

## Article

# Effect of Feeding System on Enteric Methane Emissions from Individual Dairy Cows on Commercial Farms

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**Abstract:** This study investigated the effects of feeding system on diurnal enteric methane (CH<sub>4</sub>) emissions from individual cows on commercial farms. Data were obtained from 830 cows across 12 farms, and data collated included production records, CH<sub>4</sub> measurements (in the breath of cows using CH<sub>4</sub> analysers at robotic milking stations for at least seven days) and diet composition. Cows received either a partial mixed ration (PMR) or a PMR with grazing. A linear mixed model was used to describe variation in CH<sub>4</sub> emissions per individual cow and assess the effect of feeding system. Methane emissions followed a consistent diurnal pattern across both feeding systems, with emissions lowest between 05:00 and 08:59, and with a peak concentration between 17:00 and 20:59. No overall difference in emissions was found between feeding systems studied; however, differences were found in the diurnal pattern of CH<sub>4</sub> emissions between feeding systems. The response in emissions to increasing dry matter intake was higher for cows fed PMR with grazing. This study showed that repeated spot measurements of CH<sub>4</sub> emissions whilst cows are milked can be used to assess the effects of feeding system and potentially benchmark farms on level of emissions.

**Keywords:** cattle; greenhouse gas; measurements; diet; variation

## 1. Introduction

At the United Nations Climate Change Conference in 2015, a key component of the global agreement was to protect food production whilst also reducing greenhouse gas (GHG) emissions [1]. Dairy farming contributes 20% of total global GHG emissions from the livestock sector, with enteric CH<sub>4</sub> being the largest source at 39% of dairy emissions [2]. Given the significance of CH<sub>4</sub> as a GHG, reducing enteric CH<sub>4</sub> emissions from dairy cows whilst maintaining levels of milk production could prove an important strategy for countries to meet reduction targets in global emissions. Enteric CH<sub>4</sub> is produced in the digestive tract by Archaea microorganisms as a by-product of anaerobic fermentation (methanogenesis). This process results in 3% to 14% loss in gross energy intake, which is largely dependent on composition of the animals' diet and level of feed intake [3].

Until recently, most of the methods used to quantify CH<sub>4</sub> emissions from cattle involved housing animals in respiration chambers [4–6]. Respiration chamber measurements are often costly, fixed in location so not suitable for commercial farm use, and potentially inhibit animal behaviour that would be expressed in the animals' normal environment. An alternative approach for measuring emissions from grazing animals is the sulphur hexafluoride (SF<sub>6</sub>) technique [7,8], where a small permeation tube containing the tracer gas is placed in the rumen of the animal. However, as with

chambers, the SF<sub>6</sub> technique is not suited to sampling a large population of animals on commercial farms due to restrictions on use of gas and the attachment of equipment to animals. Recent research has focused on collecting data from commercial herds through non-invasive approaches that take repeated spot measurements whilst cattle are feeding [6,9], being milked [10–12], or standing [13]. Frequent sampling of gas emissions has been found to provide repeatable measurements that allow assessment of within-cow, between-cow, diet and temporal effects on CH<sub>4</sub> emissions. Estimates of CH<sub>4</sub> made during milking have been found [10] to be correlated with total daily CH<sub>4</sub> emissions by the same cows when housed subsequently in respiration chambers. Also, the technique of repeated spot measurements can identify known high and low CH<sub>4</sub>-producing diets [10], demonstrating that the methodology was sensitive enough to assess differences in diet treatments. Crompton et al. [14] identified the relationship between the time of feeding and CH<sub>4</sub> emissions, with a rapid increase in emissions after an animal consumes food followed by a gradual decline. Several studies have observed a diurnal pattern to CH<sub>4</sub> emissions from ruminant livestock [9,14,15], which is affected by feed allowance and feeding frequency [14], with no overall influence on average daily CH<sub>4</sub> yield [16,17].

