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Implementing press cake from biorefined grass as a roughage in the diet of Holstein-Friesian dairy cows

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ABSTRACT

A feeding trial was conducted with 60 mid-lactation cows that were, on average, 116 DIM, parity 3.5, and with a fat- and protein-corrected milk (FPCM) yield of 34 kg/d. Cows were blocked based on cow traits and milk performance. Cows in each block were randomly allocated to 1 of 3 treatments that differed by replacement of half of the dietary grass silage component (involving an exchange of 20% of dietary DM). Treatments were a regular high-quality grass silage (positive control; GS-POS), an ensiled press cake obtained from grass biorefinery (GS-PC), or a lower-quality grass silage (negative control; GS-NEG). After 2 pretreatment weeks with 10% ensiled press cake in dietary DM for all cows, cows received their treatment for 10 measurement weeks in which cow performance and gaseous emissions were measured with the GreenFeed system. Digestibility was measured with TiO2 as a marker in the eighth measurement week for half of the cows (i.e., 10 cows per treatment). Cows on GS-POS and GS-PC appeared to perform equally, whereas on GS-NEG, feed intake and FPCM yield were 6.5% and 6.6% lower, respectively. After an initial decline in milk protein content for GS-PC and GS-NEG compared with GS-POS, values increased again toward levels achieved for GS-POS after 6 wk of treatment. Milk protein yield on GS-NEG was 6.4% lower compared with GS-PC (mainly due to lower milk yield) and 8.8% lower compared with GS-POS (GS-POS and GS-PC did not significantly differ). Digestibility of DM and crude fat was higher for GS-PC compared with GS-POS (and numerically for all other nutrients) corresponding to in vivo trial with wethers that was conducted to determine in vivo the NEL value of press cake according to standard protocol for energy evaluation. Methane yields (g/kg DMI) of GS-POS and GS-PC did not differ, but were 4.5% lower than GS-NEG; likewise, carbon dioxide yield and hydrogen yield were 3.7% and 17.1% lower than for GS-NEG. It is concluded that a 20% dietary DM exchange of a high-quality grass silage with ensiled press cake that contained 39% more NDF and 29% less CP did not reduce cow performance or increase enteric methane, in contrast to exchange with a low-quality grass silage that contained 21% more NDF and 34% less CP. This outcome seems to be due to the higher digestibility of ensiled press cake compared with grass silage.
Key words: dairy cow, press cake, grass biorefinery, performance, methane

INTRODUCTION

Ryegrass as a roughage component of diets in intensive dairy systems often has a relatively high CP content. This means that with high dietary inclusions of grass silage, the N utilization in particular is relatively low, leading to high rates of N excreted with urine and high ammonia emission from cow houses. Although other dietary components are used to compensate for the high CP content in grass silage (and grass herbage) in intensive dairy systems, the possibilities to substantially reduce dietary CP content to reduce ammonia emissions are limited. Furthermore, grass as a roughage generally has a high contribution to enteric methane emissions compared with an alternative roughage, such as maize silage (van Gastelen et al., 2023). Although ryegrass early in the growth season, or cutting at a very early stage of maturity, was shown to lead to substantially less enteric methane emissions (Warner et al., 2017), the CP content was higher. Without compensating for the latter, N emissions will rise and their GHG equivalent may largely outrange the lower methane emissions achieved (Dijkstra et al., 2011).

Biorefinery is a physical procedure in which grass is pressed and separated into different fractions. A liquid fraction (press juice) contains soluble proteins, free AA, sugars, dyes, enzymes, hormones, further organic sub-

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stances, and minerals (Xiu and Shahbazi, 2015), and it can be refined further into a protein, sugar, and mineral fraction to be used elsewhere in the food chain (Franco et al., 2019). For example, the protein fraction can be precipitated to serve as a valuable protein supplement in the diet of monogastric animals. The ensiled fibrous fraction (press cake) may be a valuable roughage source in dairy diets with a low and insoluble protein content and a low phosphorus content as a replacement of grass silage (Serra et al., 2023) or maize silage.

Ensiling the press cake of biorefined grass allows the harvest of grass at a young stage of maturity with a high nutritive value but also high N content, because this N is largely removed through biorefinery, and associated N excretion and N emissions can, for this part, be prevented. Nevertheless, a successful application of biorefinery would require that the nutritive value of the ensiled grass press cake remain similar to that of silage of the original grass harvested. Damborg et al. (2019) demonstrated clear beneficial effects of ensiled grass press cake (pulp) on cow performance, which were not due to higher DMI, on both a low and a high level of dietary protein. However, the specific protein-reducing potential of the ensiled grass press cake was not explored because the diets (including press cake) had a higher CP content compared with the complementary diets based on grass silage, and the dietary protein level was controlled with inclusion of soybean meal. Furthermore, enteric methane emission from ensiled grass press cake should not increase. Press cake has a higher fiber content compared with regular grass silage, and it is known that fibrous diets may lead to higher methane yields and that under in vivo conditions, the fiber fraction delivers relatively more methane compared with starch (Bannink et al., 2006). Recently, Serra et al. (2023) compared the methanogenic potential of press cake with grass silage and measured a similar acetic acid/propionic acid ratio in the rumen in vivo, but this ratio was unaffected in vitro in correspondence with a lack of observed effects on methane production. However, DM disappearance was 13% to 15% lower for the press cake, and hence methane yield per unit of DM disappeared must have been higher (numbers not given by authors), as the authors stated that the same amount of DM was introduced in the nylon bags incubated in their RUSITEC in vitro system.

The objective of this study was to investigate the effects of partial replacement of grass silage by ensiled grass press cake in the diet on the performance of lactating cows, exploring the opportunity to use ensiled grass press cake to lower dietary CP content. During a 12-wk trial, we studied feed intake, lactation characteristics, BW, enteric methane production, and (in the eighth week of treatment, i.e., wk 10) digestibility and nitrogen balance. It was hypothesized that ensiled grass press cake with a high fiber and a low CP content has a similar nutritional value compared with a regular grass silage as a positive control, and a higher value compared with a low-protein grass silage as a negative control. It is further hypothesized that ensiled press cake can be applied to lower N excretion and improve N use efficiency without negative side-effects on cow performance and enteric methane emission.

MATERIALS AND METHODS

Experimental Design

The experiment was conducted from October to December 2022 at the research facilities of Wageningen Livestock Research (Dairy Campus, Leeuwarden, the Netherlands). The study followed a completely randomized block design with 3 dietary treatments. A selection of 64 lactating Holstein-Friesian dairy cows started during the pretreatment weeks (116 ± 21.6 DIM at start of the trial; parity 3.5 ± 1.69 ; mean ± SD), of which 60 cows were blocked in advance, keeping an additional 4 cows as reserve animals. Of these 60 dairy cows, 9 were in their first lactation, 9 were in their second lactation, and 42 in their third or greater lactation. The cows were blocked in triplets based on parity (first lactation, second and higher lactation), lactation stage, and current milk yield, resulting in 20 blocks of 3 cows. In the beginning of the treatment period, 2 of the blocked cows (both parity 2) were replaced by 2 reserve animals with parity 1 and 3, resulting in 10, 7, and 43 cows in their first, second, and third or higher lactations, respectively. Within each block, cows were randomly assigned to 1 of the 3 dietary treatments. A pretrial was used to adapt all cows to the same diet for 2 wk. Only wk 2 of this pretrial phase served as a covariate period to take baseline measurements and evaluate the proper blocking of cows to treatments before starting the trial phase. Aside from the replacement of 2 cows, there were no changes in allocation of cows to blocks and treatments. The trial phase consisted of 10 wk (70 d) in which the dairy cows received 1 of the 3 dietary treatments to which they were allocated.

Dietary Treatments

During the pretrial period, all cows were fed the same basal diet according to standard operating procedures at the experimental facilities, consisting of 36% concentrates, 40% grass silage (composed of 75% regular grass silage and 25% grass press cake silage), and 24% maize silage (on DM basis). After these 2 wk, cows received a diet with similar inclusions of grass silage (41%), maize silage (27%), and concentrates (32%), but they were switched to 1 of 3 dietary treatments that differed in the type of grass silage and protein content: entirely regular grass silage (182 g CP/kg DM), serving as positive control treatment (**GS-POS**); half of the regular grass silage replaced by grass press cake silage with a lower CP content (130 g CP/kg DM; **GS-PC**; https://koeeneiwit.nl/nieuws/praktijkervaring-grassa; Grassa BV, Wageningen, the Netherlands; Koe en Eiwit, 2023); or half of the regular silage replaced by older cut grass silage with a low CP content (121 g CP/kg DM) and lower nutritional value, serving as the negative control treatment (**GS-NEG**). The chemical composition of the individual dietary components is presented in Table 1, and the inclusion of dietary components and the dietary composition effectively achieved are presented in Table 2. The chop length was 15 mm and 7 mm for grass silage and corn silage, respectively. To each of these diets, water was added to target a DM content of 330 g/kg to achieve optimal results in the mixing unit and to achieve a more homogeneous diet and prevent cows from feed selection in the diet. The concentrate meal for the partial mixed ration (**PMR**) was designed by Agrifirm (Apeldoorn, the Netherlands; Table 1) to meet the requirements for maintenance and milk production(i.e., net energy for lactation, Van Es, 1978; intestinal digestible protein and rumen degraded protein balance, van Duinkerken et al., 2011) of the dairy cows on the complete ration level for GS-POS. The same concentrate was included in all 3 dietary treatments (Table 1).

