Comparison of 3 methods for estimating enteric methane and carbon dioxide emission in nonlactating cows

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ABSTRACT: Among techniques for estimating enteric methane (CH₄) emission by ruminants, open-circuit respiration chambers (OC), the use of a gas tracer (SF₆), and the GreenFeed (GF) device are the most commonly used. In this study, we compared these techniques in 8 dry cows receiving a diet made of 70% hay and 30% concentrates given in limited and constant amounts, in a 15-wk experiment. Two periods in free stalls for SF₆ and GF and in chambers for OC were used; in addition, SF₆ was determined in chambers for 1 period. Methane emission (g/d) and CH₄ yield (g/kg DMI) were higher ($P < 0.0001$) for OC than for SF₆ and GF (367, 310, and 319 g/d for OC, SF₆, and GF, respectively). The difference between OC and GF was related to a difference in post-prandial rate of gas emission. The between-animal coefficient of variation of CH₄ emission was higher for SF₆ than for OC and GF (20.8, 13.5, and 12.0% on average, respectively). Correlation coefficients between OC and SF₆ were high and significant

for CH₄ emission and CH₄ yield ($r = 0.782$ and $r = 0.717$, respectively; $P < 0.05$), but not significant between OC and GF, or between SF₆ and GF. Correlation coefficients were highly significant for SF₆ determined either in free stalls or in chambers ($r = 0.908$ and 0.903 for CH₄ in g/d and g/kg DMI, respectively; $P < 0.01$). Carbon dioxide (CO₂) emission and CO₂ yield were similar for GF and OC (10,003 and 9,887 g/d, 752 and 746 g/kg DMI, respectively); CO₂ data obtained with SF₆ were lower (7,718 g/d and 606 g/kg DMI; $P < 0.0001$), but this technique is not relevant for CO₂ emission determination. Correlation coefficients between OC and GF were not significant for CO₂ emission and CO₂ yield. This set of results shows that differences between methods are minor for average values, but that individual correlations may limit their interchangeability for determining gas emissions of individual animals. This study also shows the reliability of GF on-farm determination of CH₄ and CO₂ emissions for groups of animals.

Key words: comparison of methods, cow, GreenFeed, methane, open-circuit respiration chamber, sulfur hexafluoride

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J. Anim. Sci. 2018.96:1559–1569

doi: 10.1093/jas/sky033

INTRODUCTION

Mitigation of enteric methane (CH₄) emissions is a major challenge for the future of livestock farming due to its large contribution to greenhouse gas emissions (Gerber et al., 2013). It is thus mandatory to determine with precision and accuracy

the extent of daily CH₄ emission and CH₄ yield per kg DMI. Among available methods, 3 of them are commonly used: open-circuit respiration chamber (OC) measures directly the whole CH₄ enteric production; the sulfur hexafluoride (SF₆) tracer gas technique measures indirectly CH₄ emissions from a continuous sampling of eructed and exhaled gas; the GreenFeed (GF) technique measures CH₄ emissions from gas spot samplings spread over day and night. In addition, these 3 methods also estimate carbon dioxide (CO₂) emissions.

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Received October 6, 2017.

Accepted February 16, 2018.

A review (Hammond et al., 2016a) evaluated these 3 techniques from methodological trials in cattle, mostly comparing 2 of these 3 techniques. Only 1 publication (Jonker et al., 2016) and 1 report (Garnett, 2012) directly compared OC, SF₆, and GF in the same experiment in cattle. There is an absence of consistency among experiments about the compared accuracy and precision of these techniques. The objective of the present trial was to compare the 3 techniques in their ability to measure CH₄ and CO₂ emissions in nonlactating cows. Experimental conditions were set to minimize differences in gas emissions between animals and across time, in order to reduce animal and day-to-day variability and to place the emphasis on the experimental error. Our hypotheses were 1) OC results in a higher CH₄ emission than SF₆ which does not account for whole enteric CH₄ production, and higher than GF which underestimates post-prandial CH₄ production due to spot sampling; and 2) the precision of GF method is similar to that of OC and SF₆ because the drawback due to spot sampling is compensated for by a higher number of days of measurement.

