ENVIRONMENTAL HEALTH | RESEARCH ARTICLE

A time-series of methane and carbon dioxide production from dairy cows during a period of dietary transition

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Abstract: Emissions from dairy farms are contributing to the increased concentrations of greenhouse gases which are linked to recent climate change. Altering diets has been proposed as a greenhouse gas mitigation strategy in dairy systems. The magnitude of mitigation and the time taken for cows to adapt to new diets has not been comprehensively quantified. Methane (CH4) and carbon dioxide (CO2) produced by dairy cows was measured for six weeks using the sulphur hexafluoride tracer technique following a change in diet; from barley straw and protein supplements to grazed grass. CH4 and CO2 production increased linearly as the animals adapted to their new diets, however, production did not reach an asymptote six weeks into the grazing period. This suggested that metabolic activity and greenhouse gas emissions may not have been at their maximum. There was substantial variation between individuals with high emitting cows producing four times more CH4 than low producing cows. Cows which produced greater amounts of CH4 consistently also produced greater CO2. We demonstrate that feeding regime plays an important role in determining greenhouse gas emissions and we highlight that transition periods in greenhouse gas models and future experiments must be sufficiently large to allow for adaptation.

ABOUT THE AUTHOR

Mark A. Lee, PhD, the lead author is an Early Career Research Fellow in Natural Capital and Plant Health at the Royal Botanic Gardens Kew. He is currently leading innovative research projects using novel approaches to investigate the sustainable intensification of soft fruit and livestock production systems. In particular, he is interested in the interactions between forage crops, livestock productivity and greenhouse gas emissions. The Royal Botanic Gardens Kew is an internationally renowned centre for plant sciences, producing research on some of the biggest issues facing the global population. The experimental work for this research article was conducted at Scotland’s Rural College (SRUC). SRUC delivers comprehensive skills, education and business support for Scotland’s land-based industries, founded on world class and sector-leading research, education and consultancy.

PUBLIC INTEREST STATEMENT

Agriculture is a major contributor to the greenhouse gas emissions that have been linked with climate change. Ruminant livestock, such as dairy cows, produce the potent greenhouse gas, methane, which predominantly comes from their breath. One way of reducing the amount of methane produced by dairy cows is to change their diets. We tested how much methane production changed when two groups of dairy cows were moved onto a diet of grazed grass from a diet of barley straw. We measured that methane production increased by an average of 42%, six weeks after the dietary change. However, methane production may not have reached maximum values during our experiment. Some individual cows produced four times more CH4 than low producing cows. Cows which produced greater amounts of CH4 consistently also produced greater CO2. We demonstrate that feeding regime plays an important role in determining greenhouse gas emissions and we highlight that transition periods in greenhouse gas models and future experiments must be sufficiently large to allow for adaptation.
1. Introduction

Atmospheric concentrations of carbon dioxide (CO₂) and methane (CH₄) have increased substantially over the past 150 years. Although CO₂ is the most influential driver of climate change, net CO₂ emissions from agriculture are small by comparison to those of CH₄ (IPCC, 2013). CH₄ is the second most influential greenhouse gas with between 21 and 25 times the global warming potential (GWP) per gram of CO₂ (IPCC, 2013). Livestock farming produces approximately 7.1 gigatonnes of CO₂ equivalents annually (GT CO₂eq)—15% of anthropogenic greenhouse gas emissions (Food and Agriculture Organisation [FAO], 2013). Enteric fermentation by livestock produces 2.8 GT CO₂eq of CH₄ each year, with 77% being produced by cattle (FAO, 2013).

Dairy farming produces approximately 2 million tonnes of CO₂eq worldwide each year (this value includes milk production, processing and transportation, and meat production from dairy-related culled animals)—4% of total anthropogenic greenhouse gas emissions (FAO, 2010). There is substantial variation between emissions from different regions, production systems and cow breeds. CH₄ produced by individual cows have been shown to range from 137 to 431 g d⁻¹ (Lassey, 2007) with approximately 96% of CH₄ production being the result of the fermentation of carbohydrates by microbes in the rumen and intestine (McGinn, Beauchemin, Iwaasa, & McAllister, 2006). CO₂ is also produced within the rumen by microbial respiration as well as by respiration by the cows themselves with one study recording CO₂ production per cow ranging from 9,900 to 14,680 g d⁻¹ (Kinsman, Sauer, Jackson, & Wolynetz, 1995). Rates of CH₄, and to a lesser extent CO₂ production are under the control of the activity rate, population size and community composition of enteric microbes (Lettat, Hassanat, & Benchaar, 2013). Factors which can modify enteric microbial activity include the composition of feed and quantity of feed intake, the breed or genotype of the animal and environmental conditions such as location or temperature (McAllister, Cheng, Okine, & Mathison, 1996). However, the direction of the response in CH₄ production to changes in temperature have been shown to be both positive and negative (McAllister et al., 1996), and is presumably context dependent.

Enteric CH₄ production can be modified by cow diet directly due to a change in microbial substrate availability or indirectly via a change in rumen pH (Bath, Morrison, Ross, Hayes, & Cocks, 2013). O’Neill et al. (2011) compared groups of cows fed either a mixed ration (containing maize silage, grass silage, concentrate, barley straw and molasses) or a diet consisting solely of grass, recording increased mean CH₄ production per cow from the mixed ration fed group compared with the grass-fed group—likely due to increased feed intake and microbial substrate availability. Reducing the digestibility of feed also increases CH₄ production (e.g. by increasing fibre content) since the residence time of feed within the rumen is increased and the opportunity for methanogenesis by the microbial population is elevated (Brask, Lund, Hellwing, Poulsen, & Weisbjerg, 2013). Conversely, increasing the digestibility of feed (e.g. by increasing starch or glucose content) reduces CH₄ production since feed moves through the digestive system more rapidly and the opportunity for methanogenesis by the microbial population is reduced (Janssen, 2010).

