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Animal factors that affect enteric methane production measured using the GreenFeed monitoring system in grazing dairy cows

K. Starsmore,[1,2]* N. Lopez-Villalobos,[2] L. Shalloo,[1] M. Egan,[1] J. Burke,[2] and B. Lahart[1]
[1]Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy Co. Cork, Ireland P31 P302
[2]Massey University, Palmerston North, Manawatu, New Zealand 4442

ABSTRACT

Similar to all dairy systems internationally, pasture-based dairy systems are under increasing pressure to reduce their greenhouse gas (GHG) emissions. Ireland and New Zealand are 2 countries operating predominantly pasture-based dairy production systems where enteric CH_4 contributes 23% and 36% of total national emissions, respectively. Ireland currently has a national commitment to reduce 51% of total GHG emissions by 2030 and 25% from agriculture by 2030, as well as striving to achieve climate neutrality by 2050. New Zealand's national commitment is to reduce 10% of methane emissions by 2030 and between 24% and 47% reduction in methane emissions by 2050. To achieve these reductions, factors that affect enteric methane (CH_4) production in a pasture-based system need to be investigated. The objective of this study was to assess the relationship between enteric CH_4 and other animal traits (feed intake, metabolic liveweight, energy corrected milk yield, milk urea concentration, and body condition score [BCS]) in a grazing dairy system. Enteric CH_4 emissions were measured on 45 late lactation (213.8 ± 29 d after calving) grazing Holstein-Friesian and Holstein-Friesian × Jersey crossbred cows (lactation number 3.01 ± 1.65, 538.64 ± 59.37 kg live weight, and 3.14 ± 0.26 BCS) using GreenFeed monitoring equipment for 10 wk. There was a training period for the cows to use the GreenFeed of 3 wk before the 10-wk study period. The average enteric CH_4 produced in the study was 352 g ± 45.7 g per day with an animal to animal coefficient of variation of 13%. Dry matter intake averaged 16.6 kg ± 2.23 kg per day, while milk solids (fat plus protein) averaged 1.62 kg ± 0.29 kg per day. A multiple linear regression model indicated that each one unit increase in energy corrected milk yield, metabolic liveweight and milk urea concentration, resulted in an increase in enteric CH_4 production per day by 3.9, 1.74, and 1.38 g, respectively. Although each one unit increase in BCS resulted in a decrease in 39.03 g CH_4 produced per day. When combined, these factors explained 47% of the variation in CH_4 production, indicating that there is a large proportion of variation not included in the model. The repeatability of the CH_4 measurements was 0.66 indicating that cows are relatively consistently exhibiting the same level of CH_4 throughout the study. Therefore, enteric CH_4 production is suitable for phenotyping.

Key words: enteric methane, grazing dairy cows, greenhouse gas emissions, methane yield, methane intensity

INTRODUCTION

Over the past decade there has been an increasing level of awareness in relation to climate change caused by increased GHG emissions (Venghaus et al., 2022). Internationally, the 3 main GHG emissions are enteric methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O; IPCC, 2014). Enteric CH_4 is the predominant GHG produced by the agricultural sector by ruminants and is the largest GHG emitted from agriculture in Ireland and New Zealand (Environmental Protection Agency, 2023; Ministry for the Environment, 2023). Enteric methane is produced through a process called methanogenesis, which converts H_2 and CO_2 into CH_4 by methanogenic archaea in the rumen (McAllister et al., 1996).

The Irish and New Zealand dairy industries are predominantly pasture-based. Intake and rumination behavior is closely linked to enteric CH_4 emissions and therefore when measuring enteric CH_4 emissions, cows should be in their normal environment (Della Rosa et al., 2021; Jonker et al., 2017). In a grazing system this applies when removing the animal from the grazing system and into a respiration chamber (Garnett, 2012; Della Rosa et al., 2021). There are 3 main techniques that directly measure enteric CH_4 emissions at an individual animal level; respiration chambers, sulfur hexafluoride (SF_6), and GreenFeed monitoring system

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*Corresponding author: k.e.starsmore@massey.ac.nz

(Hammond et al., 2015). The GreenFeed system provides the most applicable method of obtaining routine methane measurements within pasture-based dairy systems (Garnett., 2012; Jonker et al., 2018; Della Rosa et al., 2021), where the animal remains in their natural environment where they express their natural behavior.

Animal traits such as individual DMI, milk production, and liveweight (**LW**) are factors that have a large influence on performance and profitability in milk production systems (Ramsbottom et al., 2015; Hanrahan et al., 2018). Within indoor settings, multiple studies have shown that there is a relationship between DMI (Molano and Clark, 2008; Herd et al., 2014; Jonker et al., 2017), milk production (Bell et al., 2010), breed (Olijhoek et al., 2018), and LW (Herd et al., 2014) with enteric CH_4 production. There is limited research which has evaluated such effects in grazing dairy systems. It is important that when selecting animals to breed lower emitting offspring, that their offspring do not have a negative milk production, feed efficiency or in particular in a pasture-based system a lower intake response. Therefore, understanding how these animal production traits link together in a grazing setting is crucial. The objective of this study was to identify animal factors that influence enteric CH_4 emissions at an individual animal level in a pasture-based system. It is hypothesized that animals with greater milk production and LW will exhibit greater enteric CH_4 production.

