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Assessing the dry matter intake and enteric methane emissions of pre-partum dairy cows offered grass clover or grass-only silage from two different silage systems

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Over the winter period, the low grass growth and availability in pasture-based dairy systems results in animals being housed and predominantly fed a diet of grass silage. There is limited availability of methane (CH_4) data evaluating the impact of forage type on dairy cows over the pre-partum period. The objective of the current experiment was to evaluate the impact of feeding grass clover (GC) silage and grass-only (GO) silage on the dry matter intake (DMI) and enteric CH_4 emissions of dairy cows pre-partum. A complete randomised block design was utilised for the 6-week experiment over two winter periods: from December 2020 to January 2021 and from December 2021 to January 2022. In each year, 30 non-lactating pregnant dairy cows were randomly allocated to two treatments (n = 15). In both years, cows in the GC treatment were offered grass clover bale silage, while cows in the GO treatment were offered grass-only pit silage. The DMI and gaseous emissions of individual animals were monitored daily using Hokofarm RIC (roughage intake control) feed stations and the GreenFeed technology. GC silage consistently had greater (p< 0.05) organic matter (OM) digestibility and lower (p< 0.05) neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents when compared with GO silage. Cows in the GC treatment had significantly greater (p< 0.05) total DMI (TDMI) compared with cows in the GO treatment. The daily CH_4 emissions (in grams per day) were not affected by treatment; however, cows in the GC treatment had reduced (p< 0.05) CH_4 yield (in grams per kilogram TDMI). Offering dairy cows GC silage over the pre-partum period resulted in greater DMI with reduced CH_4 yield when compared with cows offered GO silage.

KEYWORDS

dry period, feed intake, gaseous emissions, non-lactating, sustainability

Introduction

Globally, agriculture is a significant source of greenhouse gas (GHG) emissions, accounting for 9.3 million tonnes of carbon dioxide (CO_2) equivalent, with 39% related to enteric methane (CH_4) from ruminant livestock (FAO, 2020). Ruminant livestock systems serve as a vital source of human edible protein and play a crucial role in food security for growing global populations, particularly in developing nations (Arndt et al., 2022). The agricultural sectors in Ireland (36.8%) and New Zealand (49%) are the largest contributors to the total GHG emissions (EPA, 2023; MOE, 2023). Within the EU, there has now been major sectoral targets implemented in an effort to sharing regulations to reduce total GHG emissions (EEA et al., 2021). The Irish agricultural sector has been tasked with lowering the total GHG by 25% relative to the 2018 levels (EEA et al., 2021; Teagasc, 2023). Effective CH_4 mitigation strategies are crucial to achieve this target as CH_4 contributes 62% to the agricultural GHG emissions in Ireland (Teagasc et al., 2019; EPA, 2023). The majority of CH_4 research in grazing dairy systems has been predominantly derived during the lactating period, with limited data available during the pre-partum period (Lahart et al., 2023).

Pasture-based dairy systems, globally, are characterised by the diet of the animal, which mainly comprises grazed grass, although variability in the grass supply due to climatic conditions is inevitable across the year (Delaby et al., 2021). During the winter period, cows are dried off, housed or kept outdoors, and then enter the non-lactating period. The non-lactating dry period is an integral part of the lactation cycle for pasture-based dairy cows due to the replenishment of the mammary glands, the body reserves, foetal maturation, and the preparation for the onset of lactation (Butler et al., 2011; Mann et al., 2015). Research over the pre-partum period has been primarily focused on periparturient health, nutritional performance, and lactation performance (Richards et al., 2020), with less focus on enteric CH_4 emissions. The quality of the diet fed during the pre-partum period is crucial for meeting the nutritional requirements and maintaining the metabolic status of dairy cows over the pre-partum and post-partum periods (Butler et al., 2011; Janovick et al., 2022). Pasture-based dairy systems such as those in Ireland and New Zealand traditionally offer cows a high-fibre diet pre-partum, predominantly grass silage (O'Brien et al., 2018) or forage crops (Pinares-Patiño et al., 2007; Jonker et al., 2017; Waghorn, 2018). The nutrient demand of cows for energy pre-partum will be dependent on the dairy system and the cow type, although it has been reported that offering dairy cows a low-energy, high-fibre diet is adequate for meeting the nutrient requirements during this time (Butler et al., 2011; Richards et al., 2020).

Grass silage is the second largest component of the diet of Irish dairy cows, accounting for 18% of the annual feed budget on a fresh weight (FW) basis (O'Brien et al., 2018). It is an inexpensive source of feed compared with concentrates (Finneran et al., 2012; Doyle et al., 2022) and aids feed security by reducing reliance on external sources of imported feed on dairy farms. Grass silage is generally harvested as precision chop or baled silage (Mceniry et al., 2011) during periods of high grass growth over the year. Traditionally, grassland swards in Ireland contain perennial ryegrass (PRG) (*Lolium perenne* L.) due to its high yield potential, nutritive value, persistency (Hearn et al., 2021), and low production cost (Doyle et al., 2022). In order to achieve adequate silage yields and forage digestibility, grass-only silage swards require a certain level of chemical nitrogen (N) (Keady et al., 2000). The inclusion of legumes such as white clover (*Trifolium repens* L.) within grassland swards has increased in recent years not only due to improvements in animal performance when offered but also to restrictions in the application of chemical N fertiliser under the EU Nitrates Directive (Council Directive 19/676/EEC) (Egan et al., 2017; Herron et al., 2021). Grass clover swards are seen as an effective mitigation strategy for environmental emissions, such as ammonia and nitrous oxide, thereby increasing their use on Irish dairy farms (Herron et al., 2021). For this reason, the current study focused on comparing the impact of feeding grass-only (GO) silage and grass clover (GC) silage over the pre-partum period to non-lactating dairy cows.

Feeding GC silage has been reported to improve the dry matter intake (DMI) and milk production over the lactation due to clover having greater organic matter (OM) digestibility compared with PRG (Johansen et al., 2017, Johansen et al, 2018). Previous research has reported that the mitigation of CH_4 is possible by altering the composition of the diet (Loza et al., 2021), forage processing (Beauchemin et al., 2008), lipid supplementation (Boland et al., 2021; Muñoz et al., 2021), and by the inclusion of feed additives (Haisan et al., 2017; Van Gastelen et al., 2024). Among these strategies, the diet composition is of primary importance as it shows a substantial difference in CH_4 production (Soder and Brito, 2023). Using high-quality forages that are more digestible can decrease CH_4 emissions as they are fermented more efficiently, leading to less CH_4 production per unit of feed intake (Van Gastelen et al., 2024). In this context, the beneficial effect of clover inclusion in silage swards on mitigating enteric CH_4 is still under investigation (Bica et al., 2022). The pre-partum period has been reported to have lower levels of CH_4 emissions when compared with the lactation period as a result of the lower energy demand for the production and suppression of DMI due to foetal maturation (Jonker et al., 2017; Lyons et al., 2018). There is a clear seasonal variation in the CH_4 emissions of dairy cows, which is related to the level of DMI achieved (Ulyatt et al., 2002; Lahart et al., 2023), although both the DMI and enteric CH_4 emissions are dependent on the digestibility of the diet that is offered to dairy cows (Jonker et al., 2017).