Changing cattle diets can influence the environmental footprint, productivity and profitability of livestock production systems (Lee & Roberts, 2015). The identity of the crops grown to feed livestock as well as farm management practices, such as soil tillage, can influence carbon fluxes and associated greenhouse gas emissions (Al-Kaisi & Yin, 2005). Weather conditions, soil erosion and leaching also modifies the carbon budgets of livestock farms (Comino et al., 2017) and can lead to a redistribution of carbon stocks (Nie, Zhang, Cheng, Gao, & Guan, 2016).

There are few studies which have measured changes to CH₄ produced by cows over time following a change in diet. One such study demonstrated that mean CH₄ increased between weeks four...
(314 g d\textsuperscript{-1}) and ten (333 g d\textsuperscript{-1}) following a change in diet (O’Neill et al., 2011). However, we are not aware of any study which has investigated how the production of CH\textsubscript{4} varies over time whilst cows adapt to grazing conditions and none which have also measured CO\textsubscript{2}. We sought to contribute to this knowledge gap by regularly measuring CH\textsubscript{4} and CO\textsubscript{2} produced by 12 non-lactating dairy cows following a change in diet; from barley straw and protein supplements fed indoors to outdoor grazing of grass. The following hypotheses were tested: (1) CH\textsubscript{4} and (2) CO\textsubscript{2} production would increase over time as cows adapted to grazing; (3) cows would produce more CH\textsubscript{4} and CO\textsubscript{2} per kg of live weight over time and (4) CH\textsubscript{4} and CO\textsubscript{2} production would asymptote within six weeks of the change in diet.

2. Materials and methods

2.1. Site and weather conditions

The study was carried out at Scotland’s Rural College (SRUC) Dairy Research Centre, Dumfries, South-West Scotland (3°35 W, 53°03 N) during May and June. Air temperatures ranged from 4.6 to 19.8°C during the seven week study period, with a mean of 6.2 ± 0.7 h of sunshine per day. Weekly mean soil temperatures (5 cm depth) increased from 12.2 °C at the start of the study to 16.3°C at the end. Rainfall varied from 0.1 mm in the driest week to 25.6 mm in the wettest (Table 1). Weather data were obtained from an on-site weather station.

2.2. Animals and experimental design

The study group consisted of 12 non-lactating Holstein Friesian dairy cattle (mean age 5.5 ± 2.8 years, mean live weight 576 kg ± 51 kg). Two of the animals were freemartin heifers, with the remaining ten cows maintained in the follicular phase of the reproductive cycle for the duration of the study to minimise any changes to the animals during the experiment. This was achieved by means of Progesterone Releasing Intra-vaginal Devices (PRIDS: Ceva Animal Health Limited, UK) administered prior to commencement of the study. Cows were housed indoors over the winter and fed a diet of barley straw in preparation for taking part in the study. In the four weeks prior to commencement of the grazing treatment, cows were fed a diet of unrestricted barley straw and each cow also received 3 kg d\textsuperscript{-1} of 18% protein concentrate. The feeding of protein supplements prior to the grazing treatment was in line with best practice for straw-fed high yielding dairy cattle.

Cows were separated into two sub-groups. This allowed a one week delay in the start date between the two sub-groups. This staggered start was incorporated in the study design as a means of reducing the impact of single-day climate effects and variation in forage quality. Cows were allocated to one of the two groups by separating the animals into matched pairs based on age and

| Week | Min air temp (°C) | Max air temp (°C) | Sunshine (h d\textsuperscript{-1}) | Rainfall (mm) |
|------|------------------|------------------|-------------------------------|--------------|
| 1    | 4.6              | 13.9             | 6.5                           | 25.6         |
| 2    | 9.1              | 16.9             | 3.8                           | 15.8         |
| 3    | 6.1              | 17.4             | 9.1                           | 7.1          |
| 4    | 7.4              | 16.8             | 6.8                           | 8.9          |
| 5    | 10.5             | 18.7             | 6.6                           | 5.3          |
| 6    | 11.8             | 19.8             | 6.2                           | 0.1          |
| 7    | 10.1             | 17.4             | 4.3                           | 2.3          |
| Mean | 8.5              | 17.3             | 6.2                           | 9.3          |
| SEM  | 1.0              | 0.7              | 0.7                           | 3.3          |

Notes: Minimum daily air temperature (min air temp), maximum daily air temperature (max air temp), hours of sunshine and total weekly rainfall. Data were obtained from an on-site weather station.
weight. Individuals were then allocated into one of the two sub-groups at random. This ensured that each sub-group was balanced for age and weight at the start of the experiment (Group 1—mean age ± standard error; 5 ± 3 years; mean live weight; 566 ± 53 kg; Group 2—mean age; 6 ± 3 years, mean live weight; 586 ± 52 kg).

