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

K. Starsmore,*† N. Lopez-Villalobos,† L. Shalloo,* M. Egan,* J. Burke,† and B. Lahart*†

*Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland
†Massey University, Palmerston North, Manawatu, New Zealand

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 CH4 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 (CH4) production in a pasture-based system need to be investigated. The objective of this study was to assess the relationship between enteric CH4 and other animal traits (feed intake, metabolic liveweight, energy corrected milk yield, milk urea concentration and body condition score) in a grazing dairy system. Enteric CH4 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 body condition score) using GreenFeed monitoring equipment for 10 weeks. There was a training period for the cows to use the GreenFeed of 3 weeks before the 10 week study period. The average enteric CH4 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 CH4 production per day by 3.91 g, 1.74 g and 1.38 g, respectively. While each one unit increase in body condition score resulted in a decrease in 39.03 g CH4 produced per day. When combined, these factors explained 47% of the variation in CH4 production, indicating that there is a large proportion of variation not included in the model. The repeatability of the CH4 measurements was 0.66 indicating that cows are relatively consistently exhibiting the same level of CH4 throughout the study. Therefore, enteric CH4 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 (CH4), carbon dioxide (CO2) and nitrous oxide (N2O) (IPCC, 2014). Enteric CH4 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 H2 and CO2 into CH4 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 CH4 emissions and therefore when measuring enteric CH4 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 CH4 emissions at an individual animal level; Respiration chambers, Sulfur
hexafluoride (SF₆), and GreenFeed monitoring system (Hammond et al., 2015). The GreenFeed system provides the most applicable method of obtaining routine methane measurements within pasture-based dairy systems (Garnett et al., 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 dry matter intake (DMI), milk production and liveweight (LW) are factors which 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 CH₄ production. There is limited research which has evaluated such effects in grazing dairy or beef 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 CH₄ 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 CH₄ production.

**MATERIALS AND METHODS**

**Experimental design**

Forty-five mid-late lactation dairy cows were used in a grazing study to measure enteric CH₄ emissions using 2 GreenFeed systems (C-Lock Inc., Rapid City, SD, USA). This study was carried out at Teagasc Moorepark, Co Cork, Ireland over a 10-week period between 3rd August 2020 and 18th October 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 22–23 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 07.00 and 09.30 in the morning and 14.30 and 16.30 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 somatic cell count and milk urea concentration. Milk solid yield (MS) was calculated as the total of fat and protein yield.

Body condition score (BCS) and liveweight (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 (Lowman and Scott, 1986); where 1 is emaciated and 5 is extremely fat.

Individual dry matter intake was estimated using the n-alkane technique (Mayes et al., 1986) as modified by Dillon and Stakelum (1989) twice over the study period (Week 1 and Week 10). The alkane method estimated individual dry matter intake 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, Karlsruhe, 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 approximately 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, Saarbrücken, 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 DMIs using the equation stated by Mayes et al. (1986).

Emissions of enteric methane (\(\text{CH}_4\)), carbon dioxide (\(\text{CO}_2\)) and hydrogen (\(\text{H}_2\)) were estimated using 2 outdoor GreenFeed systems (C-Lock Inc., Rapid City, SD, USA), 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 weeks) there was a training period (3 weeks) to ensure each animal had an adequate visitation frequency (>1 visits/day). The animals that visited the GreenFeed units enough as mentioned previously were carried through into the 10 week study period. During this 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 (10ppm hydrogen, 500ppm \(\text{CH}_4\), 5000ppm carbon dioxide and 21% oxygen and the balance of nitrogen) gases. These calibrations were carried out automatically every 3 d at 04.00. The mean \(\text{CH}_4\) factor from the standard gas calibrations was 1.076 (standard deviation; SD 0.108). A carbon dioxide 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, Saabriicken, 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 organic matter digestibility (OMD; FibertecTM Systems; Foss, Ballymount, Dublin), crude protein (CP; Leco FP-628; Leco Corporation, USA), neutral detergent fiber (NDF; AOAC, 1995, method 973.18), acid detergent fiber (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 post grazing sward height were also measured in accordance with the methods described in O’Neill et al., (2011). This was measured using a rising plate meter (Jenquip, New Zealand) over a cross section of each grazing break. It was assumed that for every cm there was 250 kg DM/ ha.

### Calculations

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

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

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):

\[
\text{Ym} = \left( \frac{((g \text{ CH}_4 \times 55.65) / 1000)}{\text{GE}_{\text{intake}}} \right) \times 100
\]

- Methane yield = g CH\(_4\) / kg DMI
- Methane intensity = g CH\(_4\) / kg milk solids
- Methane production = g CH\(_4\) / day
Statistical analysis

All statistical analyses were performed using the SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). 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-week period was normally distributed throughout the day and therefore no adjustments had to be made. All data was 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, standard deviation, and coefficient of variation) were obtained using the MEANS procedure.