Feeding, Milking, and Housing

The cows were fed a PMR (excluding the GreenFeed bait and the bait used in the milking parlor; Table 2) via an automated feeding system, consisting of the Trioliet feed mixing robot (Triomatic HP 2 300, Trioliet, Oldenzaal, the Netherlands) for mixing the diets. The PMR was fed in the roughage intake control (**RIC**) bins (Hokofarm Group BV, Marknesse, the Netherlands) to measure feed intake for each individual cow. The RIC bins were calibrated weekly using a standard weight. The Trioliet robot mixed the diets during the pretrial and trial phases, with the 3 experimental grass silage treatments mixed 4 times daily in equal portions. On a weekly basis (based on daily measurement of the DM content of each individual ration component during the previous week), the required amount of each component was determined to prepare the diets. The dietary components were cut or transported and weighed with ± 2 kg product accuracy from their bunker (silage bales or blocks), with ± 1 kg product from the silo (concentrates), or with ± 2 g product from the mini-silo (premixes), and transported into the Trioliet feed mixing robot in the order of grass silages, corn silage, premix, soybean meal, concentrates, and water. Actual weights of all products added to the mixing robot were recorded, allowing us to precisely define the composition of each individual batch of PMR produced, with each single batch being mixed weighing between 400 and 500 kg. After addition of the last dietary component to the Trioliet, the PMR was mixed for an additional 10 min before being transported to the RIC bins. The diets were offered at 10% excess to allow ad libitum feed intake. Next to the PMR, all cows received the same amount (0.89 kg) of concentrate daily in the milking parlor (van Gastelen et al., 2022).

The dairy cows were housed as a single group in a freestall barn, with 60 lying cubicles, 30 RIC bins for feeding, and having access to 3 GreenFeed system units (C-Lock Inc., Rapid City, SD) for gaseous emission measurements.

The RIC bins were positioned in one line at one side of the feed alley in the middle of the barn at the location of the feeding fence, and the PMR was automatically deposited in the RIC bins by the Trioliet mixing robot 4 times daily. The cubicles were arranged transversely to the feed fence, with RIC bins in 4 groups or islands of 16 cubicles consisting of 2 mirrored rows of 8 cubicles, with slatted floors in between. The assignment of RIC bins for each diet treatment was spread along the feed alley as much as possible, and they were allocated in the order of 3, 3, and 3 RIC bins for diets GS-POS, GS-PC and GS-NEG, respectively, followed by 4, 4, and 4 RIC bins, and finally 3, 3, and 3 RIC bins in the same order. The dietary treatments were equally distributed over the RIC bins across the barn to eliminate potential barn location effects. Cows in the same treatment had access to all RIC bins assigned to that treatment diet. At the start of the trial, the dairy cows were assigned to the RIC bins that had been allocated to one of the 3 treatments, which remained the same throughout the entire trial. The GreenFeed system units were positioned at the ends of the cubicle islands close to the outside wall of the barn, and evenly positioned along the whole length of the barn. All cows had access to each of the 3 GreenFeed system units.

The RIC bins, GreenFeed system units, and milking carousel were equipped with an automated identification system (monitor ID system based on transponders within the dairy cows' collars) to enable cow access to the RIC bins and identify cows when visiting a GreenFeed system unit, and when being weighed and scored on body condition after being milked and exiting the milking carousel. Measurements of feed intake were recorded for each visit of a cow to a RIC bin and GreenFeed system unit. Cows were milked in a milking carousel suited for 40 cows with automatic cow identification, milk recording, and milk cluster removal (AutoRotor PerFormer, Gea Farm Technologies, Leeuwarden, the Netherlands). Body weight (Gea weighing scale; Gea Farm Technologies Nederland BV, Deventer, the Netherlands) and BCS (DeLaval BCS camera, DeLaval BV, Steenwijk, the Netherlands) of each

Table 1. Chemical composition (in g/kg DM, unless otherwise stated) of dietary components used in 3 experimental diets (grass silage positive control [GS-POS], grass silage grass press cake [GS-PC], grass silage negative control [GS-NEG])

Item	Grass silage GS-POS	Grass silage GS-PC	Grass silage GS-NEG	Maize silage	Concentrates[1]	Soybean meal	GreenFeed bait[2]	Milking parlor bait[3]
DM (g/kg)	344	320	510	405	882	877	874	880
OM	873	940	884	962	925	927	924	913
CP	164	120	111	45	156	510	135	159
CP, including NH_3	182	130	121	—	—	—	—	—
Crude fat	43	29	30	30	49	20	35	46
NDF	470	655	568	370	349	307	392	314
ADF	283	425	343	199	181	66	233	173
ADL	17	40	23	11	46	5	13	38
Starch	0	0	0	412	292	15	111	258
Sugar	30	23	80	0	43	122	113	77
NEL[4] (MJ/kg DM)	6.28	5.55	5.53	7.18	7.60	8.07	7.52	7.63
DVE[5]	54	31	49	52	120	266	98	120
OEB[5]	66	37	9	−60	−11	213	−5	−11
DOM[6]	683	647	634	771	750	853	803	776

[1]Ingredient composition (g/kg DM): palm kernel flakes = 297, maize = 284, wheat semolina = 120, wheat = 101, rumen protected rapeseed meal (Mervobest, NuScience) = 75, lupine = 41, $CaCO_3$ = 24, Lucerne = 20, citrocol = 20, NaCl = 10, MgO = 5, and mineral premix = 2 (i.e., in kg/DM; vitamin A = 1,015,796 IE, vitamin D3 = 190,462 IE, vitamin E = 635 IE, iron = 3,809 mg, iodate = 190 mg, cobalt = 97 mg, copper = 1,270 mg, manganese = 3,809 mg, sink = 5,714 mg, and selenium = 38 mg).

[2]Ingredient composition (g/kg DM): beet pulp = 300, soybean hulls = 240, maize gluten feed = 90, lucerne (16%–18% CP) = 77, wheat milling byproducts = 75, barley = 50, corn = 49, molasses sugarcane = 47, soybean meal = 29, rapeseed meal = 21, bypass fat = 12.5, NaCl = 10.

[3]Ingredient composition (g/kg DM): corn = 321, palmpit kernel flakes = 291, bypass rape seed meal = 91, rape seed meal = 50, citrus pulp = 60, molasses = 98, wheat bran = 41, lime = 30, salts and premix = 18.

[4]NEL (Van Es, 1978), based on wet chemical analysis (Eurofins, Wageningen, the Netherlands).

[5]DVE = intestinal digestible protein; OEB = rumen degradable protein balance (calculations DVE/OEB-2007; van Duinkerken et al., 2011), estimates obtained by using calibration lines that were derived for regular grass silage (Eurofins, Wageningen, the Netherlands).

[6]DOM = digestible organic matter determined by in vitro incubation of grass silages (Eurofins, Wageningen, the Netherlands) according to the method of Tilley and Terry (1963), and DOM values from Dutch feed tables for concentrates and other non-grass dietary components (CVB, 2016).

Table 2. Composition of the partially mixed diet fed in RIC bins (% of dietary DM) and its chemical composition (g/kg DM, unless otherwise stated) for the pretrial diet, the positive control diet (GS-POS), the grass press cake diet (GS-PC), and the negative control diet (GS-NEG); GreenFeed and milk parlor bait were not included in the calculated diet composition and its chemical composition

Item	Pretrial	GS-POS	GS-PC	GS-NEG
Diet composition (% of DM)				
Grass silage, positive control	29.4	40.2	20.1	20.4
Grass silage, press cake	10.4	0.0	21.1	0.0
Grass silage, negative control	0.0	0.0	0.0	19.3
Maize silage	24.4	26.8	26.2	26.7
Concentrates	30.2	27.2	27.0	27.6
Premix[1]	0.5	0.5	0.5	0.5
Soybean meal	5.2	5.3	5.2	5.4
Chemical composition				
DM[2] (g/kg)	375	373	375	372
OM	916	909	923	911
CP	148	144	138	136
CP, including NH_3	157	152	145	143
Crude fat	37	40	37	38
NDF	417	401	437	418
ADF	234	222	250	232
ADL	27	22	27	24
Starch	190	191	188	192
Sugar	35	27	27	38
NEL[3] (MJ/kg DM)	6.80	6.95	6.80	6.80
DVE[4]	83	81	76	81
OEB[4]	19	16	13	6
DOM[5]	722	731	723	721

[1]Ingredient composition (g/kg DM): palm kernel flakes = 297, maize = 284, wheat semolina = 120, wheat = 101, rumen protected rapeseed meal (Mervobest, NuScience) = 75, lupine = 41, CaCO3 = 24, Lucerne = 20, citrocol = 20, NaCl = 10, MgO = 5, and mineral premix = 2 (i.e., in kg/DM; vitamin A = 1,015,796 IE, vitamin D3 = 190,462 IE, vitamin E = 635 IE, iron = 3,809 mg, iodate = 190 mg, cobalt = 97 mg, copper = 1,270 mg, manganese = 3,809 mg, sink = 5,714 mg, and selenium = 38 mg).

