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Former food and cocoa bean shells in early-lactating cows on a herbage-based diet: effects on ruminal fermentation and blood metabolites

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ABSTRACT

Rising food security concerns are driving the livestock sector to explore alternatives to cereal grains, like by–products from the food industry, but their effect on animals needs to be studied. The study assessed the impact of replacing 55% cereal grains with former food products (**FFP**) on ruminal fermentation, ruminal methane production, and blood metabolites, with or without cocoa bean shell (**CBS**) supplementation. We conducted a first (spring, **E1**) and a second (fall, **E2**) experiment, each with 17 early-lactating Holstein and Red Holstein cows. Each experiment lasted 6 weeks, including a 3-week adaptation and a 3-week experimental period. In each experiment, the animals were fed freshly cut grass as a basal diet and were balanced for milk yield, parity, and days in milk and assigned to three concentrate types (**CCT**): (i) a control concentrate (**CON**), (ii) a concentrate consisting of 55% FFP (**FFP-**), and (iii) an FFP concentrate that included an additional 5% CBS (**FFP+**). Feed intake and milk production were recorded daily during the 3-week sampling period; blood serum and ruminal fluid samples were collected twice, at the end of the adaptation and experimental periods. Statistical analyses were conducted on data from both experiments. DM, herbage, and most nutrient intakes were greater in E2 than in E1, probably because of seasonal changes in herbage quality. In E1, CON cows had lower DM intake (**DMI**) than FFP- cows, whereas in E2, CON cows had greater DMI than FFP+ cows. Across experiments, FFP- and FFP+ cows had greater water-soluble carbohydrates and fat and lower starch intakes than CON cows. The energy-corrected milk yield was greater in E1 than in E2 and unaffected by CCT. Irrespective of the experiment, the CON cows had the greatest, FFP- intermediate and FFP+ lowest milk lactose percentages and FFP + cows had greater milk fat percentages than CON cows. The mean and maximum reticular pH were lower for CON than for FFP- cows in E1 and were unaffected by CCT in E2. Irrespective of the experiment, acetate proportions in ruminal fluid of CON cows were lower than those of FFP- and FFP+ cows. Methane yield was greater in E2 than in E1 and unaffected by CCT. Serum albumin, non-esterified fatty acids and glucose levels varied by CCT in E2, but not in E1. Combining FFP and CBS with herbage could help increase the sustainability of early-lactating dairy cow nutrition without compromising health, but results need future corroboration.

Implications

Feeding human inedible or inappropriate resources, such as herbage and former food, to ruminants is crucial to reducing feed–food competition. We compared concentrates including 55% baking industry former food with or without cocoa bean shells to a cereal-based concentrate fed to early-lactating Holstein cows (mean milk yield: 36.4 kg/d) on a freshly cut herbage basal diet (zero-grazing). Concentrates with former food and cocoa bean shells had no negative consequences on ruminal fermentation, methane production, and blood metabolites. This suggests that combining herbage and former food is possible in diets for early-lactating dairy cows. Future studies should corroborate the results.

Introduction

The rising concerns about food security due to the growing global demand are leading the livestock sector to find alternatives to cereal grains that can be consumed by humans. A strategy for

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mitigating the feed–food competition for grains is the use of leftovers and by–products in the food industry (Pinotti et al., 2023), for example, former foodstuff products (**FFP**) of the baking industry (Pinotti et al., 2021). The FFP refer to food and food ingredients originally intended for human consumption but failing to comply with, for example, quality control, manufacturing processes, or market demand and are therefore unsuitable for direct human consumption (Gustafsson et al., 2013). The use of FFP in the diets of dairy cows faces several challenges. The nutrient composition in FFP often varies depending on the seasonality of the food industry leftovers. However, FFP processors have acquired experience in collecting, analysing, and mixing different ingredients and are now able to provide a final commercial product with a stable chemical composition all year round (Tretola et al., 2019). Other concerns are related to the chemical characteristics of FFP, which are rich in rapidly fermentable carbohydrates (starch: 42–73% DM, Ottoboni et al. (2019)) and fat (5–13% DM, Giromini et al. (2017)). Among carbohydrates, simple sugars (e.g., sucrose, lactose, glucose, and fructose) represent a significant part (Guo et al., 2015). Although less evident than for starch, the rapid ruminal degradation of very large amounts of simple sugars may increase the risk of subacute rumen acidosis (Plaizier et al. (2008)), depending on other dietary factors, such as particle size and physically effective NDF content (Khorrami et al., 2021). Fat and oils in FFP mainly include saturated fatty acids, which, in excessive amounts, can reduce fibre digestibility (Palmquist and Jenkins, 1980), thereby possibly lowering methane production. Another mechanism that reduces methanogenesis by saturated fatty acids may be their inhibitory effect on methanogens (Zhou et al., 2013). The impact of partially substituting cereal grains by FFP on DM intake, milk production, metabolic health, and ruminal pH of mid-lactating Simmental cows fed a total-mixed ration was recently investigated (Kaltenegger et al., 2020). Including up to 30% FFP of the baking industry in the diets did not represent a risk for the animals, increased milk performance, and, surprisingly, lowered the risk of subacute rumen acidosis by increasing the ruminal pH (Kaltenegger et al., 2020). Furthermore, feeding FFP increased the apparent total tract digestibility and digestible organic matter (OM) intake but lowered faecal microbial diversity and faecal pH (Kaltenegger et al., 2021). In both studies, mid-lactating dairy cows were used, and methane emissions were not studied.

In addition to FFP as an alternative to grains, cocoa bean shells (**CBS**), a by–product of chocolate production, could be interesting feed supplements for dairy cows. Almost 5 million tonnes of cocoa beans were processed worldwide in 2021/2022 (ICCO, 2023), resulting in tonnes of CBS that are often sent to the landfill or left on the soil, possibly promoting plant disease development (Lu et al., 2018). The chemical composition of CBS makes it an interesting feed ingredient for ruminants. It contains considerable amounts of CP (14–22% DM), energy, minerals, phosphorus, and magnesium, and is rich in structural carbohydrates (insoluble fibre content: 28–50% DM; Rojo-Poveda et al. (2020)). Due to its polyphenol content, CBS may directly affect rumen and gut ecosystems, possibly altering methane emissions, milk production, and composition (Correddu et al., 2020). However, CBS also contains around 1% of theobromine and hydrolysable tannins, which can lower palatability and apparent digestibility when fed in large amounts (Adamafio, 2013; Rojo-Poveda et al., 2020; Lazzari et al., 2023). Moreover, current EU regulations limit theobromine levels in feed material for adult cattle (700 mg/kg for complete feedingstuffs (EFSA, 2008)). Thus, careful consideration is warranted to avoid offsetting the advantages of feeding CBS polyphenols with their negative properties.

