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Corresponding author:
H. Costigan;
Email: hazel.costigan@teagasc.ie

Biorefined perennial ryegrass press cake as an alternative feed for dairy cows in late lactation and during the dry period: a demonstration

H. Costigan[1], B. Lahart[1], M. Kennedy[1,2], J. Herron[1], J. P. M. Sanders[3], J. Gaffey[4], B. Lambrechts[3] and L. Shalloo[1]

[1]Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland; [2]School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland; [3]Grassa BV, Venlo, SZ, The Netherlands and [4]Circular Bioeconomy Research Group, Shannon Applied Biotechnology Centre, Munster Technological University, Tralee, Ireland

Abstract

To investigate the potential application of replacing a proportion of a perennial ryegrass (PRG) silage diet with press cake on productivity and enteric methane (CH_4) emissions in late lactation and non-lactating spring-calving dairy cows, a study was undertaken in which control cows ($n = 21$) were offered PRG silage, while treatment cows ($n = 21$) were offered a diet consisting of 60% PRG press cake and 40% of the same PRG silage. Although treatment cows had higher group average dry matter intakes (DMI) and produced more enteric CH_4, carbon dioxide (CO_2), milk solids, protein, fat- and protein-corrected milk yield (FPCM) in late lactation, the magnitude of the difference between treatment and control cows varied from week to week ($P < 0.050$). When enteric CH_4 per kg of milk yield, milk solids and FPCM were considered, there was no significant difference between treatment and control. Absolute enteric CH_4 was higher for cows fed press cake during the non-lactating period but this tended to vary from week to week. Similarly, CO_2 ($P < 0.001$) and hydrogen (H_2; $P = 0.023$) differed from week to week for cows offered press cake, and cows offered PRG silage in the non-lactating period. Although there was no significant effect of diet on body weight (BW) and body condition score (BCS), when enteric CH_4 was expressed on a per kg BW basis, cows offered press cake tended to produce more enteric CH_4 in both late lactation and during the dry period.

Introduction

Pasture-based dairy farming is currently amongst the most sustainable in terms of food-feed competition, such that 4.92 kg of human edible protein is produced for every 1 kg of human edible protein consumed in Irish pasture-based systems (Hennessy *et al.*, 2021). Nonetheless, as drought (Emadodin *et al.*, 2021) and rainfall events (Vrac *et al.*, 2023) are more prevalent of late, it is becoming increasingly difficult to optimize the proportion of grazed grass in the diet of pasture-based animals. As such, there is concentrate feed incorporated into the diet of forage-fed dairy cows in order to alleviate the seasonal variation in grass growth and quality (O'Brien *et al.*, 2018). Green biorefinery provides a unique opportunity to further support existing native forages and as such, reduce the reliance on imported feed (Hörtenhuber *et al.*, 2011). The untapped potential of biorefined perennial ryegrass (PRG) to increase the utilization of a forage has previously been highlighted by Gaffey *et al.* (2023) whereby the outputs are a high-protein press juice, which may be used to produce feed for monogastrics (Keto *et al.*, 2020; Ravindran *et al.*, 2021; Gaffey *et al.*, 2023), and a press cake which may be used as a replacement for grass silage in the diet of ruminant livestock (Sanders *et al.*, 2023). Previous trials on press cake have been carried out at various dietary inclusion rates and at different stages of lactation, therefore it is difficult to discern the true effect of substituting grass silage with press cake on milk production performance (Damborg *et al.*, 2019; Sousa *et al.*, 2021). The enteric CH_4 abatement potential of press cake also showed promise when incorporated into a ration containing grass silage, concentrate and soybean meal and simulated using a Rusitec device (Serra *et al.*, 2023); however, to the best of the author's knowledge, this has not been evaluated *in vivo*.

In pasture-based systems such as Ireland, grazed grass accounts for 74–77% of the annual spring calving dairy cow diet, on a fresh matter basis (O'Brien *et al.*, 2018), therefore, the potential application of substituting grass silage with press cake is largely confined to the winter housing period, i.e. in either late lactation, the non-lactating period, early lactation or in autumn calving cows as is outlined by Serra *et al.* (2023). There is significant year-to-year variation in silage quality both in Ireland (Patterson *et al.*, 2021) and internationally (Nousiainen et al., 2009), which may be attributable to adverse weather conditions. In contrast, the quality of biorefined press cake is independent of adverse weather conditions and as such may have practical application both in pasture-based systems during the winter housing period, and year round in indoor feeding systems of dairying. The objective of the present study was therefore to investigate the potential application of partially replacing PRG silage with PRG press cake on productivity and enteric CH_4 emissions of late lactation and non-lactating spring calving dairy cows.

Materials and methods

The study was undertaken at Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Co. Cork, Ireland (52°09′N 8°16′W) between November 2021 and January 2022. The Teagasc Animal Ethics Committee (TAEC) (TAEC2021-313) granted ethical approval. All experiments were conducted in accordance with the Cruelty to Animals Act (Ireland 1876, as amended by European Communities regulations 2002 and 2005) and the European Community Directive 86/609/EC.