On day one of the measurement phase of the study sub-group one were turned out to pasture and allowed to graze freely for 23 h per day without supplementary feeding for a six-week period. Cows were brought inside for one hour a day. This allowed the renewal of SF₆ tracer equipment and for the cows to be weighed. One week later sub-group two was also allowed to graze the pasture under the same management regime for a period of six weeks. Measurements of CO₂ and CH₄ produced by each cow and measurement of cow weight were carried out daily for the first ten days at pasture, then three days per week from weeks three to the conclusion of the study. As a result daily greenhouse gas production and live weights for each cow was measured 22 times.

2.3. Pasture composition, productivity and nutritional quality

The grazing area was a 4 ha pasture dominated by a perennial ryegrass (Lolium perenne) sward (approximate cover >95%). The pasture was sub-divided into six smaller paddocks by means of a movable electric fence. Cows were moved between fields every two days to allow for the grass to re-grow before cows returned to graze again twelve days later. This regime aimed to retain a consistent grass height across the study period and ensured that grass availability was unrestricted and did not influence feed intakes. Sward height was measured daily using a sward stick, placed randomly at 50 locations across the pasture (mean sward height throughout the study = 10.0 ± 0.9 cm).

Each day five grass samples (~25 g) were collected from random locations across the field and harvested to ground level. Samples were bulked on a weekly basis and analysed for nutritional quality. Nutritional quality measurements were dry matter (DM), gross energy (GE), metabolisable energy (ME), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and hemicellulose content (HC). DM content was assessed by weighing 5 g of plant material, drying this material for 48 h at 60°C and comparing dry and fresh weights. CP was measured by Kjeldahl digestion using sulphuric acid and analysed by steam distillation using a Gerhardt–Vadopest system (Gerhardt Vadopest 6, Germany). NDF, ADF and HC were measured using modified neutral and acid detergent analysis following the methodology of Van Soest, Robertson, and Lewis (1991). GE and ME were measured by conventional wet chemistry, as outlined by AOAC (2002).

2.4. Methane and carbon dioxide emissions measurements

CH₄ and CO₂ production was measured using the sulphur hexafluoride (SF₆) tracer technique (Johnson, Huyler, Westberg, Lamb, & Zimmerman, 1994). A permeation tube bolus (brass 15 mm OD, 45 mm long, 55 g) with a semi-permeable Teflon membrane (5 mm diameter) and halter containing the inert tracer gas SF₆ was introduced to the rumen of the study animals. Prior to deployment, the individual release rates of SF₆ from 24 boluses were measured by weighing at daily intervals over a period of five weeks, during which time the tubes were held at 39°C in an anaerobic nitrogen environment to simulate rumen conditions (Berndt et al., 2014). Changes to bolus weight was plotted against time with the 12 boluses which exhibited the strongest linear relationship (highest r² value) being selected for use in the experiment (mean loss rate = 1.44 ± 0.04 mg SF₆ d⁻¹). Boluses were administered to the animals three weeks prior to the measurement period to allow for acclimatisation and to minimise the probability of non-linear release of SF₆ during the measurement period. After the experiment, all of the boluses were recovered post-mortem and inspected for blockages or any other damage. There was no evidence of any blockages and no evidence of any non-linearity in SF₆ release rates in the six weeks prior to the start of the experiment or during the experiment. It was therefore assumed that, once ingested by the animals, each permeation tube remained in the rumen releasing SF₆ gas at a constant rate according to its individual release signature.
CH₄ production rates ($F_{CH_4}$) were estimated using Equation 1 and CO₂ production rates ($F_{CO_2}$) were estimated using Equation 2 where $F_{SF_6}$ is the known release rate of SF₆ from the permeation tube (g s⁻¹) and where $C_{SF_6}$, $C_{CH_4}$ and $C_{CO_2}$ are the concentrations (g m⁻³) of the three gases in the exhaled air.

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F_{CH_4} = \frac{F_{SF_6} C_{CH_4}}{C_{SF_6}}
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F_{CO_2} = \frac{F_{SF_6} C_{CO_2}}{C_{SF_6}}
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Exhaled air from the animal was sampled from the area around the nostrils using flexible tubing held in place by a halter and connected via a metal capillary tube to a closed v-shaped PVC canister secured behind the cow’s head. The canisters were evacuated using a vacuum pump prior to use and the shut off valves were opened on attachment to the cows to commence air sampling. This arrangement allowed exhaled air to be sampled continuously for 24 h until the valves were closed. On removal of the canisters from the animals new evacuated canisters were attached to sample the next 24 h period. The contents of the removed canisters were diluted with nitrogen (mean dilution: 3.59 ± 0.05), decanted into subsampling tubes constructed from metal and glass, then transported to the laboratory for subsequent analysis using an HP5890 Series II gas chromatograph (detection limits: SF₆ < 0.005 ml l⁻¹, CO₂ < 0.199 ml l⁻¹ and CH₄ < 0.00126 ml l⁻¹) using an electron capture detector for SF₆ and a flame ionisation detector for CH₄ and CO₂. Dilution factors were recorded for each sample and measured CO₂, CH₄ and SF₆ concentrations adjusted accordingly.