MATERIALS AND METHODS

Experimental Design, Animals, and Diets

The experiment lasted 15 wk: 8 wk for the pre-experimental phase and 7 wk for the experimental phase. Eight Holstein dry adult cows, weighing 711 ± 76 kg at the beginning of the experiment, were used in this trial. Except during measurements in open chambers, they were housed in a single pen of a free-stall barn, together with 15 cows used in another experiment. They were tethered to the feeding fence between 0730 and 0830 h, and between 1530 and 1600 h. Cows were accustomed to being tied during the adaptation period. Animals were managed in accordance with the guidelines and regulations for animal experimentation of the French Ministry of Agriculture (Ministère de l'Alimentation, de l'Agriculture et de la Pêche, 2009) and other applicable international guidelines for animal experimentation (Canadian Council on Animal Care, 1993). The protocol was accepted by the Regional Ethics Committee on Animal Experimentation C2EA-02 with reference number 9152-2017030615511441.

During the first 4 wk of the pre-experimental phase, voluntary feed intake was determined. At the end of this phase, the daily amount of feed for each cow was set at 95% of the amount consumed

by the cow with the lowest intake, so that refusals would be very limited. The following 4 wk of the pre-experimental phase were devoted to adaptation to OC and SF₆ measurements, and to adaptation to eating concentrate in the GF system.

The diet comprised 70% natural grassland hay and 30% concentrate. The composition of the concentrate is given in Table 1. Hay was offered in 2 equal parts at 0830 and 1600 h. Concentrate was introduced for 1 part in the GF, and for the other part in the trough at 0800 h. The amount of concentrate offered in the trough was determined each week according to the amount fed in the GF during the previous week, in order to maintain a constant forage:concentrate ratio.

Measurements and Analyses

Feed intake was determined every day by weighing offered feeds, refusals of forage, and concentrates, which were separated by sorting; DM content of offered feeds and refusals was determined on an aliquot by oven-drying at 103 °C for 24 h. Chemical composition of feeds was determined in an aliquot from regular feed samplings (500 g twice a week for hay, 200 g once a week for concentrate). Ash was determined by combustion at 550 °C for 6 h, N was determined by Dumas' method (method 968-06, AOAC, 2005), NDF and ADF were determined sequentially (Van Soest et al., 1991) after treatment with amylase for concentrate, and exclusive of residual ash, and starch was determined by the Ewers polarimetric method (procedure ISO 10520:1997, www.iso.org/standard/18589.html). Chemical composition of feeds is given in Table 1.

The sequence of gas measurements using the 3 techniques is specified in Table 2. For OC, 2 periods were performed for each cow with 4 consecutive days of gas collection per period. Four OC were available in the facilities, so that 2 wk per period

Table 1. Chemical composition and nutritive value of experimental feeds

	Hay	Concentrate ^a
OM, g/kg DM	898	927
CP, g/kg DM	84	183
NDF, g/kg DM	599	257
ADF, g/kg DM	323	131
Starch, g/kg DM	—	329

^aComposition of concentrate, in g/kg DM: wheat 230, barley 200, dehydrated beet pulp 300, rapeseed meal 150, soybean meal 78, sugar-cane molasses 15, dicalcium phosphate 10, magnesium oxide 5, sodium chloride 6, mineral-vitamin premix 5, antifungal agent 0.5, aroma 0.3.

Table 2. Sequence of gas measurements with the different techniques^a throughout the experiment

	Period 1				Period 2		
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
OC	Group 1 ^b	Group 2 ^b			Group 1	Group 2	
SF _{6-OC}					Group 1	Group 2	
SF _{6-FS}			All cows			Group 1	Group 2
GF			All cows	All cows	Group 2	Group 1	All cows

^aOC = open-circuit respiration chambers; SF_{6-OC} = SF₆ tracer technique used in open-circuit respiratory chambers; SF_{6-FS} = SF₆ tracer technique used in free stalls; GF = GreenFeed system.

^bGroups 1 and 2 of 4 cows each were constituted only to define the passage of cows in chambers.

were necessary for 8 cows. Measurements were made during weeks 1 and 5 for group 1 (4 cows) and weeks 2 and 6 for group 2 (other 4 cows). For SF₆, each measurement period consisted of 5 consecutive days of gas collection. Two periods in free stalls were performed for each cow, in weeks 3 (period 1) and 6 or 7 (period 2) of the experiment. Data were noted as SF_{6-FS}. In addition, cows in OC for period 2 were also fitted with the SF₆ device, so that SF₆ was determined in chambers. Data were noted as SF_{6-OC}. For GF, measurements were made continuously during weeks 3 to 7, but could not be made in weeks 1 and 2 due to out of service of device. The 30 d of measurements were split in 2 periods of 15 d (weeks 3 and 4 for period 1, weeks 5 and 7 [group 2], or 6 and 7 [group 1] for period 2). It was considered that a 15-d period was enough to provide accurate data for CH₄ and CO₂ emission (Arbre et al., 2016).