Experimental design

For 2 weeks prior to the commencement of the experimental feeding period, 32 multiparous spring-calving dairy cows were housed and adjusted to an over-winter diet of PRG silage during which baseline data for milk production, enteric CH_4, body weight (BW) and body condition score (BCS) were collected. The animals were then divided into two homogeneous treatment groups ($n = 21$ cows per group) using a balanced randomization procedure based on breed (Holstein-Friesian or Jersey × Holstein-Friesian), parity (2.2 ± 0.87 lactations), calving date (12 February 2021 ± 18.2 days), mean daily milk yield (14 ± 2.9 kg/day), days in milk (268 ± 16.5 days), BW (559 ± 62.8 kg), BCS (3.0 ± 0.53 units) and enteric CH_4 emissions (385 ± 60.8 g/day) data that were collected during the pre-experimental period. The treatment groups comprised of cows fed a press cake and a PRG silage (GSPC) mix, and those fed an exclusively PRG silage (GS) based diet.

Feed processing

The grass silage (PRG with white clover) was grown on site in the Teagasc Moorepark Animal & Grassland Research and Innovation Centre as described by Kennedy *et al.* (in review). Grass silage was mechanically harvested in June 2021, and subsequently stored in a silage pit. The press cake fraction of the GSPC mix (English ryegrass with white clover) was grown near Afferden, Limburg, in the Netherlands, where it was cut and mechanically harvested in July and August 2021. Within 6 h of cutting, the grass for the press cake was processed using a biorefinery unit (Grassa BV, 5928 SZ Venlo, the Netherlands). The grass was crushed using an extruder, which produced two primary products, a high solid fibre fraction (press cake) and a liquid juice fraction (press juice). The press cake was then ensiled and baled, during which period the press cake had fermented sufficiently before being transported to Ireland in October 2021.

Housing management

Cows were housed in a slatted cubicle shed for the duration of the experiment. Cubicles were provided at a cow to cubicle ratio of 1:1. Cows were fed GS and GSPC through a post and rail style feed barrier. The GS and GSPC treatment groups were housed in pens at opposite ends of the cubicle shed; each group had access to a GreenFeed emissions monitoring system machine (C-Lock Inc., Rapid City, SD, USA). The treatment groups were swapped between pens once weekly to remove any pen or machine bias.

Feed management

Prior to the commencement of the study, a 2-week period of adjustment to the winter diet was imposed during which all cows were offered a PRG silage diet, in addition to approximately 3 kg concentrate/cow/day on a fresh weight basis (22% crude protein [CP; on a DM basis], Dairygold Co-Operative Society Ltd, Lombardstown Mill, Mallow, Co. Cork, Ireland); 2 kg of which was fed through the milking parlour at scheduled milking times (07.00 or 14.30 h), while the remaining 1 kg was fed through the GreenFeed machines. Data collected during the aforementioned 2-week adjustment period were used to block cows to treatment and control groups.

The trial began on 2 November 2021; from 2 November until 10 December 2021 (lactating period), cows were milked at scheduled milking times of 07.00 and 14.30 h. During the lactating period, cows were offered *ad-libitum* forage to achieve 10% refusals such that GS cows were offered PRG silage, and GSPC cows were offered a mix of PRG silage (40%) and PRG press cake (60%) on a fresh weight basis. The PRG silage and press cake were mixed using a Keenan mixing wagon (Keenan, Richard Kenan & Co. Ltd, Borris Co. Carlow, Republic of Ireland) for a 5 min period prior to feed out.

From 10 December until 20 December, a transition phase was implemented, during which all the cows were dried off under a restricted feed regime. During the 2-week transition phase, concentrates were not offered to facilitate a natural decline in milk synthesis; therefore, cows did not have access to the GreenFeed during this time. The non-lactating period of the trial began on 20 December 2021 and ended on 24 January 2022. During the non-lactating period, cows were also offered *ad-libitum* forage to achieve 10% refusals, the GS cows were offered PRG silage, and the GSPC cows were offered a mix of PRG silage (40%) and PRG press cake (60%) on a fresh weight basis, along with 1 kg/cow/day of concentrate which was fed through the GreenFeed machine. Treatment and control cows received the same concentrate supplementation throughout the trial.

Animal measurements

During the late lactation phase of the study, milk yield was recorded daily at each milking (07.00 and 14.30 h) using electronic milk meters (Dairymaster, Causeway, Co. Kerry, Ireland). Milk was sampled once weekly during successive evening and morning milkings and analysed by near-infrared reflectance spectroscopy using a MilkoScan 203 (DK 3400, Foss Electric Hillerød, Denmark). Fat- and protein-corrected milk yield (FPCM; IDF, 2015) was calculated as (milk production [kg] × [0.1226 × milk fat % + 0.0776 × milk protein % + 0.2534]). During the transition phase, all cows were dried off over the course of a 10-day period, with cows that were closest to calving and those with the lowest milk yield, i.e. less than 7 litres/cow/day, dried off first. BW and BCS were measured weekly during the lactating phase and fortnightly during the dry phase. Bodyweight was measured using electronic weighing scales (Tru-Test Ltd., Auckland, New Zealand) and

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the scales were calibrated prior to using weights. BCS was assessed on a scale of 1–5 by a trained and experienced professional (1 = emaciation and 5 = obesity; Edmonson *et al.*, 1989).

Group average dry matter intake (DMI) was calculated as the weight of the feed offered minus the weight of the feed refused, divided by the number of animals in each pen. Refusals were weighed approximately three times per week. Samples of feed were taken three times per week from along the feed face, a subsample of which (approximately 100 g) was dried at 90°C for dry matter (DM) determination. The fresh weight intake was multiplied by the corresponding DM percentage to determine the group average DMI.