MATERIALS AND METHODS

Experimental Design

Forty-five mid-late lactation dairy cows were used in a grazing study to measure enteric CH_4 emissions using 2 GreenFeed systems (C-Lock Inc., Rapid City, SD). This study was carried out at Teagasc Moorepark, Co Cork, Ireland over a 10-wk period between August 3, 2020, and October 18, 2020. There were 27 Holstein-Friesian and 18 Jersey Holstein-Friesian crossbred cows that ranged from first lactation to eighth lactation. The mean lactation number was 3.02 ± 1.67.

All cows were managed in a rotational grazing system, similar to that described by Roche et al. (2017). These dairy cows were stocked on the grazing platform at 2.6 cows per hectare. The swards mainly consisted of perennial ryegrass (*Lolium perenne* L.; PRG >85%), while the remainder consisted of meadow grasses and white clover (poa, *Festuca pratensis*, and *Trifolium repens* L., cv. Chieftain). The cows were randomized and blocked between the 2 herds with a total of 40 animals per herd. This was due to these cows being part of a previous grazing study and also to maintain the stocking rate of 22 to 23 animals per Greenfeed unit. All procedures were approved by the Teagasc Animal Ethics Committee and the Health Products Regulatory Authority (HPRA).

Animal Measurements

Individual cow milk yield was measured at each milking every day (Dairymaster, Causeway Co. Kerry, Ireland), which was typically between 0700 and 0930 h in the morning and 1430 and 1630 h in the afternoon. Milk composition samples were taken weekly from a Tuesday afternoon and Wednesday morning milking using Dairymaster milk sampling equipment (Dairymaster, Causeway Co. Kerry, Ireland). These individual milk samples were analyzed using the Pro-Foss FT 600 (MilkoScan FT) for protein, fat, and lactose percentages as well as SCC and milk urea concentration. Milk solid yield (**MSY**) was calculated as the total of fat and protein yield.

Body condition score and LW were recorded weekly. The cows were weighed at the same time every week; after morning milking before returning to the paddock. Liveweight was measured using an electronic portable weighing scale (Tru-test, Auckland, New Zealand). Body condition score was scored by a trained individual in 0.25 increments on a scale of 1 to 5 (Edmonson et al., 1989), where 1 is emaciated and 5 is extremely fat.

Individual DMI was estimated using the n-alkane technique (Mayes et al., 1986) as modified by Dillon and Stakelum (1989) twice over the study period (wk 1 and wk 10). The alkane method estimated individual DMI which included both herbage and concentrate intake. All cows were dosed twice daily, before milking, for 12 consecutive days with a paper bullet (Carl Roth GmbH, Karlesruhe, Germany) containing 500 mg of dotriacontane (C32 Alkane). From d 7 of dosing, fecal samples were collected from each cow twice daily (before both milkings) for the remaining 5 d. The fecal samples were bulked (12 g of each collected sample) and dried for 48 h at 60°C and milled through a 2-mm screen and stored for chemical analysis.

In conjunction with the fecal collection, the diet of the cows was also sampled. Two herbage samples of ~15 individual grass snips were manually collected with Gardena hand shears mimicking the grazing defoliation pattern observed on previously grazed swards, on d 6 to 11. The daily samples were stored at −18°C. The frozen herbage samples were bowl-chopped (Muller, Type MKT 204 Special, Saabrücken, Germany), freeze-dried at −50°C for 120 h, and milled through a 2-mm screen and analyzed for alkane content. The concentrate pellets fed in the GreenFeed was also subsampled and dried at 60°C and milled through a 2-mm screen. The content of C31 and C32 in the feces, concentrate, and herbage samples and the amount of the C32 dosed was used to estimate DMI using the equation stated by Mayes et al. (1986).

Emissions of CH_4 and CO_2 were estimated using 2 outdoor GreenFeed systems (C-Lock Inc., Rapid City, SD), one GreenFeed per herd. The GreenFeed units were positioned on the lane directly outside the grazing paddock. The GreenFeed constantly followed the grazing rotation allowing continual access for the cows. The GreenFeed alleyway ensured that only one animal was able to access the machine at a time. Before the beginning of the experimental period (10 wk) there was a training period (3 wk) to ensure each animal had an adequate visitation frequency (>1 visits/d). The animals that visited the GreenFeed units enough as mentioned previously were carried through into the 10-wk study period. During the training period, the visit interval was set to 2 h and animals were brought to the units once daily. Once animals began using the units independently at least once daily, the minimum visit interval was increased to 4-h intervals to ensure concentrate intake was not excessive throughout the study and diurnal pattern was captured. The mean visits per day throughout the study was 2.21 with a mean airflow of 41.03 L/s. Concentrate pellets were dispensed at a rate of 34 g in 20 s intervals and the amount of concentrate pellets consumed by each cow per day was recorded. The concentrate pellets consisted of barley (16.5 g/kg), maize (10.3 g/kg), wheat feed (5 g/kg), rapeseed extract (16.4 g/kg), maize gluten feed (14.1 g/kg), maize distillers grains (2.8 g/kg), soya hulls (12.3 g/kg), palm kernel extract (10.2 g/kg), and molasses (5.1 g/kg), delactosed permeate (3.1 g/kg), minerals and vitamins (4.2 g/kg; Dairy Pride, Dairygold Co. Kerry, Ireland). Standard gas calibrations were carried out using span (20% oxygen and 80% nitrogen) and zero (10 mg/kg hydrogen, 500 mg/kg CH_4, 5,000 mg/kg CO_2, and 21% oxygen and the balance of nitrogen) gases. These calibrations were carried out automatically every 3 d at 04.00. The mean CH_4 factor from the standard gas calibrations was 1.076 (SD 0.108). A CO_2 recovery was carried out manually every month. The mean flow coefficient from these calibrations was 0.002 (SD 0.00008). The mean percentage recovery was 102.009 (SD 2.508). These calibrations ensure the sensors do not drift away from the baseline concentrations over time.