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 $\sigma^2_c$, and residual error with mean zero and variance $\varepsilon^2$. The repeatability ($t$) of animal traits were calculated using the following formula (Manafiazar et al., 2016):

$$t = \frac{\sigma^2_c}{\sigma^2_c + \varepsilon^2}.$$

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, days in milk, LW change, and somatic cell score were fixed effects.

RESULTS

Herbage analysis

Table 1 shows the chemical composition and herbage pre and post grazing masses of the herbage consumed throughout the study. The mean herbage height removed 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/day. Therefore, the total mean DMI throughout the study was 16.33 kg DM/cow/day. This is 0.27 kg DM/cow/day less than the DMI mean reported in Table 2. The 16.33 kg DM/cow/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.

Descriptive statistics

The mean visitation frequency in the current study was 2.4 visits per day. The mean, standard deviation, and coefficient of variation values for the different traits are presented in Table 2. The cows produced 352g CH$_4$ per day. Methane emissions expressed per unit of DMI and LW averaged 20.79 g/kg and 0.65 kg, respectively. The coefficient of variation for enteric CH$_4$ (13%) was lower than milk yield (20%), milk solids yield (18%), and similar to DMI (13%). Liveweight and BCS were the only traits that had a lower coefficient of variation than enteric CH$_4$ production. Methane yield, methane intensity and the Ym value all had a greater coefficient of variation than enteric CH$_4$ production (15, 26, 14 and 13%, respectively).
Partial phenotypic correlations

The partial phenotypic correlations between CH₄ production, intensity and yield were all significant as shown in Table 3. The strongest correlations were between CH₄ production and CH₄ yield (0.57). Methane intensity (0.32), CH₄ yield (0.57), energy corrected milk yield (0.36), and DMI (0.34) had positive correlations. Body condition score had a significant negative correlation (−0.28) with CH₄ production, however, a positive significant correlation (0.35) with CH₄ intensity. Energy corrected milk yield had a strong negative correlation (−0.72) with CH₄ intensity in comparison to other significant correlations reported in Table 2. Methane yield was only significantly associated with metabolic LW (0.24), the Ym (0.24) and DMI (−0.56).

Effect of animal factors on enteric methane production

F-values for the different animal and external traits are presented in Table 4. These results indicate that metabolic LW and energy corrected milk yield are the 2 characteristics with greatest influence on enteric CH₄ emitted. Body condition score and milk urea concentration also had a significant effect on enteric CH₄. 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 energy corrected milk yield, metabolic LW, BCS and milk urea concentration has on enteric CH₄ production. The 2 external traits that showed the greatest effect on in the VIP are parity and herd. Both

Table 1. Pre and post grazing herbage masses and herbage chemical composition of pasture eaten during the study period