[2]DM (g/kg) including water that was added to PMR diet to reach a DM content of 37%.

[3]NEL according to the Dutch VEM system (Van Es, 1978).

[4]DVE = intestinal digestible protein; OEB = rumen degradable protein balance (van Duinkerken et al., 2011); estimates based on calibration lines derived for grass silages or maize silages (Eurofins, Wageningen, the Netherlands), and tables values or concentrates.

[5]DOM = calculated digestible organic matter determined by in vitro incubation of grass silages (Eurofins, Wageningen, the Netherlands) according to the method of Tilley and Terry (1963) and DOM values in Dutch feed tables for concentrates and other non-grass dietary components (CVB, 2016).

cow were recorded twice daily. Further details on procedures and methods used are described by van Gastelen et al. (2022). The lying cubicles were covered with rubber cattle mats and wood shavings as bedding, and cubicles were cleaned daily. The dairy cows were exposed to light from 0500 to 2300 h, had free access to clean drinking water, and were milked twice daily at at ~0500 and 1500 h during the entire experiment.

Sample Collection and Measurements

Representative duplo samples were taken from grass silages and maize silage to determine the DM content daily, whereas on a weekly basis, samples were taken from concentrates and baits as dry feeds from the silo and mini-silo. Based on these DM contents, the amount and proportion of components to be mixed into the PMR by the Trioliet feed mixing robot were calculated and updated in the robot weekly.

Once weekly, other duplo samples of individual ration components and GreenFeed bait were taken and stored at −20°C pending analysis. Once weekly, during 1 morning and 1 evening milking event, a milk sample (10 mL) was collected in a tube containing sodium azide (5 µL) for preservation, stored no longer than 1 d at 4°C, and analyzed for fat, protein, lactose, and urea content. A weighted average daily milk composition was calculated from milk composition and milk yield.

Digestibility measurements were obtained for a selection of 10 blocks of 3 cows (1 cow for each dietary treatment in a block) during wk 10 (the eighth week of treatment), aiming for a comparable parity, lactation stage, milk production ,and feed intake for the selected subgroup of 30 cows and the total group of cows in the trial. Soybean meal as a carrier meal was used to introduce TiO_2 as a marker for digestibility in the PMR starting 9 d before the first sampling to ensure that a steady state was achieved. A 0.25-kg allotment of soybean meal

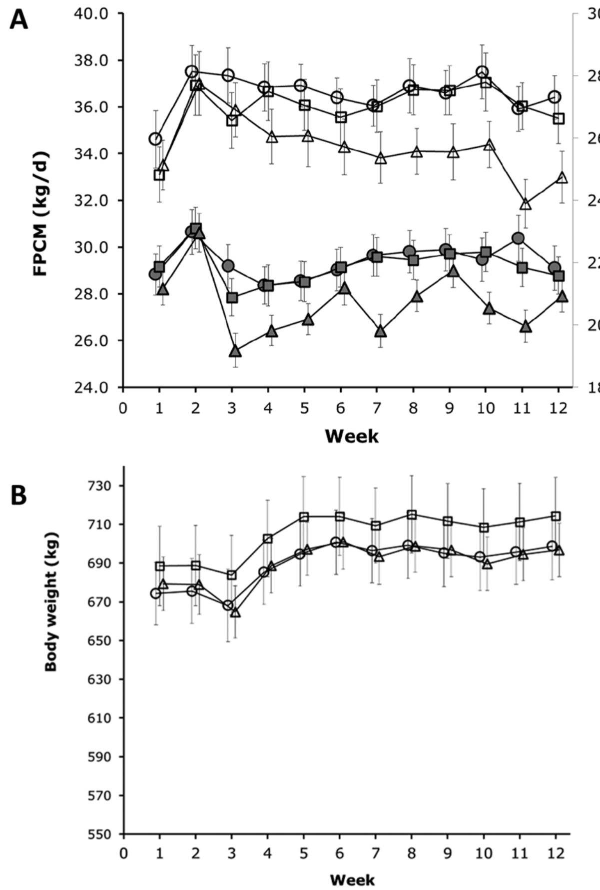

Figure 1. Evolution of (A) DMI of the PMR (kg DM/d; closed symbols; calculated average of measured values of individual cows ± SEM of these values calculated as SD divided by the square root of the number of cows) and fat- and protein-corrected milk production (kg FPCM/d; open symbols; calculated average of measured values for individual cow ± the calculated SEM of these values), and of (B) body weight of lactating dairy cows, when fed the positive control diet (GS-POS; n = 20; ○), the grass press cake diet (GS-PC; n = 20; □), or the negative control diet (GS-NEG; n = 20; △) during the subsequent 10-wk measurement period (wk 3 to 12), following an initial 2-wk pretrial period (wk 1 and 2) when all cows received the same diet (25% grass press cake and 75% of the same grass silage used in the GS-POS diet as the grass silage portion), and for which the last week served as the covariate (wk 2). The DM intake of concentrate in the GreenFeed units and milking parlor was almost the same with 2.5, 2.5, and 2.6 kg DM/d for GS-POS, GS-PC, and GS-NEG, respectively. Results depict numerical outcomes for weekly observations, and not LSM and associated SEM as outcomes of statistical analysis.

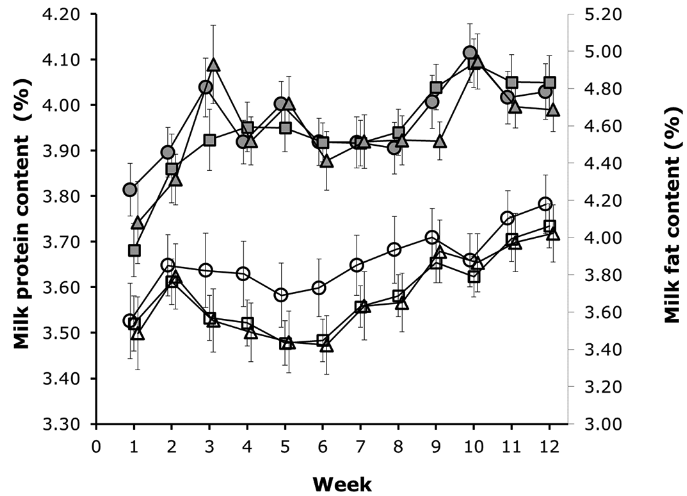

Figure 2. Evolution of milk protein (%; open symbols; calculated average of measured values of individual cows ± SEM of these values calculated as SD divided by the square root of the number of cows) and fat content (%; closed symbols; calculated average of measured values of individual cows ± the calculated SEM of these values) of lactating dairy cows fed the positive control diet (GS-POS; n = 20; circles), the grass press cake diet (GS-PC; n = 20; squares), or the negative control diet (GS-NEG; n = 20; triangles) during a 10-wk measurement period (wk 3 till 12), following an initial 2-wk pretrial period (wk 1 and 2) when all cows received the same diet (25% grass press cake and 75% of the same grass silage used in the GS-POS diet as the grass silage portion), and for which the last week served as the covariate (wk 2). Results depict numerical outcomes for weekly observations, and not LSM and associated SEM as outcomes of statistical analysis.

DM in the PMR was replaced by 0.23 kg of soybean meal DM and 0.02 kg of TiO_2. Rectal grab samples were obtained from all cows at 6 daily moments spread over 3 subsequent sampling days covering the excretion pattern of 2 full days, starting on the December 7, 2022. Grab samples were taken between 1030 h and 1130 h and between 1500 h and 1600 h on the first sampling day, between 0500 h and 0600 h, 1030 h and 1130 h, and 1500 h and 1600 h on the second sampling day, and between 0500 h and 0600 h on the third sampling day. Samples were stored at 4°C, and after the sampling days they were immediately transported, pooled, and sent for chemical analysis. For calculation of digestibility, the feed intake data (PMR from the RIC bins and concentrate bait in the GreenFeed units and milking parlor) were used from the preceding day until the end of the second day of rectal grab sampling, because the preceding day is thought to represent Ti intake and excretion more closely. The Ti intake was calculated per cow, with their own feed intake from the PMR and the realized PMR composition including TiO_2, under the assumption that the concentration in the feed orts did not differ from the composition in the fed diets (no selection of PMR components was observed during the trial).