The aim of the present study was to evaluate the effects of the replacement of 55% cereal grains in concentrate for early-lactating dairy cows by FFP on feed intake, milk production, ruminal fermentation, and methane production, with or without the use of CBS as a supplement, in a herbage-based diet. Fresh herbage was chosen as the basal diet to investigate the not-yet-studied combination of two feed resources—that is, FFP and herbage—aiming at enhancing sustainability. As the quality of fresh herbage varies largely over the growing season, we investigated this combination in both the spring and fall seasons. We chose cows in early lactation, who have a high energy demand that cannot be covered by herbage alone (Falk et al., 2018), allowing a great potential impact in terms of sustainability when feeding FFP concentrates. We tested two hypotheses: (i) feeding 55% FFP of the baking industry in early-lactating cows does not lead to any negative effect on the animals' ruminal fermentation and milk production, and (ii) CBS supplementation mitigates methane production.

Material and methods

Animals and housing

This experiment was conducted in accordance with the Swiss laws of animal protection and was approved by the cantonal veterinary office of Fribourg, Switzerland (2021-38-FR). The experiment took place between April and June (experiment 1, **E1**) and August and October (experiment 2, **E2**) 2022 at the experimental farm of Agroscope Posieux, canton of Fribourg, Switzerland. We used 34 primiparous (n = 4) and multiparous (n = 30) Holstein and Red Holstein dairy cows, 17 in E1 and E2 each. All cows underwent a health check before the experiment and were healthy at the start of the experiment. At the start of each experiment, the cows were in early lactation (mean days in milk: 33.2 ± 0.4 d, mean milk yield: 33.0 ± 2.5, and 38.3 ± 1.4 kg/d for primiparous and multiparous cows, respectively). The cows were housed in a free-stall barn equipped with access-controlled feed-weigh troughs (Insentec RIC, Hokofarm Group, Marknesse, Netherlands) and an automatic concentrate feeding station (Insentec RIC, Hokofarm Group, Marknesse, Netherlands). One GreenFeed (C-Lock Technology Inc., Rapid City, SD) unit was installed to measure the production of CH_4 as described in Denninger et al. (2019). At any time, the cows had access to fresh water and an outdoor area (except during milking). They were milked twice daily (0500 and 1600 h) in a milking parlour (SAC, A. Bertschy AG, Guschelmuth, Switzerland).

Study design

The present work was an experimental study with a one-factor factorial design with the factor concentrate type (**CCT**). Both E1 and E2 lasted for 42 d and were divided into two periods: a 21-d adaptation and a 21-d experimental period. Before the start of each experiment, the cows were grazing full time at pasture, supplemented with cereal-based concentrates. Throughout the experiments, the cows were fed an *ad libitum* freshly cut herbage in the barn to allow for the recording of the individual feed intake. The herbage originated from a mixed sward (grass:legume ratio of 70:30; grasses contained mainly *Lolium* and legumes mainly *Trifolium repens*), from which a stripe was cut each morning at a cutting height of 5 cm. After cutting, the herbage was moved to the barn and offered six times per day (between 0500 and 2200 h). Balanced for milk yield, parity, and days in milk, the cows were assigned to three CCT: (i) a control concentrate (**CON**, E1: n = 5 and E2: n = 6), consisting of corn (31%, as-fed basis), barley (31%), wheat (33%), corn gluten (3%), and minerals (2%); (ii) a concentrate consisting of FFP of the baking industry (55%), corn (31%), corn gluten (5%), untreated straw meal (5%), and minerals (3%) (**FFP-**, E1: n = 6 and E2: n = 6); and (iii) the untreated straw meal of the FFP- was replaced by CBS (**FFP+**, E1: n = 6 and E2: n = 5). The

chemical compositions of FFP and CBS raw materials are presented in Table 1. The FFP raw material was purchased as a pellet, prepelleted by the manufacturer (Pic-Mix, LANDI, Sursee, Switzerland; one batch per experiment, due to the limited maximum storage time of 6 months), whereas CBS was bought in loose form (one single batch for both experiments). Straw meal was also purchased in pelleted form. All concentrate ingredients were milled and pelleted on the experimental farm, according to the above-specified proportions for the three CCT. The 5% inclusion level of CBS corresponded to 50 g CBS/kg FFP+ concentrate, that is, 3 g theobromine/cow per day, and was chosen to fall largely below EU regulations on maximum theobromine levels (for adult cattle: 700 mg/kg complete feed; Alexander et al. (2009)). The CCT were aimed at being similar in protein and energy content (Table 2) and were offered independently of the actual milk production according to a fixed allocation scheme. In the adaptation period, the cows started with 5 kg concentrates, which gradually increased to 6 kg/cow per day (as-fed basis) at the end of the period. In the experimental period, the cows received 6 kg/cow per day. The concentrate was available at the automatic feeding station (RIC system; Insentec / Hokofarm, Marknesse, Netherlands) in portions of 1.1–1.2 kg five times a day, with a minimum interval of 4 h between meals. At the GreenFeed unit, the cows were allowed to get up to six times per day 260 g, that is, a maximum 1.6 kg/d (as-fed basis), of a bait concentrate composed, on an as-fed basis, of 79% corn (whole plant; artificially dried and ground), 12% oats, 8% molasses, and 1% salt.

Table 1
Chemical composition of former food products and cocoa bean shells used in the experimental diets for dairy cows (in g/kg DM, unless otherwise specified).

Item	Former food products		Cocoa bean shells
	Experiment 1 (E1) n = 1	Experiment 2 (E2) n = 1	n = 1
DM (%)	93.2	93.6	92.6
Ash	37.2	24.9	86.0
NDF	120	119	472
CP	86.3	98.8	192
WSC	251	281	32.6
Starch	248	205	20.0
Ether extract	159	160	38.0

Abbreviations: WSC = water-soluble carbohydrates.