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic matter digestibility (g/kg DM)</td>
<td>870.1</td>
<td>27.09</td>
</tr>
<tr>
<td>Crude protein (g/kg DM)</td>
<td>193.0</td>
<td>27.87</td>
</tr>
<tr>
<td>Acid detergent fiber (g/kg DM)</td>
<td>229.5</td>
<td>11.72</td>
</tr>
<tr>
<td>Neutral detergent fiber (g/kg DM)</td>
<td>407.6</td>
<td>17.94</td>
</tr>
<tr>
<td>Ash (g/kg DM)</td>
<td>88.81</td>
<td>12.68</td>
</tr>
<tr>
<td>Pre-grazing herbage height (cm)</td>
<td>10.60</td>
<td>2.04</td>
</tr>
<tr>
<td>Post-grazing residual height (cm)</td>
<td>4.175</td>
<td>0.247</td>
</tr>
<tr>
<td>Pre-grazing herbage mass removed (kgDM/ha)</td>
<td>1616</td>
<td>294.4</td>
</tr>
<tr>
<td>Daily herbage allowance (kgDM/cow)</td>
<td>15.53</td>
<td>1.075</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Trait</th>
<th>N *</th>
<th>Mean</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane production (g/day)</td>
<td>495</td>
<td>351.8</td>
<td>45.67</td>
<td>13</td>
</tr>
<tr>
<td>Milk yield (kg/day)</td>
<td>495</td>
<td>17.56</td>
<td>3.51</td>
<td>20</td>
</tr>
<tr>
<td>Milk solids (kg/day)</td>
<td>495</td>
<td>1.62</td>
<td>0.29</td>
<td>18</td>
</tr>
<tr>
<td>Fat yield (kg/day)</td>
<td>496</td>
<td>0.98</td>
<td>0.18</td>
<td>20</td>
</tr>
<tr>
<td>Protein yield (kg/day)</td>
<td>496</td>
<td>0.70</td>
<td>0.13</td>
<td>19</td>
</tr>
<tr>
<td>Lactose yield (kg/day)</td>
<td>496</td>
<td>0.81</td>
<td>0.17</td>
<td>21</td>
</tr>
<tr>
<td>Energy corrected milk yield (kg)</td>
<td>495</td>
<td>21.00</td>
<td>3.67</td>
<td>17</td>
</tr>
<tr>
<td>Milk urea concentration (mg/dl)</td>
<td>491</td>
<td>23.57</td>
<td>7.85</td>
<td>33</td>
</tr>
<tr>
<td>Somatic cell score ¹</td>
<td>469</td>
<td>6.30</td>
<td>1.82</td>
<td>29</td>
</tr>
<tr>
<td>Liveweight (kg)</td>
<td>494</td>
<td>540.2</td>
<td>59.37</td>
<td>11</td>
</tr>
<tr>
<td>Body condition score</td>
<td>451</td>
<td>3.14</td>
<td>0.26</td>
<td>8</td>
</tr>
<tr>
<td>Dry matter intake (kg/day)</td>
<td>86</td>
<td>16.60</td>
<td>2.23</td>
<td>13</td>
</tr>
<tr>
<td>Methane yield (g/kg)              ²</td>
<td>86</td>
<td>20.79</td>
<td>3.12</td>
<td>15</td>
</tr>
<tr>
<td>Methane intensity (g/kg)</td>
<td>495</td>
<td>224.1</td>
<td>59.06</td>
<td>26</td>
</tr>
<tr>
<td>Methane intensity (g/kg)</td>
<td>495</td>
<td>17.19</td>
<td>3.80</td>
<td>22</td>
</tr>
<tr>
<td>Gross energy intake conversion (% GEI)</td>
<td>86</td>
<td>6.08</td>
<td>0.92</td>
<td>14</td>
</tr>
</tbody>
</table>

¹Somatic cell score = log-transformed somatic cell count (Wiggans and Shook, 1987).
²Methane yield = enteric methane emissions per kg dry matter intake.
³Methane intensity = enteric methane emissions per kg milk solids produced.
⁴Methane intensity = enteric methane emissions per kg energy corrected milk yield.
* N is different between animal traits due to clean data available for each animal and week. Actual dry matter intake was only collected twice from each animal (using the alkane method) and therefore only 86 data points were available for analysis.
Table 3 and Figure 1 identify that animal traits such as energy corrected milk yield, metabolic LW, BCS and milk urea concentration have the greatest importance on CH4 production in comparison to the other traits investigated. However, when DMI was included in the analysis, this factor had the largest effect on enteric CH4. 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 an $R^2$ 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 CH4 production will increase by 3.75g, 1.80 g, 1.49 g, respectively. The partial regression of LW change on enteric CH4 production were not significant, contrastingly, the partial regression of BCS on enteric CH4 production were negative and significant ($P < 0.001$).

Repeatability of enteric methane production

The estimates of residual and cow variance components were 534.70 and 1052.07 respectively, and the estimated repeatability for enteric CH4 production was 0.66. The estimated repeatability of CO2 was 0.74. In comparison the repeatability estimates for DMI, and energy corrected milk yield were 0.52 and 0.64, respectively. Contrastingly, enteric CH4 production had a weaker repeatability than LW, milk yield and BCS by 0.16, 0.06, and 0.14, respectively. Methane yield (g CH4/kg DMI) had a similar repeatability to enteric CH4 produced (0.60), however, CH4 intensity (g CH4/kg MS) 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 CH4 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 were measured using GreenFeed units and the daily enteric CH$_4$ emissions measured of 352 g ± 45.7 g) are within the range of other pasture-based studies in mid-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 to the GreenFeed units in the current study is similar to previous grazing dairy studies (Garnett et al., 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 (253g) compared with the summer (303g) and autumn (324g) (Lahart et al., in press). 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. (in press). Dry matter intake is another factor which influences CH$_4$ emissions as greater DMI leads to an increase in material for fer-