2.5. Statistical analysis

Relationships between daily CH₄ and CO₂ production (g d⁻¹) and experimental duration as well as relationships between CH₄ and CO₂ production per gram of cow live weight (g d⁻¹ kg⁻¹, CH₄/LWt and CO₂/LWt) and experimental duration were identified for the group using maximum-likelihood linear mixed effects models (LME, Pinheiro & Bates, 2000). The relationship between CH₄ and CO₂ production was also tested using LME. In all models, each cow was treated as a random effect with duration treated as a fixed effect. This random effect structure allowed us to account for our time series, where several measurements of CH₄ and CO₂ emissions were taken from an individual animal over the course of the study. The optimal shapes of the relationships were identified by means of transforming our response data using logarithmic and quadratic transformations, comparing LME model outputs with those generated by untransformed data using Akaike’s Information Criterion (AIC). AIC represents an alternative for calculating measurements of the explained deviance to the more conventional $r^2$ values which cannot be calculated with LME models. In all cases the linear relationship had the lowest AIC value and was selected (Crawley, 2007). The equations of fitted lines from these analyses represent both the mean rate of increase in a stated parameter over time (gradient) and the mean absolute value of the stated parameter on day one following the change in diet (intercept).

Relationships between CH₄ and CO₂ production and experimental duration, and CH₄ and CO₂ production per gram of live weight and experimental duration were tested for each individual cow using linear regression (LR). Relationships between CH₄ and CO₂ production and cow weights were also tested using LR for each day since the change in ration. LR was used in these instances since these data were not nested—identifying relationships between CH₄ and CO₂ production and duration for each cow and between CH₄ and CO₂ production and live weight on each day, respectively. Relationships between grass sward quality (DM, GE, ME, CP, NDF, ADF, HC) and CH₄ and CO₂ were also tested using LR using mean weekly values for sward quality and gas production. Due to the staggered design of the experiment, separate analyses were computed for CH₄ and CO₂ production for sub-groups one and two against their respective grass sward quality measurements. The optimal shapes of the relationships were identified by means of transforming response data using logarithmic and quadratic transformations and comparing LR model outputs with those generated by untransformed data using $r^2$. In all cases the linear relationship had the highest $r^2$ value and was selected (Crawley, 2007). All analyses were computed using R v3.0.1 (R Core Team, 2013).
3. Results

3.1. Group greenhouse gas emissions
Total group production of both CO$_2$ ($t = 4.0, p < 0.001$) and CH$_4$ ($t = 7.4, p < 0.001$) increased linearly over the experimental period and following the change in diet (Figure 1). Mean production of CO$_2$ per cow increased from 11,429 g d$^{-1}$ on day one to 16,825 g d$^{-1}$ on day 38 (LME: CO$_2$ = 142d + 11,429, $p < 0.001$). This represented a mean increase in CO$_2$ production of 142 g d$^{-1}$ or a rise of 47% over the 38 day experimental period.

Mean production of CH$_4$ per individual cow was lower than CO$_2$ throughout the study, increasing from 272 g d$^{-1}$ on day one to 386 g d$^{-1}$ on day 38 (LME: CH$_4$ = 3d + 272, $p < 0.001$). Mean production of CH$_4$ per cow also increased at a slower rate than CO$_2$; increasing by 3 g d$^{-1}$ or 42% over the 38 day experimental period.

There was a positive linear relationship between CO$_2$ production and CH$_4$ production over the experiment ($t = 32.5, p < 0.001$, Figure 2). Cows which produced large amounts of CO$_2$ also produced
large amounts of CH$_4$ and days which produced large amounts of CO$_2$, also large amounts of CH$_4$, with a 1 g increase in CH$_4$ associated with a 44 g increase in CO$_2$ (LME: CO$_2$ = 44 × CH$_4$, $p < 0.001$).

### 3.2. Forage nutritive quality

Forage nutritive quality metrics generally increased by the end of the study, with DM (+19%), GE (+3%), ME (+12%), CP (+20%) and ADF (+18%) all increasing between days 1 and 38 (Table 2). However, NDF (−3%) and HC (−24%) declined over the same period. None of these metrics increased or decreased consistently over the study period. Across all of the metrics for forage quality the number of weeks in which the metric increased compared with the previous week and the number of weeks in which the metric decreased was approximately equal (range = 2–4 weeks increasing and range = 2–4 weeks decreasing).

Weekly mean CH$_4$ production was not related to any of the forage quality metrics for the first sub-group of cows, which commenced the experiment in week one ($t = -0.1$–1.5, $p = 0.2$–0.9). However, weekly mean CH$_4$ produced by sub-group two, which commenced the experiment in week two, were negatively correlated with weekly mean NDF content ($t = -2.8$, $p < 0.05$, $r^2 = 0.6$). All other forage quality metrics were not related to CH$_4$ over the experimental period for this sub-group ($t = -1.75$–1.71, $p = 0.15$–0.73). In addition, none of the forage quality metrics were related to mean weekly CO$_2$ production over the experimental period for sub-groups one ($t = -0.7$–1.4, $p = 0.1$–0.7) or two ($t = -1.5$–1.4, $p = 0.2$–0.9).

### 3.3. Cow live weights

Mean cow weight within the group increased from 576 ± 13 kg (mean ± standard error) on the first day to 583 ± 17 kg on day 38, representing a 1% increase. These increases were idiosyncratic and on a weekly basis mean group weight declined by 0.5% between weeks one and two, increased by 1% between weeks two and three, decreased by 2.4% between weeks three and four and then increased by 2.5 and 3.2% between weeks four and five, and between weeks five and six, respectively.