Research reporting on the levels of CH_4 emitted when GC silage is offered to dairy cows has been predominantly during the lactating period (Baldinger et al., 2011; Brask et al., 2013; Johansen et al., 2017), with its impact over the pre-partum period still under investigation.

The objectives of the current study were to evaluate the digestibility of GC and GO silage and to assess their impact on the animal DMI, the enteric CH_4 emission, and the post-partum milk production when offered to dairy cows during the pre-partum period. The hypothesis is that GC silage will have a greater digestibility, resulting in differences in both the animal DMI and enteric CH_4 emissions compared with GO silage during the pre-partum period.

Materials and methods

Animals and experimental design

The current experiment was conducted at the Teagasc, Animal and Grassland Innovation Centre, Moorepark, Fermoy, Co., Cork, Ireland, over two 6-week periods from December 20, 2020, to January 29, 2021 (year 1), and from December 20, 2021, to January 31, 2022 (year 2). Within each year, 30 non-lactating pregnant spring calving dairy cows were selected from the Moorepark dairy herd. In year 1, all 30 cows were multiparous, while in year 2, the group consisted of four primiparous and 26 multiparous cows. In years 1 and 2, all cows were dried off on December 7, 2020, and on December 2, 2021. The cows were then blocked for parity and breed (Holstein Friesian and Holstein × Jersey) and balanced for expected calving date, bodyweight, body condition score (BCS), and economic breeding index (EBI) (Table 1). The cows were then randomly assigned to one of two treatments: the grass and clover silage (GC) treatment or the grass-only silage (GO) treatment. Within the GC treatment, cows were offered grass and clover bale silage for the duration of the experiment in both years, whereas cows within the GO treatment were offered grass-only pit silage for the duration of the experiment. All cows were housed in a purpose-built cubicle shed for the duration of the experiment, with the cubicles fitted with a 15-mm rubber mat (Huber Technik, Erding, Germany), which were cleaned and limed daily, with cows having free access to water. All forages were fed once daily at 9 a.m. using a Keenan Mechfibre 350 mixer wagon (Alltech Farming Solutions Ltd., Borris, Co., Carlow, Ireland). Both treatments were offered silage *ad libitum* by ensuring a 5%–10% refusal rate. The cubicle shed was equipped with 16 individual feed stations used to monitor the daily feed intake of cows over the course of the experiment. In both years, a 7-day acclimation period was implemented prior to commencing the experiment to ensure adequate animal use of each feed station. All silage was placed directly into 16 individual automatic feed stations (eight for the GC treatment and eight for the GO treatment) (RIC Feed-Weigh Trough, Hokofarm Group BV, Marknesse, Netherlands), similar to those described by Kelly et al. (2019).

Cows were removed from the experiment up to 7 days prior to calving and were placed in a calving pen on a grass silage diet, with the average calving dates being February 17, 2021, and February 6, 2022, in years 1 and 2, respectively. Silage treatments were stopped post-partum, with all the cows managed as one group. Post-partum, all of the cows were kept indoors and offered *ad libitum* grass silage plus 3 kg of concentrate for an average of 1 week, depending on the weather and ground conditions. All animals were turned out to grass and managed in a rotational grazing system and offered a diet of, on average, 13 kg dry matter (DM) grazed grass plus 3 kg DM of concentrate for the first 12 weeks of lactation. During periods of inclement weather, 4 days in year 1 and 1 day in year 2, all cows were housed and offered a diet of *ad libitum* grass silage and 3 kg of concentrate.

Silage preparation

The areas harvested for the GC silage were part of a systems grazing study (Fitzpatrick, 2023). All areas cut for the GC silage were grazed previously during the first rotation (March) and then closed for a period of 8 weeks before the harvesting of silage. The GC swards received 50 kg of chemical N over two splits, in February and March, and then received a further 60 kg N/ha 8 weeks prior to cutting. The GO silage was harvested from an area used for two cut silage systems with no animal grazing. The GO swards received 80 kg chemical N/ha 8 weeks prior to cutting. Prior to harvesting, the pre-cutting herbage mass of the GC and GO swards was determined using a method described by O'donoVan and Dillon (1999), i.e., by placing a quadrat (0.25 m^2) at three random locations per hectare, cutting all grass within the quadrat to 4 cm from the ground level using a Gardena hand shears (Gardena GmbH, Ulm, Germany). Fresh cut grass from within each quadrat was placed in a bag and weighed. Once weighed, a 100-g subsample was taken and placed into an oven at 90°C for 16 h to determine the DM content. The sward clover content from the GC swards was determined using a method described by Egan et al. (2017). All of the bale GC silage for the GC treatment was harvested from swards that have been established

TABLE 1 Pre-experimental data (mean ± SD) on breed, lactation number, days in calf, expected calving date, economic breeding index, pre-experimental bodyweight, and pre-experimental body condition score of dairy cows used in year 1 (2020) and year 2 (2021) of the experiment.

Item	Mean (2020)	SD (2020)	Mean (2021)	SD (2021)
Breed (Holstein/Holstein × Jersey)	28[a]/2[b]	–	20[a]/10[b]	–
Lactation number	3.3	±1.24	2.8	±1.31
Days in calf	230	±5.9	240	±9.8
Expected calving date	February 20	±14	February 7	±13
Economic breeding index (€)	184.1	±37.94	188.1	±37.03
Pre-experimental bodyweight (kg/cow)	619	±69.5	622	±61.2
Pre-experimental body condition score (1–5)	3.1	±0.26	3.4	±0.30

[a]Holstein Friesian.
[b]Holstein × Jersey.

