Short communication: Variation in feed efficiency hampers use of carbon dioxide as a tracer gas in measuring methane emissions in on-farm conditions

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ABSTRACT

Breeding cows for low CH₄ emissions requires that the trait is variable and that it can be recorded with low cost from an adequate number of individuals and with high precision, but not necessarily with high accuracy if the trait is measured with high repeatability. The CH₄:CO₂ ratio in expired breath is a trait often used as a tracer with the production of CO₂ predicted from body weight (BW), energy-corrected milk yield, and days of pregnancy. This approach assumes that efficiency of energy utilization for maintenance and production is constant. Data (307 cow-period observations) from 2 locations using the same setup for measuring CH₄ and CO₂ in respiration chambers were compiled, and observed production of CH₄ and CO₂ was compared with the equivalent predicted production using 2 different approaches. Carbon dioxide production was predicted using a previously reported model based on metabolic BW and energy-corrected milk production and a currently developed model based on energy requirements and the relationship between observed CO₂ and heat production (models 1 and 2, respectively). Animals used were categorized (low, medium, and high efficiency) according to (1) residual feed intake and (2) residual milk production. Model 1 underestimated CH₄ production by 15%, whereas model 2 overestimated CH₄ by 1.4% for the whole database. Model 1 underestimated CO₂ production by 2.8 and 0.9 kg/d for low- and high-efficiency cows, respectively, whereas model 2 underestimated CO₂ production by 0.9 kg/d for low-efficient animals but overestimated it by 1.2 kg/d for high-efficiency cows. Efficient cows produce less heat, and consequently CO₂, per unit of metabolic body weight and energy-corrected milk than

inefficient cows, challenging the use of CO₂ as a tracer gas. Because of biased estimates of CO₂ production, the models overestimated CH₄ production of high-efficiency cows by, on average, 17% relative to low-efficiency cows, respectively. Selecting low CH₄-emitting cows using a CO₂ tracer method can therefore favor inefficient cows over efficient cows.

Key words: methane, CO₂ tracer method, breeding, dairy cow

Short Communication

Methane (CH₄) is a greenhouse gas with a global warming potential 28 times that of CO₂ (Myhre et al., 2013). Enteric CH₄ from livestock accounts for 17% of global CH₄ emissions and 3.3% of total global greenhouse gas emissions from human activities (Knapp et al., 2014). Therefore, there is significant research interest in finding strategies to mitigate enteric CH₄ emissions. The focus has been on dietary manipulation and different feed additives. Animal breeding that exploits natural animal variation in CH₄ emissions is suggested as a mitigation strategy that is inexpensive, permanent, and cumulative (Hayes et al., 2013). It has been shown that CH₄ emission is under some genetic control from the host animal (Lassen and Løvendahl, 2016), but it is controlled much more by other factors in the surrounding environment, especially feed intake and diet composition.

A successful program for selecting cows for low CH₄ emissions requires that the selection trait be variable and that the trait be easily and precisely recordable at low cost on an adequate number of animals. Several methods, often referred to as “sniffer methods,” have been developed to measure CH₄ concentration in the breath of cows during milking or feeding. The methods are based on measuring CH₄ concentration (Garnsworthy et al., 2012) or CH₄:CO₂ ratio (Madsen et al., 2010). The gas ratio method developed by Madsen et al. (2010) is based on predicted CO₂ production as a

Received March 19, 2020.

Accepted May 13, 2020.

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tracer gas for CH₄ production as $\text{CH}_4 = \text{CH}_4:\text{CO}_2 \times \text{predicted CO}_2 \text{ production}$. Production of CO₂ is predicted from metabolic BW (**MBW**), ECM yield, and days of pregnancy. This approach assumes that efficiency of energy utilization for maintenance and production is constant. However, both fasting heat production per kilogram of MBW (Yan et al., 1997) and milk energy output (and heat loss) at zero energy balance at a given ME intake (Kebreab et al., 2003) vary markedly. In addition, body energy retention or mobilization will affect CO₂ production. Between-cow variation in feed efficiency and energy balance can therefore result in biased estimates of CO₂ production and consequently affect ranking of the cows on the basis of CH₄ production as discussed earlier by Huhtanen et al. (2015). Lassen and Løvendahl (2016) also discussed that the Madsen et al. (2010) method ignores whatever animal variation there might be in CO₂ production. The objective of the present study was to examine how differences in feed efficiency expressed as residual feed intake (**RFI**) or residual ECM yield (**RECM**) influence CH₄ production estimated from CH₄:CO₂ ratio and predicted CO₂ production using data obtained from respiration chamber studies.