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Table 2. Weekly measurements and overall mean values for canopy height and herbage quality over the study period (n = 5 measurements)

| Week | Height (cm) | DM (g kg$^{-1}$) | GE (MJ kg DM$^{-1}$) | ME (MJ kg DM$^{-1}$) | CP (g kg DM$^{-1}$) | NDF (g kg DM$^{-1}$) | ADF (g kg DM$^{-1}$) | HC (g kg DM$^{-1}$) |
|------|-------------|-----------------|---------------------|---------------------|-------------------|-------------------|-------------------|-------------------|
| 1    | 10.8        | 178             | 18.6                | 11.3                | 207               | 452               | 227               | 225               |
| 2    | 10.9        | 144             | 18.8                | 11.1                | 207               | 504               | 245               | 259               |
| 3    | 9.6         | 215             | 18.3                | 10.9                | 194               | 484               | 258               | 226               |
| 4    | 8.4         | 248             | 18.3                | 10.7                | 235               | 480               | 227               | 253               |
| 5    | 9.8         | 188             | 19.2                | 11.9                | 269               | 483               | 223               | 260               |
| 6    | 9.8         | 179             | 19.1                | 12.6                | 256               | 464               | 222               | 242               |
| 7    | 10.8        | 211             | 19.2                | 12.7                | 250               | 437               | 267               | 170               |
| Mean | 10.0        | 195             | 18.8                | 11.6                | 231               | 472               | 238               | 234               |
| SEM  | 0.3         | 12.6            | 0.2                 | 0.3                 | 11                | 9                 | 7                 | 12                 |
| Weeks + | 4          | 3               | 3                   | 3                   | 2                 | 2                 | 3                 | 3                  |
| Weeks − | 2          | 3               | 3                   | 3                   | 4                 | 4                 | 3                 | 3                  |
| Barley | 841       | 18.5            | -                   | -                   | 44                | 799               | 523               | 276               |

Notes: Metrics are dry matter (DM), gross energy (GE), metabolisable energy (ME), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and hemicellulose content (HC). Weeks + indicates the number of weeks where that parameter increased compared with the previous week and week—indicates the number of times the parameter decreased compared with the previous week. Indicative values for barley straw (Barley) obtained from Moss, Givens, and Everington (1990).
There were no relationships between cow weight and CH$_4$ or cow weight and CO$_2$ production on any of the first 23 and 17 days of the study, respectively (Table 3). On day 31, CH$_4$ increased linearly with cow weight, with each 1 kg increase in cow weight representing a 1.6 g d$^{-1}$ increase in CH$_4$ emissions ($t = 3.8, p < 0.001$). CO$_2$ also increased linearly with cow weight but only on days 22, 23 and 31. On these three days, each 1 kg increase in cow weight represented a 62 g d$^{-1}$ ($t = 2.3, p < 0.05$), 70 g d$^{-1}$ ($t = 2.5, p < 0.05$) and 62 g d$^{-1}$ ($t = 3.0, p < 0.05$) increase in CO$_2$ production, respectively.

Over the study period, the mean amount of CH$_4$ ($t = 6.6, p < 0.001$, Figure 3) and CO$_2$ ($t = 3.6, p < 0.001$) produced per kg of cow live weight increased linearly. In the case of CH$_4$, the group produced a mean of 0.5 g d$^{-1}$ kg$^{-1}$ on day one rising by 0.005 g kg$^{-1}$ each day. After 38 days, the group was therefore producing mean CH$_4$ of 0.7 g d$^{-1}$ kg$^{-1}$. In terms of CO$_2$, the group produced a mean of 20.9 g d$^{-1}$ kg$^{-1}$ rising more steeply on a daily basis, by 0.25 g d$^{-1}$ kg$^{-1}$. The group was therefore producing mean CO$_2$ of 30.1 g d$^{-1}$ kg$^{-1}$ by day 38.

### 3.4. Individual cow greenhouse gas emissions

Eight of the twelve cows showed a linear relationship between experimental duration and CH$_4$ production ($t = 2.3–6.2, p ≤ 0.001–0.04$, Table 4). Two of these eight cows were the freemartin heifers. Variation between cows which produced low CH$_4$ and those which produced high CH$_4$ was substantial, by approximately four fold in terms of CH$_4$ on day one and by approximately four fold in terms of the rates of increase in CH$_4$ over the experimental period. For example, production of CH$_4$ on day one ranged from 116 g d$^{-1}$ for cow eight to 510 g d$^{-1}$ for cow nine, with the rates of increase in CH$_4$ over the 38 day experimental period ranging from 1.3 g d$^{-1}$ for cow eight to 5.3 g d$^{-1}$ for cow seven.

Rates of CH$_4$ production per kg of live weight also increased linearly for the same eight cows ($t = 2.2–6.1, all p ≤ 0.001–0.04$, Table 4) alongside absolute CH$_4$ increases. However, the rates of increase in CH$_4$ production per kg live weight increased more slowly over time and with a reduced

### Table 3. Linear regression analyses of relationships between cow weight (kg) and methane (CH$_4$) and carbon dioxide (CO$_2$) emissions for each day of the study ($n = 12$ cows)