for 5 years, with the swards containing PRG with a sown mixture of PRG (c.v *Astonenergy* and *Tyrella* in a 50:50 mixture sown at 27.2 kg/ha) and white clover (*Trifolium repens* cv. *Chieftain* and *Crusader* in a 50:50 mixture sown at 5 kg/ha). The GC was harvested in year 1 on June 8, 2020, and in year 2 on May 30, 2021. In year 1, the pre-cutting herbage mass was 3,838 kg DM/ha and the average clover content was 25% ± 13.4%. In year 2, these values were 4,107 kg DM/ha and 23% ± 11.9%, respectively. The GC silage was cut and left to wilt for 24 h to ensure that an adequate DM content (~30% DM) was achieved. Once wilted, the cut grass was raked into a 1.2-m swath and then baled and wrapped at harvesting using a McHale fusion baler (McHale, Ballinrobe, Co., Mayo, Ireland). All bale grass clover silage for the GC treatment was harvested from swards that were 5 years established, swards contained a mixture of PRG (c.v *Astonenergy, Tyrella* in a 50:50 mixture sown at 27.2 kg/ha) and white clover (*Trifolium repens* cv. Chieftain and Crusader in a 50:50 mix sown at 5 kg/ha) The GO silage was harvested in year 1 on May 20, 2020, and in year 2 on May 28, 2021. The pre-cutting herbage mass in year 1 was 5,350 kg DM/ha and that in year 2 was 5,050 kg DM/ha. The GO silage was cut and allowed to wilt for 24 h to ensure that an adequate DM content (~30% DM) was achieved at harvesting. Once wilted, the grass silage was raked into a 1.2-m swath and then harvested using a self-propelled precision chop harvester. All GO silage was ensiled in a silage clamp, rolled, and covered with a polythene sheet to ensure adequate conditions for fermentation (Teagasc, 2016).

Chemical composition

Over the course of the experiment, fresh silage samples were collected twice weekly from the RIC feed stations. From each sample, 100 g was weighed and dried at 40°C for 48 h to determine the silage DM. Fresh concentrate samples were collected twice weekly from the GreenFeed emissions monitoring (GEM) unit (C-Lock Inc., Rapid City, SD, USA). From each sample, 100 g was dried at 60°C for 48 h to determine the DM content. In both years, 12 silage and six concentrate samples were collected. Once dried, the silage and concentrate samples were milled through a 1-mm screen (Cyclotec 1093 Sample Mill, Foss Electric, Hillerød, Denmark) prior to chemical analysis. The ash content of each milled sample was determined by burning the sample at 550°C for 16 h using a muffle furnace (Nabertherm, GmbH, Lilienthal, Germany). The crude protein concentration was determined using a Leco FP-428 nitrogen analyser (Leco Australia Pty Ltd., Castle Hill, NSW, Australia) as described by Sweeney (1989). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were determined using an Ankom 2000 Fiber Analyzer as outlined by ANKOM Technology Corporation (Macedon, NY, USA) (Van Soest et al., 1991). The organic matter digestibility of the silage samples was determined using an *in vitro* NDF cellulase procedure, as described by Morgan et al. (1989). The gross energy content of the milled silage and concentrate samples was determined using a Parr 6050 bomb calorimetry system (Parr Instrument Company, Moline, IL, USA).

Animal measurements

Bodyweight and body condition score

Bodyweight and BCS were recorded at three time points in year 1 (i.e., December 6, January 8, and February 11) and four time points in year 2 (i.e., December 15, December 20, January 6, and February 5) of the experiment. The cows were individually weighed using an electronic portable weighing scale with the Animal Performance software package (Gallagher Europe, Groningen, the Netherlands). BCS was assessed by a trained professional using a scale from 1 to 5 (where 1 = emaciated and 5 = extremely fat), increasing in 0.25 increments, as described by Lowman et al. (1976).

Dry matter intake

The individual animal FW intake was recorded daily using the RIC feed-weight trough. Each feed station was equipped with an antenna above the access gate, which read a cow's radio frequency identification (RFID) ear tag (HDX EID Tag, Allflex Livestock Intelligence, Dallas, TX, USA) prior to allowing access to the silage. This ensured that GO treatment cows could not access the GC treatment silage, and *vice versa*. Once a cow visited a feed station, a feed event is recorded, with the duration of the visit, the starting weight (in kilograms FW), the ending weight (in kilograms FW), and the total roughage (in kilograms FW) removed from each feed station recorded. Prior to feeding fresh silage, all refused silage was removed from each feed bin. The daily silage dry matter intake (SDMI) for each individual cow was calculated by summing the total FW of silage consumed at each feed event per day, followed by converting the daily FW silage intake to SDMI using the corresponding DM% of the silage, which was then averaged across each week of the experiment. The fresh concentrate intake of individual cows was monitored using the GEM unit, and this was converted to dry weight using the DM% of the corresponding weekly concentrate sample to calculate the concentrate DM intake (CDMI). The individual cow SDMI was combined with the CDMI to derive the TDMI.

Methane emissions

Over the course of the experiment, the daily CH_4, CO_2, and hydrogen (H_2) emissions were monitored using the GEM unit. Cows had free access to one GEM unit for the duration of the experiment in both years. One GEM unit was allocated to all cows 7 days prior to commencing the experiment in both years. The entrance chute for cows to enter the GEM unit was modified to ensure that each individual cow had access to the unit at a given time. The GEM unit measures the spot breath samples of individual cows as they feed on a known amount of concentrate at a given time point over the day. In both years, a standard 14% crude protein dairy concentrate was used (Dairygold Co-Operative Society Limited, Cork, Ireland). The concentrate pellet consisted of barley (16.5 g/kg DM), maize (10.3 g/kg DM), wheat feed (5 g/kg DM), rapeseed extract (16.4 g/kg DM), maize gluten feed (14.1 g/kg DM), maize distillers (2.8 g/kg DM), soya hulls (12.3 g/kg DM), palm kernel extract (10.2 g/kg DM), molasses (5.1 g/kg DM), delactosed permeate (3.1 g/kg DM), and minerals and vitamins (4.2 g/kg DM).

Once a cow visited the GEM unit, 34 g FW of the concentrate was dispensed at 25-s intervals for a total of eight drops per visit. The duration between each visit was set at 6- to 8-h intervals (Figure 1), with the cows allowed to visit the GEM unit for a maximum of four visits per day to minimise the level of concentrate fed (average = 0.7 ± 0.12 kg DM/cow per day). As described by Della et al, 2021, every 3 days, automatic gas calibrations were performed, where known amounts of zero calibration gas containing nitrogen (N_2) followed by span calibration gasses containing CH_4 and CO_2 are released to ensure accuracy of the non-dispersive infrared spectrometry sensor. Prior to beginning the experiment, then once monthly and at the end of the experiment, manual CO_2 recoveries were performed to calibrate the airflow. Here, a known amount of CO_2 is released directly into the feed face of the GEM unit over three timed intervals (300 s). The change in the weight of the CO_2 canister was then compared with the amount measured by the GEM unit calculating the percentage recovered (average CO_2 recovery = 99.5% ± 0.95%.)