Data recording and sampling

Individual feed intake was recorded daily. Herbage samples were collected every morning, Monday to Friday. The freshly cut herbage was spread along the feeding alley, and samples were collected from the length of the pile of herbage using 10 cores, which were then mixed in a 40-litre container. A 60-mm-diameter probe with 10 perforations was used to obtain homogeneous 500-g samples. One sample was used for the analysis of DM (Monday to Friday); a second sample was taken on Mondays, Wednesdays, and Fridays, stored at −20 °C, and pooled per experimental week for the analysis of its chemical composition. Samples of concentrate were taken three times per experiment, namely every second week, without pooling. Milk yield was recorded daily (Pulsameter 2, SAC, A. Bertschy AG, Guschelmuth, Switzerland), and milk samples for the analysis of milk composition were collected twice weekly. For each milk sampling, two milk samples were taken, one during the evening milking and another during the subsequent morning milking. The two milk samples were pooled according to the evening and morning milk yields and filled into 50 ml tubes containing a bronopol-based preservative until the gross milk composition was analysed. The cows' BW was recorded twice daily after each milking using a walkover scale (RIC, Insentec, Hokofarm Group, Marknesse, Netherlands). The reticular pH was measured every 10 min throughout the experiments using a bolus (SmaXtec, Graz, Austria). The bolus was administered to the cows 2 d before the start of the experiment. Samples of ruminal fluid and blood were collected after the morning milking and before feeding (between 0530 and 0800 h) at three timepoints, namely in the week before the start of the experiment (baseline), in the third experimental week (end of the adaptation period), and in the sixth experimental week (end of the experimental period). Ruminal fluid samples were collected via a stomach tube (Selekt, Virbac, Kolding, Denmark) that was connected to a vacuum pump (SELEKT, Nimrod Veterinary Products Ltd., Gloucestershire, UK) into 500-ml glass bottles. The first collected approximately 300 ml were discarded because of possible saliva contamination. Immediately after sampling, the ruminal fluid samples were stored on ice. For analyses of volatile fatty acids (**VFA**) and ammonia, 0.4 mL of 50% (vol/vol) sulfuric acid and 0.2 mL of 50% (vol/vol) trichloroacetic acid, respectively, were added to 10 mL of ruminal fluid and then stored at −20 °C until analysis. After the ruminal fluid sample, blood sam-

Table 2
Chemical composition of the herbage and concentrates fed to dairy cows (in g/kg DM, unless otherwise specified).

Item	Experiment 1 (E1)					Experiment 2 (E2)				
	Herbage n = 6[1]	CON n = 3[2]	FFP- n = 3[2]	FFP+ n = 3[2]	Bait feed[1] n = 1	Herbage n = 6[1]	CON n = 3[2]	FFP- n = 3[2]	FFP+ n = 3[2]	Bait feed[3] n = 1
DM (%)	16.5 ± 1.37	88.5 ± 1.19	91.0 ± 0.99	91.0 ± 0.83	89.3	14.7 ± 2.4	88.1 ± 0.27	90.9 ± 0.41	90.8 ± 0.06	90.4
Ash	90.7 ± 5.54	53.7 ± 0.52	63.7 ± 0.14	64.7 ± 0.23	45.0	111 ± 12.9	55.0 ± 1.26	58.6 ± 0.38	61.7 ± 0.58	47.8
ADF	240 ± 41.2	41.7 ± 2.11	69.4 ± 5.70	72.5 ± 8.04	185	245 ± 16.7	45.3 ± 4.35	73.0 ± 4.93	72.7 ± 1.58	210
NDF	388 ± 55.2	137 ± 25.6	145 ± 3.6	132 ± 10.3	384	401 ± 23.0	144 ± 28.3	137 ± 2.5	124 ± 1.4	386
CP	184 ± 14.7	102 ± 1.7	110 ± 2.8	111 ± 1.0	88	223 ± 14.7	103 ± 0.2	109 ± 2.3	105 ± 0.5	78
WSC	144 ± 36.6	38 ± 1.9	137 ± 3.7	138 ± 1.6	92	97.8 ± 11.6	34 ± 1.2	165 ± 4.1	172 ± 4.7	99
Starch	–	630 ± 4.8	390 ± 2.4	386 ± 2.7	318	–	641 ± 3.3	371 ± 2.5	367 ± 5.7	327
Ether extract	–	61 ± 2.2	111 ± 2.4	115 ± 1.2	29	–	59 ± 1.1	102 ± 0.7	104 ± 4.2	24
Net energy for lactation (MJ/kg DM)[4]	6.6 ± 0.53	8.5 ± 0.03	8.8 ± 0.03	9.0 ± 0.02	6.2	6.4 ± 0.13	8.5 ± 0.03	8.8 ± 0.04	8.9 ± 0.06	6.1
Gross energy (MJ/kg DM)	–	18.4 ± 0.06	19.4 ± 0.01	19.5 ± 0.03	18.4	–	18.6 ± 0.04	19.4 ± 0.07	19.4 ± 0.07	18.3

Abbreviations: CON = control concentrate; FFP- = concentrate containing 55% bakery by-products; FFP+ = concentrate containing 55% bakery by-products and 5% cocoa bean shells; WSC = water-soluble carbohydrates.

[1] weekly samples taken in both the adaptation and experimental periods (see section Data recording and sampling).

[2] samples taken every 2 weeks in both the adaptation and experimental periods (see section Data recording and sampling).

[3] concentrate offered at the GreenFeed® station

[4] estimated for herbage and concentrates following equations for roughage and concentrates, respectively (Agroscope, 2021; see section "Calculations and statistical analysis of data").

Table 3
The effect of experiment, concentrate type, and their interaction on BW, milk production and feed intake of dairy cows.