| Day | LW (kg) | Gradient | CH$_4$ (g d$^{-1}$) | Gradient | CO$_2$ (g d$^{-1}$) |
|-----|---------|----------|---------------------|----------|---------------------|
|     |         |          | $t$    | $p$    | $t$    | $p$ |
| 1   | 576     | 0.49     | 0.64   | 0.54   | -2.30 | 0.09 |
| 2   | 564     | 0.70     | 1.31   | 0.22   | 23.91 | 1.00 |
| 3   | 556     | 1.05     | 2.23   | 0.05   | 35.27 | 1.84 |
| 4   | 558     | 1.01     | 1.73   | 0.12   | 25.97 | 0.70 |
| 5   | 556     | 0.89     | 1.06   | 0.32   | 32.14 | 0.86 |
| 6   | 572     | 0.12     | 0.12   | 0.91   | -22.88 | 0.44 |
| 7   | 562     | 0.92     | 0.69   | 0.51   | 36.13 | 0.76 |
| 8   | 571     | 0.76     | 0.90   | 0.40   | 9.03  | 0.26 |
| 9   | 556     | 1.02     | 1.39   | 0.20   | 32.57 | 0.91 |
| 10  | 558     | 0.91     | 1.45   | 0.18   | 27.79 | 1.71 |
| 15  | 563     | 0.15     | 0.18   | 0.86   | 134.80 | 0.52 |
| 16  | 573     | 1.49     | 1.77   | 0.12   | 97.76 | 1.96 |
| 17  | 563     | 0.58     | 0.70   | 0.50   | 40.55 | 1.23 |
| 22  | 554     | 1.36     | 2.15   | 0.06   | 62.40 | 2.48 |
| 23  | 557     | 1.53     | 1.77   | 0.11   | 69.76 | 2.33 |
| 31  | 570     | 1.64     | 3.77   | <0.001 | 62.13 | 2.99 |
| 38  | 583     | 1.11     | 2.22   | 0.06   | 49.26 | 1.81 |

Note: Mean live weight for each time period are also presented (LW).
range compared with absolute CH₄ production—ranging from 0.003 and 0.008 or by a factor of approximately 2.7.

CO₂ production was also linearly related to experimental duration for the same eight cows ($t = 2.6–8.9$, all $p < 0.001$). The ranges of emissions on day one were greater for CO₂ than for CH₄, ranging from 4,611 g for cow two to 20,971 g for cow six or by a factor of approximately five. Rates of increases in CO₂ production over the experimental period were also moderately greater for CO₂ than CH₄, ranging from an increase of 89 g d⁻¹ for cow eight to an increase of 367 g d⁻¹ for cow one. This represented an approximately four-fold difference.

Table 4. Regression analyses identifying linear relationships between experimental duration and methane and carbon dioxide emissions for each cow ($n = 22$ measurements)

| Cow | Sub-group | CH₄ (g d⁻¹) | CH₄/LWt⁻¹ (g kg⁻¹) | CO₂ (g d⁻¹) | CO₂/LWt⁻¹ (g kg⁻¹) |
|-----|-----------|-------------|---------------------|-------------|---------------------|
|     |           | Gradient | Intercept | $t$  | $p$       | Gradient | Intercept | $t$  | $p$       | Gradient | Intercept | $t$  | $p$       |
| 1   | 2         | 5.27     | 278.14    | 4.69 | 0.008 | 4.10     | 0.001 | 366.55 | 10,740.24 | 5.05 | 0.001 | 0.541 | 4.70 | 0.001 |
| 2   | 2         | 2.40     | 122.40    | 6.21 | 0.004 | 6.05     | 0.001 | 123.80 | 4,610.86 | 8.92 | 0.001 | 0.231 | 8.42 | 0.001 |
| 3   | 1         | 5.03     | 273.53    | 2.74 | 0.008 | 2.52     | 0.03  | 276.98 | 10,767.88 | 4.88 | 0.001 | 0.428 | 4.58 | 0.001 |
| 4   | 1         | 2.14     | 299.56    | 0.96 | 0.003 | 0.90     | 0.38  | 102.09 | 13,051.08 | 7.13 | 0.001 | 0.165 | 1.10 | 0.29 |
| 5   | 2         | 3.73     | 284.95    | 2.81 | 0.006 | 2.72     | 0.01  | 230.97 | 10,145.53 | 5.53 | 0.001 | 0.366 | 5.28 | 0.001 |
| 6   | 2         | 2.11     | 402.69    | 1.70 | 0.003 | 1.11     | 0.28  | 97.98  | 20,971.03 | 0.22 | 0.83  | 0.143 | 0.19 | 0.85 |
| 7   | 2         | 5.33     | 155.03    | 5.46 | 0.008 | 5.12     | 0.001 | 194.52 | 6,097.98 | 4.79 | 0.001 | 0.302 | 4.39 | 0.001 |
| 8   | 1         | 1.33     | 115.84    | 2.64 | 0.003 | 2.64     | 0.02  | 89.61  | 5,328.81 | 4.99 | 0.001 | 0.198 | 4.98 | 0.001 |
| 9   | 2         | 1.01     | 509.46    | 0.67 | 0.000 | 0.11     | 0.92  | 134.50 | 20,052.80 | 1.21 | 0.24  | 0.184 | 0.90 | 0.38 |
| 10  | 1         | 0.25     | 332.71    | 0.31 | 0.000 | 0.17     | 0.87  | 8.76   | 13,431.07 | 0.22 | 0.83  | 0.102 | 0.16 | 0.88 |
| 11  | 1         | 2.58     | 267.75    | 2.26 | 0.004 | 2.19     | 0.04  | 144.69 | 10,100.34 | 2.62 | 0.001 | 0.247 | 5.54 | 0.001 |
| 12  | 1         | 4.96     | 221.55    | 6.21 | 0.008 | 5.23     | 0.001 | 189.60 | 11,801.58 | 5.21 | 0.001 | 0.284 | 4.29 | 0.001 |
| All |           | 3.01     | 272.21    | 7.43 | 0.001 | 6.61     | 0.001 | 164.13 | 11,429.41 | 4.00 | 0.001 | 0.258 | 3.64 | 0.001 |

Notes: Methane emissions per day (CH₄), methane emissions per kg of cow live weight (CH₄/LWt⁻¹), carbon dioxide emissions per day (CO₂) and carbon dioxide emissions per kg of cow live weight (CO₂/LWt⁻¹) are presented. Study sub-group 2 commenced and ended the study one week after study sub-group 1.
The rank order from highest to lowest producing cow was relatively consistent over the 38 days with the standard deviation of the rank order for individual cows, representing each cows mean distance from their mean rank, ranging from 0.6 to 3.0 and from 1.3 to 2.6 for daily CH$_4$ and CO$_2$ emissions, respectively (Table 5).

