RESEARCH ARTICLE

Modified approach to estimating daily methane emissions of dairy cows by measuring filtered eructations during milking

Matt. J. Bell[1], Phil Garnsworthy[1], Dimitris Mallis[2], Richard Eckard[3], Peter Moate[4], and Tianhai Yan[5]

Received: April 24, 2019
Revised: August 3, 2019
Accepted: November 25, 2019

HIGHLIGHTS

- **Maximum eructation measured by peak height or peak amplitude can provide reliable metrics to estimate daily CH_4 emissions**
- **We recommend using peak amplitude as it removes any background emissions**

KEYWORDS **dairy cow, enteric methane, spot measurement, sampling**

Abstract

The aim of this study was to compare metrics for quantifying enteric methane (CH_4) emissions from individual cows during milking using frequent spot measurements and peak analysis methods. An infrared gas analyser was used to measure the CH_4 emitted by cows, and eructation peaks were identified using a Signal Processing Toolbox provided by Matlab. CH_4 emissions were quantified by gas peak height, peak amplitude and average concentration, and were expressed in grams per day and CH_4 yield (grams per kilogram of dry matter intake (DMI)). Peak analysis measurements of CH_4 were obtained from 36 cows during 2,474 milkings, during which cows were fed a ration containing between 39 and 70 % forage. Spot measurements of CH_4 were compared to a separate dataset of 196 chamber CH_4 records from another group of 105 cows, which were fed a ration containing between 25 and 80 % forage. The results showed that the metrics of CH_4 peak height and CH_4 peak amplitude demonstrated similar positive relationships between daily CH_4 emissions and DMI (both $r = 0.37$), and a negative relationship between CH_4 yield and DMI ($r = -0.43$ and -0.38 respectively) as observed in the chamber measurements ($r = 0.57$ for daily emissions and $r = -0.40$ for CH_4 yield). The CH_4 metrics of peak height and peak amplitude were highly repeatable (ranging from 0.76 to 0.81), comparable to the high repeatability of production traits (ranging from 0.63 to 0.99) and were more repeatable than chamber CH_4 measurements (0.31 for daily emissions and 0.03 for CH_4 yield). This study recommends quantifying CH_4 emissions from the maximum amplitude of an eructation.

1 Introduction

The process by which ruminants convert plant material into useful products such as meat and milk through rumen fermentation results in a loss of energy in the form of CH_4 emissions. The animal removes CH_4 building up in its rumen by repeated eructations of gas through its mouth and nostrils. Globally, dairy farming contributes to 20 % of total greenhouse gas emissions coming from the livestock sector, with enteric CH_4 being the largest source of dairy emissions (Gerber et al., 2013). Historically, CH_4 produced by livestock was regarded as wasted dietary energy and an inefficiency in feed utilisation. This is still the case, but CH_4 is also now seen as a pollutant and potent greenhouse gas. Although a large proportion of the variation in CH_4 emissions can be explained by diet composition and feed intake (Bell and Eckard, 2012; Niu et al., 2018), there is additional variation among animals, which may allow selective breeding (de Haas et al., 2011; Garnsworthy et al., 2012; Breider et al., 2019).

[1] The University of Nottingham, School of Biosciences, United Kingdom
[2] The University of Nottingham, School of Computer Science, United Kingdom
[3] University of Melbourne, Melbourne School of Land and Environment, Australia
[4] Agriculture Victoria Research, Australia
[5] Agri-Food and Biosciences Institute, United Kingdom
CONTACT Matt.Bell@nottingham.ac.uk

Historically, most studies to assess CH_4 emissions from cattle have been performed using respiration chambers (Blaxter and Clapperton, 1965; Mills et al., 2003; Ellis et al., 2007; Yan et al., 2010), which is seen as the 'gold' standard for measuring emissions. However, respiration chambers are impractical for estimating emissions from individual animals on a large scale in national populations and on commercial farms. Approaches such as the sniffer method to measure enteric CH_4 emissions from individual animals on commercial farms are being developed (Bell et al., 2014a; Lassen and Løvendahl, 2016) now that more portable gas analysis equipment is available, and that frequent gas sampling at the robotic milking station feed bin whilst individual cows are being milked has been found to correlate (r = 0.89) with the chamber measurements of total CH_4 production from the same cows (Garnsworthy et al., 2012). This approach of taking frequent 'spot' measurements of CH_4 within a day (expressed in various units that were measured such as the CH_4 emission rate calculated from the area under CH_4 peaks, average concentration and the ratio of CH_4 to carbon dioxide) has been found to be a repeatable measure (Huhtanen et al., 2015, Bell et al., 2014b; Negussie et al., 2017). However, to be used as a reliable measure, the data requires processing to account for error sources such as cow head position (Huhtanen et al., 2015) and the number and timing of measurements (Cottle et al., 2015; Hammond et al., 2016). The location of the animal's head relative to the gas sampling tube can be determined using a proximity sensor (Huhtanen et al., 2015), or alternatively using data filtering methods to identify CH_4 eructation peaks (Garnsworthy et al., 2012) as investigated in the current study. The current study reanalysed the dataset by Bell et al. (2014b). The hypothesis was that enhanced filtering of eructation spot measurements (i.e. individual or clusters of peaks) within a milking period could improve the reliability and repeatability of measurements used to estimate the daily CH_4 emissions of individual cows.

The objective of the current study was to compare different metrics for quantifying the CH_4 emissions of individual cows during milking using frequent 'spot' measurements and peak analysis methods. Results were compared to chamber CH_4 records for different dairy cows, as chamber measurements are considered to be the gold standard for measuring daily emissions.

2 Materials and methods

2.1 Breath sampling data

Enteric CH_4 emitted from the mouth and nostrils of 36 Holstein Friesian dairy cows was measured during milking at Nottingham University Dairy Centre (Sutton Bonington, Leicestershire, UK). The dataset covered wide ranges of milk yield (14 to 55 kg day^{-1}), lactation number (1 to 5), stage of lactation (15 to 409 days in milk) and live weight (473 to 805 kg) (*Table 1*). Cows were group housed in a freestall barn and milked individually at an automatic (robotic) milking station (Lely Astronaut A3; Lely UK Ltd., St Neots, UK). Gas concentrations (v/v) in air sampled from the milking station feed bin were measured continuously by an infrared gas analyser (Guardian Plus; Edinburgh Instruments Ltd., Livingston, UK) during 2,474 individual cow milkings throughout a sampling period of 28 days. For a full description of the study see Bell et al. (2014b), who estimated cow CH_4 emissions by calculating the area under the eructation peaks that were measured during a whole milking rather than selected peaks within a milking as in the current study. The spot sampling technique is described briefly below.