Item	Experiment 1 (E1)			Experiment 2 (E2)			SEM	*P*-values		
	CON (n = 5)	FFP- (n = 6)	FFP+ (n = 6)	CON (n = 6)	FFP- (n = 6)	FFP+ (n = 5)		Experiment	Concentrate type	Experiment × Concentrate type
BW, kg	625	629	608	663	643	661	5.4	0.30	0.76	0.61
Feed intake, kg/d										
Total DMI	19.9[a]	23.3[b]	22.1[ab]	23.8[b]	22.7[ab]	21.3[a]	0.22	<0.001	0.044	0.009
Herbage DMI	13.5[a]	17.0[b]	16.2[ab]	17.6	17.0	15.4	0.21	<0.001	0.031	0.010
Concentrate DMI	5.96	5.86	5.56	5.75	5.35	5.30	0.065	0.22	0.25	0.48
Bait feed DMI	1.12	0.97	0.92	1.14	0.84	1.07	0.025	0.87	0.41	0.33
Total CP	3.14[a]	3.81[b]	3.63[b]	4.64	4.52	4.15	0.066	<0.001	0.053	0.010
Total ADF	4.11[a]	5.17[b]	4.95[b]	4.76	4.70	4.37	0.054	0.002	0.005	0.008
Total NDF	6.93[a]	8.40[b]	7.95[ab]	7.90[b]	7.54[ab]	6.97[a]	0.090	0.002	0.025	0.006
Starch	3.80[y]	2.45[x]	2.30[x]	3.58[y]	2.06[x]	2.24[x]	0.074	0.150	<0.001	0.31
Total WSC	1.80[x]	2.72[y]	2.59[y]	1.86[x]	2.42[y]	2.34[y]	0.045	0.049	<0.001	0.073
Ether extract	0.37[x]	0.64[y]	0.63[y]	0.32[x]	0.51[y]	0.55[y]	0.013	0.120	<0.001	0.058
Total net energy for lactation, MJ[1]	129[a]	153[b]	146[ab]	154	151	141	1.6	<0.001	0.023	0.007
Milk production										
Milk yield, kg	34.9	36.6	34.2	36.7	35.8	34.9	0.20	0.010	0.46	0.44
Energy-corrected milk, kg[2]	33.4	36.0	34.2	33.9	32.6	32.9	0.31	0.033	0.27	0.21
Fat, %	3.87[x]	4.08[xy]	4.32[y]	3.56[x]	3.57[xy]	3.77[y]	0.029	0.055	0.013	0.34
Lactose, %	4.87[z]	4.68[y]	4.55[x]	4.83[z]	4.67[y]	4.52[x]	0.010	0.84	<0.001	0.97
Protein, %	3.02	3.12	3.15	3.12	2.98	3.14	0.013	0.74	0.54	0.58
Urea, mg/dL	17.1	19.2	16.7	37.7	37.7	33.4	0.58	<0.001	0.63	0.37
SCC, 10^3 cells/mL	120	55	892	27	27	52	37.4	0.80	0.32	0.54

Abbreviations: CON = control concentrate; FFP- = concentrate containing 55% bakery by-products; FFP+ = concentrate containing 55% bakery by-products and 5% cocoa bean shells; DMI = DM intake; WSC = water-soluble carbohydrates; SCC = somatic cell count.
[1] estimated for herbage and concentrates following equations for roughage and concentrates, respectively (Agroscope, 2021; see section "Calculations and statistical analysis of data").
[2] Energy-corrected milk = (0.38 x fat(%) + 0.24 × protein(%) + 0.17x lactose(%)) × milk yield(kg)/3.14.
[a,b] Means within a row with different superscripts are, by experiment, statistically different due to the season × concentrate type interaction at $P < 0.05$. Results of *posthoc* tests are only presented if significant differences were revealed.
[x,y,z] Means within a row with different superscripts are statistically different due to the concentrate type effect at $P < 0.05$. Results of *posthoc* tests are only presented if significant differences were revealed.

ples were collected by venipuncture of the vena jugularis into tubes containing a clot activator (Vacuette, Greiner Bio-One GmbH, Kremsmünster, Austria). The tubes were kept at room temperature until centrifugation at 3000 g for 15 min. The supernatant serum was stored at −20 °C for later analyses.

Laboratory analyses

Feed samples were lyophilised (only herbage samples; Delta 1–24 LSC, Christ, Osterode, Germany) and ground (1-mm sieve, Brabender mill, Brabender, Duisburg, Germany). DM and ash contents were measured gravimetrically by oven drying (prepASH 229, Precisa, Dietikon, Switzerland) for 3 h at 105 °C and subsequently incinerating at 550 °C until reaching a constant weight. The difference between DM and ash was defined as OM. The contents of the NDF and ADF (NDF: method ISO 16472:2006; ADF: ISO 13906:2008) were determined exclusive of residual ash using Fibertherm (Gerhardt, Königswinter, Germany). The total N content of the feed samples was determined using the Dumas method (ISO 16634). To calculate the CP content, the N content was multiplied by 6.25. The water-soluble carbohydrate (**WSC**) content was analysed following Hall et al. (1999). Starch content was analysed using a polarimetric method (ISO 6493 Ed. 2000-02-01). Ether extract content was analysed by extraction following hydrolysis (ISO 6492:1999). The VFA content of ruminal fluid was analysed using HPLC (Ultimate 3000, Thermo Fisher Scientific, Reinach, Switzerland) containing a refractive index detector and a Nucleogel ION column (300 OA 300 × 7.8 mm, Macherey–Nagel, Düren, Germany). The NH_3 concentrations of ruminal fluid were determined by colorimetry using a commercial test kit (Urea Liquicolor 10505, Human, Wiesbaden, Germany). Fat, protein, lactose, and urea contents and the somatic cell counts of the milk were done by Fourier-transformed IR spectrometry (Milkoscan FT 6000, Foss, Hillerød, Denmark). Serum samples were analysed enzymatically using commercial test kits for the concentrations of albumin, creatinine, glucose and phosphorus (using the corresponding kits for BT-1500, Biotecnica Instruments, Rome, Italy), beta-hydroxybutyrate and non-esterified fatty acids (both using the corresponding kits from Randox, Crumlin, United Kingdom), potassium and sodium (both by using the corresponding kits of Micro sensor Card and Stat Profile Prime Vet analyzer, Nova Biomedica, Waltham, USA).

Calculations and statistical analysis of data

In E1, one CON cow developed peritonitis in the first days of the adaptation period; therefore, she was removed from the experiment and replaced by another cow. In E2, one FFP- and one CON cow reduced their feed intake and milk production by >50% in the last week of the experimental period and were therefore removed from the experiment for this last week. Milk yield, BW, and feed intake were summed by animal and day. The energy-corrected milk was calculated as indicated in Agroscope (2021): $ECM\ (kg) = \frac{(0.38 \times fat\ (\%) + 0.24 \times protein\ (\%) + 0.17 \times lactose\ (\%)) \times milk\ yield\ (kg)}{3.14}$. The mean, minimum, maximum, range, time, and area under the curve with a pH < 6.04 according to Neubauer et al. (2018) were calculated by animal and day. Contents of net energy for lactation were calculated according to Swiss recommendations (Bickel and Landis, 1978) using the regression for the digestibility of organic matter for forage of balanced mixed stands with a predominance of ryegrass (Agroscope, 2021): $Digestibility\ of\ organic\ matter = (34.4 + 0.0863CP_{Organic\ matter} + 0.2914ADF_{Organic\ matter} -$

Table 4
The effect of experiment, concentrate type, and their interaction on reticular pH and ruminal volatile fatty acids of dairy cows.