4. Discussion

Production of CH$_4$ and CO$_2$ from both groups of cows increased over time following the shift in their diets; from straw to grazed grass. This increase was likely to have been driven by changes in feed chemical composition and increased feed intakes by the animals, as has been reported in studies elsewhere (e.g. McAllister et al., 1996; O’Neill et al., 2011). This finding is supported by comparison of the nutritive quality of barley and grass, with the DM content of grass around four times lower than that of barley straw indicating that a greater volume of grass would have been required by the cows to satisfy their nutritional demands. Since the cows were retained in the follicular phase and were not pregnant or lactating, the results obtained were unlikely to have resulted from the lifecycle of the animals during the experiment.

Elevated CH$_4$ and CO$_2$ production over the experimental period may have been partially driven by weight gains of the animals thus increasing their capacity for forage intake and metabolic activity. However, on the majority of sampling occasions there was no relationship between cow live weights and the quantity of CH$_4$ or CO$_2$ that was produced. Those occasions where significant relationships were obtained may have been statistical artefacts, since the error associated with weighing the animals was large. Although cow live weights increased between week one and week six, these gains were idiosyncratic. Despite these small live weight gains (~1%), CH$_4$ and CO$_2$ production increased rapidly and the cows became more efficient producers of CH$_4$ and CO$_2$ per kg of live weight. This suggests that weight gains were not key determinants of changes to the magnitude of CH$_4$ and CO$_2$ production and also highlights that cow weights were not good predictors of total CH$_4$ and CO$_2$ production.

Whilst nutritional differences between the two contrasting diets are likely to have been important, shifts in grass quality following the transition to grazing are unlikely to have played a major role in driving the linear increases in CH$_4$ and CO$_2$ production. An exception was a negative relationship between NDF concentrations and CH$_4$; however, this relationship was relatively weak and only significant for the second sub-group of cows. Typically NDF is positively related to CH$_4$ production (Lee,

### Table 5. Mean rank and the standard deviation of rank (SD) for each individual cow according to their methane (CH$_4$) and carbon dioxide (CO$_2$) emissions over the study period. The highest emitting cow is rank 1 and lowest emitting cow is rank 12

| Cow | CH$_4$ (g d$^{-1}$) Mean rank | SD | CO$_2$ (g d$^{-1}$) Mean rank | SD |
|-----|----------------------------|----|----------------------------|----|
| 1   | 7.0                        | 2.1| 7.4                        | 2.2|
| 2   | 1.5                        | 0.6| 1.7                        | 2.0|
| 3   | 6.4                        | 2.5| 7.0                        | 2.3|
| 4   | 5.5                        | 2.5| 6.1                        | 2.6|
| 5   | 6.3                        | 1.7| 5.7                        | 1.7|
| 6   | 8.6                        | 2.1| 8.1                        | 2.4|
| 7   | 2.6                        | 3.0| 2.2                        | 2.2|
| 8   | 3.0                        | 1.8| 2.8                        | 1.5|
| 9   | 9.8                        | 1.2| 9.4                        | 1.3|
| 10  | 6.5                        | 2.1| 6.3                        | 2.4|
| 11  | 5.5                        | 2.0| 4.4                        | 1.6|
| 12  | 5.0                        | 2.0| 6.9                        | 2.0|
Davis, Chagunda, & Manning, 2017) and therefore this relationship is also likely to be a statistical artefact. Grass quality varied throughout the study but none of the grass quality metrics increased regularly (both increasing and decreasing on a weekly basis) alongside a more consistent and linear increase in CH\textsubscript{4} and CO\textsubscript{2}. Non-linear release of SF\textsubscript{6} has been demonstrated to influence CH\textsubscript{4} measurements in studies elsewhere, particularly over longer periods (Lassey, Walker, McMillan, & Ulyatt, 2001). We tested all boluses for linear release rates over the five weeks prior to the experiment. The magnitude of change in CH\textsubscript{4} when compared with the relatively small error generated by non-linear release over the six-week measurement period and careful inspection of boluses post-mortem means that it is unlikely that non-linearity of SF\textsubscript{6} release has driven the relationships presented in this study.