The OC measurements were made using the same equipment as described by Guyader et al. (2015). Each OC was 2.2 m high, 3.6 m long, and 2.1 m wide, giving a volume of 16.6 m³. Floor dimensions gave the animal a 2 m² movement area, which was close to tie-stall conditions and equipped with a comfortable rubber mattress. The chambers were made of steel with transparent polycarbonate walls allowing sight contact between animals and with the farm personnel. Chambers had front and rear doors, with the front doors used for animal feeding and the rear doors used to enter or milk the animals, or to remove feces and urine collected once daily in a wheeled box. Before the start of the experiment, air leaks were examined in the chambers and all the conducts using a Water-based Smoke Machines (Kool Light - FOGGER 1500E; EPICAP, Saint-Symphorien d'Ozon, France). Gas concentrations (CH₄ and CO₂) in the barn and in the 4 chambers were alternately measured for 5 min every 25 min using an infrared gas analyzer (Ultramat 6; Siemens, Karlsruhe, Germany) and recorded (Nanodac Invensys recorder/controller;

Eurotherm Automation SAS, Dardilly, France). Chambers were calibrated the day before each measurement week using pure N₂ and a mixture of CH₄ (650 ppm) and CO₂ (700 ppm) in N₂. Airflow in the exhaust duct of each chamber was continuously measured (CP300 pressure transmitter; KIMO, Montpon-Ménestérol, France) and recorded every 5 min (KT-210-AO datalogger; KIMO, Montpon-Ménestérol, France) and averaged 382.9 ± 8.92 and 381.9 ± 13.31 m³/h for periods 1 and 2, respectively. Inflow air circulated in the chamber through an aperture at the bottom of the rear door (20 cm high, 2.1 m long). Outflow air was extracted from the chamber via the exhaust duct (diameter = 250 mm) situated at the top of the chamber above the head of the animal. Air flow was corrected for temperature (TomKEY temperature logger; Biomérieux, Marcy l'Etoile, France), pressure and relative humidity (Observatoire de Physique du Globe, Clermont-Ferrand, France) recorded every 5 min, according to the Wexler equation (Pinares-Patiño et al., 2012) to calculate CH₄ and CO₂ fluxes.

The SF₆ method was used as described by Johnson et al. (2007) with specifications by Arbre et al. (2016). A permeation tube with a known SF₆ gas release rate was introduced in the rumen of animals 1 mo before the experiment. In this experiment, 695.8 ± 59.9 mg of SF₆ was introduced in the tube, and the permeation rate of SF₆ from the tubes averaged 1.545 ± 0.055 mg/d. Lifetime of permeation tubes was 8.2 ± 1.7 mo, i.e., enough to maintain a constant diffusion rate of the SF₆ throughout the 15-wk experiment (Lassey et al., 2014). Animals were adapted to the halter 1 wk before the beginning of the first period of measurements. Sampling was performed using a Teflon tube held close to the nostrils and a capillary tube connected to a cylindrical gas collection device (length: 37 cm; diameter: 9 cm; volume: 2.5 liters). Each period was made of 5 successive days. Gas collection devices were changed every morning before feeding. Initial pressure in the device averaged

−0.87 atm, and final pressure before changing averaged −0.41 atm. In free-stall barn, devices were kept on the back of dry cows with a harness. This system was chosen because the more classical yoke-shaped device maintained just behind the head could not be used, due to the presence of a feeding fence in free-stall barns. When measurements were made within OC, gas collection devices were suspended at the top of the metal structure of the chamber. Gas analysis was done as described in [Arbre et al. \(2016\)](#): 2 chromatographs were used, 1 with an electron capture detector for SF₆, the other with a thermal conductivity detector for CO₂ and a flame ionization detector for CH₄. Samples were diluted with N₂ in order to increase pressure within the canister above atmospheric pressure, for a direct injection into the chromatograph. Data were corrected for background gas concentration. For SF₆, CH₄, and CO₂, background concentrations as a proportion of concentration detected in breath samples represented on average 6.8, 11.8, and 17.2% for measurements in free-stall barn, and 8.9, 8.5, and 17.1% for measurements in OC.