The data set consisted of 307 cow/period observations from 11 studies (32 diets) from respiration chamber studies conducted at the Danish Cattle Research Centre (DCRC; Foulum, Denmark; 171 cow-periods) and Natural Resources Institute Finland (LUKE; Jokioinen, Finland; 136 cow-periods). Details of respiration chambers are described by Hellwing et al. (2012). Details of the animals, diets, and designs are described in the original publications (Supplemental File S1; <https://doi.org/10.3168/jds.2020-18559>). A summary of animal and feed data used in this study is presented in Table 1.

Production of CO₂ was predicted using the method described by Madsen et al. (2010) (model 1):

$$\text{CO}_2 \text{ (kg/d)} = 180 \times (5.6 \times \text{MBW} + 22 \times \text{ECM}) \times 44/22.4 \times 0.001,$$

where 180 = liters of CO₂ per heat production unit (HPU), ECM is in kilograms per day, MBW is in kilograms, 44 is the molecular weight of CO₂, 22.4 is liters of CO₂ per mole, and 0.001 converts grams to kilograms.

Production of CO₂ was also estimated indirectly from ME requirements, assuming 0.6 MJ/kg of MBW for maintenance (Yan et al., 1997) and that production of 1 kg of ECM produces 1.9 MJ of heat (ECM = 3.14 MJ/kg and efficiency of ME utilization for milk production = 0.62). Heat production was transformed to CO₂ production using the coefficient (101.2 g/MJ) estimated from the current data [coefficient of determination (**R**²) = 0.97; root mean squared error (**RMSE**) 3.1% of observed mean] (model 2):

$$\text{CO}_2 \text{ (kg/d)} = 101.2 \times (0.6 \times \text{MBW} + 1.9 \times \text{ECM})/1,000.$$

Changes in BW or energy balance were not considered because these often cannot be measured reliably on practical farms. Residual feed intake was calculated as the difference between predicted and observed DMI, and RECM as the difference of predicted and observed ECM yield. The parameters for RFI and RECM models were estimated from the current data. Negative RFI values indicate high efficiency [cows eat less than predicted at the same ECM, MBW, and retained energy

Table 1. Summary statistics of animal and feed data used in this study

Item	Mean	SD	Minimum	Maximum
DMI, kg/d	20.2	4.50	10.1	30.9
Proportion of forage	0.58	0.141	0.36	0.99
DIM	184	103.9	11	757
Milk, kg/d	29.0	10.1	8.2	51.4
ECM, kg/d	30.3	9.41	8.2	52.0
Milk fat, g/kg	44.7	9.06	24.7	88.5
Milk protein, g/kg	36.1	4.28	28.6	53.9
BW, kg	614	95.5	404	847
Digestibility, g/kg				
OM	725	35.2	640	798
CP	674	40.9	490	766
NDF	619	82.1	392	808
Diet composition, g/kg of DM				
Ash	75	15.4	51	146
CP	170	19.8	124	233
NDF	351	49.6	224	486

(RE)], whereas for RECM positive values indicate high efficiency (cows produce more than expected at the same DMI, MBW, and RE. The MIXED procedure (SAS Institute Inc., Cary, NC) was used to predict DMI and ECM:

$$\begin{aligned} \text{Predicted DMI} = & b_0 + b_1 \times \text{ECM} \\ & + b_2 \times \text{MBW} + b_3 \times \text{RE}_p + b_4 \times \text{RE}_n + \text{Exp} \\ & + \text{Diet(Exp)} + \text{Period(Exp)}, \end{aligned} \quad [1]$$

where b_0 is the intercept, b_1 is the partial regression coefficient of DMI on ECM yield (kg/d), b_2 is the partial regression coefficient of intake on MBW (kg), b_3 is the partial regression coefficient of intake on positive energy balance (RE_p , MJ/d), b_4 is the partial regression coefficient of intake on negative energy balance (RE_n , MJ/d), and Exp, Diet(Exp), and Period(Exp) are random effects of experiment, diet within experiment, and period within experiment, respectively.

$$\begin{aligned} \text{Predicted ECM} = & b_0 + b_1 \times \text{GE intake} \\ & + b_2 \times \text{MBW} + b_3 \times \text{RE}_p + b_4 \times \text{RE}_n + \text{Exp} \\ & + \text{Diet(Exp)} + \text{Period(Exp)}, \end{aligned} \quad [2]$$

where b_1 is the partial regression coefficient of gross energy (GE) intake (MJ/d) and the other factors are as described in equation [1].