Mean CH\textsubscript{4} production increased per cow from 272 g d\textsuperscript{-1} during week one to 386 g d\textsuperscript{-1} during week six, producing quantities of CH\textsubscript{4} which were consistently greater than those produced by grass-fed cows in Ireland (251 g d\textsuperscript{-1}, O’Neill et al., 2011), Canada (270 g d\textsuperscript{-1}, McCaughey, Wittenberg, & Corrigan, 1999) and New Zealand (159–202 g d\textsuperscript{-1}, McCaughey, Wittenberg, & Corrigan, 1997). By the sixth week of the study the group was producing CH\textsubscript{4} emissions which were only moderately less than cows fed a diet of mixed ration in Ireland (397 g d\textsuperscript{-1}, O’Neill et al., 2011) and greater than all but one group of grass and clover-fed cows in New Zealand (137–431 g d\textsuperscript{-1}, Lassey, 2007). It is likely that increased feed intake and subsequent changes to the availability or chemical composition of microbial substrate played an important role in driving elevated CH\textsubscript{4} production (Kebreab, Clark, Wagner-Riddle, & France, 2006). However, it has also been demonstrated that non-lactating cows lose a greater proportion of their feed intake as CH\textsubscript{4} than lactating cows (Bell, Wall, Russell, Morgan, & Simm, 2010) and this may have contributed additionally to the high values we recorded.

Production of CO\textsubscript{2} was 42–44 times greater than CH\textsubscript{4} throughout the study and CO\textsubscript{2} also increased more rapidly than CH\textsubscript{4}. Our estimate of average CO\textsubscript{2} production over the six-week period (14,364 g d\textsuperscript{-1}) was comparable to values that were recorded using an infra-red gas analyser to measure grass-fed lactating Holstein Friesian cows in Canada (12,055 g d\textsuperscript{-1}, Kinsman et al., 1995) and greater than a previous study using the SF\textsubscript{6} tracer technique in France (8,750–10,496 g d\textsuperscript{-1}, Pinares-Patiño et al., 2007) providing additional support for the use of the SF\textsubscript{6} tracer technique to measure CO\textsubscript{2} production. The direction and magnitude of changes to CO\textsubscript{2} production provide useful insights into metabolic changes during the experiment. The rise in CO\textsubscript{2} production over the course of the study may be explained by increased respiration by the cows, digesting larger quantities of feed coupled with respiration by enteric microbes during rumen adaptation (McAllister et al., 1996). Previous studies have shown that the SF\textsubscript{6} tracer technique overestimates CO\textsubscript{2} production, with the magnitude of overestimation reported as 20–65% (Pinares-Patiño et al., 2007). Despite this, considering the 21–25 times higher GWP of CH\textsubscript{4} when compared with CO\textsubscript{2} (IPCC, 2013), the GWP of CO\textsubscript{2} produced by the cows throughout the study was approximately double (200–210%) the GWP of CH\textsubscript{4} according to our measurements—greater than the maximum proposed overestimation of 65%. Although it should be noted that CO\textsubscript{2} emissions from agriculture are considered to be balanced by subsequent plant carbon uptake in greenhouse gas inventories (IPCC, 2013), an increased efficiency of milk production per unit of CO\textsubscript{2} and CH\textsubscript{4} would reduce the overall carbon footprint of dairy farming systems.

Selective breeding studies have demonstrated that CH\textsubscript{4} production can be reduced by 19–23% if selection is based on milk production (Chagunda, Römer, & Roberts, 2009) and retaining older cows can also reduce CH\textsubscript{4} by 3%, since more productive older cows convert feed to milk more efficiently (Bell et al., 2010). Within our groups of cows there was substantial variation between individuals, with the lower producing cows producing four and five times less CH\textsubscript{4} and CO\textsubscript{2} than the high producing cows, respectively. The rank order of the highest to lowest individuals was consistent over the study and cows which produced high CH\textsubscript{4} also produced high CO\textsubscript{2}. Variation was not explained by cow live weights, cow age or grass nutritional quality and is likely to be linked to enteric conditions; where the rumen is more or less favourable for methanogenic microbial population growth and activity (McAllister et al., 1996). These data quantify the potential for reductions in greenhouse gas emissions if cow selection is based on minimising CH\textsubscript{4} production.
CH₄ and CO₂ production continued to increase linearly throughout the six-week grazing period and did not asymptote. This indicates that the increase in feed intake by the cows and/or the increase in enteric microbial activity may not have reached saturation point. Core needs to be taken in designing future livestock studies so that they are of sufficient duration to capture the full change in greenhouse gas production as animals adapt to novel feeding systems. In the absence of measured data, CH₄ production is currently estimated using predictive equations based on DM intakes, nutrient intakes and the digestibility of the diet (Mills et al., 2003). It has been shown that these equations can give accurate predictions of enteric CH₄ production (Ulyatt, Lassey, Shelton, & Walker, 2002a, 2002b). However, our data suggest that these equations should also take into account changes to the chemical composition of feed and consider the magnitude and duration of change in greenhouse gas production.

5. Conclusions

Two groups of non-lactating dairy cows were associated with increased CH₄ and CO₂ production following a change in their diet; from straw and protein supplements to grazed grass. Both CH₄ and CO₂ production increased more rapidly and consistently than cow weight gains and forage nutritive quality indicating that production of both gases may have increased as cows adapted to the new feeding system. CH₄ and CO₂ production did not reach an asymptote over the six-week grazing period, which was not expected, indicating that CH₄ and CO₂ production rates may not have reached maximum values. Predictive equations and future experiments should therefore consider the magnitude and duration of adaptation during periods of dietary transition. There was substantial variation in greenhouse gas production between individuals with our analyses highlighting that cows which produced higher CH₄ also produced higher CO₂. These data highlight that feeding regime is an important driver of greenhouse gas production, quantifies the potential for reductions in greenhouse gas production using selective breeding and also indicates that measurements of CO₂ production may serve as a useful proxy for CH₄ production by dairy cows.

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Competing Interests

The authors declare no competing interest.

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