Milk production

The post-partum milk production of individual cows was monitored over the first 12 weeks of lactation. Daily individual milk yields (in kilograms) were measured at each milking at 0700 and 1500 hours (Dairymaster, Causeway, Ireland). The milk composition was determined weekly using one milk sample taken from a weighted consecutive a.m. and p.m. milking. The milk samples were analysed for protein, fat, and lactose concentrations using mid-infrared spectroscopy analysis (Milkoscan 203, FOSS Electric, Hillerød, Denmark).

Data handling and calculations

All data cleaning and the handling of data were performed using R software version 4.2.2 (R Core Team). A total of 120,933 feed events from the feed bins were recorded over the two consecutive years. All raw intake data used were cleaned for outliers by performing regression equations of the visit duration (in seconds) *versus* the roughage intake (in kilograms) or the roughage intake (in kilograms) *versus* the visit duration (in seconds) for each feed event. A linear model was then utilized to calculate the predicted value and residuals from each of the measured values. The residuals were then divided by the standard deviation of the visit duration (in seconds) or the roughage intake (in kilograms). Feed events with a residuals/SD greater than ±3 were replaced with a predicted value from the linear model.

All raw emissions data were checked for irregularities before being finalised. Once finalised, raw emission data were checked for outliers using a regression equation described by Coppa et al.

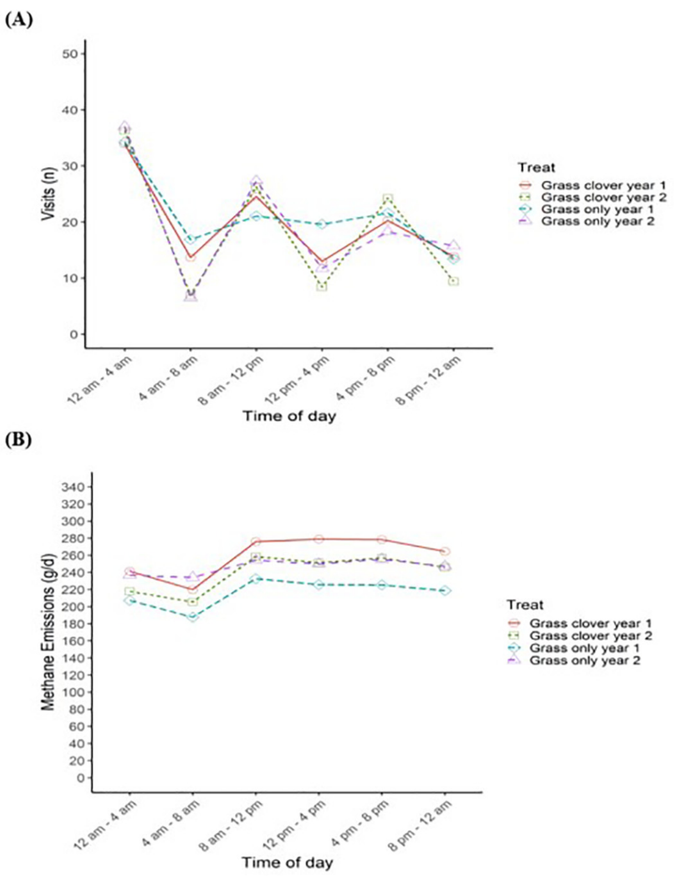

FIGURE 1
(A) Total visits (n) at each 4 hour interval to the Greenfedd emissions monitoring unit and **(B)** average daily CH_4 emissions (g/d) at each 4 hour interval over the day for pre-partum dairy cows within the grass only silage treatment in both year 1 and year 2 of the experiment.

(2021). This process involved performing regression equations of CO_2 *versus* CH_4 and CH_4 *versus* CO_2. These equations gave the predicted and residual values of each measurement. The standard deviation of each gas was determined and then used to divide the residuals of the linear model. Any measurement with residuals/SD greater than ±3 was removed from the dataset.

The methane yield (in grams per kilogram TDMI) for individual cows was derived by dividing the average daily CH_4 emissions for each cow with their corresponding TDMI. The silage and concentrate DMI measurements were multiplied by the corresponding gross energy content of the feed and summed together to calculate the gross energy intake (GEI) of individual cows. The GEI of individual cows was used to calculate the percentage of GEI converted to CH_4 energy (Eugène et al., 2019). This was calculated by multiplying each cow's daily CH_4 (in kilograms per day) by the energy (55.65 MJ) density found in a kilogram of CH_4. The total megajoules (MJ) of methane energy emitted by a cow was then divided by the cow's GEI, calculating the percentage of GEI as CH_4 energy (Liu et al., 2017).

The predicted DMI and CH_4 emissions were calculated following country-specific IPCC tier 2 methodology (IPCC, 2019), as outlined in Ireland's national inventory for the calculation of national CH_4 emissions (Herron et al., 2022; EPA, 2022). Predicted DMI (in kilograms) was calculated using the net energy (NE) system derived by Jarrige et al. (1986) and modified by O'Mara, 2006 following the INRA methodology for the calculation of dairy cow NE requirements for specific feeding systems. In Ireland, when dairy cows are housed and on a grass silage-based diet, a country-specific equation developed by Yan et al. (2000) is used to calculate the enteric CH_4 emissions, as outlined in Ireland's national inventory (O'Mara, 1996; EPA, 2022).

$$CH_4 = DEI^*[0.096 + (0.035 \times S_{DMI}/T_{DMI})] - 2.298^*(FL - 1)$$

where DEI is the digestible energy intake (in megajoules per day), S_{DMI} is the silage DM intake (in kilograms per day), T_{DMI} is the total DM intake (in kilograms per day), and FL is the feeding level (multiples of maintenance energy requirement). The digestible energy intake was derived using the OM digestibility of the corresponding offered silage during the experimental period. The individual predicted DMI and CH_4 emissions were then compared with the individual measured DMI and CH_4 emissions for each individual animal.

Statistical analysis

All data analysis was conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Data were checked for normality using PROC UNIVARIATE and analysed with a linear mixed model, which allowed for repeated measurements using PROC MIXED. Forage data were analysed using the following statistical model:

$$Y_{ijk} = \mu + T_i + W_j + Y_k + T_i \times W_j + T_i \times Y_k + e_{ijk}$$

where Y_{ijk} is the dependent variable, μ is the overall mean, T_i is the effect of treatment (i = GC or GO), W_j is the effect of week (j = 1–6), Y_k is the effect of year (k = 1 or 2), $T_i \times W_j$ is the interaction between treatment and week, $T_i \times Y_k$ is the interaction between treatment and year, and e_{ijk} is the residual error.