The CH_4 concentration (v/v) was logged at onesecond intervals on data loggers (Simex SRD-99; Simex Sp. z o.o., Gdańsk, Poland) and visualised using logging software (Loggy Soft; Simex Sp. z o.o.). The CH_4 analyser was calibrated at the start of the study using standard mixtures of CH_4 in nitrogen (0.0, 0.25, 0.50, 0.75 and 1.0 % CH_4, Thames Restek UK Ltd., Saunderton, UK). The CH_4 concentration in the gases emitted during milking was recorded in parts per million (v/v). The CH_4 concentration data measured every second were then extracted from the time-series signal using the peak analysis tools in the MatLab Signal Processing Toolbox (version R2018a; The MathWorks, Inc., Natick, United States. See https://uk.mathworks.com/help/signal/examples/peak-analysis.html for metrics). The peak analysis tools were used to identify clusters of CH_4 eructation peaks during one milking (*Figure 1*) from raw logger data, using the findpeak function. The findpeak function is a tool for extracting local maxima from two-dimensional signals. This MatLab function can be parameterised using constraints such as the number of peaks allowed, peak height, width or prominence, and the distance between peaks. The data by Garnsworthy et al. (2012) comparing chamber CH_4 measurements with spot measurements for the same cows showed that the CH_4 emission rate (g min^{-1}) and total CH_4 production (g day^{-1}) were highly correlated to CH_4 peak height (r = 0.91), CH_4 peak amplitude (r = 0.89), and less so to peak frequency (r = 0.29). Therefore, values for the following metrics were derived:

- maximum peak height (ppm)
- maximum peak amplitude (ppm)
- average CH_4 concentration (ppm)

To identify individual and clusters of peaks in CH_4 emissions from within one milking, the program extracted the data based on the following filtering criteria:

- three or more consecutive peaks (clusters)
- minimum time between peaks of 20 seconds
- minimum peak height and amplitude of 200 ppm

The average rise time for peaks (applied to the average CH_4 concentration measure) and the maximum rise time for maximum peak height and amplitude in seconds were obtained using peak analysis for each milking. The background CH_4 concentration was subtracted from measures of peak height and the average concentration during milking, with the background level assumed to be the minimum value measured. With all three metrics in ppm based on the analyser recording every second, the values were converted to emission rate in grams per minute by multiplying by 60 and assuming a CH_4 density of 0.656 x 10^{-6} g L^{-1}. This assumes

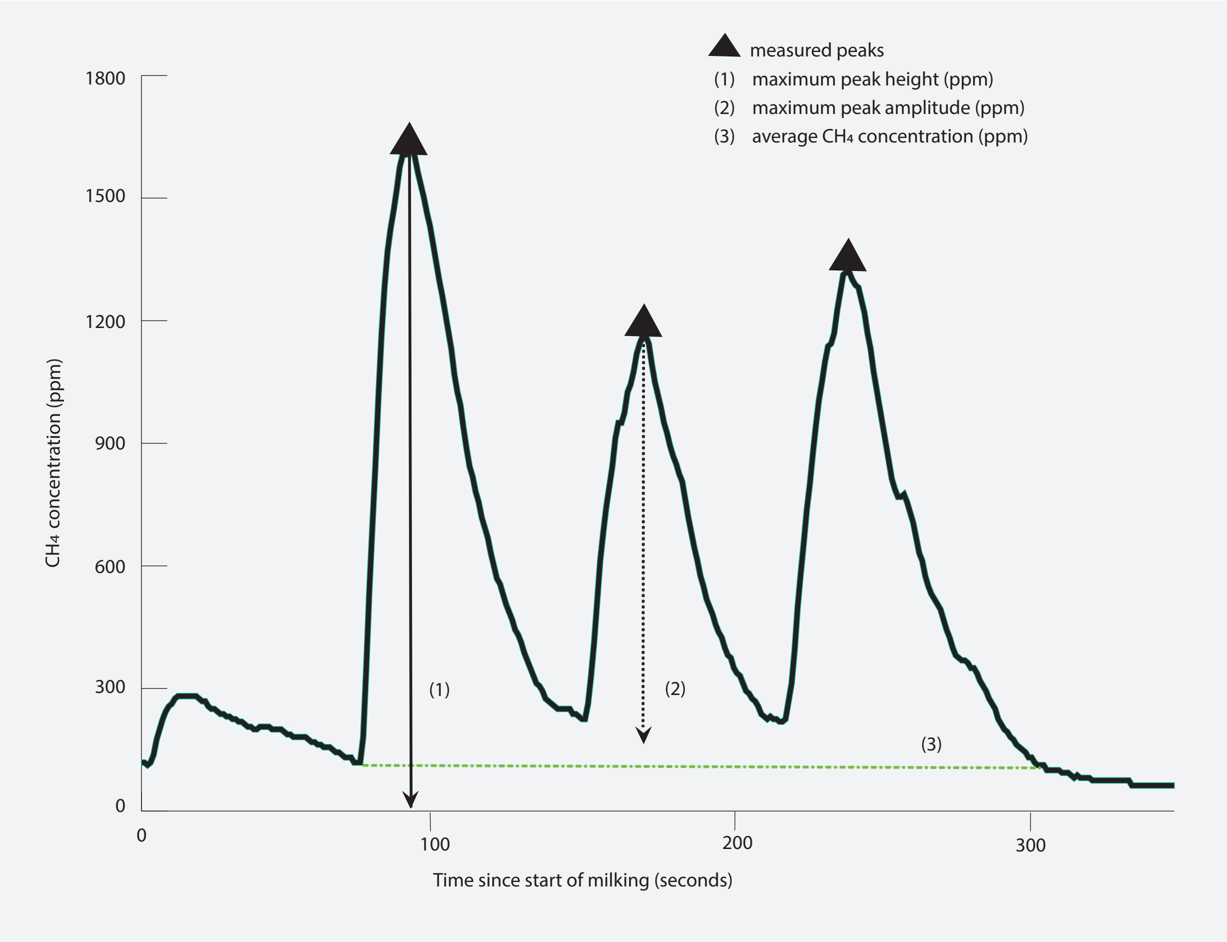

FIGURE 1
The CH_4 concentration profile in eructated gas for cow 2158 during milking showing measured peaks, maximum peak height measurements ((1) solid black line with arrow) and maximum peak amplitude measurements ((2) dotted black line with arrow). Data on the background CH_4 concentrations were extracted within each milking period ((3) dashed green line) to obtain the average concentration during this time and the minimum (i.e. background) concentration.

the analyser is sampling air at a flow rate of 1 L min^{-1}. The exponential response curve for the gas concentration measured was determined in a previous study by Garnsworthy et al. (2012). This response curve was found to relate peak rise time and amplitude to known amounts of released gas for dilution tests. All extracted emission rates (in grams per minute) during milking were scaled to the estimated emissions based on the exponential increase in gas concentration and the extracted rise time for eructation peaks using equation [1], given it takes 60 seconds for the analyser to reach the 'true' peak asymptote and fully process the gas sample:

Estimated CH_4 emission rate (g min^{-1}) = (CH_4 concentration ppm–background CH_4) [1- EXP (-(peak rise time in seconds / 60))]$^{-1}$ x 60 x 0.656 x 10^{-6} [1]

The maximum peak height and maximum peak amplitude metrics used their associated peak rise time, whereas the average concentration metric used the calculated average rise time for peaks sampled during each milking. The estimated emission rate was converted to grams per day by multiplying by 1,440. Emission values were not adjusted for potential dilution of eructated CH_4, as the study aimed to assess the potential of advanced peak analysis filtering methods to replace to need to adjust values due to gas dilution.