The current study builds on the research of Bell et al. [18], who found considerable unexplained variation in CH<sub>4</sub> emissions among farms that warranted further investigation with the addition of diet composition and feed intake data, which are known to explain a large proportion of variation in emissions [19].

The objective of the current study was to assess the effect of feeding system (PMR vs. PMR with grazing) on diurnal enteric CH<sub>4</sub> emissions from dairy cows on commercial farms.

## 2. Materials and Methods

### 2.1. Data

Data were obtained from 21,324 individual milkings of 830 cows across 12 commercial farms in the UK. Each farm was visited once during the years 2011 to 2013, with production data and CH<sub>4</sub> measurements collected for at least seven days. Farms were visited during different seasons to allow grazing and non-grazing systems to be monitored. Cows in this study were milked individually at automatic (robotic) milking stations which recorded cow ID, time of milking, duration of milking, stage of lactation, lactation number, milk yield, robot concentrate intake, and live weight at each milking for each individual cow (Table 1).

**Table 1.** Mean herd size, number of milking stations, feeding system category (Partial mixed ration (PMR) or PMR with grazing), month of year for sampling, and mean (s.d.) lactation number, days in milk, milk yield, live weight, dry matter intake and methane emissions per cow for each herd.

Farm No.	Number of Cows	Number of Milking Stations	Feeding System	Month of Sampling	Lactation No.	Days in Milk	Milk Yield	Live Weight	Dry Matter Intake	Methane Emissions
							kg/day	kg	kg/day	mg/L
A	65	1	PMR + Grazing	10	4.1 (2.4)	79 (51)	24.3 (8.6)	586 (74)	16.9 (2.5)	1.9 (1.2)
B	53	1	PMR + Grazing	9	3.2 (1.9)	173 (92)	28.2 (10.0)	622 (31)	18.3 (1.4)	2.6 (1.5)
C	51	1	PMR + Grazing	4	3.6 (1.8)	168 (99)	28.5 (10.3)	642 (60)	18.9 (1.8)	3.5 (2.5)
D	47	1	PMR + Grazing	4	2.3 (1.2)	161 (113)	27.7 (11.2)	611 (59)	18.1 (1.9)	2.5 (1.7)
E	66	1	PMR + Grazing	5	4.0 (3.3)	130 (86)	28.8 (9.4)	625 (57)	18.5 (1.8)	3.7 (3.2)
F	45	1	PMR + Grazing	6	3.5 (2.3)	135 (80)	27.0 (9.2)	598 (72)	17.7 (2.3)	4.0 (2.2)
G	116	2	PMR	6	2.6 (1.6)	159 (90)	26.1 (8.8)	625 (73)	18.2 (2.1)	4.0 (2.6)
H	96	2	PMR	8	2.9 (2.0)	163 (102)	27.1 (9.9)	593 (75)	17.5 (2.2)	3.9 (2.2)
I	46	1	PMR	11	1.0 (0.0)	99 (31)	25.2 (5.4)	547 (44)	16.2 (1.3)	0.6 (0.5)
J	55	2	PMR	11	3.7 (1.8)	136 (111)	28.9 (10.9)	690 (63)	20.1 (2.0)	2.4 (1.1)
K	110	2	PMR	2	2.4 (1.4)	156 (92)	35.6 (12.6)	603 (74)	18.6 (2.4)	2.4 (1.3)
L	80	2	PMR	2	2.8 (1.8)	158 (87)	19.1 (8.3)	578 (71)	16.4 (1.9)	3.7 (3.1)
Mean <sup>1</sup> PMR + Grazing					3.7 (0.03)	143 (14.3)	26.9 (1.7)	612 (15.6)	17.9 (0.5)	
Mean <sup>1</sup> PMR					2.6 (0.04)	145 (13.9)	26.5 (1.6)	607 (15.4)	17.8 (0.5)	
SED					0.05	19.8	2.3	21.9	0.7	
<i>P value</i>					<0.001	0.912	0.854	0.809	0.835	

<sup>1</sup> Predicted mean  $\pm$  s.e. presented for both feeding systems. Linear mixed model with unique cow ID within farm, milking station within farm and month of sampling added as random effects and covariates centred to a zero mean. SED means standard errors of differences.