The GF system has been developed by a private company (C-Lock Inc., Rapid City, SD, USA). It was used as described by [Arbre et al. \(2016\)](#). Briefly, the GF device is an automatic feeder filled with a concentrate available for each animal up to 6 times a day with a 4-h interval. Each visit to the feeder allowed the intake of 300 g of concentrate in 6 successive 50-g drops. A device for air extraction allows the measurement of air outflow and of CH₄ and CO₂ concentrations in the extraction pipe by a nondispersive infrared sensor. The calibration of the nondispersive infrared sensor of the GF was automatically performed twice a day by injecting gas mixture of certified concentrations of CH₄ (1,003.4 ppm) and CO₂ (9,997 ppm) in N₂ (Air Liquide, Mitry-Mory, France). Methane and CO₂ emissions (g/d) were calculated from CH₄ and CO₂ concentrations and air flow during the animal's visits to the feeder, corrected by background CH₄ and CO₂ concentration and air flow, and by air temperature. Data were transferred to the C-lock server in a blind manner, and handled by C-lock before return to the laboratory. Two paired devices were present in the pen. Throughout the experiment, the daily number of visits to the feeder was 2.8 ± 0.4 on average between animals and days. The total time per visit was $3 \text{ min } 55 \text{ s} \pm 16 \text{ s}$, and the total time per day was $11 \text{ min } 33 \text{ s} \pm 1 \text{ min } 48 \text{ s}$. If some data were incomplete (visits too short, for example), they did not appear in data transmitted by C-lock.

Pretreatment of Data and Statistical Analysis

Some daily data were lost due to technical issues. For OC, no missing data were observed. For SF₆, 12% of the expected data were lost because of a breath gas sample collected from animals that was nonrepresentative due to leaks or blocking in the gas collection device. For GF, only 3.2% of daily data on CH₄ and CO₂ were lost, but as mentioned above incomplete data leading to a possible bias (e.g., too short visits to the feeder) were removed due to the data pretreatment by C-lock. As feed intake was constant among cows and days, daily data for the 8 cows were pooled, and outliers were removed prior to statistical analysis. After a residual normality test, a q–q plot test, and the Henry line, the data were processed by calculating the distance of each point to the mean. If this distance was above ± 2 times SD, data were eliminated. The proportion of outliers was 2.5, 1.8, and 3.6% for CH₄ emission, and 2.5, 2.7, and 6.9% for CO₂ emission, for OC, SF₆ and GF, respectively. Repeatability of gas emission data with GF for measurements over the 15-d period was 92 and 82% for CH₄ emission and yield, respectively, and 91 and 66% for CO₂ emission and yield, respectively.

Statistical analyses were performed using the mixed model of [SAS \(2004\)](#) version 9.1. For the comparison of the 3 techniques (OC, SF_{6-FS}, and GF), the model included the fixed effects of technique and of period, the interaction between technique and period, and the random effect of the animal. For the comparison between OC and SF_{6-OC} in period 2, the model included the fixed effects of the technique and the random effect of the animal. Means adjusted by the model were reported in the tables. Significance was considered when $P < 0.05$. The number of animals needed to highlight a difference of 20% between 2 treatments, when applicable, was calculated using a level of significance of difference of 5%, a statistical power of 80% according to [Arbre et al. \(2016\)](#). Pearson's coefficients were used for calculating correlations.

RESULTS

Dry matter intake was 13.50 kg/d on average. Between animals ($n = 8$), the coefficient of variation was 4.7%, and between days ($n = 46$) the coefficient of variation was 2.6% for DMI (data not shown). [Table 3](#) shows significant differences among the 3 techniques for CH₄ and CO₂ emission and yield, and for CO₂/CH₄ ratio. For CH₄ emission (g/d) and yield (g/kg DMI) were higher for OC than for

SF_{6-FS} and GF ($P < 0.001$). For CO₂, emission (g/d) and yield (g/kg DMI) were higher for OC and GF than for SF_{6-FS} ($P < 0.001$). The CO₂/CH₄ ratio was higher for OC and SF_{6-FS} than for GF ($P < 0.0001$). Table 4 shows that emission and yield were higher for OC than for SF_{6-OC} for CH₄ ($P < 0.05$) and for CO₂ ($P < 0.001$); the opposite was observed for CO₂/CH₄ ratio ($P < 0.001$). Data were not significantly different between SF_{6-FS} and SF_{6-OC} (data not shown). Diurnal variations in CH₄ and CO₂ emission are shown for OC and GF (Fig. 1). The extent of diurnal variations in gas emissions was higher for OC than for GF, due to less marked peaks of emission for GF after mean meals.