Throughout the experimental period, enteric CH_4, hydrogen (H_2) and carbon dioxide (CO_2) were measured using the GreenFeed emissions monitoring system (C-Lock Inc.), as described in detail by Hammond *et al.* (2015). In brief, cows were offered a small quantity of bait concentrate as an incentive to visit the GreenFeed machine, during which a sample of the animal's breath was taken and analysed for CH_4, H_2 and CO_2 concentration. Animals were trained to the units during a 3-week acclimatization period, which took place prior to the 2-week pre-experimental period. The minimum time between GreenFeed visits was set to 6 h for the duration of the study, with concentrate dispensed every 25 s, to a maximum of six concentrate drops per visit for each animal. The mean (SD) weight of the concentrate drops was 33.8 g (SD = 0.67 g). The mean (SD) of GreenFeed visits of GSPC and GS cows throughout the experiment was 2.8 (0.94) and 2.8 (0.97) and visits per cow per day, respectively. Each individual visit lasted an average of 3 min and 17 s (SD = 43.2 s). Auto calibrations were performed every 3 d, whereas manual CO_2 recoveries, which were performed monthly, averaged 96% (SD = 2.9).

Feed analysis

Composite samples of GS and of GSPC were freeze-dried for approximately 72 h at −50°C before being milled through a 1 mm sieve to determine DM percentage, chemical composition and digestibility. Prior to sample analysis, samples were bulked by treatment and by week. Grass silage, GSPC and concentrate samples were analysed by wet chemistry for organic matter digestibility (OMD; Foss, 151 Ballymount, Dublin 12, Ireland; Morgan *et al.*, 1989), CP (Leco Australia Pty Ltd., Baulkham Hills, New South Wales, 150 Australia), neutral detergent fibre (NDF) and acid detergent fibre (Van Soest *et al.*, 1991), and ash concentrations (FBA laboratories Ltd, Cappoquin Co., Waterford, Ireland). Dried, milled GS, GSPC and concentrate samples were shipped to Dairy One Cooperative Inc. (Ithaca, NY, USA) and analysed for gross energy using an adiabatic calorimeter (IKA – C5000, IKA® Works, Staufen, Germany; https://dairyone.com/download/forage-forage-lab-analytical-procedures).

Y_m calculation

Greenhouse gas emissions (GHG) in Ireland are currently estimated using tier 2 methodology (IPCC, 2019) in which average daily feed intake (in terms of gross energy content, MJ/d) and CH_4 conversion rates (Y_m) are used to estimate CH_4 emissions. The Irish national GHG inventory and life cycle assessments of Irish cattle systems have used an equation derived by Yan *et al.* (2000) to calculate enteric methane emissions from cattle during the housing season (Herron *et al.*, 2022; EPA, 2023). The proportion of gross energy consumed converted to CH_4, and the absolute CH_4 emissions from enteric fermentation from the GS and GSPC treatments were calculated using the equation by Yan *et al.* (2000).

Statistical analysis

All statistical analyses were conducted using SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). The UNIVARIATE procedure was used to screen data for normality and the presence of outliers. Outliers (i.e. values that were more than 3 times ± the mean) were identified and excluded from further analysis; 98.6% of data remained after outliers had been deleted. As BW was measured on a fortnightly basis during the dry period, interim BW was predicted by non-parametric local regression using PROC LOESS. Pre-experimental DMI was predicted using an equation (NRC, 2001);

$$\text{DMI (kg / d)} = (0.372 \times \text{FPCM} + 0.0968 \times \text{BW}^{0.75}) \times (1 - e^{(-0.192 \times (\text{WOL}+3.67)})$$

where FPCM was fat- and protein-corrected milk yield, BW was body weight and WOL was week of lactation. In order to include the pre-experimental data for each metric as a covariate in the respective model, data were centred within breed and parity for pre-experimental values for enteric CH_4, CO_2, H_2, predicted DMI, BW and BCS. Data were also centred within breed and parity for pre-experimental milk yield, milk solids, FPCM, CH_4 per kg of milk (g/kg), CH_4 per kg milk solids (g/kg) and CH_4 per kg FPCM (g/kg). Enteric CH_4, milk yield, milk solids, FPCM, group average DMI and BW data were all averaged by experimental week and used to calculate CH_4 output per unit of milk yield, milk solids, FPCM, group average DMI and BW, respectively. Somatic cell count was normalized to somatic cell score (SCS) by taking the natural logarithm of SCC/1000 in animals. The effects of treatment (i.e. GS or GSPC) on CH_4, CO_2, H_2, BW, BCS, milk yield, milk solids, FPCM, fat (g/kg), protein (g/kg), lactose (g/kg), SCS, CH_4 per kg milk yield (g/kg), CH_4 per kg milk solids (g/kg), CH_4 per kg FPCM (g/kg) and CH_4 per kg BW (g/kg) were analysed using linear mixed models (PROC MIXED). In all models, cow was included as a random effect, while week was included as a repeated effect. Fixed effects included in the models were treatment, breed and parity. The corresponding pre-experimental values centred within breed and parity and calving day of year were included in the models as covariates. The interaction between treatment and week was tested in all models. Only interaction terms that improved ($P < 0.050$) the fit to the data were retained. In all models, different covariance structures were tested, with the best overall model fit assessed by the Akaike information criterion value. Significant associations were confirmed when $P < 0.050$ and least-square means were assessed. Mean (standard deviation) feed chemical composition, group average DMI and CH_4 per kg group average DMI (g/kg) data are presented.