The cows were categorized into 3 efficiency groups of equal size ($n = 102\text{--}103$) on the basis of RFI (low, medium, and high RFI) and on the basis of RECM (low, medium, and high RECM). Differences between the efficiency classes were tested with MIXED model analysis with efficiency class as a fixed factor and Exp, Diet(Exp), and Period(Exp) as random factors.

Overall variability of CH₄ production was large [coefficient of variation (CV) = 23.2%] reflecting variation in DMI and diet composition (Table 2). The variability was greater than that (17%) reported by Yan et al. (2010), probably because the current data set included fat supplementation studies and slightly greater variability in DMI. When the effects of Exp, Diet(Exp), and Period(Exp) were taken into account in variance component analysis, the between-cow CV decreased to 11.7%. Most of this variation was due to differences in DMI, as indicated by a much smaller (5.7%) CV in CH₄ yield (g of CH₄/kg of DMI). Between-cow variability was even less for the CH₄:CO₂ ratio (4.9%). Because of the small between-cow variability with the gold standard method, high precision of on-farm methods is required for correct ranking of cows based on CH₄ emissions. In previous studies (breath sampling “sniffer” methods based on either CH₄ concentration or CH₄:CO₂ ratio), between-cow variability in these variables was much greater (Garnsworthy et al., 2012; Lassen et al., 2012; Bell et al., 2014; Negussie et al., 2017; van Engelen et al., 2018). In the study of Bell et al. (2014), between-cow CV in CH₄ concentration varied between 22 and 67% on different farms. Much greater between-cow variability with sniffer methods indicates a large contribution of random measurement errors in observed values. Some reported gas ratios have been outside of the expected biological range; for example, values of 0.17 on a volume basis (62 g/kg on weight basis) in the studies of Negussie et al. (2017) and van Engelen et al. (2018).

The model of Madsen et al. (2010) (model 1) clearly underestimated CO₂ production by approximately 15%, whereas the model based on heat production (model 2), using the current energy requirements for maintenance and ECM production, estimated CO₂ production ac-

Table 2. Summary statistics of gas production data

Item	Mean	SD	Between-cow CV, %	Minimum	Maximum
Observed CH ₄ , g/d	414	96.0	11.7	208	628
Predicted CH ₄ , ¹ g/d	354	87.2	11.2	154	549
Predicted CH ₄ , ² g/d	420	100.3	10.9	188	640
Observed CO ₂ , kg/d	13.5	2.43	11.2	7.4	18.6
Predicted CO ₂ , ¹ kg/d	11.5	2.27	10.9	6.5	16.7
Predicted CO ₂ , ² kg/d	13.7	2.58	10.3	7.9	19.5
CH ₄ /CO ₂ , g/kg	30.6	3.67	4.9	17.2	38.2
CH ₄ , kJ/MJ of gross energy	60.8	8.91	5.7	35.8	86.5
CH ₄ , g/kg of ECM	14.4	3.53	11.0	7.5	31.6
RFI, ³ kg/d	0.13	1.528	0.55	−4.35	4.85
RECM, ⁴ kg/d	−0.13	3.449	1.40	−9.54	9.62

¹Predicted using the equation of Madsen et al. (2010) (model 1).

²Predicted using heat production estimated from maintenance + heat increment from ECM production (model 2).

³RFI = residual feed intake.

⁴RECM = residual ECM yield.

curately, as indicated by a small mean bias (<2% of observed mean). The following relationships between observed and predicted CO₂ (pCO₂) production were estimated:

$$\text{Model 1: CO}_2 \text{ production (kg/d)} = 2.46 \pm 0.36 \\ + 0.95 \pm 0.030 \times \text{pCO}_2 \text{ (R}^2 = 0.773, \text{RMSE} = 1.16),$$

$$\text{Model 2: CO}_2 \text{ production (kg/d)} = 1.91 \pm 0.37 \\ + 0.84 \pm 0.026 \times \text{pCO}_2 \text{ (R}^2 = 0.780, \text{RMSE} = 1.14).$$

The lack of correlation from unity indicates that there is considerable variation between animals in the efficiency of nutrient utilization for both maintenance and production.