The total number of animals required in experiments has been calculated for evidencing a 20% difference in CH₄ emission and yield between treatments. When knowledge of the direction of the difference between treatments (2-sided test) is not known, the number of animals is 16, 26, and 12 for CH₄ emission using OC, SF_{6-FS}, and GF, respectively, and 12, 34, and 16 for CH₄ yield using OC, SF_{6-FS}, and GF, respectively. The total number of animals to use in a comparison of 2 diets when the direction of the difference between treatments, i.e., when a mitigating agent is studied (1-sided test), is 12, 20, and 10 for CH₄ emission using OC, SF₆, and GF, respectively, and 10, 28, and 14 for CH₄ yield using OC, SF_{6-FS}, and GF, respectively. Between-animal coefficients of variation (i.e., SD/mean) are given for each technique (Table 5). For CH₄ emissions, they were lower for GF and OC than for SF_{6-FS} (on average 12.0, 13.5, and 17.6%, respectively). For CO₂ emissions, they were lower for GF than for OC and SF_{6-FS} (on average 6.0, 10.1, and

11.8%, respectively). For CO₂/CH₄ ratio, they were lower for OC than for GF and SF_{6-FS} (on average 4.5, 12.0, and 15.8%, respectively). Between-animal coefficients of correlation between techniques are shown in Table 6. For CH₄ emission and yield, they were significant only for correlations between OC and SF_{6-FS} ($P < 0.05$), and between SF_{6-FS} and SF_{6-OC} ($P < 0.01$). They were especially low for correlations between GF and other techniques. For CO₂, correlation coefficients between techniques were not significant.

DISCUSSION

Between-Animal Coefficient of Variation for the 3 Techniques of Gas Emission Determination

The between-animal coefficient of variation for CH₄ emission and yield was lower for GF and OC than for SF_{6-FS}. For SF₆, our coefficients of variation are in the range of those of Arbore et al. (2016) in dry cows (23 and 16% for emission and yield, respectively) and Grainger et al. (2007) in lactating cows (20% for emission), whereas Vlaming et al. (2008) reported coefficients of variation of 8 and 18% for yield with 2 different diets in dry cows; higher coefficients of variation (30 and 35% for emission and yield, respectively) were found by Hammond et al. (2015) in dairy heifers on pasture with 12 animals and 2 periods of 4-d measurements. Recently, a low coefficient of variation between animals (6.5%) was found by Deighton et al. (2014) who used a modified SF₆ technique using flow restrictors instead of capillary tubes for a constant sampling collection rate over the day. This should improve the precision of the technique. For GF, our coefficients of variation are lower than in previous experiments: between 15 and 20% for emission or yield according to Manafiazar et al. (2017) in beef heifers, Doreau et al. (2018) in fattening bulls, and Arbore et al. (2016) in lactating cows. Huhtanen et al. (2015) reported coefficients of variation of 11 and 18% for yield in 2 different trials in dairy cows, and Hammond et al. (2015) reported coefficients of variation of 10 and 15% for emission, and 10 and 20% for yield. The low variability in the present experiment may be related to our experimental conditions, which were standardized: the same amount of feed was given in limited amount to all cows throughout the experiment. For OC, our results showed a lower between-animal variability of CH₄ emission than that reported by Grainger et al. (2007) in lactating cows (18%). Conversely, the results of Hammond

Table 3. Methane and carbon dioxide emission and yield using 3 techniques of gas emission measurement in nonlactating cows ($n = 8$)

	Technique ^a			SEM	<i>P</i> -value ^b
	OC	SF _{6-FS}	GF		
Methane (CH ₄)					
g/d	367 ^a	310 ^b	319 ^b	14.5	<0.0001
g/kg DMI	27.7 ^a	24.1 ^b	23.7 ^b	1.19	0.0006
Carbon dioxide (CO ₂)					
g/d	9887 ^a	7718 ^b	10003 ^a	259.1	<0.0001
g/kg DMI	746 ^a	606 ^b	752 ^a	18.4	<0.0001
CO ₂ /CH ₄ ratio	27.1 ^b	25.8 ^b	31.8 ^a	0.95	<0.0001

^aOC = open-circuit respiration chambers; SF_{6-FS} = SF₆ tracer technique used in free stalls; GF = GreenFeed system. Values are averages of 2 periods.

^bWithin a row, means with a common superscript did not differ ($P > 0.05$).