Item	Experiment 1 (E1)			Experiment 2 (E2)			SEM	*P*-values		
	CON (n = 5)	FFP- (n = 6)	FFP+ (n = 6)	CON (n = 6)	FFP- (n = 6)	FFP+ (n = 5)		Experiment	Concentrate type	Experiment × Concentrate type
Reticular pH										
Mean	6.16[a]	6.54[b]	6.32[ab]	6.61	6.37	6.44	0.031	0.12	0.014	0.004
Minimum	5.94	6.17	5.96	6.31	5.99	6.05	0.030	0.29	0.34	0.038
Maximum	6.40[a]	6.92[b]	6.65[ab]	6.89	6.62	6.71	0.037	0.14	0.013	0.010
Range	0.46	0.75	0.69	0.58	0.63	0.66	0.019	0.36	0.027	0.33
Time $_{pH < 6.04}$ (min)	274	40	369	103	143	252	34.2	0.15	0.098	0.077
Area under the curve $_{pH < 6.04}$	19.1	5.4	51.0	10.6	16.2	40.9	5.19	0.20	0.42	0.34
Ruminal fluid analytes										
Ammonia N (mM)	4.13	4.15	6.69	7.68	11.6	9.87	0.609	0.029	0.091	0.058
Total VFA (mM/L)	99.5	89.6	114.1	78.6	85	83.1	3.18	0.012	0.008	0.059
Acetate (mol %)	62.3[x]	64.9[y]	63.4[y]	66.5[x]	68.9[y]	68.9[y]	0.51	<0.001	<0.001	0.37
Propionate (mol %)	23.8	19.5	21.6	20.3	16.4	16.2	0.60	0.13	0.007	0.77
Acetate:Propionate	2.66[x]	3.37[y]	3.02[y]	3.33[x]	4.21[y]	4.27[y]	0.125	0.12	0.031	0.51
n-Butyrate (mol %)	10.4	12.2	11.5	10.0	10.7	11	0.23	0.52	0.13	0.63
Isobutyrate (mol %)	0.8	0.86	0.86	1.00	1.36	1.34	0.045	0.86	0.51	0.36
n-Valerate (mol %)	1.48	1.32	1.4	0.94	0.94	0.91	0.048	<0.001	0.095	0.47
Isovalerate (mol %)	1.19	1.18	1.27	1.29	1.68	1.64	0.048	0.80	0.70	0.41

Abbreviations: CON = control concentrate; FFP- = concentrate containing 55% bakery by-products; FFP+ = concentrate containing 55% bakery by-products and 5% cocoa bean shells; VFA = volatile fatty acids.
[a,b] Means within a row with different superscripts are, by experiment, statistically different due to the season × concentrate type interaction at $P < 0.05$. Results of *posthoc* tests are only presented if significant differences were revealed.
[x,y] Means within a row with different superscripts are different due to the concentrate type effect at $P < 0.05$. Results of *posthoc* tests are only presented if significant differences were revealed.

$0.000133CP^2_{Organic\ \ matter} - 0.000647ADF^2_{Organic\ \ matter}$). Correction factors for the digestibility of organic matter according to growth (primary growth vs. regrowth) and growth stage were used as indicated in Agroscope (2021; Table 15.1). Data were used according to the measured or calculated frequency (daily data for BW, feed intake, milk yield, and reticular pH; twice weekly data for milk composition; once per experimental period for blood and ruminal fluid analytes). Values for VFA were presented as molar percentages of total VFA. For gas measurements, weekly means of cows that visited the GreenFeed at least 10 times per week were used (Lazzari et al., 2023). The methane yield was calculated by dividing the total individual CH_4 amount by DMI and NDF intake, and CH_4 intensity was obtained by dividing by energy-corrected milk. Baselines were calculated for feed intake, milk production, and reticular pH by averaging the data of the 3 days (for feed intake and milk production) and of the day (for reticular pH) preceding the start of the adaptation period, when all cows were fed the same diet. Baselines of milk composition, blood metabolites, and ruminal fluid analytes corresponded to the baseline measurements in the week before the start of the experiment. For statistical analysis, only data of the experimental period and baselines and the R software were used (R Core Team, 2023), particularly the lme4 package (Bates et al., 2015). Linear (mixed) models on raw data were constructed for outcome variables measured repeatedly (i. e., feed intake, milk production, reticular pH, CH_4 production). The cow was the experimental unit. The linear mixed models included the random effect cow and the fixed effects CCT, experiment, their interaction, experimental week, and—except for BW and gas measurements, because not available—baselines. The linear models for blood metabolites and ruminal fermentation analytes, which were measured only once during the experimental period, included the fixed effects CCT and baselines. *Posthoc* tests were conducted for significant F-tests ($P < 0.05$), with, for the interaction experiment × CCT, pairwise comparisons by experiment (emmeans package (Lenth, 2024), multivariate t-distribution adjustment). Only effects with $P < 0.05$ are reported, effects of baselines are not shown.

Results

Feed chemical composition, BW, feed intake, and milk production

The descriptive analysis of the chemical composition of former food and herbage showed that the latter differed by the experiment. In E1, FFP contained slightly less CP and WSC and more ash and starch than in E2 (Table 1). Herbage in E1 had a greater DM percentage and contents of ADF, NDF, and WSC and contained less CP and net energy for lactation than herbage in E2 (Table 2). The BW of the cows was not affected by the experiment, CCT, or their interaction ($P \geq 0.30$; Table 3). The total DMI and, accordingly, intake of most nutrients was greater in E2 than in E1 ($P \leq 0.002$). In E1, the total DMI and, accordingly, NDF intake were lower in CON cows than in FFP- cows, with FFP+ cows having values similar to CON and FFP- cows, whereas in E2, total DMI and NDF intakes were higher for CON than for FFP+ cows (interaction: $P \leq 0.009$). Herbage DMI and net energy for lactation intake were greater for FFP- than for CON cows in E1 and were similar, as indicated by non-significant *posthoc* comparisons, for CCT in E2 (interaction: $P \leq 0.010$). The CP and ADF intakes were lower for CON than for FFP- and FFP+ cows in E1, and unaffected by CCT in E2 (interaction $P \leq 0.010$). Irrespective of the experiment (interaction: $P \geq 0.058$), the CON cows ingested less WSC and ether extract and more starch than the FFP- and FFP+ cows (all $P < 0.001$). The concentrate and bait feed intakes were not influenced by the CCT, experiment or their interaction ($P \geq 0.22$). The energy-corrected milk was lower and milk yield and urea content greater in E1 than in E2 ($P \leq 0.033$), and they were unaffected by CCT ($P \geq 0.27$). Across the experiments, the fat percentage was greater in the milk of FFP+ cows than in that of CON cows with FFP- cows having similar levels to CON and FFP+ cows ($P = 0.013$), and lactose percentages were greatest in the milk of CON cows, intermediate in that of FFP- cows and lowest in that of FFP+ cows ($P < 0.001$). The milk protein content and SCC were not influenced by experiment, CCT, or their interaction ($P \geq 0.32$).