Measurements of enteric CH_4 during milking were conducted during two consecutive feeding periods of 14 days, during which cows were fed either a grass or maize silage partial mixed ration (PMR) *ad libitum* plus concentrates at milking. A 14-day crossover design experiment during which cows were each fed a diet containing between 39 and 70 % forage (*Table 1*) was conducted. A concentrate was dispensed into the feed bin throughout the milking period, which helped to keep the cow's head within suitable proximity of the gas sampling tube. Daily concentrate allowance fed during milking was 1.5 kg plus 0.16 kg per litre of milk yield above 23 L d^{-1}. Total daily DMI of concentrate from the PMR and AMS combined was calculated. Milk yield and live weight were recorded automatically at each milking. Feed intake was recorded automatically by electronic feeders (Roughage Intake Control feeders; Fullwood Ltd., Ellesmere, UK).

TABLE 1
Average production values and composition of diets fed to cows (n = 36) with spot samples and cows (n = 105) with chamber methane (CH_4) measurements

Component	Units	Spot		Chamber	
		Mean (sd)	Range	Mean (sd)	Range
Observations		*n=72*		*n=196*	
Forage	%	54 (10)	39–70	50 (13)	25–80
Dry matter intake (DMI)	kg day^{-1}	19.7 (3.2)	12.4–26.1	18.0 (2.8)	11.4–24.5
Forage DMI	kg day^{-1}	10.5 (2.2)	6.6–16.0	8.8 (2.6)	2.9–15.2
Concentrate DMI	kg day^{-1}	9.2 (2.3)	5.1–13.0	9.2 (2.8)	3.6–16.9
Milk yield	kg day^{-1}	33.3 (9.4)	14.4–55.2	25.9 (6.9)	14.1–49.1
Live weight	kg	646 (68)	473–805	572 (60)	385–733
Crude protein	g kg^{-1} DM	170 (1.1)	166–173	188 (21)	127–250
Ether extract (oil)	g kg^{-1} DM	22.4 (2.5)	17.9–27.5	55.2 (8.4)	25.2–63.5
Starch	g kg^{-1} DM	187 (0.9)	158–206	129 (30)	72–216
Sugar	g kg^{-1} DM	40.9 (3.2)	34.9–47.7	56.5 (16.7)	39–137
Neutral detergent fibre	g kg^{-1} DM	313 (12)	292–337	390 (50)	264–554
Ash	g kg^{-1} DM	14.5 (0.8)	13.1–16.3	84.4 (8.6)	57–111
Metabolisable energy	MJ kg^{-1} DM	12.1 (0.03)	12.0–12.2	12.1 (0.7)	10.3–14.4
Milkings per day		3.3 (0.8)	1.8–6.0	2	–
Milking duration	s	395 (100)	242–778	–	–
Average peak rise time	s	10.4 (3.0)	6.5–15.6		
Maximum peak rise time	s	15.5 (2.3)	10.8–20.3		
Minimum CH_4 concentration	ppm	185 (33)	113–251		
Maximum CH_4 height	ppm	1,253 (208)	744–1,736		
Maximum CH_4 amplitude	ppm	1,042 (208)	535–1,497		
Average CH_4 concentration	ppm	568 (91)	344–814		
Daily CH_4 production					
Peak CH_4 height	g day^{-1}	288 (59)	152–431		
Peak CH_4 amplitude	g day^{-1}	282 (63)	135–431		
Average CH_4 concentration	g day^{-1}	177 (72)	66–344	387 (64)	202–541
CH_4 yield					
Peak CH_4 height	g kg^{-1} DM	14.8 (3.1)	9.1–23.4	–	–
Peak CH_4 amplitude	g kg^{-1} DM	14.5 (3.2)	8.8–23.3	–	–
Average CH_4 concentration	g kg^{-1} DM	9.0 (3.2)	3.6–17.9	21.8 (3.4)	13.8–33.5

2.2 Chamber data

The chamber dataset consisted of a total of 196 measurements from 105 lactating dairy cows of different breeds (Holstein Friesian, Norwegian Red and Jersey Holstein) taken during energy metabolism studies conducted at the Agri-Food and Biosciences Institute (Yan et al., 2010) and Ellinbank (Williams et al., 2013) research centres. The dataset covered wide ranges of milk yield (14.1 to 49.1 kg day^{-1}), lactation number (1 to 9), stage of lactation (early to late) and live weight (385 to 733 kg) (*Table 1*). All cows were offered a diet of between 25 and 80 % forage (either fresh cut grass, grass silage or alfalfa hay) *ad libitum*. The concentrate portion of the diet was offered either as part of a complete diet mixed with the forage or as a separate feed, and when the concentrates were fed they consisted of cereal grains (barley, wheat or maize), by-products (maize gluten meal, molassed or unmolassed sugar-beet pulp, citrus pulp or molasses) or protein supplements (fish meal, soybean meal or rapeseed meal). Prior to commencing CH_4 measurements, all cows were offered experimental diets for at least two weeks. In the metabolism unit, each cow spent at least four days in metabolism stalls followed by three days in a chamber (indirect open-circuit calorimeter) for CH_4 measurements, with the CH_4 measurements from the final 48 h period being used for analysis.

2.3 Statistical analysis

Data from spot sampling and chamber measurements were analysed using a linear mixed model in Genstat Version 19.1 (Lawes Agricultural Trust, 2018). Average emissions per day and average CH_4 yield (grams per kilogram DMI) were calculated for each cow during each feeding period (two-weeks for spot measurement values and two days for chamber values) and used in the analysis. Equation [2] was used to calculate variance components for feed intake (DMI, forage DMI and concentrate DMI), milk production, live weight and various metrics for CH_4 per individual cow:

$$y_{ijkl} = \mu + P_i + D_j + L_k + L_k.C_l + E_{ijkl} \quad [2]$$

where y_{ijk} is the dependent variable; μ = overall mean; P_i = fixed effect of measurement period; D_j = fixed effect of diet; L_k = fixed effect of lactation number (k = 1, 2 or 3 and more); $L_k.C_l$ = random effect of individual cow; E_{ijkl} = random error term.