Both models predicted CH₄ emissions precisely (R² = 0.86; RMSE 8.6–8.8% of observed mean). Model 1 (Madsen et al., 2010) underestimated CH₄ production by 15%, whereas the model based on feeding recommendation was accurate (overpredicted CH₄ by 1.4%). A close relationship between observed and predicted values is not surprising because the CH₄:CO₂ ratio used was measured in respiration chambers. In on-farm conditions, precision is likely less because CH₄:CO₂ ratios measured by the sniffer were not strongly correlated with gas ratios measured by GreenFeed (Huhtanen et al., 2015) or respiration chambers (Difford et al., 2018). In the current study, predicted CO₂ production predicted CH₄ emissions better than the gas ratio (R² = 0.60 for both CO₂ models vs. 0.40) despite relatively large between-Diet(Exp) variation in the gas ratio (CV = 7.7%), probably arising from fat supplementation that reduces CH₄ production (Grainger and Beauchemin, 2011). In the study of Difford et al. (2018), predicted CO₂ production explained variation in observed CH₄ production measured in respiration chambers as well as CH₄ production predicted from CH₄:CO₂ using CO₂ as a tracer gas (r = 0.74 vs. 0.77); that is, measurements of CH₄:CO₂ did not improve precision CH₄ prediction.

The high-efficiency cows, categorized based on RECM, produced 6.6 kg/d (*P* < 0.001) more ECM than the low-efficiency cows at the same DMI, BW, and RE (Table 3). The high-efficiency cows produced 25 (*P* = 0.02) and 21 g/d (*P* = 0.03) less CH₄ than the low- and the medium-efficiency cows, respectively, but the reverse was true for predicted CH₄ production, which was 40 and 42 g/d greater (*P* < 0.001) for the high- than the low-efficiency cows when estimated using model 1 and model 2. The difference between observed and predicted CH₄ production (model 2) was 62 g/d (*P* < 0.001), ranging from underprediction of 26 g/d (6.7%) for the low-efficiency cows to overprediction of 36 g/d

(9.9%) for high-efficiency cows. With model 1, underprediction of CH₄ production decreased (*P* < 0.001) from 83 g/d in the low-efficiency cows to 27 g/d in the high-efficiency cows. Because the differences between the efficiency groups in the CH₄:CO₂ ratio were small, the biases in predicted CH₄ production were due to errors in predicting CO₂ production. In the low-efficiency cows, observed CO₂ production was, on average, 0.9 kg/d more than the predicted value, whereas for the high-efficiency cows, it was 1.2 kg/d less than predicted (model 2). Differences between low- and high-efficiency cows in predicted CO₂ production were similar (2.1 kg/d) when estimated by model 1 or by model 2. Efficient cows produce less heat, and consequently less CO₂, per unit of MBW and ECM than inefficient cows, violating the hypothesis of using CO₂ as a tracer gas. Based on regression analysis, 1 g of bias in predicted CH₄ production was associated with 0.065 ± 0.0028 (*P* < 0.001) kg/d difference in ECM yield. Our findings are consistent with Huhtanen et al. (2015), who discussed the possible problems of using CO₂ as a tracer gas in quantification of CH₄ production.

Residuals (observed – predicted) of both CO₂ (16.0 g/MJ) and CH₄ production (0.52 g/MJ) increased (*P* < 0.001) with observed energy balance. When cows are in positive energy balance, true CO₂ production is greater than CO₂ production predicted from MBW and ECM yield, resulting in underestimation of CH₄ production. In contrast, when cows are in negative energy balance, CH₄ production is overestimated, because milk production promoted by the mobilization of body tissues increases predicted CO₂ production but does not result in a concurrent production of CH₄. However, under on-farm conditions, accurate estimates of energy balance are difficult. In addition to random errors in measuring BW change, the poor relationship between BW change and actual energy balance may impede improvements in predictions of CO₂ production. The difference between the low- and high-RECM groups in CH₄ intensity (CH₄/ECM, g/kg) was 4.3 and 2.3 g/kg when calculated using observed and predicted CH₄, respectively.

Average DMI differed (*P* < 0.001) among the high-, medium-, and low-efficiency cows when categorized according to RFI, with no differences (*P* > 0.11) in ECM, BW, or energy balance (Table 4). Predicted CH₄ production was similar among the efficiency groups, because CH₄:CO₂ and factors influencing predicted CO₂ production (ECM yield, MBW) did not differ between the groups. In contrast, observed CH₄ production decreased (*P* < 0.001) with improved efficiency (reduced RFI) due to decreased DMI. Because of the biases in predicted CO₂ production, CH₄ production was over-

Table 3. Milk and gas production characteristics of low-, medium- and high-efficiency cows ranked according to residual ECM production (RECM)