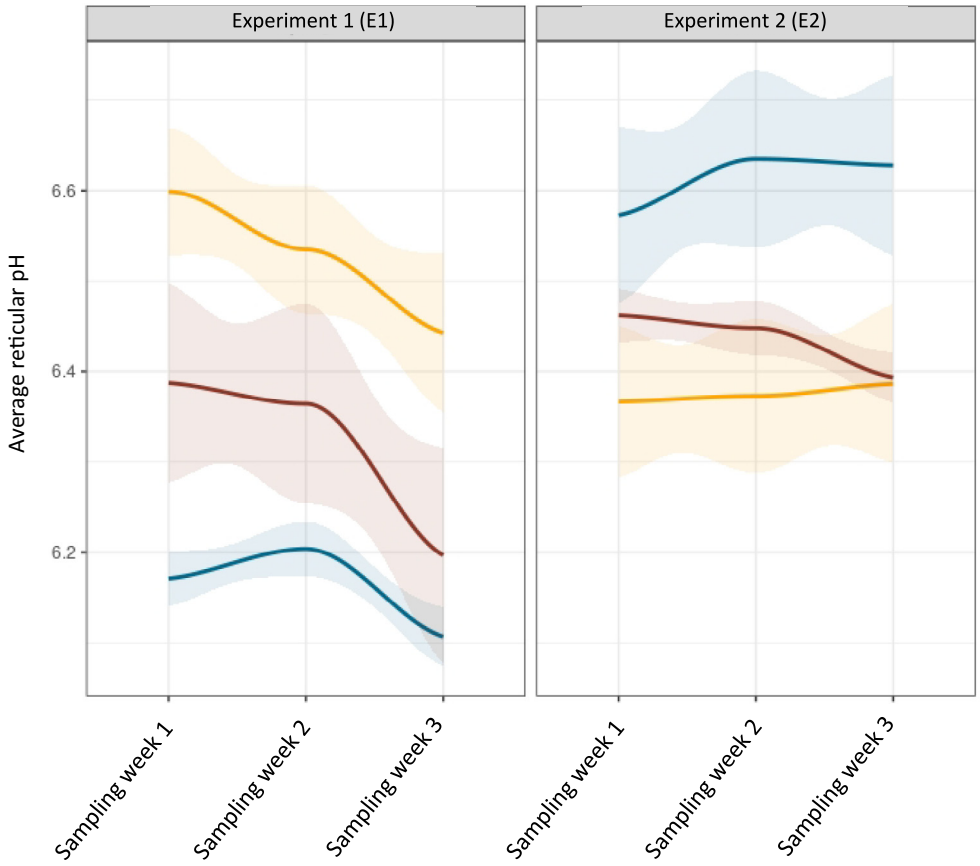

Fig. 1. Temporal evolution of the smoothed average reticular pH as affected by the concentrate type. Blue, yellow and brown lines represent the values of dairy cows receiving the CON, FFP- and FFP+ concentrate. Abbreviations: CON = control concentrate; FFP- = concentrate containing 55% bakery by-products; FFP+ = concentrate containing 55% bakery by-products and 5% cocoa bean shells.

Reticular pH and ruminal fermentation and methane production

In E1, the mean and maximum of reticular pH were lower in CON cows than in FFP- cows, with FFP+ cows having similar values to CON and FFP- cows, whereas in E2, reticular pH was unaffected by the CCT (interaction $P \leq 0.010$; Table 4; Fig. 1). The minimum reticular pH varied also by CCT depending on the experiment (interaction $P = 0.038$), but *posthoc* comparisons did not reach significance. Independently of the experiment, the range of reticular pH was affected by the CCT ($P = 0.027$). Although *posthoc* comparisons did not reveal significant differences, CON cows had numerically lower ranges than FFP- and FFP+ cows. The time and area under the curve with a pH < 6.04 were not influenced by experiment, CCT, or their interaction ($P \geq 0.077$). Compared with E1, the ammonia concentration and acetate proportion of ruminal fluid were higher, and total VFA and n-valerate proportion were lower in E2 ($P \leq 0.029$; Table 4). Independently of the experiment, the CCT affected total VFA, acetate and propionate proportions and their ratio ($P \leq 0.031$). Acetate proportions and the acetate:propionate ratio were lower in CON than in FFP- and FFP+ cows ($P < 0.001$ and $P = 0.031$, respectively), and, despite non-significant *posthoc* differences, CON cows had numerically lower values of total VFA than FFP+ cows and numerically higher proportions of propionate than FFP- and FFP+ cows. Proportions of n-butyrate, isobutyrate and isovalerate were not affected by experiment, CCT, or their interaction ($P \geq 0.13$). The CH_4 production was lower in E1 than in E2 ($P = 0.007$) and influenced by the CCT ($P = 0.017$), despite non-significant *posthoc* comparisons (Table 5). The CH_4 intensity (g CH_4/kg energy-corrected milk) was lower in E1 than in E2 ($P = 0.034$), independently of the CCT. The CH_4 yields per kg DMI and NDF intake were not affected by the experiment, CCT, or their interaction ($P \geq 0.45$).

Blood metabolites

Blood metabolites are presented in Table 6. Albumin levels were lower in E1 than in E2 ($P < 0.001$) and were unaffected by CCT in E1, whereas in E2, they were lower for FFP- than for FFP+ cows, with CON cows having similar values to FFP- and FFP+ cows (interaction $P = 0.002$). Levels of non-esterified fatty acids were unaffected by CCT in E1 and lowest for CON, intermediate for FFP- and greatest for FFP+ cows in E2 (interaction $P < 0.001$). Glucose levels were also unaffected by CCT in E1 and greater for CON than for FFP- cows in E2, with levels of FFP+ cows not differing from CON and FFP- cows (interaction: $P = 0.037$). Other metabolites were not influenced by the experiment, CCT, or their interaction ($P \geq 0.064$).