Repeatability of animal production variables and gas emission measures were assessed by σ^2 animal (σ^2 animal + σ^2 residual)$^{-1}$, where σ^2 is the variance. The between-cow and residual coefficient of variation (CV) were calculated from variance components as root mean square error divided by the mean. The Pearson correlation coefficient was used to assess the association between CH_4 emission metrics and total DMI, forage DMI, concentrate DMI, milk yield and live weight across all individual cow records. The results for the three metrics of CH_4 from peak analysis (peak height, peak amplitude and average concentration) were compared with each other after converting to daily emissions and CH_4 yield, which allowed comparison to CH_4 emissions from chamber measurements. Significance was attributed at $P<0.05$. Equation 1 was validated on peak analysis data from spot measurements and chamber measurements from the same ten cows from the study by Garnsworthy et al. (2012). The maximum peak amplitude (mean ± sd of 1054 ± 313 ppm and ranging from 625 to 1592 ppm) and peak rise time (mean ± sd of 10.9 ± 0.4 seconds and ranging from 10.2 to 11.5 seconds) were derived within milking periods and the total daily CH_4 production (mean ± sd of 370 ± 28 g day^{-1} and ranging from 332 to 407 g/day) whilst in the chamber. For this data, the Pearson correlation coefficient (r), Lin's bias correction factor (C_b) and concordance correlation coefficient (*CCC*) were used to test the association between total CH_4 production estimated from spot measurements using Equation 1 and chamber measurements from the same cows. Coefficient r was multiplied by Lin's bias correction factor (C_b), which measures how far the best-fit line deviates from the 45° line through the origin, in order to derive the *CCC* (Lin, 1989).

3 Results and discussion

3.1 Methane and its association with production traits

After filtering spot measurements for peaks in emissions during milking, ranges of 66 to 431 g CH_4 day^{-1} and 3.6 to 23.4 g CH_4 kg^{-1} DM were observed across CH_4 metrics from peak analysis (*Table 1*). The average CH_4 concentration values (177 g day^{-1} and 9 g kg^{-1} DM) were lower than those for peak height (288 g day^{-1} and 14.8 g kg^{-1} DM) and peak amplitude (282 g day^{-1} and 14.8 g kg^{-1} DM) metrics, which were all lower than the average CH_4 emissions measured for dairy cows in the chamber data (387 g day^{-1} and 21.8 g kg^{-1} DM). After deriving CH_4 emission metrics it is noticeable that the peak height and peak amplitude metrics produce similar results. Both metrics have been found to be associated with total CH_4 production (Garnsworthy et al., 2012). Furthermore, using input data of maximum peak amplitude and peak rise time from the study by Garnsworthy et al. (2012) in Equation 1 of the current study, found that estimates of total CH_4 production (mean ± sd of 388 ± 31 g day^{-1} and ranging from 334 to 430 g day^{-1}) are correlated to chamber CH_4 values ($r = 0.75$ and $CCC = 0.62$; mean ± sd of 370 ± 28 g day^{-1} and ranging from 332 to 407 g day^{-1}) (*Figure 2*).

Although peak analysis can help to identify when the animal's head is in close proximity to the gas sampling tube (i.e. from maximum peak height and peak amplitude during one milking), the difference in average daily CH_4 emissions (*Figure 3*) and CH_4 yield (*Figure 4*) between spot measurements and chamber measurements would suggest that some dilution or loss of spot measurement CH_4 emissions occurred between the emissions being expelled by the cow and sampled by the gas analyser. Metrics for spot measurement CH_4 were not adjusted for any dilution effect. Further refinement of the breath sampling approach to capture more of the eructation produced by the animal may improve estimates and is worth comparing to the current proposed approach.

This study found a positive relationship between total DMI (*Figure 3*), forage DMI and CH_4 emissions per day (*Table 2*), and a negative relationship between DMI and CH_4 yield (*Figure 4* and *Table 2*) estimated from peak height and peak amplitude. The magnitude of the correlation between DMI and CH_4 yield estimated from peak height ($r = -0.43$) and peak amplitude ($r = -0.38$) were noticeably similar to the correlation between DMI and CH_4 yield from chamber measurements ($r = -0.40$). As observed in chamber measurements, CH_4 yield declined with increasing concentrate DMI but not forage DMI for metrics of peak height and peak amplitude.

When a highly energy-dense diet is formulated to meet the nutrient requirements of a high milk yielding animal (with spot sampled cows averaging 33 kg milk day^{-1} compared to 26 kg milk day^{-1} for chamber cows), often through feeding a higher proportion of concentrates in the diet, the CH_4 yield can be 19 g kg^{-1} DMI or less (Mills et al., 2003 and *Figure 3* for cows with high DMI). The CH_4 yield would be expected to be higher (21 g kg^{-1} DM or more, see Moate et al, 2011) for predominantly forage-based diets. Bell and Eckard (2012) found that in lactating dairy cows fed a diet with a high or low proportion of forage content, the relationship between CH_4 production and DMI appears to be linear up to an average intake of 15 kg DMI day^{-1}. Above this level of intake (as the majority of cows in this study), the CH_4 yield declines, with the lower CH_4 yield for spot measurements potentially being influenced by the allocation of concentrates during milking (*Figure 3*).

The improved relationship between DMI, forage DMI and CH_4 emissions found in the current research compared to the results published in a previous study by Bell et al. (2014b) ($r = 0.19$ to 0.29) can be attributed to the extraction and