Table 4. Methane and carbon dioxide emission and yield measured with open chambers and with SF₆ used in open chambers in nonlactating cows (*n* = 8)

	Technique ^a			<i>P</i> -value
	OC	SF _{6-OC}	SEM	
Methane (CH ₄)				
g/d	369	318	14.8	0.018
g/kg DMI	27.2	23.7	0.99	0.017
Carbon dioxide (CO ₂)				
g/d	9782	7054	351.8	<0.001
g/kg DMI	722	522	22.9	<0.001
CO ₂ /CH ₄ ratio	22.6	26.6	0.57	<0.001

^aOC = open-circuit respiration chambers; SF_{6-OC} = SF₆ tracer technique used in open-circuit respiration chambers. Values are for period 2.

et al. (2015) were in the same range as in our trial for CH₄ emission (11 and 15% in 2 trials), but lower (6 and 11%) for CH₄ yield than in our trial. Taken together, the literature data in cattle show moderate differences of animal variability between OC and GF, within experiment. For OC, differences between experiments in variability may be related to material and mastery of the technique which can differ among research teams. For GF, increasing

total number of spot samples used for measuring emissions is mainly achieved by increasing the number of days and/or of animals, and contributes to decrease the variability (Cottle et al., 2015) and to increase the repeatability of the measurement (Arbre et al., 2016).

Here we report lower coefficients of variation for CO₂ emission and yield than for CH₄ for the 3 methods, but they remained high for SF₆. A part of this variation may be explained by a lack of precision of the thermal conductivity detector used for CO₂ determination; the use of flame ionization detector may have increased the precision of the determination. We hypothesize that variability is higher for CO₂ emission of metabolic origin than for CO₂ emission of ruminal origin, which represents a minor part of total CO₂ emission. However, this hypothesis remains to be checked, and to our knowledge, there is scarce information on CO₂ emission variability among animals: Arbre et al. (2016) noted a between-animal variability of 12% for CO₂ emission and yield with GF, and 23 and 18% for emission and yield in another experiment with SF₆.

Figure 1. Diurnal variation of methane (a) and carbon dioxide (b) emissions in nonlactating cows (*n* = 8) measured with open-circuit respiration chambers (in gray) and with the GreenFeed system (in black). Thick lines are means, dotted lines are SDs for values of periods 1 and 2.

Table 5. Between-animal coefficient of variation (%) of gas emissions for 3 techniques^a in nonlactating cows ($n = 8$)

	OC		SF _{6-FS}		GF	
	Period 1	Period 2	Period 1	Period 2	Period 1	Period 2
Methane (CH ₄)						
g/d	13.4	13.6	16.8	18.4	12.7	11.3
g/kg DMI	12.8	11.4	18.8	22.8	14.2	14.3
Carbon dioxide (CO ₂)						
g/d	9.6	10.6	11.8	14.6	6.5	5.5
g/kg DMI	8.6	8.4	14.0	16.3	6.2	5.0
CO ₂ /CH ₄ ratio	5.3	3.8	15.8	16.1	12.8	11.3

^aOC = open-circuit respiration chambers; SF_{6-FS} = SF₆ tracer technique used in free stalls; GF = GreenFeed system.

Comparison Between the 3 Techniques: Average Data and Kinetics of Gas Emissions

Comparisons between techniques for CH₄ daily emissions have been summarized and analyzed by Hammond et al. (2016a) in a review; most comparisons have been published since 2012. Two recent papers complete this panel of comparisons (Jonker et al., 2016, Rischewski et al., 2017). Only 2 studies compared the 3 techniques simultaneously (1 trial by Garnett, 2012; 5 studies by Jonker et al., 2016), but complementary information is given by paired comparisons between 2 techniques. The 5 studies cited by Hammond et al. (2016a) and 4 among the 5 trials of Jonker et al. (2016) did not show significant differences in CH₄ emission between GF and OC. Comparing these techniques in dairy cows at 3 lactation stages, Rischewski et al. (2017) did not find differences in emissions, but noted a higher yield for chambers at the 3 stages due to lower DMI in chambers. Among the 5 comparisons between OC and SF₆ cited by Hammond et al. (2016a), 4 did not show any difference and 1 (Muñoz et al., 2012) resulted in a higher value for SF₆ (+10%); among the 5 trials cited by Jonker et al. (2016), 4 did not show differences, whereas 1 resulted in a higher value for OC. Comparisons between SF₆ and GF provide

less consistent results: most of them did not show any difference or a higher value for GF (Dorich et al., 2015; Garnett, 2012); only 1 found a higher emission with SF₆ (+12%; Hammond et al., 2015). Our experiment showed a lower CH₄ emission for GF and SF₆ than for OC (−14% on average). The lower emission for SF₆ and GF can be due to the occurrence of flatulence. According to Murray et al. (1976), CH₄ from flatus and feces represents less than 2% of enteric production. However, this figure is assessed only by 1 study, and the extent of CH₄ from flatus and feces may be higher. From a literature analysis, Ulyatt et al. (1999) suggest a proportion of 4% of enteric production. In any case, the difference between SF₆ and OC cannot be explained only by flatulence. Another explanation of the difference between OC and SF₆ could be the principle of SF₆ measurement. Indeed, a major part of CH₄ production in the large intestine is exhaled, which accounts for CH₄ arising from both eructation and exhalation, but it is not known whether the SF₆/CH₄ ratio determined in the sample is biased by a differential rate of absorption of the 2 gases by the rumen wall, and thus their ratio in exhaled gases is the same as in eructed gases. However, it is not possible to know the consequences of such a possible bias.