Results

The chemical composition of GS, GSPC and concentrate in late lactation and the dry period is outlined in Table 1. Mean (standard deviation) DMI of concentrate from the GreenFeed was 0.9 (0.26) kg/cow/day.

There was no association between diet in late lactation and enteric H_2 emissions, SCS, milk lactose (g/kg), BW and BCS (Table 2). There was a treatment-by-week interaction for enteric

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Table 1. Mean and standard deviation (SD) chemical composition of a perennial ryegrass silage and perennial ryegrass press cake mix (GSPC) and perennial ryegrass silage only (GS) offered to dairy cows during late lactation ($n = 5$ per treatment) and during the dry period ($n = 4$ per treatment)

	GSPC	SD	GS	SD
Late lactation				
Dry matter (g/kg DM)	250	18.2	219	9.70
Organic matter digestibility (g/kg DM)	723	11.3	721	20.50
Metabolizable energy (MJ/kg)	10.0	0.17	9.9	0.43
Neutral detergent fibre (g/kg DM)	564	14.1	534	24.4
Acid detergent fibre (g/kg DM)	364	7.5	346	17.0
Crude protein (g/kg DM)	123	11.5	112	4.5
Ash (g/kg DM)	84	1.8	100	7.5
Dry period				
Dry matter (g/kg DM)	254	16.3	204	22.2
Organic matter digestibility (g/kg DM)	727	14.5	722	18.0
Metabolizable energy (MJ/kg)	10.1	0.21	10.4	0.27
Neutral detergent fibre (g/kg DM)	574	8.0	552	31.5
Acid detergent fibre (g/kg DM)	365	7.0	357	23.7
Crude protein (g/kg DM)	115	9.6	109	3.6
Ash (g/kg DM)	78	2.6	76	8.1

CH_4 ($P = 0.030$; Fig. 1) and CO_2 emissions in late lactation. The patterns of enteric CH_4 and CO_2 emissions were similar such that the GS cows experienced a steadier decline in emissions week-by-week compared to that of the GSPC cows. The percentage week-on-week change in enteric CH_4 for the GSPC cows was between 0.5 and 15.3%, while the enteric CH_4 output of the GS cows reduced by between 6.5 and 11.5% week on week. Similarly, the percentage week-on-week change in CO_2

Table 2. The effect of grass silage (GS) and grass silage press cake (GSPC) diets in late lactation on least squares means (pooled standard error; SEM), estimated using linear mixed models, for production parameters

				P value	
	GSPC	GS	SEM	Treatment	Treatment × week
CH_4 (g/day)	334.4	313.3	6.46	0.022	<0.001
CO_2 (g/day)	9614	9349	108.4	0.087	<0.001
H_2 (g/day)	0.5	0.5	0.03	0.765	0.329
BW (kg)	568	560	4.0	0.229	0.956
BCS	3.0	3.1	0.05	0.115	0.643
Milk yield (kg)	11.7	11.1	0.44	0.278	<0.001
Milk solids (kg)	1.2	1.1	0.03	0.010	<0.001
FPCM (kg)	15.2	13.7	0.39	0.012	0.001
Fat (g/kg)	58	57	1.2	0.802	0.024
Protein (g/kg)	42	42	0.7	0.478	<0.001
Lactose (g/kg)	47	47	0.4	0.425	0.165
SCS	4.3	4.3	0.34	0.926	0.236
CH_4/milk yield (g/kg)	29	30	1.4	0.877	0.071
CH_4/milk solids yield (g/kg)	293	307	12.0	0.392	0.183
CH_4/FPCM (g/kg)	22.6	23.7	0.97	0.423	0.182
CH_4/BW (g/kg)	0.6	0.6	0.02	0.024	0.063

CH_4, methane; CO_2, carbon dioxide; H_2, hydrogen; BW, body weight; BCS, body condition score; FPCM, fat- and protein-corrected milk; SCS, somatic cell score.