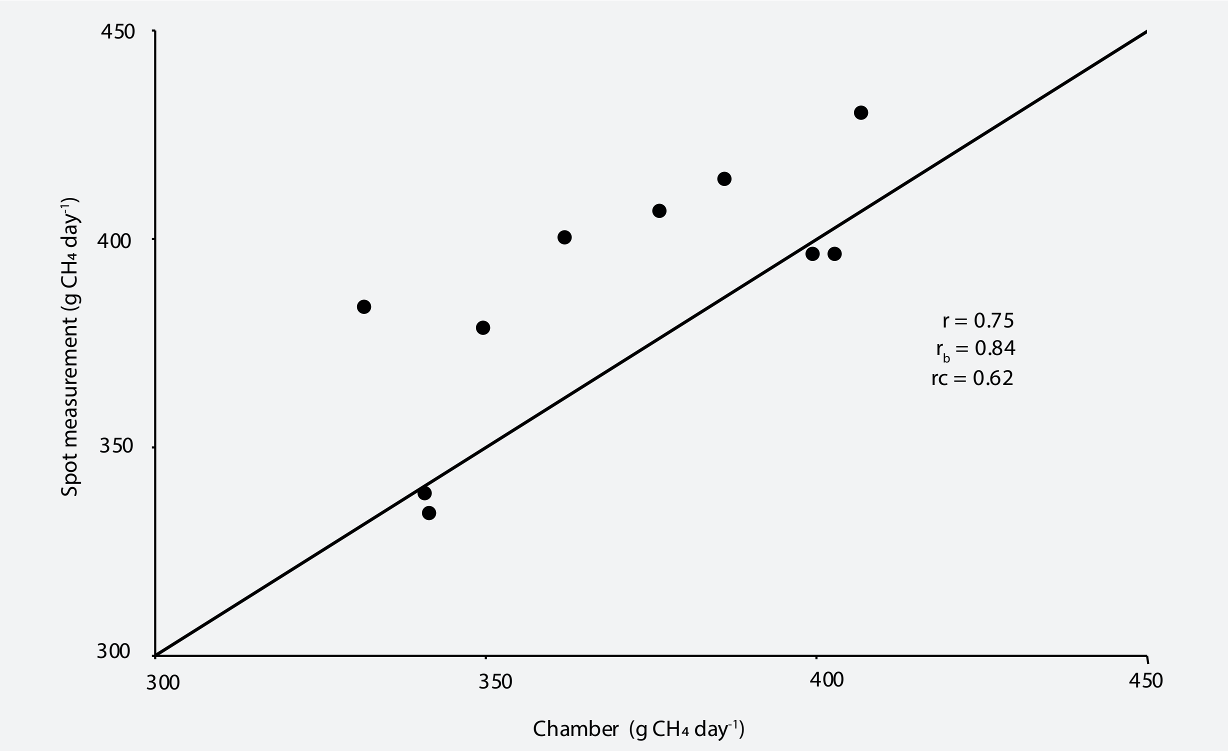

FIGURE 2
Relationship between spot measurements of CH_4 emissions estimated using Equation 1 and chamber measurements for the same cows

improved identification of clusters and of individual eructation peaks in CH_4 emissions during each milking, rather than extracting measurements from across the whole milking period as before. The benefit of extracting the amplitude of eructation peaks is the potential to easily remove background emissions and any buildup in gas that may occur in the feed bin during milking. Milk yield was negatively associated with CH_4 yield from both spot measurements and chamber measurements (*Table 3*). The allocation of concentrate was different between the spot sampled cows and cows in chambers. The high correlation (r = 0.833 for spot sampled cows compared to r = 0.609 for chamber cows) between milk yield and concentrate DMI for spot sampled cows may explain the lower CH_4 yield in these cows compared to the CH_4 yield of cows in chambers. Increased intake of more digestible feeds such as concentrate results in a reduction in CH_4 yield (Yan et al., 2010). There was no association between liveweight and CH_4 yield from spot or chamber measurements (*Table 2* and *Table 3*), but daily chamber CH_4 emissions were positively associated with live weight.

3.2 Repeatability and variability of methane measures

The CH_4 metrics from peak analysis were highly repeatable for metrics of peak height and peak amplitude (ranging from 0.76 to 0.81), and comparable to the high repeatability of production traits for the same cows (ranging from 0.63 to 0.99) (*Table 4*). These instances of high repeatability for CH_4 emissions from spot measurements have been observed in several other studies (0.72 to 0.87 by Huhtanen et al., 2015) and confirm findings from our previous work (0.74 to 0.75 by Bell et al., 2014b). There was little difference in the residual CV observed for the CH_4 metrics derived from peak height and peak amplitude (ranging from 8 to 9 % for daily CH_4 emissions and CH_4 yield) compared to chamber CH_4 measurements (11 %), and in the feed intake traits for spot sampled cows and cows in chambers (CV ranged from 7 % to 15 %). These findings are consistent with the results of Huhtanen et al. (2013), and the modified approach used in the current research to identify eructation peaks within each milking – rather than throughout the whole milking – has improved the reliability of the technique compared to our previous research (Bell et al., 2014b).

The between-cow CV for both daily emissions and CH_4 yield derived from peak analysis metrics in the current study were within the range of 3 to 34 % found in studies using respiration chambers to measure emissions in research herds (Grainger et al., 2007; Ellis et al., 2007; Yan et al., 2010). The between-cow CVs ranged from 16 % to 18 % across peak analysis metrics for CH_4 (*Table 4*) and were higher than the values observed for chamber between-cow CVs of 8 % for daily emissions and 2 % for CH_4 yield. The approach of extracting eructation peak height and peak amplitude to quantify daily CH_4 emissions and CH_4 yield resulted in similar variation between-cows (CV ranging from 16 to 18 %), residual variation (CV ranging from 8 to 9 %) and repeatability (ranging from 0.76 to 0.81) for spot measurements compared to variation between-cow (CV = 12 % for spot but CV = 8 % for chamber