Discussion

In the present study, the inclusion of FFP (55% of the concentrate on an as-fed basis; ~17% of the whole diet on DM basis) of the baking industry in concentrate, fed in combination with fresh herbage as a basal diet, did not have negative consequences on the ruminal fermentation and blood metabolites of early lactating cows. This confirms our hypothesis and the general feasibility of combining two sustainable feed resources, at least for a period of 3 weeks, in diets for lactating dairy cows at the FFP inclusion level and animal production level studied. The observed effects of FFP on

Table 5
The effect of experiment, concentrate type, and their interaction on ruminal gas emissions of dairy cows.

Item	Experiment 1 (E1)			Experiment 2 (E2)			SEM	*P*-values		
	CON (n = 5)	FFP- (n = 6)	FFP+ (n = 6)	CON (n = 6)	FFP- (n = 6)	FFP+ (n = 5)		Experiment	Concentrate type	Experiment × Concentrate type
CH_4 production (g/d)	406	484	456	483	482	455	5.9	0.007	0.017	0.062
CH_4 yield										
g CH_4/kg energy-corrected milk	12.8	14.0	13.2	15.1	14.0	13.5	0.26	0.034	0.49	0.27
g CH_4/kg DMI	20.5	20.8	20.6	20.3	21.3	21.4	0.23	0.97	0.93	0.86
g CH_4/kg NDF intake	58.9	58.0	58.1	60.9	64.2	65.4	0.74	0.47	0.88	0.45

Abbreviations: CON = control concentrate; FFP- = concentrate containing 55% bakery by-products; FFP+ = concentrate containing 55% bakery by-products and 5% cocoa bean shells; DMI = DM intake.

Table 6
The effect of experiment, concentrate type, and their interaction on blood metabolites of dairy cows.

Item	Experiment 1 (E1)			Experiment 2 (E2)			SEM	*P*-values		
	CON (n = 5)	FFP- (n = 6)	FFP+ (n = 6)	CON (n = 6)	FFP- (n = 6)	FFP+ (n = 5)		Experiment	Concentrate type	Experiment × Concentrate type
Albumin (g/L)	29.4	29.8	28.3	32.7[ab]	30.2[a]	32.2[b]	0.422	<0.001	0.055	0.002
Beta-hydroxybutyrate (mmol/L)	0.30	0.45	0.38	0.39	0.54	0.67	0.030	0.34	0.17	0.17
Creatinin (µmol/L)	70.2	68.6	68.1	64.2	67.5	64.6	1.49	0.19	0.76	0.47
Non-esterified fatty acids (mmol/L)	0.19	0.10	0.09	0.10[a]	0.23[b]	0.35[c]	0.021	0.64	0.86	<0.001
Glucose (mmol/L)	3.64	3.72	3.48	3.53[b]	3.18[a]	3.16[ab]	0.052	0.51	0.24	0.037
Potassium (mmol/L)	4.13	4.28	3.97	4.40	4.46	4.37	0.076	0.89	0.42	0.80
Sodium (mmol/L)	139	139	139	140	138	139	0.2	0.25	0.67	0.22
Phosphorus (mmol/L)	1.19	1.03	1.20	1.50	1.62	1.43	0.055	0.064	0.54	0.17

Abbreviations: CON = control concentrate; FFP- = concentrate containing 55% bakery by-products; FFP+ = concentrate containing 55% bakery by-products and 5% cocoa bean shells.
[a,b,c] Means within a row with different superscripts are, by experiment, statistically different due to the season × concentrate type interaction at $P < 0.05$. Results of *posthoc* tests are only presented if significant differences were revealed.

ruminal pH in E1 were not observed in E2 and need to be further elucidated. Lastly, supplementing polyphenol-rich CBS did not mitigate methane production with the tested dosage, contrary to our hypothesis.

In this study, all cows, irrespective of CCT, had access to 6 kg of pelleted concentrate per day at a feeding station. The CCT did not alter the concentrate intake, which confirms that the palatability of a concentrate containing 55% FFP of the baking industry is as good as that of a cereal-based concentrate. Because of the inherent nature of the CCT's ingredients, cows receiving a cereal-based concentrate ingested less WSC and fat and more starch than cows receiving concentrate, including FFP of the baking industry. This shift was also observed in an earlier study including FFP of the baking industry in the total-mixed ration of mid-lactating dairy cows (Kaltenegger et al., 2020). The greater starch intake of CON cows lowered their ruminal acetate:propionate ratio, probably because of the influence of starch-rich diets on the ruminal microbiota, namely the enhancement of propionate-producing bacteria (Ellis et al., 2008).

Cows receiving CBS had the greatest milk fat percentage. Cocoa bean shells contain high contents of insoluble fibre (28–50% on a DM basis, Rojo-Poveda et al. (2020)) that make them similar to cotton seed by–products, and other effective NDF sources (Mertens, 2002). The latter have been proposed as counteractors to milk fat depression, which is commonly caused by diets containing too little fibre content. An alternative explanation might be a polyphenol-driven effect on microbial metabolism and consequent alterations of milk fat synthesis, similar to the reported changes in the rumen microbiota and milk fatty acid profile (but not milk fat percentage) of goats supplemented with CBS (Renna et al., 2022). In both experiments, the CON cows had the greatest milk lactose percentages, the FFP+ cows had the lowest, and the FFP- cows had intermediate levels. Although unassociated with changes in milk yield, the differing lactose levels between CON and the other two CCT groups are probably related to the according intakes of starch, the main provider of glucose and glucose precursors. The reason for the greater milk lactose levels of FFP- compared with FFP+ cows remains unclear. Despite the CCT's effects on milk composition, no effect on the energy-corrected milk was observed. This contrasts with reported milk production increases from incorporating up to 30% baking industry FFP, possibly due to the FFP-induced higher DM intake noted in that study (Kaltenegger et al., 2020).