Sward Measurements

Herbage quality samples were taken randomly in each grazing throughout the study. Herbage sample analysis methods are as described by Ganche et al. (2013) and Looney et al. (2021). Briefly, herbage samples were taken using hand-held Gardena shears, the sward samples were cut to grazing height and stored at −18°C. The frozen herbage samples were bowl chopped (Muller, Type MKT 204 Special, Saabrücken, Germany), freeze-dried at −50°C for 120 h and subsequently milled through a 1-mm screen using a Cyclotech 1093 Sample Mill (Foss, DK-3400 Hillerød, Denmark). The chemical composition was analyzed through wet chemistry for OM digestibility (**OMD**; FibertecTM Systems; Foss, Ballymount, Dublin), CP (Leco FP-628; Leco Corporation), NDF (AOAC, 1995, method 973.18), ADF (AOAC, 1995, method 973.18), and ash (AOAC, 1995, method 942.05) concentrations. Individual chemical components were analyzed according to the methods of Looney et al. (2021). Herbage mass and pre- and postgrazing sward height were measured using a rising plate meter (Jenquip, New Zealand) over a cross section of each grazing break. It was assumed that each centimeter above 4 cm equated to 250 kg DM/ha.

Calculations

Energy-corrected milk yield (**ECMY**) was calculated through the following formula (Sjaunja et al., 1991):

$$\text{ECMY (kg)} = \text{Milk yield} \times [(383 \times \text{fat\%} + 242 \times \text{protein\%} + 165.4 \times \text{lactose\%} + 20.7)/3140]$$

and metabolic live weight (mLW) was calculated as:

$$\text{mLW} = \text{LW}^{0.75}$$

Percentage of gross energy (**GE**) intake consumed through methane production (**Ym**) was calculated using the following formula (Herron et al., 2022):

$$\text{Ym} = \{[(\text{g CH}_4 \times 55.65)/1{,}000)] \,/\, \text{GE}_{\text{intake}}\} \times 100$$

- Methane yield = g of CH_4/kg DMI
- Methane intensity = g of CH_4/kg of milk solids
- Methane production = g of CH_4/d

Statistical Analysis

All statistical analyses were performed using the SAS software version 9.4 (SAS Institute Inc., Cary, NC). Individual estimated enteric CH_4 production values were excluded from the study if the average visits frequency was ≤1 visit per day for each 7-d period, which was 2.8% of the data collected. During the study period the cows averaged 15.5 (SD 4.90) visits per week. The diurnal pattern for the 10-wk period was normally distributed throughout the day and therefore no adjustments had to be made. All data were averaged per week of the experiment. As LW was measured weekly, daily weight estimations were made through a polynomial of order 3 for each cow using the REG procedure. Daily LW change was estimated from the predicted live weights at each day of the lactation. Descriptive statistics (mean, SD, and CV) were obtained using the MEANS procedure. Partial correlation coefficients were calculated between the differing animal traits using the GLM procedure. The model included herd, breed, lactation number, and week of trial as class effects, and visitation frequency and deviation from median calving date as covariates.

Within and between individual cow variances was calculated using MIXED procedure in SAS. The model included the fixed effect of parity, breed, herd, week, and the deviation from median calving date of the herd as covariates and the random effect of cow. In the model, the random effect of the cow was assumed with mean zero and variance σ_c^2, and residual error with mean zero and variance σ_e^2. The repeatability (t) of animal traits were calculated using the following formula (Manafiazar et al., 2016):

$$t = \sigma_e^2 \Big/ \left(\sigma_c^2 + \sigma_e^2\right).$$

Daily enteric CH_4 emissions were modeled with a linear multiple regression model that included herd, breed, lactation number and week of trial as class effects and visitation frequency, energy corrected milk, metabolic LW, BCS, predicted LW change and deviation from median calving date as covariates, and the residual error. Daily measures of all variables were averaged per week. Other effects were considered in the multiple regression such as milk composition and DMI but were not included because they introduced multicollinearity in the model. Factors that had variance inflation factors greater than 4.0 were not included in the model (Hair et al., 2010). F factors were calculated using the same class effects and covariates through GLM procedure in SAS.

The variables importance plots were calculated using the PLS (partial least square) procedure in SAS. The model included parity and breed as class effects and ECMY, LW, BCS, milk urea concentration, DIM, LW change, and SCS were fixed effects.