All cows were fed a partial mixed ration (PMR) containing forage and concentrates ad libitum, with additional concentrates fed whilst milking. Of the 12 farms studied, half the farms allowed the cows access to grass (PMR + grazing) during the day. Dry matter intake of individual cows was predicted from their milk yield and live weight using the equation by MAFF [20] as: Dry matter intake (kg/day) =  $0.025 \times \text{live weight (kg)} + 0.1 \times \text{milk yield (kg/day)}$ . Records on the composition of diet and forage (Table 2) and concentrate feeds (Table 3) were obtained from each farm, with feed samples analysed by a commercial analytical laboratory (Sciante Analytical Services, Cawood, UK). Cows used in this study were mainly Holstein-Friesian breed and remained on the same feeding regime throughout the measurement period.

**Table 2.** Forage percentage (grass percentage in the diet and in parentheses) in the diet and forage nutrient content for each farm.

Farm	Forage	Dry Matter (DM)	Starch	Neutral Detergent Fibre	Crude Protein	Oil	Metabolisable Energy
	%	g/kg	g/kg DM	g/kg DM	g/kg DM	g/kg DM	MJ/kg DM
A	68.7 (4.5)	316	133	424	132	40	10.2
B	68.4 (58.8)	172	0	362	237	31	11.0
C	48.5 (3.8)	398	6.1	291	74	22	11.2
D	57.9 (1.3)	344	186	452	109	26	11.2
E	62.6 (1.4)	494	74	507	143	51	10.7
F	75.6 (46.7)	304	0	426	153	26	11.1
G	60.2	263	156	333	79	18	10.0
H	45.8	351	6	470	132	32	10.6
I	57.1	570	0	592	104	18	9.8
J	49.4	313	124	414	128	29	11.2
K	58.3	394	56	474	116	18	11.0
L	68.0	283	45	440	124	48	10.3

**Table 3.** Concentrate percentage in the diet and concentrate nutrient content for each farm.

Farm	Concentrate	Dry Matter (DM)	Starch	Neutral Detergent Fibre	Crude Protein	Oil	Metabolisable Energy
	%	g/kg	g/kg DM	g/kg DM	g/kg DM	g/kg DM	MJ/kg DM
A	31.3	874	187	246	193	57	12.7
B	31.6	880	127	285	162	57	12.2
C	51.5	879	124	321	195	57	12.1
D	42.1	872	91	230	207	52	12.1
E	37.4	870	139	262	181	47	11.5
F	24.4	886	320	169	178	49	12.4
G	39.8	885	191	126	252	46	12.1
H	54.2	870	131	200	150	52	13.0
I	42.9	867	290	157	180	42	12.7
J	50.6	888	143	259	140	36	12.6
K	41.7	868	190	213	187	81	13.4
L	32.0	873	220	226	178	58	12.0

## 2.2. Measurements of Enteric CH<sub>4</sub>

The CH<sub>4</sub> concentration of eructed gas from cows was measured using the methodology devised by Garnsworthy et al. [10]. During milking, air was continually sampled from the feed bin in a robotic milking station at 1 L per minute through a polythene tube, whilst cows received concentrate feed dispensed in small amounts. Continual allocation of feed kept the cow's mouth and nose within the bin for the duration of milking. Concentration of CH<sub>4</sub> in the breath of cows was measured using an infrared gas analyser (Guardian Plus; Edinburgh Instruments Ltd., Livingston, UK). Concentration of CH<sub>4</sub> was logged at 1-s intervals on data loggers (Simex SRD-99; Simex Sp. Z o.o., Gdańsk, Poland) and visualised using logging software (Loggy Soft version 1.5.7.78; Simex Sp. Z o.o.). The CH<sub>4</sub> analyser was calibrated using standard mixtures of CH<sub>4</sub> in nitrogen (0.0, 0.25, 0.50, 0.75, and 1.0% CH<sub>4</sub>, Thames