Item	RECM			SEM	P-value
	Low	Medium	High		
RECM, kg/d	−3.2	0.1	3.3	0.25	<0.001
ECM, g/d	23.2	26.7	29.8	2.12	<0.001
DMI, kg/d	18.5	18.8	18.4	1.01	0.57
BW, kg	592	595	583	20.4	0.60
Retained energy, MJ/d	7.8	8.7	5.2	3.58	0.47
Observed CH ₄ , g/d	388	384	363	22.5	0.05
Predicted CH ₄ , ¹ g/d	301	325	341	19.6	<0.001
Predicted CH ₄ , ² g/d	360	387	402	22.7	<0.001
Observed − predicted CH ₄ , ¹ g/d	83	57	27	5.6	<0.001
Observed − predicted CH ₄ , ² g/d	26	−3	−36	5.7	<0.001
CH ₄ /DMI, g/kg	21.0	20.5	20.0	0.05	0.04
CH ₄ /CO ₂ , g/kg	29.8	30.2	30.3	0.76	0.45
Observed CO ₂ , kg/d	12.9	12.7	12.1	0.57	0.02
Predicted CO ₂ , ¹ kg/d	10.0	10.7	11.2	0.53	<0.001
Predicted CO ₂ , ² kg/d	12.0	12.7	13.2	0.60	<0.001
Observed − predicted CO ₂ , ¹ kg/d	2.8	1.9	0.9	0.17	<0.001
Observed − predicted CO ₂ , ² kg/d	0.9	−0.1	−1.2	0.19	<0.001
CO ₂ /DMI, g/kg	699	679	611	11.1	<0.001
CH ₄ /ECM, g/kg	17.1	15.0	12.9	0.73	<0.001
CO ₂ /ECM, g/kg	589	510	442	25.1	<0.001

¹Predicted using the equation of Madsen et al. (2010) (model 1).²Predicted using heat production estimated from maintenance + heat increment from ECM production (model 2).

estimated (30 g/d) for the high-efficiency (low-RFI) cows and underestimated (28 g/d) for the low-efficiency (i.e., high-RFI) cows (model 2). When estimated using model 1 (Madsen et al., 2010), similar biases between

low- and high-efficiency cows were observed between observed and predicted CH₄ and CO₂ productions.

In conclusion, the use of CO₂ as a tracer gas combined with CH₄:CO₂ ratio depends upon the underlying

Table 4. Milk and gas production characteristics of low-, medium- and high-efficiency cows ranked according to residual feed intake (RFI)

Item	RFI			SEM	P-value
	Low	Medium	High		
RFI, kg/d	−1.4	0.1	1.5	0.11	<0.001
ECM, g/d	26.9	26.7	25.5	2.41	0.28
DMI, kg/d	17.3	19.0	19.5	1.01	<0.001
BW, kg	583	602	581	20.5	0.11
Retained energy, MJ/d	7.7	8.9	4.2	3.34	0.28
Observed CH ₄ , g/d	350	384	407	21.3	<0.001
Predicted CH ₄ , ¹ g/d	321	323	315	21.3	0.58
Predicted CH ₄ , ² g/d	383	386	374	24.4	0.43
Observed − predicted CH ₄ , ¹ g/d	30	58	87	5.2	<0.001
Observed − predicted CH ₄ , ² g/d	−30	3	28	6.4	<0.001
CH ₄ /DMI, g/kg	20.7	20.2	20.9	0.66	0.06
CH ₄ /CO ₂ , g/kg	30.2	29.9	30.2	0.76	0.63
Observed CO ₂ , kg/d	11.7	12.8	13.4	0.54	0.02
Predicted CO ₂ , ¹ kg/d	10.5	10.8	10.4	0.58	<0.001
Predicted CO ₂ , ² kg/d	12.6	12.8	12.4	0.66	<0.001
Observed − predicted CO ₂ , ¹ kg/d	1.0	2.0	2.9	0.17	<0.001
Observed − predicted CO ₂ , ² kg/d	−1.0	−0.1	0.9	0.21	<0.001
CO ₂ /DMI, g/kg	684	674	686	12.3	0.19
CH ₄ /ECM, g/kg	14.2	15.2	16.2	0.88	<0.001
CO ₂ /ECM, g/kg	487	518	552	31.7	<0.001

¹Predicted using the equation of Madsen et al. (2010) (model 1).²Predicted using heat production estimated from maintenance + heat increment from ECM production (model 2).

assumption that there is no variation in efficiency of energy utilization for maintenance or milk production among the cows. However, the results of the present study showed large differences in CO₂ production between low- and high-efficiency cows at similar maintenance and milk production levels. Because of the biased estimates of CO₂ production due to differences in the efficiency of nutrient utilization, this method favors inefficient cows over efficient cows in ranking of them according to their CH₄ phenotypes. The method in its current form also favors selection of cows that partition nutrients to body energy retention rather than milk production.

ACKNOWLEDGMENTS

This project had no external funding. The authors have not stated any conflicts of interest.

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