The bodyweight, average daily gain, and BCS were analysed using the following model:

$$Y_{wjimnk} = \mu + B_w + P_j + T_i + C_m + P_n + Y_k + T_i \times P_n + T_i \times Y_k + X_{wjimnk} + e_{wjimnk}$$

where Y_{wjink} is the dependent variable, μ is the overall mean, B_w is the breed (w = 1 or 2), P_j is parity (j = 1 or 2 or 3+), T_i is the effect of treatment (i = GC or GO), C_m denotes cow within treatment (m = 1–15), P_n is the effect of week (n = 1–6), Y_k is the effect of year (k = 1–2), $T_i \times W_n$ is the interaction between treatment and week, $T_i \times Y_k$ is the interaction between treatment and year, X_{wjimnk} denotes the pre-experimental bodyweight variables, and e_{wjimnk} is the residual error.

The enteric CH_4 emissions, SDMI, CDMI, TDMI, and the predicted CH_4 emissions and predicted DMI were analysed using the following model:

$$Y_{wjimnk} = \mu + B_w + P_j + T_i + C_m + W_n + Y_k + T_i \times W_n + T_i \times Y_k + DTC_{wjimnk} + e_{wjimnk}$$

where Y_{wjink} is the dependent variable, μ denotes the overall mean, B_w is the breed (w = 1 or 2), P_j is parity (j = 1 or 2 or 3+), T_i is the effect of treatment (i = GC or GO), C_m denotes the cow within treatment (m = 1–15), W_n is the effect of week (n = 1–6), Y_k is the effect of year (k = 1–2), $T_i \times W_n$ is the interaction between treatment and week, $T_i \times Y_k$ is the interaction between treatment and year, DTC_{wjimnk} denotes the days until calving, and e_{wjimnk} is the residual error.

The post-partum milk production, bodyweight, and BCS were analysed using the following model:

$$Y_{wjimnk} = \mu + B_w + P_j + T_i + C_m + W_n + Y_k + T_i \times Y_k + e_{wjimnk}$$

where Y_{wjink} is the dependent variable, μ is the overall mean, B_w represents breed (w = 1 or 2), P_j denotes parity (j = 1 or 2 or 3+), T_i is the effect of pre-partum treatment (i = GC or GO), C_m denotes cow within the pre-partum treatment (m = 1–15), W_n is the effect of week of lactation (n = 1–12), Y_k is the effect of year (k = 1–2), $T_i \times Y_k$ is the interaction between pre-partum treatment and year, and e_{wjimnk} is the residual error.

Differences between the predicted and the measured DMI and enteric CH_4 emissions were analysed using a PROC TTEST procedure for individual measurements within each treatment.

All results reported are least square means values, with significant effect declared when $p \leq 0.05$ and tendencies declared from $0.05 \leq p \geq 0.10$.

Results

Chemical composition

There were significant (p< 0.001) effects of silage treatment on all chemical composition variables, except for the crude protein content (Table 2). The chemical composition of silage was not consistent across experimental years, and the DM content of GC silage was lower (p< 0.05) in year 2 when compared with year 1 of the experiment (199.1 and 374.1 g/kg, respectively). GC silage had greater (p< 0.001) OM digestibility when compared with GO silage across both years of the experiment (716.6 and 676.8 g/kg DM, respectively). The ash content was not significantly (p > 0.05) different for each silage treatment; however, the ash contents of both silage types increased (p< 0.001) in year 2 when compared with year 1. The crude protein content of the GO silage decreased by 35.2 g/kg DM from year 1 to year 2, resulting in a significant (p< 0.05) treatment-by-year interaction in year 2. The GC silage had a lower (p< 0.001) NDF content when compared with the GO treatment silage (484.3 and 558.6 g/kg DM, respectively). The acid detergent fibre content was greater (p< 0.001) in the GO silage when compared with that in the GC silage (335.1 and 292.3 g/kg DM, respectively). The gross energy content of the silage was not affected (p > 0.05) by treatment; however, the GE content was lower (p< 0.001) in year 2 compared with that in year 1 (17.7 and 18.6 MJ/kg DM, respectively).

Bodyweight, body condition score, and dry matter intake

The bodyweight, BCS, and the DMI parameters are reported in Table 3. Bodyweight tended to be greater (p = 0.071) for cows within the GC treatment when compared with cows in the GO treatment. Over the course of the experiment, the average daily gain was not affected by treatment. The BCS were not affected by treatment (p > 0.05) across both years of the experiment.

There was no effect of treatment on CDMI. Silage DMI was affected by treatment, with the GC treatment having greater (p< 0.05) SDMI compared with the GO treatment (10.9 and 8.1 kg/cow per day, respectively). There were differences in SDMI across treatments and years: in year 1, the GC treatment consumed 3.7 kg of additional (p< 0.001) SDMI when compared with the GO treatment. In year 2 of the experiment, the SDMIs were similar between treatments, highlighting a significant (p< 0.001) treatment-by-year interaction. The GO treatment cows had greater (p< 0.05) SDMI (+1.4 kg) in year 2 compared with year 1 (11.0 and 8.7 kg/cow per day, respectively). The total DMI was affected by treatment, with the key differences across treatments and years similar to those of SDMI (Table 3). The GC treatment had greater (p< 0.05) GEI compared with the GO treatment (214.6 and 191.6 MJ/cow per day, respectively). There were differences in the GEI across treatments and years, with the GO treatment cows having greater (p< 0.001) GEI in year 2 compared with year 1 (229.9 and 183.8 MJ/cow per day, respectively), while the GC treatment cows showed a decline in GEI (p< 0.001) from year 1 to year 2 (252.8 and 196.1 MJ/cow per day, respectively).

The predicted DMIs for both treatments are reported in Table 3. There was no difference for predicted DMI between treatments (9.5 and 9.9 kg, respectively). The predicted DMI was greater (p< 0.05) for all animals in year 2 when compared with the animals in year 1 of the experiment (9.1 and 10.3 kg/cow per day, respectively). For both treatments, the predicted DMIs were significantly lower (p< 0.05) than the measured DMIs. For the GC treatment, the predicted DMI was reduced (p< 0.001) by 2.4 kg, while the GO treatment exhibited a predicted DMI reduction (p< 0.05) of 0.5 kg.

Enteric methane emissions

There was no effect (p > 0.05) of treatment on the daily CH_4 emissions. The methane yield (in grams per kilogram TDMI) was affected (p< 0.05) by silage treatment, with the cows offered GC silage having lower CH_4 yields (in grams per kilogram TDMI) compared with the cows in the GO treatment (20.9 and 22.0 g/kg TDMI, respectively). There was a significant (p< 0.001) effect of year for both treatments due to the greater CH_4 yield (in grams per kilogram TDMI) in year 2 when compared with that in year 1 of the experiment (20.0 and 22.9 g/kg TDMI, respectively). Across the 2-year experiment, the greatest difference in CH_4 yield was evident in year 1 of the experiment, with the GC treatment having a 17% lower CH_4 yield, with no difference reported in year 2, highlighting a significant (p< 0.001) treatment-by-year interaction. There was a significant (p< 0.001) treatment-by-year interaction for the percentage of GEI as CH_4 energy, with the GC treatment having

TABLE 2 Chemical composition of grass clover and grass-only silages offered to dairy cows over the pre-partum period in years 1 and 2 analysed for treatment, year, and their relative interaction.