RESULTS

Herbage Analysis

Table 1 shows the chemical composition and herbage pre- and postgrazing masses of the herbage offered throughout the study. The mean herbage height removed (pre − post height) was 6.4 cm, equating to 15.5 kg DM per day of herbage being offered for each animal. The mean concentrate pellet intake through the GreenFeed system was 0.83 ± 0.07 kg DM/cow per day. Therefore, the total mean DMI throughout the study was 16.33 kg DM/cow per day. This is 0.27 kg DM/cow per day less than the DMI mean reported in Table 2. The 16.33 kg DM/cow per day calculated above is the average estimated individual DMI for every week in the study, whereas DMI reported in Table 2 is the mean DMI for wk 1 and 10 from individual DMI intake samples that were collected and analyzed using the alkane method. The chemical composition of the herbage in this study has slightly greater OMD, and lower CP, ADF and NDF than the typical Irish mid-late lactation grass quality analysis, (OMD 841–859 g/kg DM, CP 211–236 g/kg DM, ADF 252–285 g/kg DM, NDF 411–497 g/kg DM, Ash 69–92 g/kg DM; Wims et al., 2010; O'Neill et al., 2012). The CP (149 g/kg DM) and NDF (259 g/kg DM) of the concentrate pellets were lower than the herbage offered to the cows during this study. However little effect from the concentrate pellets is expected due to the concentration making up only 5% of the animals DMI.

Table 1. Pre- and postgrazing herbage masses and herbage chemical composition (g/kg DM) of pasture eaten during the study period

Item	Mean	SD
OM digestibility	870.1	27.09
CP	193.0	27.87
ADF	229.5	11.72
NDF	407.6	17.94
Ash	88.81	12.68
Pregrazing herbage height (cm)	10.60	2.04
Postgrazing residual height (cm)	4.175	0.247
Pregrazing herbage mass (kg DM/ha)	1,645	507.0
Daily herbage allowance (kg DM/cow)	15.53	1.075

Descriptive Statistics

The mean visitation frequency in the current study was 2.4 visits per day. The mean, SD, and coefficient of variation (**CV**) values for the different traits are presented in Table 2. The cows produced 352 g of CH_4 per day. Methane emissions expressed per unit of DMI and LW averaged 20.79 g/kg and 0.65 kg, respectively. The CV for enteric CH_4 (13%) was lower than milk yield (20%), MSY (18%), and similar to DMI (13%). Liveweight and BCS were the only traits that had a lower CV than enteric CH_4 production. Methane yield, methane intensity, and the Ym value all had a greater CV than enteric CH_4 production (15%, 26%, 14%, and 13%, respectively).

Table 2. Number of observations (n), mean, SD, and CV for different animal traits and methane production traits measured through the GreenFeed monitoring system in late lactation grazing dairy cows

Trait	n[1]	Mean	SD	CV (%)
Methane production (g/d)	495	351.8	45.67	13
Milk yield (kg/d)	495	17.56	3.51	20
Milk solids (kg/d)	495	1.62	0.29	18
Fat yield (kg/d)	496	0.98	0.18	20
Protein yield (kg/d)	496	0.70	0.13	19
Lactose yield (kg/d)	496	0.81	0.17	21
ECM yield (kg)	495	21.00	3.67	17
Milk urea concentration (mg/dL)	491	23.57	7.85	33
SCC[2]	469	6.30	1.82	29
Liveweight (kg)	494	540.2	59.37	11
BCS	451	3.14	0.26	8
DMI (kg/d)	86	16.60	2.23	13
Methane yield[3] (g/kg)	86	20.79	3.12	15
Methane intensity[4] (g/kg)	495	224.1	59.06	26
Methane intensity[5] (g/kg)	495	17.19	3.80	22
Gross energy intake converted to CH_4 (% GEI)	86	6.08	0.92	14

[1]Number is different between animal traits due to clean data available for each animal and week. Actual DMI was only collected twice from each animal (using the alkane method) and therefore only 86 data points were available for analysis.

[2]Log-transformed SCC (Wiggans and Shook, 1987).

[3]Methane yield = enteric methane emissions per kg DMI.

[4]Methane intensity = enteric methane emissions per kilogram of milk solid produced.

[5]Methane intensity = enteric methane emissions per kilogram of ECM yield.

Partial Phenotypic Correlations

The partial phenotypic correlations between CH_4 production, intensity, and yield were all significant as shown in Table 3. The strongest correlations were between CH_4 production and CH_4 yield (0.57). Methane intensity (0.32), ECMY (0.36), and DMI (0.34) also had positive correlations. Body condition score had a significant negative correlation (−0.28) with CH_4 production, however, a positive significant correlation (0.35) with CH_4 intensity. Energy corrected milk yield had a strong negative correlation (−0.72) with CH_4 intensity in comparison to other significant correlations reported in Table 3. Methane yield was only significantly associated with metabolic LW (0.24), the Ym (0.99) and DMI (−0.56).