Gas Emissions

Emissions of CH_4, H_2, and CO_2 were measured on an individual cow level for the duration of the trial by using the GreenFeed system (C-Lock Inc.; Zimmerman et al., 2011). The GreenFeed system measured airflow as well as CH_4, H_2, and CO_2 concentrations in the exhaust air. Each GreenFeed unit was equipped with a head position sensor, and gas emission data were rejected when the head position criteria were not met. Each individual cow could

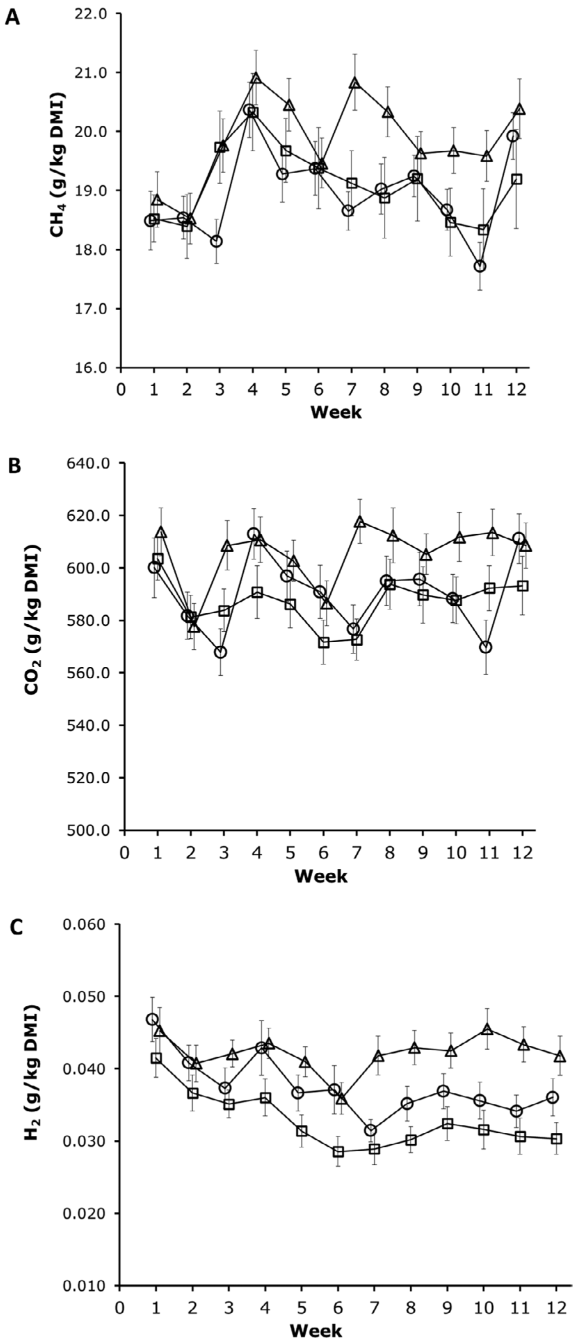

Figure 3. Evolution (calculated average of measured values of individual cows ± SEM of these values calculated as SD divided by the square root of the number of cows), of (A) CH_4 yield (g CH_4/kg DMI), (B) CO_2 yield (g CO_2/kg DMI), and (C) H_2 yield (g H_2/kg DMI) of lactating dairy cows fed the positive control diet (GS-POS; n = 20; ○), the grass press cake diet (GS-PC; n = 20; □), or the negative control diet (GS-NEG; n = 20; △), during the subsequent 10-week measurement period (wk 3 to 12), following an initial 2-wk pretrial period (wk 1 and 2) when all cows received the same diet (25% grass press cake and 75% of the same grass silage used in the GS-POS diet as the grass silage portion), and for which last week served as covariate (wk 2). Total DMI was used for calculations of CH_4 yield including DM intake of the PMR and concentrate bait in GreenFeed units and milking parlor. Results depict numerical outcomes for weekly observations, and not LSM and associated SEM as outcomes of statistical analysis.

visit the GreenFeed units every 3 h (with a maximum of 8 visits/d), and data collection was dependent on the cows' voluntary visits to the GreenFeed units, with a maximum of 9 so-called cup drops per visit (1 cup drop per 25 s, and 41.0 ± 3.1 g of feed per cup drop). By working with a 10-wk period, sufficient measurements per week (26.2 ± 9.2) were obtained for each individual cow to obtain reliable CH_4, H_2, and CO_2 measurements. The same procedures were followed with the GreenFeed system measurements as previously described in detail by van Gastelen et al. (2022), who performed a trial at the same positions in the same barn. We ensured that sufficient cow visits and emissions recordings were available to allow for a weekly estimate of gas emissions (for details, see van Gastelen et al., 2022 and de Mol et al., 2024).

Chemical Analysis

All feed samples collected during the feeding trial were air-dried by Eurofins at 60°C for 16 h and ground to pass a 1-mm screen (Eurofins Agro, Wageningen, the Netherlands). Separate feed and fecal samples collected to determine digestibility, were thawed at room temperature, freeze-dried until constant weight, and ground to pass a 1-mm screen by using a cross beater mill for both silages (Peppink 100AN) and an ultra-centrifugal mill for all other ration components (Retsch ZM200, Retsch GmbH). The samples were analyzed by using wet chemistry for DM, ash, N, starch (except for grass silage), reducing sugars (i.e., all carbohydrates with reducing properties and soluble in 40% ethanol; except for corn silage), crude fat (NEN-ISO 6492; ISO, 1999), NDF, ADF, and ADL (NEN-EN-ISO 13906; ISO, 2008) by Eurofins (Eurofins Agro, Wageningen, the Netherlands) and as described by Abrahamse et al. (2008). Crude protein was calculated as N × 6.25, where N was determined using the Kjeldahl method with $CuSO_4$ as catalyst (ISO 5983; ISO, 2005), and the fraction of ammonia determined by near-infrared spectroscopy (Eurofins Agro, Wageningen, the Netherlands) to distinguish between ammonia-N and CP-N.

The rectal grab samples were freeze-dried and milled, and then analyzed for DM, ash, N, crude fat, starch, NDF, ADF, and ADL. The Ti content of the Ti-containing soybean meal, as well as the rectal grab samples, was determined using the procedure described by Nichols et al. (2018). Before analysis, the 6 fecal grab samples per cow were pooled and a representative fecal sample was taken for analysis of Ti content, and wet chemical analysis was conducted comparable to the feed analysis (DM, ash, N, crude fat, NDF, ADF, and ADL).

Milk samples were analyzed for fat, protein, and lactose content, as well as SCC, by mid-infrared spectroscopy using MilkoScan FT 6000 equipment (Foss, Hillerød, Denmark) with the manufacturer-supplied basic calibration

Table 3. Feed intake, protein intake, and feed efficiency adjusted for covariate values of lactating dairy cows fed the positive control diet (GS-POS; n = 20), the grass press cake diet (GS-PC; n = 20), and the negative control diet (GS-NEG; n = 20)

Item	Treatment			Significance[1]			
	GS-POS	GS-PC	GS-NEG	SED	*P*-value T	*P*-value W	*P*-value T × W
DMI GreenFeed bait (kg/d)	1.69	1.62	1.75	0.108	0.447	<0.001	0.057
DMI milk parlor bait (kg/d; allowance was constant)	0.86	0.86	0.86	0.004	0.363	<0.001	0.731
DMI partially mixed ration (kg/d)	22.1[a]	21.7[a]	20.5[b]	0.27	<0.001	<0.001	<0.001
Total DMI (kg/d)	24.6[a]	24.2[a]	23.1[b]	0.23	<0.001	<0.001	<0.001
Total CP intake (kg/d)	3,537[a]	3,357[b]	3,140[c]	32.7	<0.001	<0.001	<0.001
Total CP intake, including NH_3 (kg/d)	3,721[a]	3,507[b]	3,283[c]	34.8	<0.001	<0.001	<0.001
Total DVE intake[2] (g/d)	2,044[a]	1,923[b]	1,922[b]	18.0	<0.001	<0.001	<0.001
Total OEB intake[2] (g/d)	340[a]	267[b]	111[c]	5.9	<0.001	<0.001	<0.001
Feed efficiency (kg FPCM/kg total DMI)	1.48	1.49	1.47	0.022	0.346	<0.001	<0.001
Feed N efficiency (g milk N/g total N intake)	0.337	0.346	0.346	0.004	0.145	<0.001	<0.001
Feed N efficiency (g milk N/g total N, including NH_3 intake)	0.321[a]	0.331[b]	0.331[b]	0.004	0.042	<0.001	<0.001

[a–c]Least squares means within a row with a different superscript indicate a significant difference ($P < 0.05$) between treatments.

[1]SED = standard error of difference; T = treatment effect; W = week effect; T × W = interaction of treatment and week effect.