Table 6. Correlation coefficients^a between techniques^b for methane (CH₄) and carbon dioxide (CO₂) emissions in nonlactating cows ($n = 8$)

	CH ₄ (g/d)	CH ₄ (g/kg DMI)	CO ₂ (g/d)	CO ₂ (g/kg DMI)
OC–GF ^c	0.373	0.320	0.573	0.221
OC–SF _{6-FS} ^c	0.782*	0.717*	0.104	−0.140
SF _{6-FS} –GF ^c	0.265	0.456	0.282	0.164
OC–SF _{6-OC} ^d	0.398	0.329	0.275	0.292
SF _{6-FS} –SF _{6-OC} ^d	0.908**	0.903**	0.497	0.603

^a** $P < 0.05$; ** $P < 0.01$.

^bOC = open-circuit respiration chambers; SF_{6-FS} = SF₆ tracer technique used in free stalls; SF_{6-OC} = SF₆ tracer technique used in open-circuit respiration chambers; GF = GreenFeed system.

^cAverage of periods 1 and 2.

^dPeriod 2.

A higher emission with SF₆ is not biologically sound. Muñoz et al. (2012) explained their results by the characteristics of the sampling tube and by the decrease in SF₆ permeation rate with time. Such a decrease was not likely in our experiment, because the time lapse between introducing the bolus in the rumen and the end of the experiment was less than 4 mo; according to Lassey et al. (2014), a constant permeation rate is maintained for more than 6 mo after introducing the bolus in the rumen. Muñoz et al. (2012) found that CH₄ emission determined with SF₆ was higher by 3% when measured in OC than when measured in a barn, and suggest that flatulence accounts for 3% of total CH₄ emission because in chambers all produced gases are taken up by the SF₆ device. We did not find such a difference between locations. In our opinion, it is not sure that all gases produced in OC (flatulence and CH₄ produced by manure fermentation) are taken up by the SF₆ sampling tube, because of the high rate of gas extraction system from the chamber. Differences between methods in estimates of daily CH₄ emission average 15%, and the ranking between methods is not consistent among experiments. This may be explained by some inherent differences between the 3 gas emission techniques (representativeness of gas sample collection, method of gas concentration analyses, calibration method, standard gases...).

Diurnal variations of CH₄ and CO₂ emissions were of lower magnitude with GF than with OC, due to lower peaks of emission after main meals. Our results are in line with observations made by Hammond et al. (2015) for CH₄. The CO₂ pattern shows an absence of diurnal variation with GF in our trial as in a study conducted by Manafiazar et al. (2017). Spot sampling is a cause of underestimation of CH₄ emissions where there is a peak of CH₄ emission even when visits to GF are evenly distributed. In our experimental conditions, cows in free stalls were tethered at the gate during the main meals, and the pattern of visits to GF is not representative of feeding behavior pattern: the proportion of visits to GF is low during main meals (data not shown) so that the underestimation of CH₄ emissions is increased. The difference in daily average CH₄ emissions between GF and OC is likely due for a large part to these differences observed after main meals. The underestimation of CH₄ emission with GF when there is a post-prandial peak of emission, i.e., when there is a limited number of meals per day and when animals are fed in restricted amounts, may bias comparison of treatments in the case of agents which reduce CH₄ specifically after main meals, such as nitrate

(Guyader et al., 2015) or 3-nitrooxypropanol (Reynolds et al., 2014). In order to overcome this issue, Hammond et al. (2016b) proposed a weighing of spot data according to time of the day; this remains to be developed then evaluated accounting for animal differences in feeding behavior. A major constraint remains the collection of gas spot samples representative of post-prandial peaks of gas emissions.