Effect of Animal Factors on CH_4 Production

F-values for the different animal and external traits are presented in Table 4. These results indicate that metabolic LW and ECMY are the 2 animal characteristics with greatest influence on enteric CH_4 emitted. Body condition score and milk urea concentration also had a significant effect on enteric CH_4. The only environmental trait that had a significant *F*-value was herd.

Figure 1 displays the animal and external traits in a variable of importance plot (**VIP**). This highlights the strong effect that ECMY, metabolic LW, BCS, and milk urea concentration has on enteric CH_4 production. The 2 external traits that showed the greatest effect in the VIP are parity and herd. Both Table 4 and Figure 1 identify that animal traits such as ECMY, metabolic LW, BCS and milk urea concentration have the greatest importance on CH_4 production in comparison to the other traits investigated. However, when DMI was included in the analysis, this factor had the largest effect on enteric CH_4. This was not included in the analysis due to this trait being very difficult to measure in a grazing system and is therefore not a readily available trait across the full time period.

Partial Regression Coefficients

The multiple regression model had a coefficient of determination ($\mathbf{R^2}$) of 0.47 and a relative prediction error of 9.76%. The estimated regression coefficients from this model are presented in Table 5. The regression coefficients showed, that for every unit increase in energy corrected milk, metabolic LW, and milk urea concentration, it is expected that CH_4 production will increase by 3.91 g, 1.74 g, 1.38 g, respectively. The partial regression of LW change on enteric CH_4 production were not significant, contrastingly, the partial regression of BCS on enteric CH_4 production were negative and significant ($P < 0.001$).

Table 3. Partial phenotypic correlations for different animal traits and methane production traits measured through the GreenFeed monitoring system in late lactation grazing dairy cows

Item	Methane production (g/d)		Methane intensity[1]		Methane yield[2]		Ym[3]	
	r	*P*-value	r	*P*-value	r	*P*-value	r	*P*-value
Methane production (g/d)							0.563	<0.0001
Methane intensity[1]	0.321	0.0039					0.421	0.0001
Methane yield[2]	0.567	<0.0001	0.418	0.0001			0.989	<0.0001
ECM yield (kg/d)	0.358	0.0013	−0.722	<0.0001	−0.061	0.5752	−0.073	0.5531
Metabolic liveweight (kg)	0.212	0.0633	0.279	0.0112	0.238	0.0321	0.237	0.0333
Milk urea concentration (mg/dL)	0.320	0.0044	0.111	0.3203	0.149	0.2007	0.142	0.2082
BCS	−0.281	0.0116	0.349	0.0014	0.071	0.5447	0.071	0.5348
Liveweight change (kg/d)	−0.052	0.666	0.161	0.152	0.160	0.1531	0.160	0.1443
DMI (kg/d)	0.343	0.002	−0.160	0.1647	−0.564	<0.0001	−0.564	<0.0001

[1]Methane intensity = enteric methane emissions per kg of milk solid produced.
[2]Methane yield = enteric methane emissions per kg DMI.
[3]Ym = percentage of gross energy intake lost through methane production.

Table 4. *F*-values and *P*-values for different animal and environmental traits relating to enteric methane production measured through the GreenFeed monitoring system in late lactation grazing dairy cows

Item	*F*-value	*P*-value
Herd	44.15	<0.0001
Week of study	2.43	0.0108
Visitation frequency	2.66	0.1039
Breed	0.01	0.9057
Parity	1.12	0.3260
DIM	0.63	0.4272
ECM yield (kg/d)	24.59	<0.0001
Metabolic liveweight (kg)	46.00	<0.0001
Liveweight change (kg/d)	2.94	0.0872
BCS	21.86	<0.0001
Milk urea concentration (mg/dL)	20.69	<0.0001
SCC[1]	1.74	0.1879

[1]Log-transformed SCC (Wiggans and Shook, 1987).

Repeatability of CH_4 Production

The estimates of residual and cow variance components were 534.70 and 1,052.07 respectively, and the estimated repeatability for enteric CH_4 production was 0.66. The estimated repeatability of CO_2 was 0.74. In comparison the repeatability estimates for DMI, and ECMY were 0.52 and 0.64, respectively. Contrastingly, enteric CH_4 production had a weaker repeatability than LW, milk yield and BCS by 0.16, 0.06, and 0.14, respectively. Methane yield (g of CH_4/kg of DMI) had a similar repeatability to enteric CH_4 produced (0.60); however, CH_4 intensity (g CH_4/kg MSY) had a much weaker repeatability of 0.13.

DISCUSSION

Dairy systems worldwide are under increasing pressure to reduce GHG emissions, particularly in countries such as Ireland and New Zealand where agriculture is the largest emitting sector within their economies (Environmental Protection Agency, 2023; Ministry for the Environment, 2023). Both these countries have similar challenges as the dairy industries are predominantly pasture-based. To date there is a lack of understanding as to what factors affect enteric CH_4 production in pasture-based dairy cows. The objective of this study was to identify the relationship that animal factors have on different enteric CH_4 production traits in grazing dairy cows.