![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-week study period.](image)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Partial regression coefficient$^1$</th>
<th>Standard error</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy corrected milk (kg/day)</td>
<td>3.91</td>
<td>0.847</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body condition score</td>
<td>−39.03</td>
<td>9.612</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Metabolic live weight (kg)</td>
<td>1.74</td>
<td>0.283</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Live weight change (kg/day)</td>
<td>4.07</td>
<td>4.526</td>
<td>0.3689</td>
</tr>
<tr>
<td>Milk urea concentration (mg/dl)</td>
<td>1.38</td>
<td>0.390</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$^1$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.
mentation in the rumen (Woodford et al., 1988) and is positively correlated with CH₄ emissions in the current study. Increased DMI can also increase ruminal passage rate of material and result in less CH₄ being produced per kg DMI (Janssen., 2010). This is supported by the inverse correlation between DMI and CH₄ yield within the current data set. Methane intensity (g CH₄/kg MS) is another metric that is used commonly to compare CH₄ emissions and efficiency (Niu et al., 2018). The CH₄ intensity in the current study (224 g CH₄/kg MS) is within the lower range (174 g to 336 g CH₄/kg MS) of previous grazing studies carried out in Ireland (Wims et al., 2010; O’Neill et al., 2011) and New Zealand (Waghorn et al., 2016; Garrett et al., 2019). The relatively lower CH₄ 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 CH₄ 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 CH₄ intensity reported in this study. Therefore, a higher yielding animal is likely to have a lower CH₄ intensity in comparison to its contemporaries, albeit based on the positive correlation observed between CH₄ intensity and CH₄ emissions within the current data set, total CH₄ 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 CH₄ emissions and routinely available animal production traits. The F values demonstrate metabolic LW, BCS, energy corrected milk yield and milk urea concentration all have a relationship with total CH₄ 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 CH₄ production (McAllister et al., 1996; Shibata and Terada, 2010). For every 1 unit increase in ECMY, there was a 3.75 g increase in CH₄ 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 CH₄ 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 CH₄ intensity; meaning lighter animals will simultaneously produce less CH₄ per cow and per unit of MS output. The Irish economic breeding index (EBI) 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 CH₄ intensity.

Milk urea concentration was also associated with CH₄ 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 et al., 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 CH₄ will increase by 1.49 g per day. 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 et al., 1999). Ammonia is then absorbed or diffused across all sections of the digestive tract by associating with bicarbonate or volatile fatty acids (VFA) anions for transportation to liver and kidneys (Parker et al., 1995; Huntington and Archibeque et al., 1995). Ammonia is removed from the blood by the liver and converted into urea, which helps prevent excess N becoming toxic (Huntington and Archibeque et al., 1995). Once urea is released into the bloodstream it is either excreted in the urine and/or milk (Reynolds, 1992; Nousianinen et al., 2004) or re-enters into the digestive system through being diffused into saliva (Huntington and Archibeque et al., 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 CH₄ production reported in this study. The VFA breakdown process plays a role in the methanogenesis process (Rouviere and Wolfe, 1988) by producing hydrogen gas. This hydrogen gas is then converted to methane through Archaea (Janssen et al., 2010). Hence, the digestive processes are likely linking the milk urea concentration and enteric CH₄ production through anaerobic fermentation.

Contrastingly, BCS had a negative relationship with daily enteric CH₄ production. For each unit increase in BCS, daily enteric CH₄ production is expected to decrease by 41.54 g. There was also a negative correlation between BCS and CH₄ yield (−0.28). Thinner cows are lacking body fat stores and therefore are unable to utilize 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 CH₄ production (Jonker et al., 2017), a thin cow eating more DMI is expected to have higher enteric CH₄ 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 CH₄ production (Molano and Clark., 2008; Herd et al., 2014; Bird-Gardiner et al., 2017). When solely including DMI in the multiple regression model, the R² 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 CH₄ 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 CH₄ 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 CH₄ 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 CH₄ 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 MS. There may also be metabolic issues as a result of fatter animals.

Selection for CH₄ could also be considered in breeding indexes in the future. The repeatability of enteric CH₄ emissions measured using the GreenFeed monitoring system indicates there is phenotypic potential. This means that the enteric CH₄ 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 CH₄ from Holstein lactating dairy cows (Arbre et al., 2016; Coppa et al., 2021) and crossbred beef heifers (Manafiazar et al., 2016) on a total mixed ration 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 CH₄ 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 CH₄ 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, cognisance 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.

**CONCLUSION**

The animals in the current study provide a deeper understanding to what factors are driving enteric CH₄ production in grazing late lactation cows. The results demonstrate that enteric CH₄ production has a positive relationship with feed intake, metabolic LW, energy corrected milk yield, 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 CH₄. Indicating that se-
lecion for lower yielding and fatter animals may be a strategy to reduce CH4 output. These results also suggest that if only focusing on one factor for estimating CH4 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 CH4 estimation models would likely result in a more representative estimated enteric CH4 production than concentrating on one trait. Further research should be conducted assessing the effect of environmental factors on grazing enteric CH4 production.

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ORCIDS

K. Starsmore https://orcid.org/0000-0001-6199-493X
L. 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/0000-0003-2093-3295
B. Lahart https://orcid.org/0000-0002-0341-2030