Restek UK Ltd., Saunderton, UK). To enable CH<sub>4</sub> concentrations to be adjusted to relative amounts released by the cow, the dilution factor was determined at the end of each sampling period at each robotic milking station and varied from 12.8 to 48.7. To do this, a fixed volume (2.7 L) of 1.0% CH<sub>4</sub> in nitrogen was released at two locations in the feed bin of the milking station, which were at the base of the trough and at the centre of the feed bin level with the sample tube. Release of CH<sub>4</sub> was replicated three times at each location, with the dilution factor being the mean ratio of six values of CH<sub>4</sub> concentrations in released and sampled gas [18]. Concentration of CH<sub>4</sub> in the air sampled followed a pattern of peaks and troughs demonstrating that a pulse release of CH<sub>4</sub> was eructated by the cow. A custom-made program was then used to identify and quantify peaks in concentration when each cow visited the milking station (using cow ID and time of visit information), and extract the area and frequency of peaks. The peak frequency per minute was multiplied by the area under each peak to calculate the milligrams of CH<sub>4</sub> per litre of air sampled. An eructation peak was defined as the time from the start of a rapid rise in concentration, until the following rise or return to baseline concentration. Milkings with less than three eructation peaks for CH<sub>4</sub> concentration and peaks where the cow's head was not within the feed bin were excluded from the analysis. The CH<sub>4</sub> emissions during each milking were calculated as: CH<sub>4</sub> (mg/L) = (average integral of CH<sub>4</sub> per peak × frequency of peaks) × dilution factor.

### 2.3. Statistical Analysis

Data were analysed using a linear mixed model in Genstat Version 18.1 (Lawes Agricultural Trust, 2012) to assess the effect of feeding system on log-transformed CH<sub>4</sub> emissions (mg/L). Previous studies [18,21] have identified important explanatory variables describing CH<sub>4</sub> emissions per individual cow as being time of year, stage of lactation, time of day, and effect of farm. The following model was used to describe emissions from individual cows with the inclusion of explanatory variables for feeding system effects:

$$Y_{ijklmn} = \mu + aDIM + S_i + bI + c_iI \times S_i + H_j + S_i \times H_j + F_l.A_k + F_l + F_l.R_m + M_n + E_{ijklmn} \quad (1)$$

where  $Y_{ijklmn}$  is the dependent variable of log-transformed CH<sub>4</sub> emissions;  $\mu$  is the overall mean;  $aDIM$  is the linear regression of  $Y$  on days in milk;  $S_i$  is the fixed effect of feeding system (PMR or PMR + grazing);  $bI$  and  $c_iI$  are the linear regressions of  $Y$  on estimated dry matter intake (kg/day);  $H_j$  is the fixed effect of time of day (categorised as six time periods of 01:00 to 04:59, 05:00 to 08:59, 09:00 to 12:59, 13:00 to 16:59, 17:00 to 20:59, and 21:00 to 00:59);  $F_l.A_k$  is the random effect of individual cow within farm;  $F_l$  is the random effect of farm (A to L);  $F_l.R_m$  is the random effect of robot within farm;  $M_n$  is the random effect of month of sampling;  $E_{ijklmn}$  is the random error term. The terms  $a$ ,  $b$ , and  $c_i$  are regression coefficients.

The following diet components were also included in the analysis: forage intake, concentrate intake, starch, neutral detergent fibre (NDF), crude protein, oil (all percentage in diet), and metabolisable energy content (MJ/kg DM). Each component was added to Equation (1), but none was found to be significant ( $P > 0.05$ ). Difference in lactation number, days in milk, milk yield, live weight, and estimated dry matter intake between feeding systems were obtained using Equation (1) with only the feeding system (PMR and PMR + grazing) included as the fixed effect.