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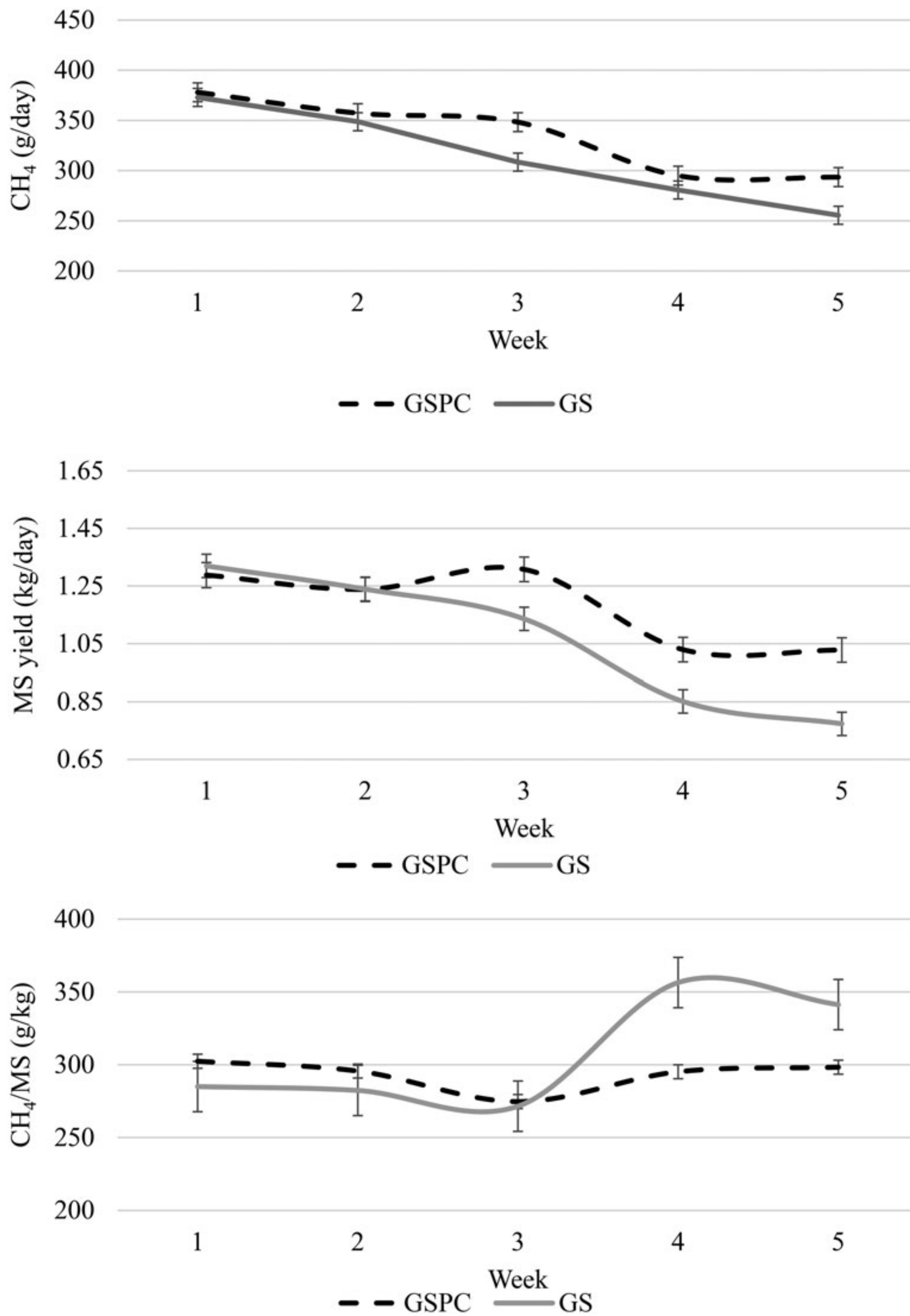

Figure 1. Least square means (standard error bar represents ± 1 SE unit) of weekly enteric methane emissions (CH_4; g/day), milk solids (MS; kg) and methane per kg milk solids (CH_4/MS; g/kg) of spring-calving dairy cows fed grass silage (GS) and grass silage press cake (GSPC) diets during late lactation.

for the GSPC cows was between −0.2 and +9.3%, while the CO_2 output of the GS cows reduced by between 4.1 and 8.9% week-on-week. In terms of milk yield, milk solids yield and FPCM, GSPC cows had greater milk yield, milk solids and FPCM yields compared to GS cows, with considerable variation observed from week-to-week ($P \leq 0.001$). Cows consuming grass silage only had higher milk yield, milk solids (Fig. 1) and FPCM in the first 2 weeks of the study. From weeks 3 to 4, the productivity of the GSPC cows surpassed that of the GS, producing between 10.4 (week 3) and 25.0% (week 5) more milk yield, milk solids and FPCM. When enteric CH_4 production was expressed on a milk yield, milk solids (Fig. 1) and FPCM basis, there was no significant difference between GS and GSPC cows. There was also an interaction between diet in late lactation and week for fat, protein (g/kg; Table 2). While the GSPC cows produced consistently more milk protein (g/kg) (ranging from +0.6 to +2.9%), the difference in milk fat (g/kg) between GSPC and GS cows was inconsistent from week-to-week (ranging from −5.3 to +6.2%).

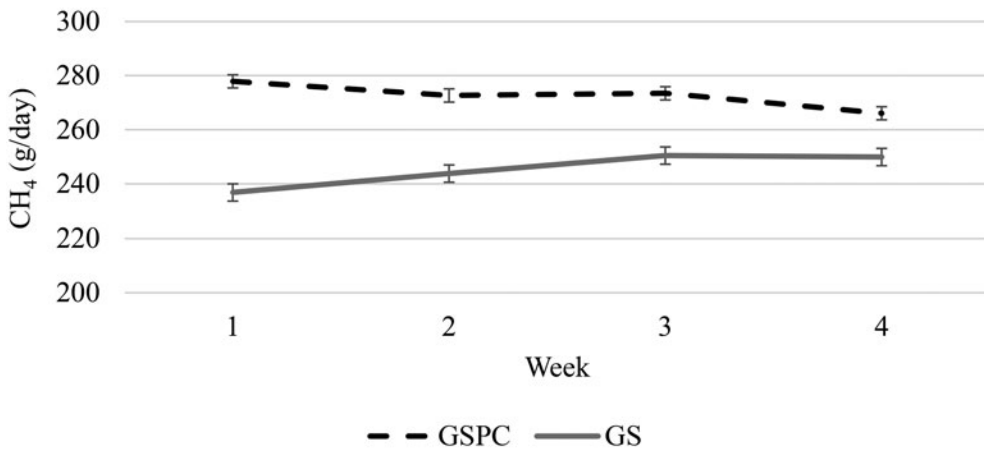

Figure 2. Least square means (standard error bar represents ± 1 SE unit) of enteric methane emissions (CH_4; g/day) of spring-calving dairy cows fed grass silage (GS) and grass silage press cake (GSPC) diets during the dry period.