[2]DVE = intestinal digestible protein; OEB = rumen degradable protein balance (van Duinkerken et al., 2011).

models according to THE manufacturer's recommended procedures (ISO 9622; ISO, 2013). Urea content was determined using the pH difference technique (ISO 14637; ISO, 2004). Milk composition was corrected for differences in milk yield between milking events on the same day, and the weighed milk composition on a daily basis was used for data analysis. Fat- and protein-corrected milk (**FPCM**) yield was subsequently calculated to milk containing 4.00% fat and 3.30% protein according to the equation FPCM (kg/d) = (0.337 + 0.116 × fat % + 0.06 × protein %) × milk yield (kg/d) (CVB, 2016).

Digestibility Trial with Sheep

In August 2023, Van Wesemael (unpublished data) determined in vivo digestibility of another batch of GS-PC at the Animal Science Unit of ILVO (Melle, Belgium) according to the protocol of CVB (1996) and approved by the Ethic Commission of ILVO (EC2023/440). This batch had a DM content of 316 g/kg, containing 936 g OM, 583 g NDF, 200 g CP, and 6 g sugars per kilogram of DM, AND thus less NDF but more CP than the batch used for the dairy trial. The GS-PC was fed as sole feedstuff to 5 almost-mature castrated male sheep (±70 kg BW) individually housed in metabolic crates. They received a fixed amount of 1 kg DM daily during an adaptation period of 18 d and an experimental period of 10 d, during which feces was collected in total daily and frozen. During the trial, there were no leftovers. After 10 d, the collected feces of each of the 5 animals was weighed, mixed, and sampled. The GS-PC and the 5 feces samples were analyzed for DM, ash, crude fiber, CP, crude fat, and NDF to obtain digestion coefficients. The NEL was calculated from determined gross energy and metabolizable energy based on the content of digestible nutrients (Van Es, 1978).

Statistical Analysis

Data analysis was performed on 60 cows, 20 cows from each of the 3 dietary treatments. During the pretrial period, 2 cows were replaced by reserve cows. One cow on GS-PC in wk 3, which was a small cow that did not use the cubicles well, was replaced by a reserve cow for which data from wk 3 are hence missing. Another cow on GS-POS had a knee accident in wk 5 and was replaced by another reserve cow, and as a result, data for this cow are missing in wk 3, 4 and 5. Further, a cow on GR-NEG had a reduced feed intake due to digestive disorders in wk 6 and 7, and a cow on GS-PC had digestive disorders in wk 5 and 6, and these data were indicated as outliers. Another cow on GS-POS suffered from mastitis, and wk 3 was indicated as outlier, and a cow on GS-NEG suffered from mastitis at the end of wk 8 and beginning of wk 9, and only these days were indicated as outliers (with the average of the remaining data no longer appearing to be an outlier).

All parameters related to feed intake, lactation performance, and gaseous exchange were averaged per cow per week during the 2 wk of the pretrial period, of which the last week was used as covariate, as well as in the subsequent 10 wk of the measurement period. Cow was considered as the experimental unit for all variables (all RIC bins allocated to a treatment were available for every cow on that specific treatment across the entire length of the barn, i.e., cows were not restricted in their use of allocated RIC bins, or other cows allocated to that same treatment). Data were analyzed with the REML procedure including adjustment for covariate in GENSTAT (GENSTAT Release 22.1, 2022, VSN International Ltd.) using the model $Y_{ijk} = \mu + C_i + T_j + W_k + (T \times W)_{jk} + Cov_i + \varepsilon_{ijk}$, with Y_{ijk} as the response variable, μ as the means of the parameters of interest, C_i as

Table 4. Milk production, BW, and BCS adjusted for covariate values of lactating dairy cows fed the positive control diet (GS-POS; n = 20), the grass press cake diet (GS-PC; n = 20), and the negative control diet (GS-NEG; n = 20)

	Treatment			Significance[1]			
Item	GS-POS	GS-PC	GS-NEG	SED	*P*-value T	*P*-value W	*P*-value T × W
Milk yield (kg/d)	33.6[a]	33.1[a]	31.0[b]	0.58	<0.001	<0.001	<0.001
Milk fat content (g/100 g)	4.61	4.68	4.71	0.075	0.434	<0.001	0.121
Milk protein content (g/100 g)	3.64	3.60	3.60	0.029	0.096	<0.001	0.037
Milk lactose content (g/100 g)	4.49	4.50	4.47	0.017	0.132	<0.001	0.026
Milk urea content (mg/dL)	18.2[a]	17.0[b]	16.9[b]	0.536	0.013	<0.001	0.001
Milk fat yield (g/d)	1,535[a]	1,534[a]	1,444[b]	29.0	0.003	<0.001	0.022
Milk protein yield (g/d)	1,214[a]	1,185[a]	1,108[b]	22.2	<0.001	<0.001	<0.001
Milk lactose yield (g/d)	1,507[a]	1,490[a]	1,385[b]	28.7	<0.001	<0.001	<0.001
FPCM[2] (kg/d)	36.4[a]	36.1[a]	33.9[b]	0.70	<0.001	<0.001	0.002
SCC (× 1,000 cells/mL)	280	241	260	113.0	0.903	0.070	0.618
BW (kg)	699a	701[a]	694[b]	2.7	0.028	<0.001	0.043
BCS	3.09	3.09	3.08	0.07	0.983	<0.001	0.788
BW change (kg/wk)	2.3	2.6	1.6	0.50	0.438	<0.001	0.007

[a,b]Least squares means within a row with a different superscript indicate a significant difference ($P < 0.05$) between treatments.

[1]SED = standard error of difference; T = treatment effect; W = week effect; T × W = interaction of treatment and week effect.

[2]Fat- and protein-corrected milk = [0.337 + 0.116 × fat (g/100 g) + 0.06 × protein (g/100 g)] × milk yield (kg/d) (CVB, 2016).

the effect of cow within block (i = 1… 20), T_j as the effect of dietary treatment j (j = 1…3), W_k as the effect of week (k = 1…10), $(T \times W)_{jk}$ as the interaction between T_j and W_k effect, Cov_i as the covariate for each cow, and ε_{ijk} as the residual term. Because observations of nutrient digestibility and N balance data were obtained for a single week, an ANOVA analysis was used without a covariate, without a week effect, and without the effect of interaction between treatment and week. All results are reported as LSM and significance of effects was based on the LSD, with significance declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS

Average DMI (PMR) and FPCM appeared very similar during the pretrial period (initial 2 wk); on average 22.3 ± 3.1 (mean ± SD), 22.5 ± 3.0, and 22.1 ± 2.7 kg DM/d and 35.9 ± 5.3, 34.8 ± 5.8, and 34.9 ± 5.7 kg FPCM/d, respectively, for the 3 groups to be allocated to the 3 treatments while on the same diet (including 25% GS-PC and 75% of a regular grass silage in the grass silage portion of the diet). Results obtained in the second week of the pretrial period that served as the covariate preceding the treatment period demonstrated that individual cows appeared well-blocked over the 3 treatments. The results in wk 2 (Figures 1A and 2) that served as the covariate demonstrated an average DMI of the PMR of 23.0 ± 3.2, 23.1 ± 3.0, and 23.0 ± 2.8 kg DM/d; an FPCM production of 37.5 ± 4.9, 36.9 ± 5.8, and 37.0 ± 6.1 kg/d; a milk protein content of 3.65% ± 0.30%, 3.61% ± 0.23%, and 3.62% ± 0.32%; and a milk fat content of 4.46% ± 0.59%, 4.37% ± 0.83%, and 4.31% ± 0.61%, for the groups to be allocated later to treatments GS-POS, GS-PC, and GS-NEG, respectively. All cows hence responded similarly to treatment during the covariate period, and no changes appeared necessary with respect to the initial blocking of cows at the start of the pretrial period. Also the observed CH_4 yield (Figure 3A) was very similar between treatments with 18.4, 18.1, and 18.2 g CH_4/kg DMI for GS-POS, GS-PC, and GS-NEG, respectively (differing less than 2%). The small differences remaining in wk 2 between the 3 groups of cows were taken into account as a covariate with statistical analysis and the results presented here (except for digestibility and N balance data).

The intended differences in grass silage characteristics were achieved (Table 1), with the CP content of GS-PC and GS-NEG silage being 27% and 32% lower, respectively, than the GS-POS silage (resulting in ~1% lower CP content of the total diet), and the NDF content was 39% and 21% higher (Table 1). The highest NEL value was estimated for the grass silage applied in the GS-POS diet (serving as positive control), whereas the value was 12% lower for the grass silage used in GS-NEG (serving as negative control) as well as for the ensiled grass press cake used in GS-PC.

The average composition of the single experimental diet applied during the pretrial period (including the covariate week), as well as the 3 treatment diets during the 10-wk treatment period, remained close to what was planned (Table 2). Differences in the average proportion of grass silage in dietary DM remained between 39.8% on a DM basis (during adaptation and covariate weeks) and 41.4% for GS-PC during the treatment period. The average proportion of the other dietary components (maize silage, concentrates, premix, soybean meal) remained within 1% on a DM basis of what was intended (Table 2).