### 3. Results and Discussion

Cows fed a PMR with grazing had a higher mean lactation number ( $P < 0.001$ ) compared to cows fed a PMR (Table 1). There was no difference in number of days in milk, milk yield, live weight, or dry matter intake between cows fed a PMR or PMR with grazing. Therefore, any effects on CH<sub>4</sub> emissions from cows could be assumed to be related to feeding system. Grandl et al. [5] found that CH<sub>4</sub> emissions per unit intake changed in dairy cattle with age and was associated with changes in the efficiency of fibre digestibility with increasing age. The current study found no effect of lactation number on

CH<sub>4</sub> emissions, which is consistent with other studies [22]. As with others studies [19], the most important drivers of CH<sub>4</sub> emissions were daily dry matter intake ( $P < 0.001$ ) and variables related to changes in intake (i.e., days in milk and time of day, both  $P < 0.001$ ) (Table 4). The effect of diet, i.e., intake and composition, has been found to account for a large proportion of variation in enteric CH<sub>4</sub> emissions from dairy cows [10,23]. Important components of a diet that influence methane emissions are known to be fermentable carbohydrate, fibre, fat, and digestible energy intake [19], but no effect of nutrient composition was found in the current study. Across feeding systems, increasing dry matter intake increased emissions by 0.02 mg/L per kilogram dry matter intake. The response in emissions to increased dry matter intake was higher for cows on a PMR with grazing at 0.03 mg/L per kilogram dry matter intake compared to a PMR system at 0.02 mg/L per kilogram dry matter intake ( $P < 0.001$ ; Table 4). Increasing forage content of diets is known to increase ruminal acetate production, which promotes CH<sub>4</sub> production [3].

From the total of 21,324 milkings across all farms studied, 3106 were between 01:00 to 04:59, 3410 were between 05:00 to 08:59, 3490 were between 09:00 to 12:59, 3612 were between 13:00 to 16:59, 3893 were between 17:00 to 20:59, and 3813 were between 21:00 to 00:59 within a 24-h day. Therefore, the highest number of measurements were obtained between 17:00 to 20:59. A diurnal pattern was observed for CH<sub>4</sub> emissions (Table 4), which is consistent with other studies [9,14,15]. Across feeding systems, emissions were lowest between 05:00 and 08:59, which would relate to a typical time to allocate feed to dairy cows, after which emissions increased to a peak concentration between 17:00 to 20:59. Differences in diurnal pattern were found between feeding systems (SED = 0.08,  $P < 0.001$ ). Notably, between 21:00 and 00:59, emissions of cows on a PMR with grazing system remained high and similar to the previous time period, whereas emissions of cows on a PMR feeding system were reduced compared to the previous time period. The diurnal pattern is dependent on the time, frequency, and amount of food consumed [14], and has no overall influence on average daily CH<sub>4</sub> emissions [16,17], which is consistent with the lack of an overall difference in emissions between feeding systems in the current study (log-transformed mean of 0.3 mg/L for PMR and 0.36 mg/L for PMR with grazing, SED = 0.14,  $P > 0.05$ ). The precise timing of feed allocation at each farm was not known, but would add to the interpretation of the results.

A number of studies have demonstrated techniques for obtaining measurements of CH<sub>4</sub> emissions from individual cattle in their normal environment using repeated spot measurements [9,11–13,15,18]. The positive correlation between spot measurements of CH<sub>4</sub> obtained during milking and total daily CH<sub>4</sub> emissions by the same cows when housed subsequently in respiration chambers in a previous study [10], and the ability of the technique to detect the effect of diet [10,24], has led to considerable research into the spot measurement technique. The approach is reliant on several spot measurements within a day and over several days (at least seven days) of measurements to be able to rank cows as low or high producers of CH<sub>4</sub> [25]. Further comparison of spot measurements on-farm and with the same cows in a respiration chamber are needed to validate or determine the limitations of the technique. The method used in the current study demonstrates the potential for benchmarking cattle or farms and selecting individual animals based on their emissions.