In the current study, enteric CH_4 emissions was measured using GreenFeed units and the daily enteric CH_4 emissions (measured at 352 g ± 45.7 g) are within the range of other pasture-based studies in mid to late lactation grazing perennial ryegrass pasture using a combination of SF_6 techniques and GreenFeed (278–384 g; Wims et al., 2010; O'Neill et al., 2012; Jonker et al., 2018). Also, the average visit frequency of animals

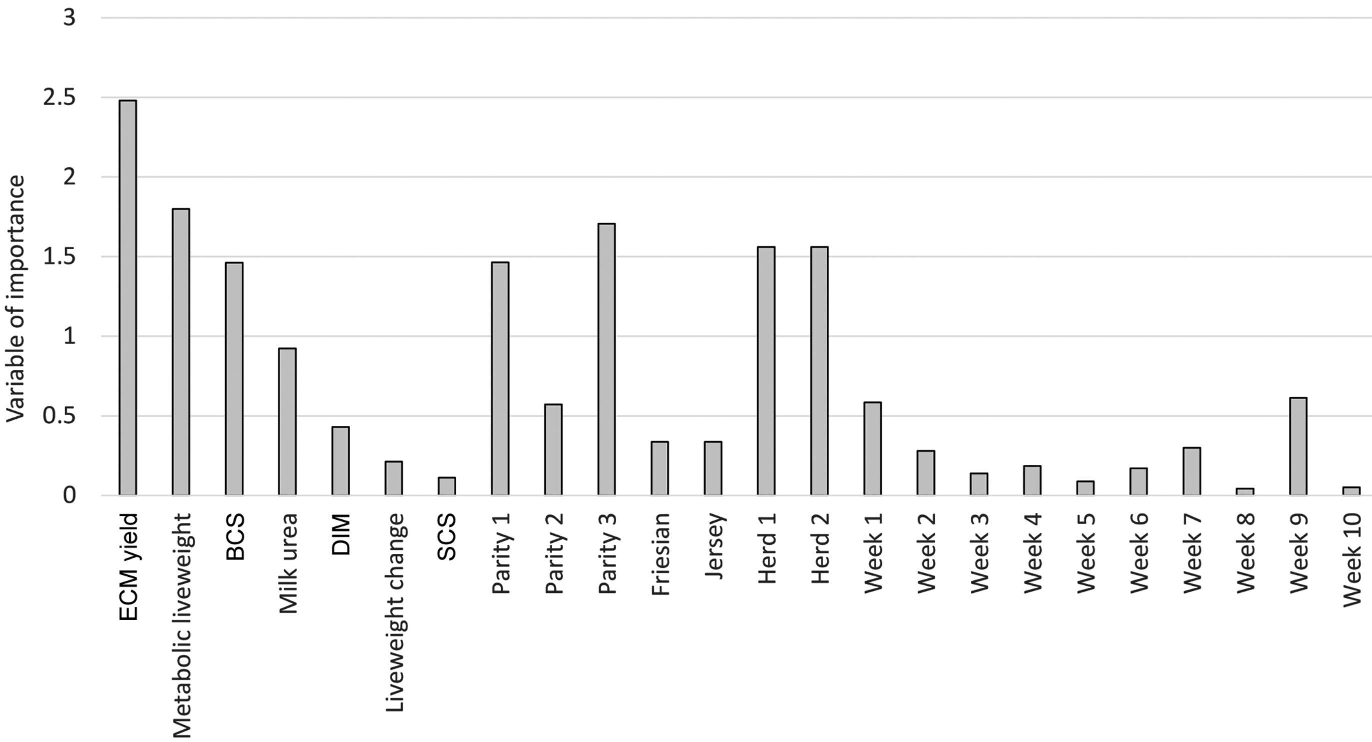

Figure 1. Variable of importance plot with different animal and environmental factors relating to enteric methane emissions measured through the GreenFeed monitoring system in late lactation grazing cows over a 10-wk study period.

to the GreenFeed units in the current study is similar to previous grazing dairy studies (Garnett, 2012). The discrepancies in CH_4 emissions across studies may be due to differences in pasture quality and type (e.g., sward composition), or season. Research has demonstrated that CH_4 emissions have a seasonal nature in spring calving grazing dairy systems, with lower CH_4 emissions observed in the spring (253 g) compared with the summer (30 3g) and autumn (324 g; Lahart et al., 2024). Direct comparisons of daily CH_4 emissions between the studies cannot be made due to differences in breed composition (and associated differences in DMI across the 2 studies), however, when expressed per unit of DMI, $CH_{4,}$ emissions in the current study (20.79 g/kg) are comparable to the autumn results (19.79 g/kg) of Lahart et al. (2024). Dry matter intake is another factor which influences CH_4 emissions as greater DMI leads to an increase in material for fermentation in the rumen (Woodford and Murphy, 1988) and is positively correlated with CH_4 emissions in the current study. Increased DMI can also increase ruminal passage rate of material and result in less CH_4 being produced per kg DMI (Janssen, 2010). This is supported by the inverse correlation between DMI, and CH_4 yield within the current dataset. Methane intensity (g CH_4/kg MSY) is another metric that is used commonly to compare CH_4 emissions and efficiency (Niu et al., 2018). The CH_4 intensity in the current study (224 g CH_4/kg MSY) is within the lower range (174–336 g CH_4/kg MSY) of previous grazing studies carried out in Ire-