Item	Year 1		Year 2		SEM	p-value[a]		
	Grass clover	Grass only	Grass clover	Grass only		Treatment	Year	Treatment × year
Dry matter (g/kg)	374.1	218.3	199.1	217.2	0.80	0.001	0.001	0.001
Organic matter digestibility (g/kg DM)	726.0	680.8	707.2	672.7	0.47	0.001	0.062	0.450
Ash (g/kg DM)	92.2	96.6	132.1	101.9	5.44	0.066	0.023	0.037
Crude protein (g/kg DM)	148.6	164.2	149.5	130.4	2.13	0.302	0.001	0.001
Neutral detergent fibre (g/kg DM)	483.6	547.9	488.0	571.3	6.08	0.001	0.148	0.942
Acid detergent fibre (g/kg DM)	283.0	336.6	303.3	333.3	4.57	0.001	0.449	0.033
Gross energy (MJ/kg DM)	18.8	18.6	17.6	17.9	0.04	0.140	0.001	0.001

[a]For p-value, significant effect is declared when $p \leq 0.05$, while tendencies are declared from $0.05 \leq p \geq 0.10$.

TABLE 3 Bodyweight, average daily gain, body condition score, silage dry matter intake (DMI), total DMI, gross energy intake (GEI), and predicted DMI of dairy cows within the grass clover and grass-only treatments during the pre-partum period analysed for treatment, year, and their relative interaction.

Item	Year 1		Year 2		SEM	*p*-value[a]		
	Grass clover	Grass only	Grass clover	Grass only		Treatment	Year	Treatment × year
Bodyweight (kg)	633	612	623	623	5.4	0.071	0.885	0.096
Average daily gain (kg/day)	1.1	0.9	1.1	1.0	0.47	0.689	0.002	0.723
Body condition score (1–5)	3.3	3.3	3.3	3.3	0.06	0.935	0.506	0.648
Concentrate DMI (kg/cow per day)	0.7	0.7	0.7	0.7	0.02	0.612	0.008	0.202
Silage DMI (kg/cow per day)	12.4	8.7	9.8	11.0	0.40	0.001	0.001	0.001
Total DMI (kg/cow per day)	13.1	9.4	10.5	11.7	0.40	0.001	0.001	0.001
GEI (MJ/cow per day)	252.8	183.8	196.1	229.9	7.35	0.003	0.202	0.001
Predicted DMI (kg/cow per day)[b]	9.0	9.2	9.0	10.5	0.3	0.235	0.002	0.520

[a]For *p*-value, significant effect is declared when $p \leq 0.05$, while tendencies are declared from $0.05 \leq p \geq 0.10$.
[b]Calculated using the net energy requirement system (O'Mara, 1996).

a lower ($p< 0.05$) percentage of GEI as CH_4 energy when compared with the GO treatment in year 1. For cows on both treatments, the percentage of GEI as CH_4 energy increased from year 1 to year 2 (5.7% and 6.6%, respectively).

The predicted CH_4 emissions were not significantly ($p > 0.05$) affected by silage treatment (Table 4). The predicted CH_4 emissions were greater ($p< 0.05$) than the measured CH_4 emissions, with 10.9 and 26.6 g/cow greater predicted CH_4 emissions in the GC and GO treatments, respectively, compared with the measured CH_4 emissions.

Post-partum bodyweight, body condition score, and milk production

The post-partum milk production was not affected by the silage treatment imposed in the pre-partum period for all measured variables (Table 5). In addition, the silage treatment during the pre-partum period did not have a significant ($p > 0.05$) effect on the bodyweight and BCS during the first 12 weeks of lactation post-partum (Table 5). However, there was a significant ($p< 0.05$) effect of year on post-partum BCS.

Discussion

The findings of the current experiment support the hypothesis that the silage system impacts the forage digestibility, which in turn impacts both the animal DMI and enteric CH_4 emissions in pre-partum dairy cows. Grass silage is well acknowledged to be a cost-effective source of feed in the Irish pasture-based dairy system and is subsequently fed over the housed winter period (Butler, 2014; Doyle et al., 2022). During the pre-partum period, the digestibility of the diet offered to dairy cows impacts the level of DMI and the metabolic status (Richards et al., 2020); however, the level of CH_4 emissions during this time has received less attention. It is widely acknowledged that the digestibility of the offered diet dictates the level of DMI achieved, which is a key driver of the CH_4 emissions in dairy cows (Parnian-Khajehdizaj et al., 2023).

TABLE 4 Methane emissions, carbon dioxide emissions, methane yield, percentage gross energy intake (GEI) as methane energy, and predicted methane emissions of dairy cows within the grass clover and grass-only treatments during the pre-partum period analysed for the effect of treatment, year, and their relative interactions.

Item	Year 1		Year 2		SEM	*p*-value[a]		
	Grass clover	Grass only	Grass clover	Grass only		Treatment	Year	Treatment × year
Methane (g/cow per day)	244	200	233	254	8.1	0.112	0.010	0.002
Carbon dioxide (kg/cow per day)	9.5	8.7	8.6	9.0	0.19	0.170	0.183	0.002
Methane/DMI (g/kg TDMI)[b]	18.4	21.6	23.5	22.4	0.48	0.041	0.001	0.001
Methane/GEI (%)[c]	5.3	6.1	7.0	6.3	0.15	0.090	0.001	0.001
Predicted methane (g/cow)[d]	255	245	260	271	10.7	0.976	0.225	0.313

[a]For *p*-value, significant effect is declared when $p \leq 0.05$, while tendencies are declared from $0.05 \leq p \geq 0.10$.
[b]Methane yield = methane divided by dry matter intake.
[c]Megajoules of methane per megajoule of gross energy intake (in percent).
[d]Calculated using the IPCC (2019) tier 2 methodology.

TABLE 5 Bodyweight, body condition score, and milk production of dairy cows over the first 12 weeks of the lactation within the pre-partum grass clover and grass-only treatments analysed for treatment, year, and their relative interaction.