**Table 4.** Results from multivariate analysis <sup>1</sup> showing effect of partial mixed ration (PMR) or PMR with grazing feeding system on log-transformed CH<sub>4</sub> emissions (mg/L) from dairy cows.

Variable		Mean (s.e.) <sup>2</sup>					Effect (s.e.)	Degrees of Freedom	F Statistic	s.e.d.	P Value
Days in milk							0.0003 (0.00007)	1	22.8		<0.001
Feeding system		PMR 0.30 (0.1)	PMR + grazing 0.36 (0.1)					1	0.19	0.14	0.672
Time of day		01:00 to 04:59 0.31 <sup>a</sup> (0.07)	05:00 to 08:59 0.26 <sup>b</sup> (0.07)	09:00 to 12:59 0.33 <sup>c</sup> (0.07)	13:00 to 16.59 0.35 <sup>d</sup> (0.07)	17:00 to 20.59 0.37 <sup>e</sup> (0.07)	21:00 to 00.59 0.35 <sup>d</sup> (0.07)	5	84.9	0.01	<0.001
Feeding system × time of day		01:00 to 04:59 PMR 0.28 <sup>a</sup> (0.1)	05:00 to 08:59 PMR + grazing 0.24 <sup>b</sup> (0.1)	09:00 to 12:59 0.31 <sup>c</sup> (0.1)	13:00 to 16.59 0.32 <sup>c,d</sup> (0.1)	17:00 to 20.59 0.34 <sup>d</sup> (0.1)	21:00 to 00.59 0.30 <sup>c</sup> (0.1)	5	4.3	0.08	<0.001
Predicted dry matter intake		0.34 <sup>a</sup> (0.1)	0.29 <sup>b</sup> (0.1)	0.35 <sup>a</sup> (0.1)	0.38 <sup>c</sup> (0.1)	0.40 <sup>d</sup> (0.1)	0.39 <sup>c,d</sup> (0.1)	0.02 (0.003)	1	196.6	<0.001
Feeding system × predicted dry matter intake		PMR						0.02 (0.003)	1	12.6	<0.001
PMR + grazing								0.03 (0.007)			

<sup>1</sup> Linear mixed model with unique cow ID within farm, milking station within farm and month of sampling added as random effects and covariates centred to a zero mean. <sup>2</sup> Means within a row with different superscript letters (i.e., a,b,c,d,e) differ significantly and attributed at  $P < 0.05$ . SED means standard errors of differences.



#### 4. Conclusions

This is the first study to explore differences in CH<sub>4</sub> emissions among commercial farm feeding systems (PMR vs. PMR with grazing and diet components). Similar overall mean levels of emissions were found for both feeding systems; however, differences were found in the diurnal pattern of CH<sub>4</sub> emissions between feeding systems. The response in emissions to increasing dry matter intake was higher for cows fed PMR with grazing. Differences in emissions among farms were explained largely by factors associated with changes in individual feed intake over time. Measurement of CH<sub>4</sub> emissions from cows during milking not only provides a method of comparing individual cows, but also benchmarking levels of emissions from different farming systems. Understanding this will aid the development of strategies that could contribute to reductions in emissions from the dairy sector whilst maintaining milk output.

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**Author Contributions:** Max Eckert analysed the data and wrote the paper; Matt Bell, Jim Craigon, and Phil Garnsworthy contributed to the analysis and writing of the paper; Sarah Potterton, Neil Saunders, Ruth Wilcox, and Morag Hunter collated the data.