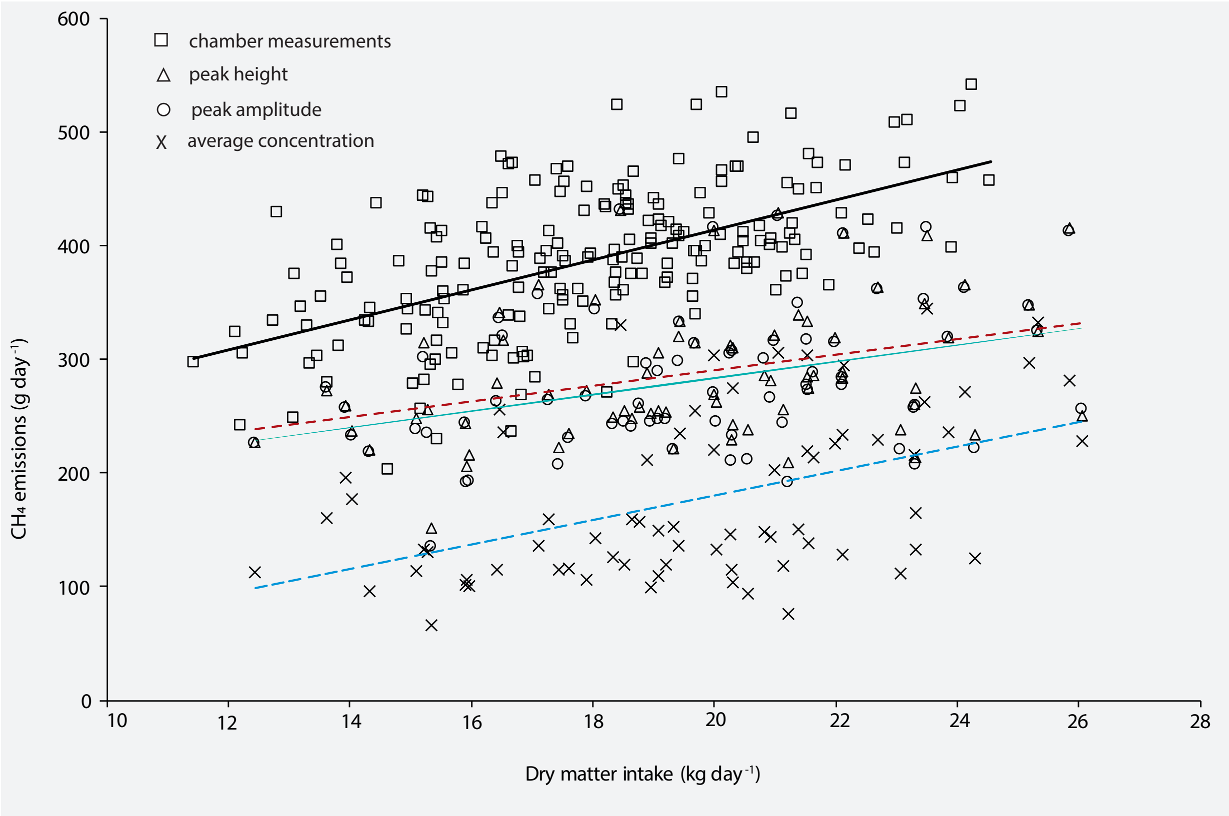

FIGURE 3
Observed dry matter intake and CH_4 emissions per day for chamber measurements and spot sample CH_4 metrics of peak height, peak amplitude and average concentration. The line of best-fit is shown for chamber measurements (black solid line, $y = 149 + 13.2x$, $r = 0.57$, $P < 0.001$) and CH_4 metrics of peak height (red dashed line, $y = 153 + 6.8x$, $r = 0.37$, $P < 0.01$), peak amplitude (green solid line, $y = 138 + 7.3x$, $r = 0.37$, $P < 0.01$) and average concentration (blue long dashed line, $y = -33.8 + 10.7x$, $r = 0.47$, $P < 0.001$).

TABLE 2
Pearson correlation coefficients and significance of the relationship between feed dry matter intake (DMI), milk production, live weight and daily methane emissions as estimated from peak related parameters (below diagonal) and methane yield as estimated from peak related parameters (above diagonal) for cows measured using spot sampling.

Variable[1]	Units	DMI	Forage DMI	Concentrate DMI	Milk yield	Live weight	Peak height	Peak amplitude	Average concentration
		kg day^{-1}				kg	g CH_4 kg^{-1} DMI		
DMI	kg day^{-1}	1					-0.432 (<0.001)	-0.380 (<0.001)	0.056 (0.640)
Forage DMI	kg day^{-1}	0.698 (<0.001)	1			-0.015 (0.901)	0.043 (0.718)	0.431 (<0.001)	
Concentrate DMI	kg day^{-1}	0.726 (<0.001)	0.015 (0.903)	1		-0.588 (<0.001)	-0.572 (<0.001)	-0.335 (<0.01)	
Milk yield	kg day^{-1}	0.618 (<0.001)	0.031 (0.799)	0.833 (<0.001)	1	-0.524 (<0.001)	-0.515 (<0.001)	0.300 (<0.05)	
Live weight	kg	-0.03 (0.800)	0.355 (<0.01)	-0.383 (<0.001)	-0.186 (0.118)	1	0.011 (0.929)	0.0002 (0.999)	0.007 (0.953)
Peak height	g CH_4 day^{-1}	0.366 (<0.01)	0.550 (<0.001)	-0.017 (0.891)	-0.056 (0.638)	-0.028 (0.818)	1		
Peak amplitude	g CH_4 day^{-1}	0.367 (<0.01)	0.568 (<0.001)	-0.033 (0.785)	-0.074 (0.539)	-0.037 (0.758)	0.993 (<0.001)	1	
Average concentration	g CH_4 day^{-1}	0.470 (<0.001)	0.691 (<0.001)	-0.007 (0.950)	-0.031 (0.798)	-0.011 (0.927)	0.792 (<0.001)	0.814 (<0.001)	1