Table 5. Estimates of partial regression coefficients[1] of animal traits on enteric methane production in late lactation grazing dairy cows

Item	Partial regression coefficient[1]	SE	P-value
ECM (kg/d)	3.91	0.847	<0.001
BCS	−39.03	9.612	<0.001
Metabolic live weight (kg)	1.74	0.283	<0.001
Live weight change (kg/d)	4.07	4.526	0.3689
Milk urea concentration (mg/dL)	1.38	0.390	<0.001

[1]This partial regression coefficients are obtained through a multiple regression model that includes week of study, herd, breed, parity, calving date and visitation frequency to the GreenFeed machine during trial.

land (Wims et al., 2010; O'Neill et al., 2011) and New Zealand (Waghorn et al., 2016; Garrett et al., 2019). The relatively lower CH_4 intensity observed within the current study may be due to greater productivity, due to genetic improvement that has occurred over time. Greater productivity leads to a dilution effect and less CH_4 being produced per unit of milk solids output (Capper et al., 2009). This is supported by the strong negative correlation (−0.72) between milk production and CH_4 intensity reported in this study. Therefore, a higher yielding animal is likely to have a lower CH_4 intensity in comparison to its contemporaries, albeit based on the positive correlation observed between CH_4 intensity and CH_4 emissions within the current data set, total CH_4 production will increase. Methane intensity is an important trait when marketing and selling dairy products internationally. Customers are becoming more aware of their environmental footprint which is leading to consumers demanding lower footprint food products (Stampa et al., 2020). However, to meet specific climate targets total emissions must also be reduced.

Of major interest in the current study were the associations between total CH_4 emissions and routinely available animal production traits. The F values demonstrate metabolic LW, BCS, ECMY, and milk urea concentration all have a relationship with total CH_4 production. These traits were also identified in the VIP graph. Multiple studies have shown an increase in milk production will result in an increase in enteric CH_4 production (McAllister et al., 1996; Shibata and Terada, 2010). For every 1 unit increase in ECMY, there was a 3.91-g increase in CH_4 in the current study. Greater milk production results in a greater energy demand for the animal (Nicol and Brookes, 2007), meaning the animal must consume more feed to meet this demand. Metabolic LW also had an influence on enteric CH_4 production which is consistent with findings from other studies (Molano and Clark, 2008; Herd et al., 2014; Bird-Gardiner et al., 2017) and is likely due to an increase in DMI, as a result of a greater maintenance energy demand (Niu et al., 2018). Interestingly, metabolic LW showed an inverse correlation with CH_4 intensity; meaning lighter animals will simultaneously produce less CH_4 per cow and per unit of MSY output. The Irish economic breeding index is currently selecting animals that have a lower LW (Irish Cattle Breeding Federation, 2021), which in turn, is also selecting for animals with a reduced CH_4 intensity.

Milk urea concentration was also associated with CH_4 in the current study. It has been reported that diet composition, intake and productive properties of the animal have a direct effect on the urea production, excretion, and recycling to the gut (Huntington and Archibeque, 1999). Milk urea concentration gives an indication of the level of N or protein that is going into the cow's diet (Roy et al., 2011). For every unit increase in milk urea concentration, it is expected that enteric CH_4 will increase by 1.38 g/d. Generally, the higher the milk urea concentration, the higher N or protein intake (Spek et al., 2013). Through the breakdown of protein and non-protein nitrogen, ammonia is produced as a by-product in the rumen. It is estimated that at least half to all of the N supply to the rumen enters the ammonia pool (Huntington and Archibeque, 1999). Ammonia is then absorbed or diffused across all sections of the digestive tract by associating with bicarbonate or VFA anions for transportation to liver and kidneys (Parker et al., 1995; Huntington and Archibeque, 1995). Ammonia is removed from the blood by the liver and converted into urea, which helps prevent excess N becoming toxic (Huntington and Archibeque, 1999). Once urea is released into the bloodstream it is either excreted in the urine or milk (Reynolds, 1992; Nousianinen et al., 2004) or re-enters into the digestive system through being diffused into saliva (Huntington and Archibeque, 1999). Both ammonia/urea synthesis and methanogenesis are started in the rumen through breakdown of proteins, which may be the explanation to the link between milk urea concentration and enteric CH_4 production reported in this study. The VFA breakdown process plays a role in the methanogenesis process (Rouvière and Wolfe, 1988) by producing hydrogen gas. This hydrogen gas is then converted to methane through Archaea (Janssen, 2010). Hence, the digestive processes are likely linking the milk urea concentration and enteric CH_4 production through anaerobic fermentation.

Contrastingly, BCS had a negative relationship with daily enteric CH_4 production. For each unit increase in BCS, daily enteric CH_4 production is expected to decrease by 39.03 g. There was also a negative correlation between BCS and CH_4 yield (−0.28). Thinner cows are lacking body fat stores and therefore are unable to use any energy deposits within the body (Wathes et al., 2013). It is likely that thinner animals have increased DMI to achieve energy intake equilibrium as fat stores are unavailable to the animal (Hayirli et al., 2002). As DMI is closely linked with enteric CH_4 production (Jonker et al., 2017), a thin cow eating more DMI is expected to have higher enteric CH_4 production. However, solely selecting animals with greater BCS may also have a negative effect on milk production. As fatter cows are eating less, they also generally produce less milk (Roche et al., 2007), and therefore, caution should be exercised when BCS is solely selected for.