Table 5. Nutrient intake, feces composition, and fecal nutrient digestibility in lactating dairy cows during the measurement week when part of the soybean meal was replaced by soybean meal including TiO_2 marker in the positive control diet (GS-POS; n = 10), the grass press cake diet (GS-PC; n = 10), and the negative control diet (GS-NEG; n = 10)

	Treatment			Significance	
Item	GS-POS	GS-PC	GS-NEG	SED[1]	*P*-value
Diet					
Total DM intake (kg/d)	25.7	25.0	23.9	1.34	0.435
Roughage (% of total DMI)	59	60	59	0.9	0.146
CP (kg/d)	3.93	3.65	3.45	0.201	0.078
Feces composition					
DM (g/kg freeze-dried feces)	131	128	132	3.9	0.667
OM (g/kg DM)	859[b]	873[a]	865[b]	3.0	<0.001
N (g/kg DM)	31	30	30	0.6	0.397
Crude fat (g/kg DM)	36	35	36	1.3	0.309
NDF (g/kg DM)	457[b]	486[a]	475[ab]	9.5	0.017
ADF (g/kg DM)	274[b]	295[a]	288[a]	6.0	0.005
ADL (g/kg DM)	73[b]	83[a]	76[b]	2.7	0.002
Digestibility (% of intake)					
DM	68.2[b]	70.4[a]	69.7[ab]	0.82	0.035
Ash	50.8	53.1	54.1	1.48	0.089
OM	70.0	71.9	71.2	0.80	0.063
CP	60.2	61.7	60.9	0.88	0.250
Crude fat	69.7[b]	72.9[a]	69.9[b]	1.21	0.025
NDF	62.2	65.0	63.4	1.48	0.174
ADF	60.1	62.9	60.8	1.46	0.160
ADL	−7.1	−4.8	−3.1	3.70	0.562

[a,b]Least squares means within a row with a different superscript indicate a significant difference ($P < 0.05$) between treatments.

[1]SED = standard error of difference.

In general, most cow performance variables for which a significant treatment effect was established also demonstrated a simultaneously significant effect of measurement week, and often a significant interaction between treatment and week as well. The latter indicates that next to treatment differences, the cow response variables also depended on week of treatment. Furthermore, the time course (week effects) of differences in cow performance due to treatment significantly differed between treatments ($P < 0.001$, except for SCC). In the following sections, treatment effects will be presented in more detail.

Feed Intake and Feed Efficiency

There was a significant effect of grass silage type on PMR intake, with a 7.3% and 5.6% lower ($P < 0.001$) DMI with GS-NEG compared with GS-POS and GS-PC, respectively, whereas DMI of the GreenFeed and milking parlor bait did not differ between treatments (Table 3). All 3 treatments differed ($P < 0.001$) from each other in CP intake and rumen protein balance (Table 3), decreasing in the order of GS-POS, GS-PC, and GS-NEG. Intake of estimated intestinal digestible protein (Table 3) was 6.3% higher ($P < 0.001$) for GS-POS compared with GS-PC and GS-NEG respectively. No differences ($P > 0.05$) in feed efficiency were established, but N use efficiency was 3.1% higher for both GS-PC and GS-NEG (on average 33.1% N use efficiency) compared with GS-POS.

Lactation Characteristics and Body Measures

Treatments groups differed ($P < 0.001$) in milk yield, with a yield for GS-NEG 7.7% and 6.4% lower compared with GS-POS and GS-PC, respectively (Table 4). Milk composition did not differ ($P > 0.05$) between treatments. Milk protein, fat, and lactose yields were lower ($P < 0.001$) for GS-NEG compared with GS-PC (6.5%, 5.9%, and 7.0% lower, respectively) and GS-POS (8.7%, 5.9%, and 8.1% lower, respectively), whereas GS-PC and GS-POS did not differ (Table 4). In addition, FPCM was 6.1% and 6.9% lower ($P < 0.001$) for GS-NEG compared with GS-PC and GS-POS, without differences between the latter two. Although the automatically monitored BCS did not differ between the start and end of the trial, the recorded BW was lower ($P = 0.028$) for GS-NEG by 5 to 7 kg compared with GS-POS and GS-PC, respectively (Table 4). There was a small increase in BW, which numerically barely differed, however, among treatments (24, 21, and 18 kg in total with GS-POS, GS-PC, and PS-NEG, respectively), and the small differences in BW were very consistent throughout the measurement period (Figure 1B) with no significant differences in BW change per week (Table 4).

Table 6. Nitrogen intake and N excretion in milk, feces, and urine, and associated apparent N use efficiency in lactating dairy cows during the measurement week when part of the soybean meal was replaced soybean meal including TiO_2 marker to determine fecal digestibility for the positive control diet (GS-POS; n = 10), the grass press cake diet (GS-PC; n = 10), and the negative control diet (GS-NEG; n = 10); calculations were made under assumption observed N digestibility and zero N retention in the cow body

	Treatment			Significance	
Item	GS-POS	GS-PC	GS-NEG	SED[1]	*P*-value
N balance (g/d)	628	584	552	32.2	0.078
N intake (observed)					
N feces (observed)	249	224	217	14.4	0.078
N milk (observed)	193	191	182	10.9	0.556
N urine (calculated)	185	168	153	12.8	0.059
N excreted (calculated)	435[a]	393[ab]	370[b]	24.6	0.042
Partitioning ingested N (%)					
N feces	39.8	38.3	39.2	0.88	0.250
N milk	30.9	32.9	33.0	1.22	0.171
N urine (calculated)	29.3	28.8	27.8	1.42	0.594
N excreted (calculated)	69.1	67.1	67.0	1.22	0.171
N efficiency (g milk N/g N intake × 100)	30.9	32.9	33.0	1.22	0.166

[a,b]Least squares means within a row with a different superscript indicate a significant difference ($P < 0.05$) between treatments.

[1]SED = standard error of difference.

Digestibility, Use Efficiency, and Excretion of Nitrogen

Two weeks before ending the treatment period (i.e., wk 10 or the eighth week of treatment), digestibility was determined making use of the recorded composition of the PMR and the recorded intake of this PMR as well as of concentrate bait consumed in the GreenFeed units and milking parlor. Due to the smaller amount of data used (from 10 instead of the total of 20 cows per group) for analysis during the week of rectal grab sampling, treatment differences in total DMI remained insignificant (Table 5), but outcomes for treatments closely resembled the differences observed for the whole treatment period. Numerically, the DMI of GS-NEG was 1.8 and 1.1 kg DM/d lower compared with that of GS-POS and GS-PC when digestibility was measured, compared with 1.5 and 1.1 kg DM/d lower values established for the whole treatment period (Table 3). The treatment differences in CP intake ($P = 0.078$) also resembled those for the whole treatment period, with GS-POS being 7.0% and 12.1% lower in this sampling week than GS-PC and GS-NEG, respectively (Table 5), compared with 5.7% and 11.7% lower values that were obtained for the whole treatment period (Table 3). The roughage percentage achieved in the PMR and in total DMI did not differ, and treatment differences remained within 1% of DM in this particular sampling week (Table 5).

The chemical composition of feces collected for the 3 treatment groups significantly differed in OM and ADL content, with higher values for GS-PC compared with GS-POS, as well as GS-NEG. Feces NDF and ADF content were significantly lower for GS-POS compared with GS-PC and only for ADF compared with GS-NEG (Table 5). Calculated digestibility of DM and crude fat was higher ($P = 0.025$) for GS-PC compared with GS-POS, with only a trend for higher values with GS-PC for ash ($P = 0.089$) and OM ($P = 0.063$) compared with GS-POS. Numerically, a 1- to 2-percentage-units higher digestibility of CP, NDF, and ADF was also observed for GS-PC compared with GS-POS and GS-NEG. For GS-NEG, only the digestibility of crude fat was significantly lower compared with GS-PC, but not that of DM (Table 5).

Based on estimates of CP digestibility, a N balance was calculated (Table 6) which only showed treatment differences ($P < 0.05$) for the total amount of N excreted. For N intake, N excreted in feces, and N excreted in urine, only trends were obtained. For all, the amounts of N involved were significantly higher for GS-POS compared with GS-NEG, with GS-PC showing intermediate values not significantly differing. When expressing the partitioning of N as a percentage of N intake, and for calculated N efficiency, treatments did not differ.