While there tended to be an interaction between treatment and week throughout the dry period ($P = 0.081$), the difference between treatment and control cows lessened from the first week of the dry period (+14.7%) to week 4 of the dry period (+6.1%; Fig. 2). The interaction between treatment and week for CO_2 ($P < 0.001$; Table 3) output followed a similar pattern whereby the GSPC cows produced 7.7% more CO_2 than GS in week 1 of the dry period but by the third week of the dry period this difference had lessened to 4.4%, and in week 4 of the dry period the CO_2 output of the GS cows had surpassed that of GSPC such that they produced 4.6% more CO_2. The interaction between treatment and week for H_2 was also significant ($P = 0.023$); however, the pattern was variable such that in the first week of the dry period GSPC produced 10.2% more H_2 than GS, while from weeks 2 to 4 of the dry period GSPC produced between 4.7 and 8.8% less H_2 than GS. There was no effect of diet during the dry period on BW or BCS. When enteric CH_4 was expressed relative to BW the GSPC group tended to produce more enteric CH_4 per kg BW across the weeks of the dry period compared to GS cows ($P = 0.078$). Nonetheless, this effect lessened from week 1 of the dry period (+12.8%) through to week 4 of the dry period (+3.7%).

The differences in DMI are outlined in Table 4 such that group average DMI and CH_4 per kg group average DMI were 3.4 and 3.0% higher, respectively, for GSPC cows relative to GS cows in late lactation. During the dry period group average DMI and CH_4 per kg group average DMI were 11.3 and 5.5% higher, respectively, for GSPC cows relative to GS cows. Mean methane conversion factors (Y_m) were similar for GSPC (7.0%) and GS cows (6.9%) in late lactation, while during the dry period the cows offered GSPC had a mean Y_m of 6.2% and the GS cows had a mean Y_m of 6.6%.

Discussion

PRG silage is the predominant feed on pasture-based dairy farms in Ireland during the winter housing period (O'Brien *et al.*, 2018); however, silage quality is often variable (Patterson *et al.*, 2021), one of the reasons of which may be due to delays in harvesting as a result of adverse weather (Ferris *et al.*, 2022). Due to the nature of the green biorefinery process, which is outlined in detail by Kromus *et al.* (2005), fractionation is independent of adverse weather conditions and provides an alternative way in which to increase the feed value of PRG by producing a press cake, which can replace grass silage in the over-winter diet of a pasture-based ruminant (McEniry *et al.*, 2012) and year-round in the diet of an indoor animal. The outputs of the fractionation process are a high-protein press juice, which has the potential to displace 50% of soya bean meal if used to produce a leaf protein concentrate for monogastric animals (Ravindran *et al.*, 2021; Gaffey *et al.*, 2023), while at the same time producing the press cake by product. In previous research documenting the substitution of a PRG silage with PRG press cake in the diet of dairy cows, there are differing reports on the effect of PRG press cake on animal performance (Damborg *et al.*, 2019; Sousa *et al.*, 2021; Serra *et al.*, 2023). While Damborg *et al.* (2019) reported benefits in terms of feed

Table 3. The effect of grass silage (GS) and grass silage press cake (GSPC) diets during the dry period on least squares means (and the weighted pooled standard error; SEM), estimated using linear mixed models, for production parameters

	GSPC	GS	SEM	Treatment	Treatment × week
CH_4 (g/day)	272	245	6.8	0.006	0.081
CO_2 (g/day)	8694	8374	147.7	0.130	<0.001
H_2 (g/day)	0.2	0.2	0.01	0.620	0.023
BW (kg)	606	585	9.2	0.117	0.730
BCS	3.3	3.2	0.09	0.402	0.306
CH_4/BW (g/kg)	0.5	0.4	0.01	0.028	0.078

CH_4, methane; CO_2, carbon dioxide; H_2, hydrogen; BW, body weight; BCS, body condition score.

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Table 4. Mean and standard deviation (SD) group average dry matter intake[a] and methane (CH_4) per kg dry matter intake of cows offered a perennial ryegrass silage and perennial ryegrass press cake mix (GSPC) and perennial ryegrass silage only (GS) during late lactation and during the dry period

	GSPC	SD	GS	SD
Late lactation				
Dry matter intake (kg)	14.5	1.48	14	1.40
CH_4/dry matter intake (g/kg)	23.2	3.34	22.5	3.65
Dry period				
Dry matter intake (kg)	13.3	1.57	11.4	0.92
CH_4/dry matter intake (g/kg)	20.6	3.75	21.8	3.23

[a]Fresh weight group average intake was calculated as the weight of the feed offered minus the weight of the feed refused, divided by the number of animals in each pen. The fresh weight group average intake was multiplied by the corresponding dry matter percentage to determine the group average dry matter intake.

efficiency in dairy cows that consumed press cake, another study reported the complete replacement of grass silage with press cake to have adverse effects on milk production thereafter (Sousa *et al*., 2021), which was likely attributable to lower DMI. Serra *et al*. (2023) documenting similarities in terms of milk yield and quality in cows supplemented with press cake and those without, despite noting a reduction DMI in press cake supplemented cows. It is important to note that the aforementioned trials were undertaken at different stages of lactation and incorporated different dietary inclusion rates. The current study evaluated the impact of partially substituting press cake to dairy cows in late lactation (last 5 weeks of lactation) and over the dry period under Irish conditions where animals are generally only housed during these time periods (Dillon *et al*., 1995). Findings from the present study indicate that there may be merit in the partial replacement of grass silage with press cake during the over-winter period in terms of late lactation milk production. In agreement with previous research, the merit of including press cake in the diet of dairy cows is contingent on inclusion rate, quality of the basal diet and stage of lactation in which the press cake is fed.