Dry matter intake was not directly included in the multiple regression model because the trait is strongly associated with milk production and LW (Madilindi et al., 2022), which could result in multi-collinearity (Hair et al., 2010). Multiple studies have reported a direct relationship between DMI and enteric CH_4 production (Molano and Clark, 2008; Herd et al., 2014; Bird-Gardiner et al., 2017). When solely including DMI in the multiple regression model, the R^2 was 0.38 (results not shown) suggesting that that DMI alone accounts for 38% of the animal to animal variation. Partial least squares regression allows for the investigation into various traits which may be highly correlated (Ahmed et al., 2006). When using partial least squares regression during only the alkane DMI weeks, DMI was shown to have the greatest VIP (results not shown). Nonetheless, information on individual DMI is not routinely available on commercial farms at present, and if DMI is available they are often estimates based on the herd. Therefore, the model incorporating the energy sinks is the most appropriate model for capturing routine data on CH_4 output on-farm. All these traits identified in this study explained 47% of the variation, therefore other environmental and animal factors are likely influencing the quantity of enteric CH_4 emitted. There is sparse literature on potential environmental factors in a pasture-based system and therefore should be investigated in further research. However, factors such as sward quality, digestibility, passage rate, weather, and animal behavior should be given more prominence for investigations in the future. Based on the collective output of this model it is clear that a low CH_4 emitting cow will likely to be on average, lighter, lower yielding, have a lower milk urea concentration and fatter than her contemporaries. Selecting for these traits within a breeding index may offer the potential to indirectly reduce CH_4 output. However, this may lead to an industry producing less milk output and leading to reduced overall food production. Not only would this lead to less dairy products available for human consumption worldwide, but there would also be financial repercussions for the average NZ and Irish dairy farm. Having an animal with the above traits could result in less milk being produced and hence sold resulting in less income for the average farming family. There is potential for unintended genetic changes to occur when selecting for a cow that is fatter and producing less, such as poorer feed conversion efficiency which would mean that the milk produced would cost the farmer more to produce as more dry matter would be required to produce 1 kg MSY. There may also be metabolic issues as a result of fatter animals.

Selection for CH_4 could also be considered in breeding indexes in the future. The repeatability of enteric CH_4 emissions measured using the GreenFeed monitoring system indicates there is phenotypic potential. This means that the enteric CH_4 trait has variance that was not associated with the experimental statistical factors included in the model and therefore is exhibiting variation between animals in this study (Coppa et al., 2021). The repeatability (0.66) in this study is consistent with other studies that have been carried out in indoor systems (Huhtanen et al., 2013; Arbre et al., 2016; Manafiazar et al., 2016; Coppa et al., 2021). These studies measure enteric CH_4 from Holstein lactating dairy cows (Arbre et al., 2016; Coppa et al., 2021) and crossbred beef heifers (Manafiazar et al., 2016) on a TMR diet consisting of either grass or maize silage as the base diet. Arbre et al. (2016) reported repeatability for a 5-d period of 0.72 and for 10-d period of 0.77. Coppa et al. (2021) reported a repeatability 0.06 less than the current study over 7-d period increments. However, Manafiazar et al. (2016) reported 0.69 repeatability of enteric CH_4 production over a 7-d period. These studies are both carried out in an indoor system, indicating this study's findings are consistent with current literature despite being conducted in an outdoor grazing system. As a result of this, it is evident that enteric CH_4 production has the ability to be phenotyped in both indoor and outdoor systems using the GreenFeed monitoring system (Arbre et al., 2016; Coppa et al., 2021). However, cognizance needs to be taken of other important traits such as feed conversion efficiency and DMI. Therefore, further investigation needs to be carried out to establish an improved selection criterion.

CONCLUSIONS

The animals in the current study provide a deeper understanding to what factors are driving enteric CH_4 production in grazing late lactation cows. The results demonstrate that enteric CH_4 production has a positive relationship with feed intake, metabolic LW, ECMY, and milk urea concentration, as well as a negative relationship with BCS. Liveweight and metabolic LW contributed toward the largest source of variation to enteric CH_4. Indicating that selection for lower yielding and fatter animals may be a strategy to reduce CH_4 output. These results also suggest that if only focusing on one factor for estimating CH_4 production, the outcome is likely to be inaccurate as only a small portion of variation is accounted for in the model. Therefore, including these factors identified in this study to grazing late lactation cow enteric CH_4 estimation models would likely result in a more representative estimated enteric CH_4 production than concentrating on one trait. Further research should be conducted assessing the effect of environmental factors on grazing enteric CH_4 production.

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ORCIDS

K. Starsmore https://orcid.org/0009-0001-6199-493X
N. Lopez-Villalobos https://orcid.org/0000-0001-6611-907X
L. Shalloo https://orcid.org/0000-0003-1714-672X
M. Egan https://orcid.org/0000-0003-3990-8035
J. Burke https://orcid.org/0000-0003-2019-3295
B. Lahart https://orcid.org/0000-0002-0341-2030