Correlations Between the 3 Techniques of Gas Emission Determination

Correlations among methods are in the same range for CH₄ emission and yield. This is a consequence of the low variation in intake among animals. For CH₄, correlations between SF_{6-FS} and SF_{6-OC} were very high (0.90). This shows that measurement in chambers does not create a bias in the estimation of CH₄ emission and yield despite the difference in environment between cows free in stalls and tied in chambers; this suggests low differences in feeding behavior between the 2 housing conditions. Curiously, the correlation coefficient was higher between OC and SF_{6-FS} (0.78 and 0.72 for emissions and yield, respectively) than between OC and SF_{6-OC} (0.40 and 0.33 for emissions and yield, respectively). More logically, a high coefficient of correlation (0.83) between OC and SF₆ emissions was found by Muñoz et al. (2012) when SF₆ was determined in chambers, and a low correlation (0.36) was found by Garnett, (2012) when SF₆ was measured out of chambers. The low correlations between GF and SF_{6-FS} observed in the present work (0.26 and 0.46 for CH₄ emissions and yield, respectively) are in line with previous data of our group in bulls: 0.27 and 0.52 for CH₄ emission and yield, respectively (Doreau et al., 2018) but lower than was reported by Hammond et al. (2015) in heifers on pasture: 0.63 for CH₄ emission; this difference between this latter experiment and the present study is especially marked because Hammond et al. (2015) calculated concordance correlation coefficients, which integrate precision and accuracy of the method. Correlations between GF and OC were low as well (0.37 and 0.32). The low correlations observed in our experiment may be due to the standardized feeding conditions, as all animals were fed the same amount of feed, resulting in a small range of variation of CH₄ emission. Data reported in the literature on the correlation between GF and OC are inconsistent, with values varying between 0.60 and 0.85 in cattle (Velazco

et al., 2015) and between 0.10 and 0.24 in heifers (Hammond et al., 2015). The higher correlation observed by the former authors may be due to the higher number of animals than in the trial of the latter authors and in our trial. Low correlations whereas precision is correct may mean that GF is suitable to compare treatments with groups of animals, but that conditions for determination of individual CH_4 emissions may require specification for the number of animals and of days of measurement (Cottle et al., 2015).

To our knowledge, there is no published study of the correlation between methods for CO_2 emission and yield. The very low correlation between SF_6 and other methods is logical because using SF_6 as tracer is not suitable to estimate CO_2 emission, which is from both ruminal and metabolic origin (Pinares-Patiño et al., 2007; Martin et al., 2012). The principle of SF_6 method is the use of a tracer of gases produced in the rumen. SF_6 is eructed at the same rate as methane and CO_2 from ruminal origin, these 3 gases are absorbed to a minor extent then exhaled at a rate which is different from eructated gases rate, so that SF_6 is not a suitable marker for measuring CO_2 emission of metabolic origin. The correlation between OC and GF is nonsignificant but numerically lower for CO_2 yield than for CO_2 emission. This suggests that feed intake is not a major driver of CO_2 emission which is mainly of metabolic origin and is related to maintenance requirements (Madsen et al., 2010).

CONCLUSION

This experiment carried out in standardized conditions with dry cows provides new information on differences between GF, OC, and SF_6 techniques for quantifying gas emissions. Inter-animal variability was lower with GF and OC than with SF_6 . Correlations between individual CH_4 emissions and yields were better between OC and SF_6 than between GF and the other 2 techniques. Differences between techniques in CH_4 emissions and yield were moderate, confirming the literature data. The minor difference between OC and GF could be due to differences in post-prandial period, as assessed by the analysis of daily kinetics; the absence of difference between OC and SF_6 suggests that SF_6 method accounts for the whole production of enteric CH_4 . This experiment also confirms that the GF technique can provide reliable information in field conditions for groups of animals.

ACKNOWLEDGMENTS

This experiment is part of a large collaborative project led by INRA with grant funding from a consortium of 11 R&D institutes and private companies: Adisseo France SAS (Antony, France), Agrial (Caen, France), Apis-Gene (Paris, France), Deltavit (Janzé, France), DSM Nutritional Products AG (Kaiseraugst, Switzerland), Institut de l'Élevage (Paris, France), Lallemand (Blagnac, France), Moy Park Beef Orléans (Fleury-les-Aubrais, France), Neovia (Saint-Nolff, France), Techna France Nutrition (Couëron, France), Valorex (Combournillé, France). This project aims to reduce enteric methane emission by nutrition. Animals were managed in the experimental facilities of the INRA Experimental Unit Herbipôle, of which the personnel is sincerely acknowledged, especially S. Rudel, D. Roux, L. Mouly, and V. Tate. Authors sincerely thank G. Renand (INRA, GABI Unit, 78350 Jouy en Josas, France) for his relevant comments on the Discussion section.

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