reported as LSM with significance declared at $P \leq 0.05$. A minimum of 5 measurements per cow per period were required for inclusion of a cow into period emission averages. One CH₄ and 2 SF₆ outliers were removed from background concentrations during period 1. One cow refused to use the GQS during both periods.

Intake of DM averaged 23.8 and 22.4 kg/d (SEM = 0.68 kg/d) and was highest ($P < 0.01$) for the AL treatment (data not shown). Conversely, CH₄ emissions, using period averages, did not differ significantly between treatments and averaged 472 and 458 g/d (SEM = 18 g/d) for AL and RFI treatments, respectively (data not shown). This lack of difference in CH₄ emissions may be explained by the relatively modest difference (-1.4 kg/d) in DMI between treatments. The CV observed for CH₄ measurements were up to 5-fold greater for the SF₆ method compared with the GQS (Table 1). Large variability in emissions has been reported in enteric CH₄ studies partially due to inter- and intra-animal variability, but in general a higher variability has been observed in studies using SF₆ (Pinares-Patino et al., 2011; Storm et al., 2012). Average emissions based on all individual measurements were 468 ± 105 and 467 ± 519 g/d for GQS and SF₆, respectively (Table 1). However, when values outside the range of 150 to 800 g/d of CH₄ were removed from the data set, the average per cow per period CH₄ emissions decreased from 464 ± 213 to 405 ± 65 g/d for the SF₆ technique but did not change for the GQS method (Table 1). It is important to note that data-filtered CH₄ emissions per cow per period were within the range of values reported elsewhere (Aguerre et al., 2011; Hassanat et al., 2013; Hristov et al., 2013).

Table 1. Description of data set used to assess CH₄ emissions measured by the portable automated open-circuit gas quantification system (GQS) and the sulfur hexafluoride (SF₆) tracer technique

Item	Individual CH ₄ measurements				Average per cow/period			
	All		150 to 800 g/d ¹		All		150 to 800 g/d ¹	
	GQS	SF ₆	GQS	SF ₆	GQS	SF ₆	GQS	SF ₆
Samples discarded, ² no.	0	28	0	28	0	0	0	1
Samples out of data filter range, no.	0	0	0	81	0	0	0	0
Samples used, no.	396	385	396	304	30 ³	32	30 ³	31
Minimum, g/d of CH ₄	168	6.95	168	150	328	225	328	297
Maximum, g/d of CH ₄	751	5,647	751	792	572	1,240	572	541
Mean, g/d of CH ₄	468	467	468	405	466	464	466	405
SD, g/d of CH ₄	105	519	105	167	65.9	213	65.8	64.6
CV, %	22.4	111	22.4	41.1	14.1	46.0	14.1	16.0

¹Range of CH₄ emissions (g/d) used to filter the data set.

²Twenty-eight negative CH₄ measurements were discarded and 1 average/cow per period was discarded because only 3 CH₄ measurements were available to calculate the average.

³One cow refused to use the GQS during both periods.

Methane emissions were linearly related to DMI ($P < 0.01$; Figure 1), with a better fit for the GQS ($n = 30$; $R^2 = 0.42$) compared with the SF₆ technique ($n = 31$; $R^2 = 0.17$). Based on the lower CV calculated from individual measurements and the stronger relationship between CH₄ emissions and DMI, the GQS appears to be more reliable than the SF₆ technique under the poor barn ventilation and high background gas concentration conditions of the current study.

Our data corroborate previous studies in which large variability in background gas concentrations were detected in experiments conducted with SF₆ (Williams et al., 2011). Measured LBG values ranged from 21.3 to 77.6 µg/kg of CH₄ (mean = 44.8 ± 10.1 µg/kg), whereas those of SF₆ ranged from 24.9 to 390 ng/kg (mean = 120.5 ± 60.1 ng/kg) during the 2 periods (data not shown). The barn windows remained closed most of the time during the study due to low outdoor temperatures, resulting in poor ventilation. Although the presence of 80 lactating dairy cows and a manure gutter with daily flushing also contributed to background gas concentrations higher than previously reported in SF₆ studies (Vlaming et al., 2007), current background concentrations were consistent with those reported for a naturally ventilated barn (Ngwabie et al., 2011). It is noteworthy, however, that CH₄ emissions are not expected to be affected by recycling of self-inhaled gases (Lassey, 2013). This indicates that the large background variability between samplings and LBG canisters is likely more concerning than a high background gas concentration itself. It is well known that the precision of the SF₆ tracer technique is partially dependent on maintaining low background concentrations and variability, which allows differences in CH₄ emissions to be detected (Williams et al., 2011; Lassey, 2013). Permeation rates

ranging from 1.13 to 4.97 mg/d of SF₆ (mean = 2.67 ± 1.09 mg/d) likely contributed to the large variation in our data set, as background gas concentrations can be influenced by neighboring cows with different permeation rates (Lassey, 2013). Whereas the GQS measures a background concentration before sampling the animal, the effects of ventilation and background gas concentration have not yet been examined to the best of our knowledge.

One of the main benefits of the SF₆ method is the ability to sample a large number of animals particularly in outdoor settings (Storm et al., 2012). The GQS was also designed with the intent of sampling a large number of animals (Hegarty, 2013). However, the GQS sampling protocol is still under development and will be optimized by emerging research. Even though the GQS sampling protocol used herein aimed to account for diurnal variation in CH₄ emissions, the low sampling frequency (i.e., twice daily) may not have fully captured the daily fluctuation in CH₄ emissions. In addition to low sampling frequency, CH₄ emissions may be biased by sampling collection time as CH₄ emissions are generally highest after feeding and during rumination bouts (Storm et al., 2012; Hegarty, 2013). However, this potential bias was likely minor in our study, as sampling was scheduled to accommodate a diurnal pattern rather than random animal visits (Hegarty, 2013). An additional constraint of the GQS is the requirement for delivering a bait to attract cows to the manger and keep animals for 3 to 6 min while CH₄ emissions from eructated gas are measured (Hegarty, 2013). This procedure may require up to 1 kg of feed (usually pelleted feed) to be delivered over 4 to 6 visits daily, which increases total dietary energy intake potentially affecting CH₄ emissions by the measurement process itself (Hegarty,

Figure 1. Relationships between CH_4 emissions and DMI for the portable automated open-circuit gas quantification system (GQS; $R^2 = 0.42$, $P < 0.01$; closed circles and continuous line) and the sulfur hexafluoride (SF_6) tracer technique ($R^2 = 0.17$, $P = 0.02$; open circles and dashed line).

2013). Animals in the current study consumed (as fed basis) approximately 500 g of alfalfa pellets daily and the potential interference on CH_4 emissions was likely minor and distributed across both treatments.

Overall, data from the GQS (i.e., relatively low CV and significant relationship between DMI and CH_4 emissions with a moderate R^2) indicate that this methodology can be used to obtain reliable enteric CH_4 emissions data with minimal animal disturbance. Even though the SF_6 tracer technique showed high variability and a poor relationship between DMI and CH_4 emissions, the high concentration of background gases combined with poor barn ventilation were likely more prejudicial to the SF_6 than the GQS methodology. Research with improved barn ventilation systems or outdoors is warranted to further assess these 2 methodologies. Future studies should also report a detailed description of the methodology and data management protocol used to enable unbiased comparisons of enteric CH_4 emissions across sites and experiments.

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