High-quality feed is essential to sustain milk production and support maintenance requirements of the cow. Some of the most important determinants of feed quality are OMD, NDF and CP, the optimization of which will enhance DMI and the utilization thereof (Fernández *et al*., 2011). A meta-analysis undertaken by Nousiainen *et al*. (2009) of ~500 grass silage-based diets highlighted that there is a significant variation in silage quality, with the NDF of the PRG silage used in the present study similar to that of the lowest quality silages in the meta-analysis. Suboptimal silage quality may be as a result of poor weather which may delay harvesting, insufficient wilting and conditions at ensiling (Ferris *et al*., 2022). The quality of the reference material (i.e. silage) may therefore be a limitation of the present study and it may be beneficial to incorporate silages of different qualities in future research on the potential application of press cake as a partial replacement for grass silage during the winter housing period. The nutritive value of the herbage presented for fractionation, which can be influenced by grass species, can impact the nutritive value of the press cake after fractionation; therefore, a further limitation of the present study is that the GS and GSPC were not produced from the same parent material. Further research should be undertaken in which GSPC and GS are produced on the same site to allow for direct comparison between the two feeds.

Despite the higher NDF content in GSPC diet in the present study compared to the GS diet, which is also noted in the literature (Damborg *et al*., 2019; Santamaria-Fernandez *et al*., 2020; Sousa *et al*., 2021), the group average DMI of the GSPC cows was approximately 3.5 and 14.3% higher than that of the GS cows in late lactation, and during the dry period, respectively. It is likely that the mechanical fractionation of the grass during biorefinery may have resulted in loss of soluble, fermentable organic matter and as such an increase in DM and NDF in the solid fraction (press cake; McEniry *et al*., 2012). Fractionation is also reported to enrich (Wachendorf *et al*., 2009) and improve the degradability of the fibre in the GSPC diet (Damborg *et al*., 2018; Savonen *et al*., 2020), resulting in superior intakes, and consequently productivity. In the present study, cows offered GSPC in late lactation produced more milk, milk solids and FPCM compared to those offered GS, with the gap between the aforementioned milk production parameters of GS and GSPC cows widening week-on-week until the cows were dried off. Given the increasing difference in milk production between GSPC and GS cows as the experimental period progressed, it is conceivable that evaluating the diets longer than the 5-week period in the current study may have been beneficial. This may not be applicable to standard practice under intensive grazing systems practised in Ireland where animals generally only spend a fraction of late lactation housed indoors fulltime before the animals are dried off. Feeding press cake in early lactation may also have application in pasture-based systems as cows are often housed for a period until grazing conditions are optimal. There may be further scope to feed press cake throughout the lactation in indoor feeding systems and in other enterprises such as beef systems in which animals are housed for a period close to finishing.

Over the 5-week late lactation period, the milk production benefits in cows offered diets partially substituted with press cake in the present study may be related to the CP content of the forage, which was 9.8% higher in GSPC than in GS during late lactation. Other studies report the reverse, i.e. a higher CP concentration in GS relative to GSPC (Santamaria-Fernandez *et al*., 2020; Serra *et al*., 2023). The CP content of the grass silage in the present study (10.9–11.2%) is considerably less than the mean CP content outlined in the aforementioned meta-analysis of silage diets (15.2%; Nousiainen *et al*., 2009). Future research comparing GS and GSPC should ensure the criterion for growing the rudimentary forages is similar, unlike in the present study in which the GS and GSPC were produced in different geographical locations (Ireland and the Netherlands), which is undoubtedly a limitation and stemmed from logistical issues within the study. Nutrition of the dairy cow during late lactation and the non-lactating period should optimize body reserves prior to calving so that the cow has sufficient condition to support productivity in early lactation (Roche *et al*., 2015); increasing the nutrient density of the diet, and consequently DMI is one way in which to do this (Grummer, 1995; Hayirli *et al*., 2002). Supplementing diets with press cake resulted in superior DMI throughout the present study. Although this did not translate to significant differences in BW and BCS, the magnitude of the rate of BCS increase between late lactation and the dry period was greater for GSPC cows whereby their BCS increased by 10.0%, while the equivalent increase in GS cows was 4.0%. The difference in the rate of BCS increase from late lactation into the dry period may be attributable to the superior DMI of GSPC cows, particularly in the non-lactating period. Nonetheless, the mean BCS for GSPC and GS cows during the dry period was consistent with the target BCS

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at calving of 3.25 units as recommended by Buckley *et al.* (2003). Furthermore, benefits of nutrition of the cow in late lactation and during the dry period are often realized in the subsequent lactation (Van Saun, 1991; Mann *et al.*, 2015); monitoring milk production in the successive lactation was outside the scope of the present study. Future research evaluating the effect of including press cake in the diet of the late lactation and non-lactating dairy cow should incorporate a period in which the carry-over effects of feeding treatment are monitored.