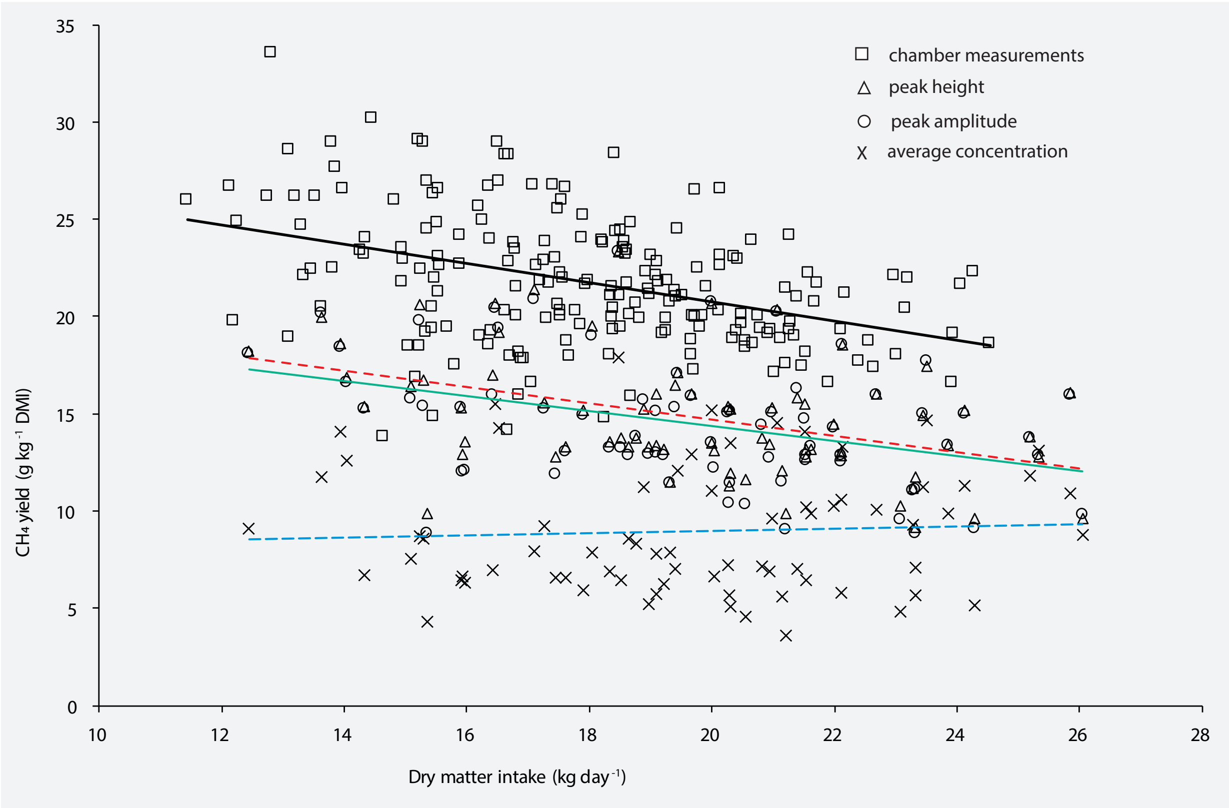

FIGURE 4
Observed dry matter intake and CH_4 yield for chamber measurements and CH_4 metrics of peak height, peak amplitude and average concentration. The line of best-fit is shown for chamber measurements (black solid line, $y = 30.5 - 0.49x$; $r = -0.40$, $P < 0.001$) and CH_4 metrics of peak height (red dashed line, $y = 23.1 - 0.42x$; $r = -0.43$, $P < 0.001$), peak amplitude (green solid line, $y = 22.1 - 0.39x$; $r = -0.38$, $P < 0.001$) and average concentration (blue long dashed line, $y = 7.8 + 0.06x$, $r = 0.06$, $P = 0.640$).

measurements), residual variation (CV = 8 % for spot and CV = 9 % for chamber measurements) and repeatability (0.70 for spot but different at 0.40 for chamber measurements) for DMI, which was also found by Huhtanen et al. (2013) using a spot sampling approach.

The frequent 'spot' sampling of enteric CH_4 emissions from cows has come about due to the need to measure CH_4 emissions from large numbers of commercial animals for farm benchmarking, improving national greenhouse gas inventories and for selecting low CH_4 producing animals. Methods that are more mobile, non-invasive to the animal and can fit into the animal's normal environment are of great interest, such as the technique used in this study. Furthermore, identification of eructation peaks and clusters of peaks can provide a repeatable and reliable metric that is consistent with cow chamber records, which is the gold standard measure for measuring CH_4 emissions. Cows in the current study were milked on average three times per day and had spot measurements of CH_4 recorded for two weeks during two feeding periods to obtain individual cow enteric CH_4 emission rates. Duration of spot sampling depends on the frequency and number of spot measurements being obtained (Cottle et al., 2015). This approach of taking spot measurements over at least a week is longer than the three days animals spend in a chamber to measure CH_4 emissions. However, this approach can be implemented on commercial farms unlike the use of chambers.

In conclusion, this study showed that quantifying enteric CH_4 emissions using eructation peaks (maximum peak height or maximum peak amplitude) detected within a milking can provide a highly repeatable metric for quantifying daily CH_4 emissions and daily CH_4 yields. The association between DMI and metrics for estimating methane emissions derived from peak height and peak amplitude were similar for cows studied using spot sampling and in a respiration chamber. The extraction of eructation CH_4 peaks can provide a repeatable and reliable method for quantifying CH_4 emissions and assessing variation among cows. We recommend estimating daily CH_4 emissions by measuring the maximum peak amplitude of an eructation during one milking.

TABLE 3
Pearson correlation coefficients and significance of the relationship between feed dry matter intake (DMI), milk production, live weight and daily methane emissions and methane yield for cows measured in chambers.

Variable[1]	Units	DMI	Forage DMI	Concentrate DMI	Milk yield	Live weight	CH_4 yield
		kg day^{-1}				kg	g CH_4 kg^{-1} DMI
DMI	kg day^{-1}	1					-0.402 (<0.001)
Forage DMI	kg day^{-1}	0.445 (<0.001)	1				-0.018 (0.808)
Concentrate DMI	kg day^{-1}	0.578 (<0.001)	-0.474 (<0.001)	1			-0.379 (<0.001)
Milk yield	kg day^{-1}	0.583 (<0.001)	-0.039 (0.588)	0.609 (<0.001)	1		-0.439 (<0.001)
Live weight	kg	0.454 (<0.001)	0.262 (<0.001)	0.207 (<0.01)	0.142 (0.05)	1	0.088 (0.219)
CH_4 emissions	g CH_4 day^{-1}	0.574 (<0.001)	0.425 (<0.001)	0.177 (<0.05)	0.146 (<0.05)	0.509 (<0.001)	0.509 (<0.001)

TABLE 4
Variability and repeatability (standard error in parentheses) of dry matter intake (DMI), milk production, live weight and methane (CH_4) emissions, and of yields as derived from peak analysis for spot sampled cows and by traditional methods for cows in chambers.

Variable	Units	Spot			Chamber		
		Between-cow CV (%)	Residual CV (%)	Repeatability	Between-cow CV (%)	Residual CV (%)	Repeatability
DMI	kg day^{-1}	12.0	7.8	0.70 (0.21)	7.5	9.2	0.40 (0.16)
Forage DMI	kg day^{-1}	13.7	10.5	0.63 (0.21)	8.2	15.4	0.22 (0.14)
Concentrate DMI	kg day^{-1}	23.1	7.3	0.91 (0.24)	4.0	10.0	0.14 (0.18)
Milk yield	kg day^{-1}	23.7	10.0	0.85 (0.23)	16.4	10.4	0.71 (0.15)
Live weight	kg day^{-1}	9.8	1.1	0.99 (0.24)	9.3	3.6	0.87 (0.15)
Daily CH_4 production[1]							
Peak CH_4 height	g day^{-1}	16.1	9.1	0.76 (0.22)	–	–	–
Peak CH_4 amplitude	g day^{-1}	17.6	8.6	0.81 (0.22)	–	–	–
Average CH_4 concentration	g day^{-1}	16.4	18.0	0.45 (0.19)	7.8	11.7	0.31 (0.15)
CH_4 yield[1]							
Peak CH_4 height	g kg^{-1} DMI	16.9	8.2	0.81 (0.22)	–	–	–
Peak CH_4 amplitude	g kg^{-1} DMI	17.7	8.8	0.80 (0.22)	–	–	–
Average CH_4 concentration	g kg^{-1} DMI	16.5	14.6	0.56 (0.20)	1.8	11.4	0.03 (0.12)

[1] Spot sampling metrics for daily emissions and CH_4 yield were estimated from emission rate.

Acknowledgements

This work was funded by Defra, the Scottish Government, DARD, Welsh Government (as part of the UK's Agricultural GHG Research Platform project), the Australian Department of Agriculture, Dairy Australia, Meat and Livestock Australia, the University of Melbourne, DARD and AgriSearch. The authors are grateful for the assistance from technicians and farm staff.