Item	Year 1		Year 2		SEM	p-value[a]		
	Grass clover	Grass only	Grass clover	Grass only		Treatment	Year	Treatment × year
Bodyweight (kg)	525	522	541	543	8.6	0.913	0.074	0.800
Body condition score (1–5)	3.3	3.3	3.1	3.1	0.04	0.822	0.005	0.716
Milk yield (kg/cow)	23.9	22.0	21.8	22.4	1.26	0.602	0.577	0.358
Milk solids (kg/cow)	2.2	2.0	2.0	2.0	0.11	0.356	0.523	0.360
Milk fat (kg/cow)	1.3	1.2	1.2	1.2	0.07	0.400	0.192	0.363
Milk protein (kg/cow)	0.8	0.8	0.8	0.8	0.04	0.538	0.609	0.227
Milk lactose (kg/cow)	1.2	1.1	1.1	1.1	0.05	0.310	0.896	0.201

[a]For p-value, significant effect is declared when $p \leq 0.05$, while tendencies are declared from $0.05 \leq p \geq 0.10$.

Throughout the experiment, key differences in the silage quality became evident between treatments and years. The greater DM content in the GC silage resulted in the GC treatment cows having greater DMI, which resulted in greater CH_4 emissions in year 1. The inclusion of clover in swards has been reported to increase the OM digestibility due to the lower NDF content (Egan et al., 2018), which was clear in the current experiment findings. Offering forages with a lower fibre content can impact the physical effective NDF, which is a key driver of DMI and rumen fermentation (Grant, 2023). This is attributed to the differing lignin contents and compositions, resulting in variations in the cell wall concentrations between PRG and clover (Jung, 1989). GC silage has been reported to increase DMI, particularly due to the lower fibre content increasing the rate of digestion (Lind et al., 2020). In year 2 of the experiment, no differences were evident in the SDMI, as both silage treatments had similar DM, NDF, and OM digestibility, highlighting that forage digestibility had the greatest impact on individual animal SDMI, over sward species or silage system (Bica et al., 2022). To maintain a high level of white clover in grazing swards, it is recommend to keep a lower herbage mass (Barthram and Grant, 1994; Murray et al., 2022), resulting in a lower pre-cutting herbage mass in the current study for the GC silage sward as the GC sward was grazed prior to being closed for silage harvesting (Egan et al., 2018). In contrast, as the GO silage sward was not grazed before silage harvesting, it had a greater pre-cutting herbage mass. This resulted in the GO silage having lower OM digestibility and greater fibre content (Humphreys and O'Kiely, 2006; Pang et al., 2021; Álvarez et al., 2022). Offering forages with greater fibre content has been reported to have lower rumen degradability within the rumen, which in turn increases the rumen retention times, thus reducing the animal DMI (Gregorini et al., 2013; McCarthy et al., 2023). This response was clear in cows on the GO treatment, as the GO silage had greater fibre content and lower OM digestibility, which reduced the DMI (−3.7 kg DM/cow), whereas the GC treatment silage had a greater level of OM digestibility, which is associated with greater rumen passage rates, resulting in greater propionate concentrations within the rumen (Van Gastelen et al., 2019). Legumes such as white clover, due to their different plant morphology, have been reported to have a greater rumen passage rate due to the smaller particle size in the rumen when compared to PRG with long fibres that reduce the passage rate and the rumen effective degradability (Dewhurst et al., 2009).

It is well acknowledged that, during the pre-partum period, dairy cows have lower levels of DMI and enteric CH_4 emissions when compared with cows in the lactating period (Ulyatt et al., 2002; Lyons et al., 2018; Meese et al., 2020). The levels of CH_4 emissions reported over the dry period in the current experiment, 200–254 g/cow per day, are similar to those reported by Ferris et al. (2017) and Jonker et al. (2017), who reported CH_4 emissions of 173–215 g/cow per day when cows were offered grazed grass or grass silage and beet. In the current experiment, similar to those in Pinares-Patiño et al. (2007), the enteric CH_4 emissions were correlated (R^2 = 0.46) with the DMI over the dry period (Figure 2). This was evident in year 1 of the experiment, where the GC treatment emitted 18% greater daily CH_4 emissions compared with the GO treatment, due to a 26% increase in the TDMI. In year 2, differences in the CH_4 emissions and the CH_4 yields were not as apparent due to reductions in the DM content, the OM digestibility, and the NDF and ADF contents in both silage treatments. Greater intake of digested OM is positively correlated with daily CH_4 due to the greater rumen fill and rumen passage rate when feeding forage-based diets (Parnian-Khajehdizaj et al., 2023). The overall contribution of legumes to CH_4 emissions in ruminant livestock is still under investigation, with contrasting responses dependent on the animal species, the production system, and their proportions in the diet (Bica et al., 2022). Numerous studies have reported elevations in the performance of dairy cows with the inclusion of clover in the diet, mainly due to the enhanced forage digestibility, which increases ruminal passage (Bica et al., 2022), thus increasing the intake capacity of the ruminant animal (Brask et al., 2013; Van Dorland et al., 2007; Dineen et al., 2018). Similar to the current experiment, Van Dorland et al. (2007) reported a 64% lower fibre content in white clover silage, elevating the DMI of dairy cows, which resulted in greater CH_4 emissions, although no difference in the CH_4 yield was evident (in litres per kilogram OM digested) when compared with red clover and PRG silage.

It has been reported that the digestibility and energy content of the diet offered during the pre-partum period affect the subsequent