Although production is an important factor when considering ruminant feeding strategies, there has been a strong focus of late on the environmental impact of dairy farming, most of which has been centred on enteric CH_4 emissions. Consequently, there has been much interest in strategies to reduce enteric CH_4 emissions in order to achieve the emissions reduction targets set out by the Irish government (Madden *et al.*, 2022). Contrary to findings from a Rusitec simulation study (Serra *et al.*, 2023), the GSPC cows in the present study did not experience a reduction in absolute CH_4 emissions. While the GS cows experienced a more gradual reduction in CH_4 and CO_2 as they neared the end of the lactating phase, the enteric CH_4 and CO_2 output of GSPC cows fluctuated. This was likely due to increased DMI, which also sustained their milk production in late lactation. As a result of the significant improvement in milk yield in GSPC cows in weeks 4 and 5, the interaction between diet in late lactation and week tended to be significant for CH_4 per kg milk yield, and when CH_4 was expressed based on milk solids and FPCM output, there was no significant difference between GS and GSPC. Although cows offered a diet consisting of press cake also produced more enteric CH_4 and CO_2 emissions relative to the control, the difference between the two groups lessened as the dry period progressed. This indicates that perhaps had the length of the experimental feeding period been extended, the enteric CH_4 and CO_2 output may ultimately have been similar for GS and GSPC cows; investigating this was outside the scope of the present study. The relationship between experimental feed treatment and week for enteric H_2 followed a different pattern such that in the first week of the dry period GSPC cows produced 10% more H_2 than GS cows; however, the GS cows produced between 5 and 9% more H_2 than the GSPC cows for the remainder of the non-lactating period. Hydrogen is a substrate required by methanogens for the production of CH_4 (Mackie *et al.*, 2024), and can vary with diet quality due to partitioning to alternative hydrogen sinks (Ungerfeld, 2020), which may have occurred across the current study.

The enteric CH_4 output of the GSPC cows in late lactation, as measured in the present study (334.4 g/day), was lower than that predicted by an IPCC tier 2 method for the same cows (367.0 g/day; Yan *et al.*, 2000). The same was also true during the non-lactating period whereby the measured CH_4 emissions of GSPC cows (272.5 g/day) and GS cows (245.3 g/day) were lower than that predicted by an IPCC tier 2 method for cows consuming GSPC (358.7 g/day) and GS diets (304.0 g/day), respectively. The aforementioned IPCC tier 2 method that is widely used to calculate enteric CH_4 during housing period was developed for animals consuming grass silage diets and as such, may need to be refined to account for alternative forages such as PRG press cake.

In addition to press cake, the benefits of which are outlined above, green biorefinery also generates a high value commodity in the form of press juice, which may be used to produce a grass protein concentrate for monogastrics (Stødkilde *et al.*, 2019; Ravindran *et al.*, 2021). It is therefore important that when examining the environmental impact of biorefinery, all outputs are considered. One of the biggest challenges facing the agriculture sector is to feed the growing global population sustainably and, with 4.92 kg of human edible protein produced for every 1 kg of human edible protein consumed (Hennessy *et al.*, 2021), Irish dairy farming is well positioned to do so. Nonetheless, during periods when grass growth is insufficient to meet demand of the herd, the sector is reliant on imported concentrate to sustain production (Wallace, 2020). This action ultimately has environmental ramifications whereby a reduction in concentrate supplementation on Irish dairy farms has been highlighted as one of the ways in which to improve the sustainability (Herron *et al.*, 2022). Herein lies one of the attractions in green biorefinery; as is evident from the present study, and consistent with Sanders *et al.* (2023), feeding GSPC to dairy cows increased milk solids production in late lactation and as such may be a suitable replacement for grass silage during the winter housing period, while the press-juice component may also be used to produce a concentrate for monogastrics (Ravindran *et al.*, 2021; Stødkilde *et al.*, 2021), which will reduce the reliance on imported protein. A life cycle assessment, with land use change incorporated, of protein derived from biorefinery, showed an 80% reduction in GHG relative to soybean meal (Tallentire *et al.*, 2018) (dependent on if land use change was associated with the soya). Utilising biorefined PRG press cake and juice as a source of feed for both ruminants and monogastrics thus has potential to increase food production from pasture, further improve land use efficiency of pasture based systems, reduce feed food competition in our food system, while also reducing GHG emissions from the Irish agricultural sector.

Conclusion

If produced from good quality PRG, press cake can be utilized as a feedstuff to increase productivity during the over-winter period on seasonal calving pasture-based dairy farms. Although findings indicated an increase in absolute CH_4 emissions in cows offered press cake, when CH_4 was expressed based on milk production, emissions were similar for cows both with and without press cake supplementation. As such, in the present study there were overarching production and environmental benefits associated with feeding biorefined PRG press cake to cows during the winter housing period as a partial replacement for grass silage; however, the differences in the nutritive value of the grass used to produce press cake and grass silage are a limitation. The value proposition associated with the press cake will therefore be associated with the costs of the press cake by product, which will be related to the value that can be achieved from the biorefined protein juice.

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Competing interests. Grassa BV (5928 SZ Venlo, the Netherlands) supplied the press cake that was used for the trial. J. P. M. Sanders and B. Lambrechts are employed by Grassa, the remaining authors declare no conflict of interest.

Ethical standards. The Teagasc Animal Ethics Committee (TAEC) (TAEC2021-313) granted ethical approval. All experiments were conducted in accordance with the Cruelty to Animals Act (Ireland 1876, as amended by European Communities regulations 2002 and 2005) and the European Community Directive 86/609/EC.

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