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#### References

1. UNFCCC. Available online: [https://unfccc.int/files/essential\\_background/convention/application/pdf/english\\_paris\\_agreement.pdf](https://unfccc.int/files/essential_background/convention/application/pdf/english_paris_agreement.pdf) (accessed on 17 January 2018).
2. Gerber, P.J.; Steinfield, H.; Henderson, B.; Mottet, A.; Opio, C.; Dijkman, J.; Falcucci, A.; Tempio, G. *Tackling Climate Change through Livestock—A Global Assessment of Emissions and Mitigation Opportunities*; FAO: Rome, Italy, 2013; ISBN 925107920X.
3. Jouany, J.-P. Enteric methane production by ruminants and its control. In *Gut Efficiency; The Key Ingredient in Ruminant Production*; Andrieu, S., Wilde, D., Eds.; Wageningen Academic Publishers: Wageningen, The Netherlands, 2008; pp. 35–59. ISBN 9789086860678.
4. Blaxter, K.L.; Clapperton, J.L. Prediction of the amount of methane produced by ruminants. *Br. J. Nutr.* **1965**, *19*, 511–522. [[CrossRef](#)] [[PubMed](#)]
5. Grandl, F.; Amelchanka, S.L.; Furger, M.; Clauss, M.; Zeitz, J.O.; Kreuzer, M.; Schwarm, A. Biological implications of longevity in dairy cows: 2. Changes in methane emissions and efficiency with age. *J. Dairy Sci.* **2016**, *99*, 3472–3485. [[CrossRef](#)] [[PubMed](#)]
6. Hammond, K.J.; Jones, A.K.; Humphries, L.A.; Crompton, L.A.; Reynolds, C.K. Effects of diet forage source and neutral detergent fiber content on milk production of dairy cattle and methane emissions determined using GreenFeed and respiration chamber techniques. *J. Dairy Sci.* **2016**, *99*, 7904–7917. [[CrossRef](#)] [[PubMed](#)]
7. Granger, C.; Clarke, T.; McGinn, S.M.; Auldist, M.J.; Beauchemin, K.A.; Hannah, M.C.; Waghorn, G.C.; Clark, H.; Eckard, R.J. Methane emissions from dairy cows measured using the Sulfur Hexafluoride (SF<sub>6</sub>) tracer and chamber techniques. *J. Dairy Sci.* **2007**, *90*, 2755–2766. [[CrossRef](#)] [[PubMed](#)]
8. Storm, I.; Hellwing, A.L.; Nielsen, N.; Madsen, J. Methods for Measuring and Estimating Methane Emission from Ruminants. *Animals* **2012**, *2*, 160–183. [[CrossRef](#)] [[PubMed](#)]
9. Manafiazar, G.; Zimmerman, S.; Basarab, J.A. Repeatability and variability of short-term spot measurement of methane and carbon dioxide emissions from beef cattle using GreenFeed emissions monitoring system. *Can. J. Anim. Sci.* **2017**, *97*, 118–126. [[CrossRef](#)]
10. Garnsworthy, P.C.; Craigon, J.; Hernandez-Medrano, J.H.; Saunders, N. On-farm methane measurements during milking correlate with total methane production by individual dairy cows. *J. Dairy Sci.* **2012**, *95*, 3166–3180. [[CrossRef](#)] [[PubMed](#)]
11. Lassen, J.; Lovendahl, P. Heritability estimates for enteric methane emissions from Holstein cattle measured using noninvasive methods. *J. Dairy Sci.* **2016**, *99*, 1959–1969. [[CrossRef](#)] [[PubMed](#)]