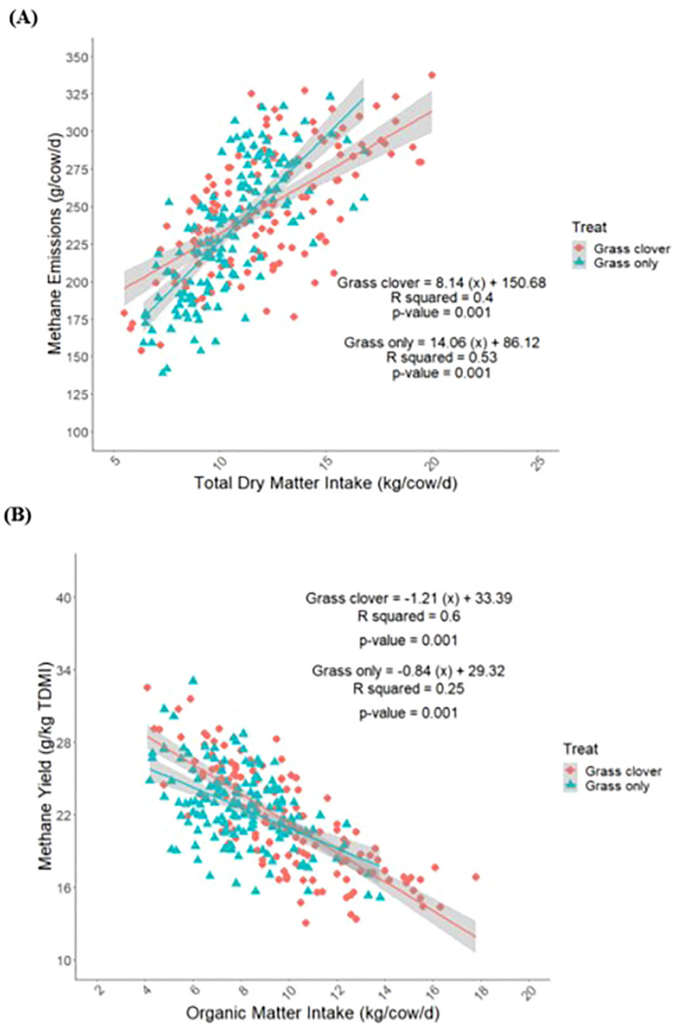

FIGURE 2
Relationship between **(A)** methane emissions (g/cow/d) and total dry matter intake (kg/cow/d) and **(B)** methane yield (g/kg TDMI) and organic matter intake (kg/cow/d) of dairy cows within both the grass clover and grass only treatments with each ● and ▲ represents one measurement point for each animal across the pre-partum experimental period in both years.

post-partum milk production and the metabolic status of dairy cows (Butler et al., 2011; Mann et al., 2015). Janovick et al. (2022) reported that dairy cows fed above their energy requirement pre-partum showed a reduced milk production post-partum, with greater incidences of metabolic disorders due to the greater lipid deposition on the liver on day 14 post-partum as these cows had greater BCS at calving. In the current experiment, the GC treatment had greater levels of TDMI over the experimental period, particularly in year 1; however, no effects on post-partum milk production were evident mainly due to there being no differences in the BCS during the experimental period and over the first 12 weeks of lactation. In pasture-based dairy systems, the diet offered during the pre-partum period does not have the main goal of improving milk production (Butler, 2014) when compared with confined production systems (Mann et al., 2015; Richards et al., 2020). The benefit in the DMI reported in the present study would suggest that offering high-quality GC silage to dairy cows is more beneficial during the lactation to help improve milk production, particularly in the spring or autumn, when grass growth is reduced, in pasture-based dairy systems (O'Brien et al., 2018; Walsh et al., 2023). Post-partum dairy cows experience negative energy balance due to the onset of milk production, therefore supporting the nutrient demands with highly digestible forages being key to maximising production in pasture-based dairy cows (Butler, 2014; Delaby et al., 2021). According to reports, the supplementation of grass silage over highly digestible grazed grass during the lactation has been shown to reduce animal performance, particularly with regard to the milk protein concentration (Claffey et al., 2019; Walsh et al., 2023). Therefore, improving the quality of the offered silage could potentially improve the DMI and, in turn, improve or maintain animal performance when low grass availability necessitates silage supplementation.

The substantial positive association between DMI and enteric CH_4 emissions renders the estimation of DMI indispensable for accurately predicting enteric CH_4 emissions (Appuhamy et al., 2016). Ferris et al. (2017) reported a stronger relationship between DMI and CH_4 emissions compared with that between bodyweight and CH_4 emissions when predicting CH_4 from non-lactating dairy cows offered grazed grass. The NE system used to predict DMI (Jarrige et al., 1986) does not take into account the rumen fill or the intake capacity of the cow with the corresponding forage digestibility, which can result in an overestimation of the prediction of CH_4 emissions, as reported in the current study. The use of predicted DMI to calculate daily CH_4 emissions resulted in 11% greater predicted CH_4 emissions in the GO treatment when compared with the measured CH_4 emissions (Table 4). The methodology used in the current experiment is highly dependent on the fill value and the digestible energy content of the consumed forages (Binggeli et al., 2022). Moraes et al, 2014 highlighted that models that account for dietary NDF and ether extract are effective in accurately predicting enteric CH_4 over the non-lactating period in dairy cows. Greater levels of DMI result in increased rumen fermentation, which can lead to higher CH_4 emissions (Hristov et al., 2013). The current study demonstrated a positive correlation between TDMI and daily CH_4 (R^2 = 0.47) (Figure 2). However, both silage types had contrasting OM digestibility content, which would result in different rumen passage rates (McCarthy et al., 2023). The rumen fermentation kinetics and rumen fill are highly dictated by both the animal bodyweight and the digestibility of the offered forages (Moraes et al., 2014), explaining the contrasting results between the predicted and the calculated DMIs and enteric CH_4 emissions in the current experiment.

Data on forage digestibility and herd-averaged DMI have the potential to be commercially available in ruminant livestock systems in Ireland (O'Mara, 2006). The estimation of CH_4 emissions using IPCC methods is dependent on the data availability in a specific country. The use of tier 2 calculations requires information on animal categories, feeding systems, production systems, and manure management (IPCC, 2019). When all elements connected to CH_4 are understood, a country-specific tier 3 methodology can be utilised to create in-depth descriptive data on feeding systems that link the conversion factors to the calculated energy requirements of the specific animal (Eugène et al., 2019). The current experiment offers valuable insights into the impact on the animal DMI and CH_4 emissions when GC bale silage or GO pit silage is offered to dairy cows during the pre-partum period in pasture-based dairy systems. The use of GC bale silage during the pre-partum period is not advantageous as the substantial increase in DMI due to greater digestibility has no benefit on milk production, but results in greater CH_4 during the pre-partum period. The digestibility of the offered silage plays a pivotal role in the levels of DMI and CH_4 emissions in dairy cows; therefore, considerations should be taken when feeding silage types with superior digestibility.

Conclusions

This study evaluated the digestibility of GC silage and GO silage, focusing on their effects on the DMI, the enteric CH_4 emissions, and the post-partum milk production of dairy cows during the pre-partum period. GC silage had greater OM digestibility and lower fibre content, which resulted in greater DMI when offered to GC treatment cows. This resulted in the GC treatment cows having higher CH_4 emissions, but a lower CH_4 yield, than the GO treatment cows. No benefit in post-partum milk production was evident from offering GC silage during the pre-partum period. The study also highlighted discrepancies in the current national inventory calculations, which overestimated CH_4 emissions by up to 10% for both silage types. Overall, the study provides valuable insights into the impact of silage systems and forage quality on enteric CH_4 emissions and offers essential data on DMI and CH_4 emissions over the pre-partum period, which are crucial for the development of effective CH_4 mitigation strategies in dairy systems.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by Teagasc Animal Ethics Committee (TAEC) (TAEC247/2019). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

MK: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Visualization, Writing – original draft, Writing – review & editing. BL: Data curation, Formal analysis, Writing – review & editing. JH: Data curation, Formal analysis, Writing – review & editing. TB: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. CF: Data curation, Investigation, Writing – review & editing. ME: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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