Gas Emissions

Measurement of gaseous emissions throughout the whole treatment period demonstrated significant treatment differences for CO_2, CH_4, and H_2 (Table 7). The production of CO_2 (g/d) was significantly lower for GS-NEG compared with GS-POS, but CO_2 yield (g/kg DMI) was significantly higher for GS-NEG compared with GS-POS, as well as GS-PC (3.8% on average). The CH_4 production did not differ between treatments, but CH_4 yield was significantly higher for GS-NEG compared with GS-POS and GS-PC (4.1% and 5.8%,

Table 7. Gaseous emissions measured with the GreenFeed system units (GF) adjusted for covariate values of lactating dairy fed the positive control diet (GS-POS; n = 20), the grass press cake diet (GS-PC; n = 20) and the negative control diet (GS-NEG; n = 20)

Item	Treatment			Significance[1]			
	GS-POS	GS-PC	GS-NEG	SED	*P*-value T	*P*-value W	*P*-value T × W
GF visits per cow per day	3.9	3.5	4.0	0.26	0.085	<0.001	0.614
CO_2 emission							
Production (g/d)	14,427[a]	14,154[ab]	14,001[b]	144.0	0.014	<0.001	0.003
Yield (g/kg DMI)	589[b]	586[b]	610[a]	6.6	0.002	<0.001	<0.001
CH_4 emission							
Production (g/d)	465	467	462	8.5	0.907	<0.001	0.117
Yield (g/kg DMI)	19.0[b]	19.3[b]	20.1[a]	0.28	<0.001	<0.001	<0.001
Intensity (g/kg FPCM[1])	13.0[b]	13.1[b]	13.8[a]	0.22	0.001	<0.001	0.010
H_2 emission							
Production (g/d)	0.86[ab]	0.79[b]	0.94[a]	0.039	0.002	0.002	0.146
Yield (g/kg DMI)	0.035[b]	0.033[b]	0.041[a]	0.0017	<0.001	0.001	0.050
Intensity (g/kg FPCM)	0.024[b]	0.023[b]	0.028[a]	0.0012	<0.001	0.009	0.038
CH_4 to CO_2 ratio (×100)	3.22	3.29	3.29	0.047	0.224	<0.001	0.002
H_2 to CH_4 ratio (×1,000)	1.85[b]	1.70[b]	2.05[a]	0.082	<0.001	<0.001	0.550

[a,b]Least squares means within a row with a different superscript indicate a significant difference ($P < 0.05$) between treatments.

[1]SED = standard error of difference; T = treatment effect; W = week effect; T × W = interaction of treatment and week effect.

respectively), which also holds for CH_4 intensity (5.3% and 6.2%, respectively). Values for H_2 (production, yield, and intensity) were significantly lower for GS-POS and GS-PC compared with GS-NEG (except H_2 production for GS-POS). The CH_4/CO_2 ratio did not differ between treatments, but the H_2/CH_4 ratio was significantly higher for GS-NEG compared with GS-POS and GS-PC (on average 18.8%).

We found no significant differences between treatments in the number of cow visits to the GreenFeed system units (Table 7), and despite a significant (but numerically small) effect of measurement week, there were no interactions between treatment and week. Sufficient visits were available for cows to allow for weekly estimates of gas emissions.

DISCUSSION

There is a need to reduce dietary CP content if current dairy production systems are to reduce N emissions substantially. Grass products are in many dairy systems the main dietary component and producing grass with a lower CP content, can be a N mitigation measure by preventing high rates of N excretion with urine and preventing ammonia emissions in confined systems in particular, and nitrous oxide emissions in grazing systems in particular. However producing grass with a lower CP content is associated with less N fertilization and less productive grasslands or with grass harvested or grazed at a later stage of maturity. Such measures directly affect the grass nutritive value, its digestibility, and cow performance, and as a trade-off it is likely to increase enteric CH_4 emission as well as manure CH_4 emissions due to a lower fiber digestibility. A study of Warner et al. (2017) clearly demonstrated these effects when feeding lactating cows silages (70% on DM basis) of grass cuts at drastically different stages of maturity. With an increase of maturity, N intake decreased 42% and total N excreted decreased 40%, whereas milk N efficiency (N milk/N intake) increased 47%. Therefore, increased grass maturity or an extensification of grassland management appears to be an effective N mitigation measure. However, this was at the cost of a decline in NEL by 16%, of OM digestibility by 12%, and of FPCM production by 12%, whereas CH_4 yield increased 21% when expressed per kilogram of DMI and 34% when expressed per kilogram of OM digested. These effects are huge trade-offs that must be to be taken into account. Other studies with grass-based diets demonstrated similar outcomes with different stages of maturity of ryegrass-clover silage (Brask et al., 2013), grass herbage in a grazing system (Wims et al., 2010) and early- and late-cut grass herbage (Hansen et al., 2022). Thus, there are serious consequences of N mitigation through extensification of grassland management and harvesting grass with a low CP content.

In the present study, biorefinery of grass herbage as a N mitigation measure was evaluated as an alternative to changing grassland management. Previous studies reported that press cake from biorefined grass herbage is a valuable roughage feed (Serra et al., 2023; Damborg et al., 2019). The aim of the present study was to investigate this value and the potential application of ensiled press cake as a rest stream from biorefinery of grass herbage as a roughage in diet of lactating dairy cows, as well as to compare ensiled press cake with a more extensively produced grass silage as a N mitigation measure. The ensiled press cake was studied by partial replacement of the regular grass silage part of the diet, which is considered to be a realistic amount for farming practice, and control on diet composition was ensured by offering the diet as a mixed diet. The realized proportion of grass silage DM exchange (50% of total grass silage DM) was close to planning, and the DM proportion of the other dietary components also appeared to have been kept at the intended DM proportion throughout the trial. The present trial was hence conducted according plan, and the small differences in portion of diet components are mainly to be explained by slight deviations from the intended exchange of ~20% of ensiled grass product in dietary DM (Table 2).

Using existing grass silage NIRS calibrations to predict cow performance (as commercial dairy farmers do), or even in vitro digestibility measurements, would lead to lower expectations of GS-PC than observed in the present study (Table 8), and lower expectations compared with regular grass silages, represented by GS-POS. The reason for this is thought to be the comminution and improved disclosure of the fibrous material for rumen microbial breakdown compared with regular grass silage, either of a regular (GS-POS) or poorer (GS-NEG) quality. An indicative chop length for GS-PC would rather be 5 to 10 mm, in contrast to 15 mm for GS-POS. Tayyab et al. (2019) reported that silage of short chopped grass increased NDF digestibility by 5 percentage units compared with long chopped grass. Results from Kammes and Allen (2012) for long compared with short chopped orchard grass were less clear, however, perhaps due to the exchange of all grass silage (50% OM, DM basis) in contrast to only 20% of DM in the present study. The results of Zebeli et al. (2007) also showed that the effect of particle size of grass hay on DM and NDF digestibility differed with the level of concentrate feeding. Although results in the literature on the effect of chopping of grass silage may not always be conclusive, it is important to consider that the material in the press cake from the grass biorefinery process is both finer (increased surface) and has an opened fiber structure, which is different from regular grass cutting, which leaves the fiber structure basically intact. Other studies on press cake from grass biorefinery also report the positive effect and disclosure of the grass fiber fraction with 35% of dietary DM exchanged (Damborg et al., 2019), and with 65% of dietary DM exchanged (Hansen et al., 2022). For the latter study, an interaction was obtained for the effect of grass processing on NDF digestibility and the stage of maturity of the harvested grass, with a 7-percentage-unit and 1-percentage-unit higher NDF digestibility for late-cut and early-cut grass, respectively. This demonstrates that with more fibrous and less digestible grass, the disclosure effect of the pressing process on digestibility compared with regular ensiling of unprocessed grass is most prominent.

Cow Performance

Although a 12% lower NEL value of 5.55 and 5.53 MJ/kg DM was estimated for GS-PC and GS-NEG with regular (grass silage) NIRS analysis (Table 1), compared with GS-POS (6.28 MJ/kg DM), cow performances did not differ between GS-POS and GS-PC, and DMI was similar (Table 3). This finding is supported by the digestibility measurements, which indicated a higher DM digestibility for GS-PC compared with GS-POS despite the 39% higher fiber content in the exchanged silage with the former treatment. A 2-percentage-unit higher DM digestibility was established for GS-PC compared with GS-POS, and numerically, the digestibility of all other fractions was also highest for GC-PC, which indicates an apparently better disclosure for microbial degradation and digestion. Cows performed equally well on GS-POS and GS-PC in terms of DMI and FPCM yield, whereas they consumed 5% less and produced 7% less on GS-NEG (Figure 1A; Tables 3 and 4). Because digestibility of GS-NEG was rather similar to that of GS-POS (Tables 5 and 8), this result appears to be mainly due to a lower DMI, and as a result a lower NEL intake, leading to a lower FPCM yield. The numerical differences in DMI and FPCM yield with GS-NEG compared with GS-POS appeared to be consistent (1.5 kg of DMI and 2.5 kg FPCM, respectively). Effects on feed efficiency remained too small to become statistically significant but were numerically consistent with the digestibility results.

In addition to the cow trial, an in vivo study in wethers was performed with a batch of ensiled GS-PC other than that used in the present cow study in order to determine the NEL value according to the protocol (CVB, 1996) for the Dutch VEM system (Van Es, 1978). Table 8 shows the comparison between results of the present in vivo study in lactating cows and the results for wethers. Overall, results on digestibility from the wether study further substantiate the indications from the cow study that the nutrient digestibility and energy value of grass press cake is higher than currently estimated with feed analysis in practice (either by in vitro digestibility or use of NIRS grass silage calibration lines).