REFERENCES

Bell MJ, Eckard R (2012) Reducing enteric methane losses from ruminant livestock – Its measurement, prediction and the influence of diet. In: Javed K (ed) Livestock Production, Rijeka, Croatia: InTech Publishing, 135–150, doi:10.5772/50394

Bell MJ, Potterton SL, Craigon J, Saunders N, Wilcox RH, Hunter M, Goodman J, Garnsworthy PC (2014a) Variation in enteric methane emissions among cows on commercial dairy farms. Animal 8(9):1540–1546, doi:10.1017/S1751731114001530

Bell MJ, Saunders N, Wilcox RH, Homer EM, Goodman JR, Craigon J, Garnsworthy PC (2014b) Methane emissions among individual dairy cows during milking quantified by eructation peaks or ratio with carbon dioxide. J Dairy Sci 97(10):6536–6546, doi:10.3168/jds.2013-7889

Blaxter KL, Clapperton JL (1965) Prediction of the amount of methane produced by ruminants. Br J Nutr 19(4):511–522, doi:10.1079/bjn19650046

Breider IS, Wall, E, Garnsworthy PC (2019) Heritability of methane production and genetic correlations with milk yield and body weight in Holstein-Friesian dairy cows. J Dairy Sci 102(8):7277–7281, doi:10.3168/jds.2018-15909

Cottle DJ, Velazco J, Hegarty RS, Mayer DG (2015) Estimating daily methane production in individual cattle with irregular feed intake patterns from short-term methane emission measurements. Animal 9(Suppl 12):1949–1957, doi:10.1017/S1751731115001676

de Haas Y, Windig JJ, Calus MPL, Dijkstra J, de Haan M, Bannink A, Veerkamp RF (2011) Genetic parameters for predicted methane production and the potential for reducing enteric emissions through genomic selection. J Dairy Sci 94(12):6122–6134, doi:10.3168/jds.2011-4439

Ellis JL, Kebreab E, Odongo NE, McBride BW, Okine EK, France J (2007) Prediction of methane production from dairy and beef cattle. J Dairy Sci 90(7):3456–3467, doi:10.3168/jds.2006-675

Garnsworthy PC, Craigon J, Hernandez-Medrano JH, Saunders N (2012) On-farm methane measurements during milking correlate with total methane production by individual dairy cows. J Dairy Sci 95(6):3166–3180, doi:10.3168/jds.2011-4605

Gerber PJ, Steinfeld H, Henderson B, Mottet A, Opio C, Dijkman J, Falcucci A, Tempio G (2013) Tackling climate change through livestock – A global assessment of emissions and mitigation opportunities; Rome: Food and Agriculture Organization of the United Nations (FAO), 115 p

Grainger C, Clarke T, McGinn SM, Auldist MJ, Beauchemin KA, Hannah MC, Waghorn GC, Clark H, Eckard RJ (2007). Methane emissions from dairy cows measured using the sulfur hexafluoride (SF6) tracer and chamber techniques. J Dairy Sci 90(6):2755–2766, doi:10.3168/jds.2006-697

Hammond KJ, Crompton LA, Bannink A, Dijkstra J, Yáñez-Ruiz DR, O'Kiely P, Kebreab E, Eugenè MA, Yu Z, Shingfield KJ, Schwarm A, Hristov AN, Reynolds CK (2016) Review of current in vivo measurement techniques for quantifying enteric methane emission from ruminants. Anim Feed Sci Technol 219:13–30, doi:10.1016/j.anifeedsci.2016.05.018

Huhtanen P, Krizsan SJ, Hetta M, Gidlund H, Cabezas Garcia EH (2013) Repeatability and between cow variability of enteric CH_4 and total CO_2 emissions. Proceedings of the 5th Greenhouse Gases and Animal Agriculture conference (GGAA 2013), Dublin, Ireland, 23–27 June 2013, Adv Anim Biosci 4(2):588, doi:10.1017/S2040470013000125

Huhtanen P, Cabezas-Garcia EH, Utsumi S, Zimmerman S (2015) Comparison of methods to determine methane emissions from dairy cows in farm conditions. J Dairy Sci 98(5):3394–3409, doi:10.3168/jds.2014-9118

Lassen J, Løvendahl P (2016) Heritability estimates for enteric methane emissions from Holstein cattle measured using noninvasive methods. J Dairy Sci 99(3):1959-1967, doi:10.3168/jds.2015-10012

Lawes Agricultural Trust (2018) Genstat 19, Version 19.1 Reference Manual. London: Clarendon Press, UK

Lin LI (1989) A concordance correlation coefficient to evaluate reproducibility. Biometrics 45(1):255–268, doi:10.2307/2532051

Mills JAN, Kebreab E, Yates CM, Crompton LA, Cammell SB, Dhanoa MS, Agnew RE, France J (2003) Alternative approaches to predicting methane emissions from dairy cows. J Anim Sci 81(12):3141–3150, doi:10.2527/2003.81123141x

Moate PJ, Williams SRO, Grainger C, Hannah MC, Ponnampalam EN, Eckard R (2011) Influence of cold-pressed canola, brewers grains and hominy meal as dietary supplements suitable for reducing enteric methane emissions from lactating dairy cows. Anim Feed Sci Technol 166–167:254–264, doi:10.1016/j.anifeedsci.2011.04.069

Negussie E, Lehtinen J, Mäntysaari P, Bayat AR, Liinamo AE, Mäntysaari EA, Lidauer MH (2017) Non-invasive individual methane measurement in dairy cows. Animal 11(5):890–899, doi:10.1017/S1751731116002718

Niu M, Kebreab E, Hristov AN, Oh J, Arndt C, Bannik A, Bayat AR, Brito AF, Boland T, Casper D et. al. (2018) Prediction of enteric methane production, yield, and intensity in dairy cattle using an intercontinental database. Global Change Biol 24(8):3368–3389, doi:10.1111/gcb.14094

Williams SRO, Clarke T, Hannah M, Marett LC, Moate PJ, Auldist MJ, Wales WJ (2013) Energy partitioning in herbage-fed dairy cows offered supplementary grain during an extended lactation. J Dairy Sci 96(1):484–494, doi:10.3168/jds.2012-5787

Yan T, Mayne CS, Gordon FG, Porter MG, Agnew RE, Patterson DC, Ferris CP, Kilpatrick DJ (2010) Mitigation of enteric methane emissions through improving efficiency of energy utilization and productivity in lactating dairy cows. J Dairy Sci 93(6):2630–2638, doi:10.3168/jds.2009-2929