12. Vanlierde, A.; Vanrobays, M.-L.; Gengler, N.; Dardenne, P.; Froidmont, E.; Soyeurt, H.; McParland, S.; Lewis, E.; Deighton, M.H.; Mathot, M.; et al. Milk mid-infrared spectra enable prediction of lactation-stage-dependent methane emissions of dairy cattle within routine population-scale milk recording schemes. *Anim. Prod. Sci.* **2016**, *56*, 258–264. [[CrossRef](#)]
13. Pickering, N.K.; Chagunda, M.G.G.; Banos, G.; Mrode, R.; McEwan, J.C.; Wall, E. Genetic parameters for predicted methane production and laser methane detector measurements. *J. Anim. Sci.* **2015**, *93*, 11–20. [[CrossRef](#)] [[PubMed](#)]
14. Crompton, L.; Mills, J.; Reynolds, C.; France, J. Fluctuations in methane emission in response to feeding pattern in lactating dairy cows. In *Modelling Nutrient Digestion and Utilisation in Farm Animals*; Sauvant, D., Van Milgen, J., Faverdin, P., Friggens, N., Eds.; Wageningen Academic Publishers: Wageningen, The Netherlands, 2011; pp. 176–180. [[CrossRef](#)]
15. Kinsman, R.; Sauer, F.D.; Jackson, H.A.; Wolynetz, M.S. Methane and carbon dioxide emissions from dairy cows in full lactation monitored over a six-month period. *J. Dairy Sci.* **1995**, *78*, 2760–2766. [[CrossRef](#)]
16. Jonker, A.; Molano, G.; Antwi, C.; Waghorn, G. Feeding lucerne silage to beef cattle at three allowances and four feeding frequencies affects circadian patterns of methane emissions, but not emissions per unit of intake. *Anim. Prod. Sci.* **2014**, *54*, 1350–1353. [[CrossRef](#)]
17. Brask, M.; Weisbjerg, M.R.; Hellwing, A.L.F.; Bannink, A.; Lund, P. Methane production and diurnal variation measured in dairy cows and predicted from fermentation pattern and nutrient or carbon flow. *Animal* **2015**, *9*, 1795–1806. [[CrossRef](#)] [[PubMed](#)]
18. Bell, M.; Potterton, S.; Craigon, J.; Saunders, N.; Wilcox, R.; Hunter, M.; Goodman, J.; Garnsworthy, P. Variation in enteric methane emissions among cows on commercial dairy farms. *Animal* **2014**, *8*, 1540–1546. [[CrossRef](#)] [[PubMed](#)]
19. Bell, M.J.; Eckard, R.J. Reducing enteric methane losses from ruminant livestock—Its measurement, prediction and the influence of diet. In *Livestock Production*; Javed, K., Ed.; In Tech Publishing: Rijeka, Croatia, 2012; pp. 135–150. [[CrossRef](#)]
20. MAFF. Energy allowances and feeding systems for ruminants. In *MAFF Reference Book 433*; HMSO: London, UK, 1984; ISBN 0112426425.
21. Garnsworthy, P.C.; Craigon, J.; Hernandez-Medrano, J.H.; Saunders, N. Variation among individual dairy cows in methane measurements made on farm during milking. *J. Dairy Sci.* **2012**, *95*, 3181–3189. [[CrossRef](#)] [[PubMed](#)]
22. Ramírez-Restrepo, C.A.; Clark, H.; Muetzel, S. Methane emissions from young and mature dairy cattle. *Anim. Prod. Sci.* **2015**, *56*, 1897–1905. [[CrossRef](#)]
23. Mills, J.A.N.; Kebreab, E.; Yates, C.M.; Crompton, L.A.; Cammell, S.B.; Dhanoa, M.S.; Agnew, R.E.; France, J. Alternative approaches to predicting methane emissions from dairy cows. *J. Anim. Sci.* **2003**, *81*, 3141–3150. [[CrossRef](#)] [[PubMed](#)]
24. Bell, M.J.; Saunders, N.; Wilcox, R.; Homer, E.; Goodman, J.R.; Craigon, J.; Garnsworthy, P.C. Methane emissions among individual dairy cows during milking quantified by eructation peaks or ratio with carbon dioxide. *J. Dairy Sci.* **2014**, *97*, 6536–6546. [[CrossRef](#)] [[PubMed](#)]
25. Cottle, D.J.; Velazco, J.; Hegarty, R.S.; Mayer, D.G. Estimating daily methane production in individual cattle with irregular feed intake patterns from short-term methane emission measurements. *Animal* **2015**, *9*, 1949–1957. [[CrossRef](#)] [[PubMed](#)]