Neither including FFP nor CBS had an effect on ruminal fermentation or methane production. An explanation for the lacking methane lowering effect by concentrate containing FFP and thus greater amounts of fat (almost twice as high as in the CON concentrate) might be the type of fat. It seems that mostly polyunsaturated fats are efficient in lowering methane production, even when fed in relatively low amounts (Beauchemin et al., 2020). In the present study, the fat in FFP corresponded very likely to mainly saturated fats, as in the presumably used raw ingredients of the FFP (e.g., biscuits, dough, chocolate). A reason for the absence of an effect on methane production with CBS supplementation might be the too–low ingested amount of polyphenols/cow per day. In particular, the methane-mitigating effects of tannins at low dietary concentrations are highly variable and need further investigation (reviewed by Beauchemin et al. (2020)).

Furthermore, the CCT had only a marginal effect on blood metabolites in E2, but none in E1. The measured metabolite concentrations reflect the general metabolism of the cows participating in the study. For example, the relatively high values of glucose and those of beta-hydroxybutyrate, non-esterified fatty acids, creatinine, and minerals being within the reference range suggest a good energy and mineral supply of the early-lactating cows, mostly irrespective of the CCT. The slightly greater albumin

levels of FFP+ compared with FFP- cows in E2 seem of no clinical relevance to us, and the greatest glucose and lowest non-esterified fatty acids values of CON cows likely reflect their slightly greater feed and starch intake.

Other effects of the CCT, namely those on feed intake and reticular pH levels depended on the experiment. The herbage's nutrient composition and its seasonal changes are comparable to those of earlier studies (Dohme-Meier et al., 2014; Thanner et al., 2014; Klevenhusen and Zebeli, 2021). The lower DMI in E1 compared with that in E2 might be related to both greater fibre and lower protein contents in spring herbage, as greater fibre and lower protein contents were shown to limit the feed intake (Mertens, 1987) and reviewed by Peyraud and Astigarraga (1998)). The lower DMI and protein intake and presumably a lower protein solubility may explain the lower milk yield, milk urea content, ruminal ammonia and acetate, and blood albumin levels and the greater ruminal n-valerate levels in E1. The season-dependent herbage quality and consequently feed intake and rumen metabolism seem to be key factors in the animals' responses to the three CCT. In general, most effects were found for E1, and not for E2. For example, the CCT influenced reticular pH in E1 but had no effect in E2. An explanation for the inconsistent effects between experiments—with CON cows having the lowest mean pH and reticular pH range, with FFP- cows having the greatest mean, minimum, and maximum pH, and with FFP+ cows having intermediate levels in E1—might be the cows' herbage intake. The latter was lowest and highest for CON and FFP- cows, respectively, and intermediate for FFP + cows in E1 and did not differ between diet groups in E2, while the concentrate intake was similar for all CCT. The resulting slightly lower forage:concentrate ratios may partly explain lower reticular pH levels in E1, as lower forage:concentrate ratios have been shown to shorten chewing and rumination times, thereby decreasing saliva production and ruminal buffer capacity while increasing lactate formation in the rumen (Lechartier and Peyraud, 2010). Notably, low ruminal pH levels may also lower feed intake (Dijkstra et al., 2012). Therefore, the causal direction of the association between low ingestion and low reticular pH may be both-sided.

An alternative explanation for the experiment-dependent effects of the CCT on reticular pH might be season-dependent herbage and concentrate compositions, especially WSC fractions. Monosaccharides, disaccharides, and oligosaccharides have different effects on ruminal fermentation and pH (Oba et al., 2015). Monosaccharides and disaccharides are quickly captured by microbes and used as energy sources, but when ingested in excessive amounts, they enhance lactate and VFA formation and increase subacute rumen acidosis risk, whereas oligosaccharides and fructans are less rapidly fermented, mostly to acetate and only little to lactate (Klevenhusen and Zebeli, 2021). Other season-dependent concentrate variations might concern heat treatment and processing of the used FFP, which can influence their fermentability, thereby affecting ruminal functions and activities. As the prior history of the FFP is unknown and soluble carbohydrate fractions were not measured in the present study, it is possible that they varied between seasons. In conclusion, although the manufacturers of finished FFP products try to produce a consistently high-quality feed, future studies should focus on the effects of soluble carbohydrate fractions in FFP.

A conclusive interpretation of the results in view of forestomach health is difficult. Importantly, in terms of reticular pH, we can state that the mean reticular pH was adequate for lactating dairy cows in early lactation, as time periods with a pH < 6.04 did not exceed 5–6 h/d (Neubauer et al., 2018), independent of the CCT fed. Concerning pH variations, the reticular pH of CON cows in E1 was more stable, as indicated by their lower pH range, compared with FFP- and FFP+ cows. For E2, a similar (numerical) trend was observed. In earlier studies, a lower pH range was associated with a lower subacute rumen acidosis risk (Villot et al., 2018). Regarding the ruminal fermentation pattern, the acetate:propionate ratio in ruminal fluid was more favourable in FFP- and FFP + cows compared with CON cows, as a greater acetate:propionate ratio (FFP- and FFP+ cows averaged 3.1 and 4.2 in E1 and E2, respectively) is indicative of a lower subacute rumen acidosis risk (Golder et al., 2023). We conclude that the cows in this study were in general not in subacute rumen acidosis, whether fed with a cereal-based concentrate or a concentrate containing 55% FFP. Detailed effects on rumen health, including effects on microbial populations, potentially in view of greater FFP inclusion levels or feeding FFP over a longer period, require further investigation involving greater sample sizes.

Conclusion

Including FFP in the concentrate (55% of the concentrate on an as-fed basis; ~17% of the whole diet on DM basis) of dairy cows fed fresh herbage over a period of 6 weeks without negatively affecting ruminal fermentation and blood metabolites suggests that combining herbage and FFP is feasible in diets for early lactating dairy cows with a production level of around 35 kg/d. The inclusion of FFP and CBS had no effect on the cows' methane production. The numerous interactions between experiment and CCT underline that the effects of FFP depend on seasonal changes in feed intake, herbage and possibly FFP composition. The ruminal degradation pattern of combinations of herbage and FFP, especially nutrient and VFA delivery and their use by the whole organism including the mammary gland, merits further investigation. Given the general various composition of FFP available on the market, further studies are necessary to better understand their effects and those of soluble carbohydrate fractions on dairy cow nutrition and performance.

Ethics approval

This experiment was in accordance with the Swiss laws of animal protection and was approved by the cantonal veterinary office of Fribourg, Switzerland (2021-38-FR).

Data and model availability statement

None of the data were deposited in an official repository. Data are available upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

None.

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