The lower intake of N with GS-PC and GS-NEG did not result in a significantly lower milk protein content. Nevertheless, inspecting the time course of milk protein content (Figure 2) indicates a numerical drop during the first 6 wk for GS-PC, as well as for GS-NEG compared with GS-POS (Figure 2), which was reversed again from the seventh week of treatment onward. This result seems to indicate an adaptation of the cows to the diets with a lower N content, so that no differences remained toward

Table 8. In vivo digestibility and NEL value of a batch ensiled grass press cake (GS-PC) fed at 100% of DMI to wethers (ILVO trial) versus the in vivo digestibility of GS-PC fed at 20% of DMI to lactating cows (Wageningen trial) estimated from the increase in digestibility of the diet compared with either the diet in which this 20% of DMI was high-quality grass silage (GS-POS) and the diet in which it was low-quality grass silage (GS-NEG); see Materials and Methods for further details on diets

	Wether study	Cow study				
Item	100% GS-PC	20% GS-PC and 20% GS-POS	40% GS-POS	GS-PC digestibility estimated from increase compared with GS-POS and GS-NEG treatments[1]		20% GS-NEG and 20% GS-POS
Diet digestibility, in vivo (%)						
DM	75.6	70.4	68.2	79.2	73.2	69.7
OM	78.0	71.9	70.0	79.5	74.7	71.2
NDF	83.5	65.0	62.2	76.2	71.4	63.4

		Energy value of grass silages		
	GS-PC	GS-PC	GS-POS	GS-NEG
In vivo digestibility[2]				
NEL vivo (MJ/kg DM)	6.68	—	—	—
In vitro digestibility[3] (%)	—	64.7	68.3	63.4
NEL vitro (MJ/kg DM)	—	5.55	6.28	5.53
NIRS				
NEL NIRS (MJ/kg DM)	—	5.31	6.31	5.46

[1]Digestibility of DM, OM and NDF in GS-PC as a product was estimated under assumption that the increased digestibility can be entirely attributed to the exchange with of 20% of DM with GS-PC. The digestibility was estimated by (digestibility of the GS-PC diet − digestibility of the GS-POS diet or of the GS-NEG diet)/0.2 + digestibility of the GS-POS diet or of the GS-NEG diet.

[2]Energy value (NEL) of GS-PC in the ILVO-study was not estimated by NIRS, but was calculated based on chemical composition and the in vivo digestion coefficients (Van Es, 1978).

[3]Digestible Organic Matter determined by in vitro incubation of grass silages (Eurofins, Wageningen, the Netherlands) according to the method of Tilley and Terry (1963). Estimated NEL by NIRS using grass silage calibrations (Eurofins, Wageningen, the Netherlands).

the end of the trial. A possible reason for this adaptation to GS-NEG could be that the lower silage quality caused a lower DMI, FPCM yield, and milk protein yield, also reducing the requirement for metabolizable protein, which may have contributed to this adaptation. For the GS-PC, DMI and FPCM and milk protein yield were rather unaffected (Tables 4 and 5). It is possible that adaptation processes in cow metabolism and urea recycling gradually enabled a larger amount of metabolizable protein to be formed from the disclosed grass press cake during the first 6 wk of treatment, allowing more microbial protein synthesis, which contributes to the delivery of metabolizable protein. Digestibility appeared highest for the GS-PC diet during the eighth week of treatment (wk 10 in Figure 2) which may not reflect digestibility at the onset of the treatment period. With numerically similar DMI for GS-POS and GS-PC (Figure 1A; apart from the first week), the FPCM yield appeared to be lower for GS-PC during the first 4 wk of treatment only, which may indicate an improvement of microbial activity, microbial protein synthesis, and utilization of the GS-PC diet for milk synthesis over time.

N Mitigation, Digestion, Feeding Values, and Feed and N Efficiency

The N-mitigating effect remained marginal because only 20% of dietary DM was exchanged, and the CP content in the grass products exchanged differed by only 5% to 6% of DM (Table 1), leaving a 1% lower CP content in GS-PC and GS-NEG compared with GS-POS (Table 2). Nevertheless, N use efficiency was significantly improved by 3% with GS-PC and GS-NEG compared with GS-POS, without a loss of FPCM yield with GS-PC compared with GS-POS, but with 7% less FPCM yield with GS-NEG. For the N digestibility measurements, the effects were numerically in the same direction but too variable to become significant (Table 6), and the same holds for N balance data, except for calculated total N excreted (which was significantly lower for GS-NEG compared with GS-POS). The present findings demonstrate no trade-off in terms of cow performance when using GS-PC as a N mitigation measure, although this clearly was the case for GS-NEG, which corresponds with literature on feeding varying grass silage quality and comparing different stages of maturity (Brask et al., 2013; Warner et al., 2017; Hansen et al., 2022).

Gas Emissions

Gaseous emissions observed included CO_2, which most directly reflects DMI and cow metabolism. There appeared to be consistent differences between treatments in CO_2 emission (Figure 3B), with GS-NEG generating lower emissions rates due to a lower DMI compared with GS-POS and GS-PC, but with a 4% higher CO_2 yield. Because diet digestibility was rather similar for all treatments with no significant difference between GS-NEG and GS-PC, this result reflects that relatively more CO_2 was formed per unit of digested DM, which may be due to the relatively greater contribution of cow metabolism (maintenance as well as productive functions) per unit of milk synthesized. No clear patterns in time were observed in CO_2 yield, and the variation observed is probably due to variation and changes in weekly DMI and GreenFeed measurements.

The emission of CH_4 reflects enteric fermentation and silage degradation in the rumen (and large intestine), in particular of the dietary NDF fraction, as this can only be digested through microbial fermentation processes. The emission rate of CH_4 did not differ between treatments because the lower DMI with GS-NEG compensated for its higher CH_4 yield (Figure 3A). This means that the lower quality grass silage (GS-NEG) delivered relatively more CH_4 per unit of DM and per unit of digested OM compared with the regular quality grass silage (GS-POS). Attributing the differences in CH_4 yield entirely to the portion of 20% DM grass silage exchanged, the present study demonstrates a 29% higher CH_4 yield for GS-NEG compared with GS-POS (Table 7), which compares well to results from Warner et al. (2017). Although not significantly differing from GS-POS, the CH_4 yield appeared numerically 8% higher for GS-PC (Table 7), which is modest considering the 39% higher fiber content of the exchanged grass portion with GS-PC (Table 1) and the normally higher CH_4 yields attributed to fiber fermentation (Niu et al., 2018).

The reason for the similar CH_4 yield probably lies in the more disclosed fiber in this material, enabling a faster rate of fermentation, and the more fine material as a roughage, which allows a faster meal intake, affecting the H_2 production rate and fermentation pattern (with less H_2 formed if the pattern shifts from an acetate to a propionate fermentation) compared with the exchanged material in GS-POS. An indication for this is the numerically higher DM digestibility of the GS-PC diet, even though only 20% of DM was exchanged (Table 5). Attributing this higher digestibility entirely to this exchanged material, the press cake in GS-PC was 16% more digestible compared with the grass silage in GS-POS. Another indication is the numerically lower H_2 yield with GS-PC compared with GS-POS, as demonstrated by the 8% lower H_2/CH_4 ratio (Table 7). Hence, the results all appear to indicate grass press cake to be a well-disclosed highly fibrous material that is consumed well, ferments well, and contributes to a faster rumen fermentation, with an increase in CH_4 emission that is less than theoretically expected. Notwithstanding the fact that the H_2 measurements also support this view, some reservation must be made on the H_2 measurements because confounding with differences in feed intake patterns and GreenFeed visiting behavior of cows can also play an important role (de Mol et al., 2024) and cannot entirely be excluded here.

CONCLUSIONS

Reduction of dietary CP content by partially replacing a regular grass silage with either a low-CP grass silage evaluated as of less nutritive value, or a low-CP ensiled grass press cake obtained with grass biorefinery, demonstrated that cow performance only could be maintained with the latter. This result is explained from an improved digestibility and nutritive value of ensiled press cake, which was seriously underpredicted with the current methodology of grass silage analysis. Despite the relatively high fiber content of ensiled grass press cake, methane yield was not increased, whereas it was significantly higher for the poor-quality grass silage. The present results indicate that ensiled press cake retrieved from grass biorefinery is a high-potential roughage in ration formulation for lactating cows due to its high nutritive value and low CP content. The use of grass press cake appeared to be an effective measure to decrease N intake and excretion while increasing N use efficiency of cows, without compromising cow performance, in contrast to silage of late-cut grass with a low CP content.

NOTES

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Nonstandard abbreviations used: DOM = digestible organic matter; DVE = intestinal digestible protein; FPCM = fat- and protein-corrected milk; GS-NEG = negative control lower quality grass silage; GS-PC = ensiled press cake obtained from grass biorefinery; GS-POS = positive control regular quality grass silage; OEB = rumen degradable protein balance; PMR = partial mixed ration; RIC = roughage intake control; SED = standard error of difference; T = treatment effect; W = week effect.

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