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Impact of corn silage or corn silage plus protein supplementation on the ingestive and rumination behaviours, ruminal fermentation characteristics and efficiency of grazing dairy cows

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Abstract

This study's objective was to compare two options of pasture supplementation: corn silage (CS) alone or corn silage mixed with protein concentrate. The experiment was conducted with 18 lactating Holstein cows in mid-lactation in a crossover design that included three treatments and three data collection periods. All cows had access to pasture for 17 h/day with an average herbage allowance of 16 kg dry matter (DM)/cow/day and were offered in-barn corn silage, corn silage mixed with protein concentrate, or no supplementation. Cows were equipped with pH sensors residing in the reticulum and, during the 7-day data collection periods, with a jaw movement recorder. Nonsupplemented cows produced 21.3 kg energy-corrected milk (ECM) and ate 13.3 kg DM herbage at pasture. Cows supplemented with corn silage and corn silage plus protein produced 2.5 and 4.5 kg/day more ECM, respectively, consumed 3.4 and 3.3 kg/day more DM in total, respectively, ate for a shorter period of time, and ruminated longer than their nonsupplemented peers. Supplemented cows were almost able to cover their energy requirements and mobilised less body mass in contrast to the nonsupplemented cows. Cows offered corn silage plus protein showed increased ECM production, increased milk urea content and lower nitrogen use efficiency (NUE) compared to cows supplemented with corn silage only. Nonsupplemented dairy cows had the highest milk urea content and performed worst in terms of NUE. The best feed conversion efficiency resulted from the nonsupplemented dairy cows and those supplemented with corn silage plus protein. In nonsupplemented cows, the high feed conversion efficiency seemed to be due to the increased mobilisation of body mass. As a result of the starch-rich supplementations, the ruminal acetic:propionic acid ratio became smaller, and the proportions of *n*-butyric acid increased. The mean reticular pH values did not substantially vary across the three feeding treatments. For the choice of a supplementation option, herbage allowance and cost of supplement will have to be considered, but aspects of feed–food competition as well as animal welfare should not be ignored.

KEYWORDS
feed efficiency, Holstein, maize silage, nitrogen use efficiency, pasture, ruminal pH

1 | INTRODUCTION

The continuing erosion of profit margin per unit of milk is a challenge for milk producers (Reijs et al., 2013). In regions where conditions are favourable for herbage growth, increasing the proportion of herbage in dairy cow rations can be economically beneficial (Holshof et al., 2015). The supplementation of grazing dairy cows often increases the total dry matter (DM) intake (DMI), energy intake and animal performance compared to pasture only (Bargo et al., 2003; Peyraud & Delaby, 2001). However, supplements are usually more expensive than grazed herbage, which is widely recognised as the cheapest source of nutrients for dairy cows (Peyraud & Delaby, 2001). An increase in the proportion of grazed herbage in the ration also promises ecological benefits (Guyader et al., 2016) and reduces feed–food competition (Wilkinson, 2011).

However, high-yielding dairy cows are often unable to meet their energy and nutrient requirements when fed all-herbage rations (Schori & Münger, 2021). Further, the botanical composition and vegetation stage of herbage show seasonal and management-related changes; this leads to variations in the nutrient density and composition of the offered herbage (Bovolenta et al., 2008; Guy et al., 2018). Supplementation offers the possibility of supporting more consistent and higher milk production on pasture, although it has often been shown to be inefficient (Bargo et al., 2002; Heublein et al., 2017), leading to a reduction in grazing time and pasture intake. Previous studies on supplementation of grazing dairy cows focused on energy (Bargo et al., 2002), recognising that energy supply is the first limiting factor in feeding systems based on herbage, especially in grazing systems where intake is additionally limited (Hills et al., 2015). Crude protein (CP), by contrast, is often in excess, requiring cows to transform and excrete surplus nitrogenous products which also requires energy supply. Supplements that increase fermentable energy in the rumen enable cows to capture more nitrogen as microbial protein, in addition to improving their energy status. However, this is associated with a higher risk of acidosis (Krause & Oetzel, 2006), especially with high-quality pasture, which can contain elevated concentrations of soluble carbohydrates (O'Grady et al., 2008). Acidosis at different degrees can lead to reduced nutrient utilisation and lower intake, both affecting production and feed efficiency. Another drawback of balancing supplementation is that it is not eaten simultaneously with the pasture part of the ration. On the one hand, supplement uptake in a full-time grazing system is usually limited to a relatively short time frame around milking, and on the other hand, the different degradation rates of carbohydrates and proteins from the grazed herbage and the supplements may also affect the effect of the supplemented feeds (Dickhoefer et al., 2022). However, feeding a supplement nutritionally balanced with respect to energy and protein requirements may primarily serve to cover the gap between the cow's energy and nutrient requirements and uptake from grazed herbage. The effect of a nutritionally balanced supplement is potentially more independent from the distribution pattern and a milk production response may occur in case of high levels of energy intake (Kellaway & Harrington, 2004)—for example, with corn silage. An intrinsically nutritionally balanced supplement certainly adds more nitrogen to the nitrogen pool and may further reduce nitrogen use efficiency (NUE) in excess situations (Huhtanen et al., 2015). The supplementation strategy that is most efficient in terms of overall nutrient or energy efficiency remains unclear and depends on the nutrient content of grazed herbage and the production level of the animals, among other factors (Kellaway & Harrington, 2004). In the present study with grazing dairy cows, two supplementation strategies were compared with each other and with a nonsupplemented treatment. We used whole-plant corn silage, which is generally low in CP, as a balancing supplement or a mix of corn silage and protein concentrate as a balanced supplement. The treatments were compared in terms of milk production, feed intake, ingestion and rumination behaviours, ruminal fermentation characteristics and efficiency measures. Our hypothesis was that under intensive grazing conditions in temperate climates, feeding a balancing supplement would have a beneficial effect on production and efficiency characteristics but might increase the risk of ruminal acidosis. The intrinsically balanced supplement may improve milk production, but would entail a CP overfeeding, resulting in a lower NUE.

2 | MATERIALS AND METHODS

2.1 | Experimental design, animals and housing

The study was carried out at Agroscope in Posieux, Switzerland (46°45′59.59″ N; 7°6′14.477″ E), from 28 July to 27 September 2014. All experimental procedures were in accordance with the Swiss guidelines for animal welfare and were approved (No. 2014_38_FR) by the Animal Care Committee of the Canton Fribourg, Switzerland.

Prior to the selection of the experimental animals, all cows passed a veterinary check.

The experiment was set up as a crossover design, including three feeding treatments and three data collection periods. Each period lasted 21 days, consisting of 14 days for the adaptation of the cows to the feeding treatments and the measuring devices and 7 days of data collection. Each cow underwent all three treatments and thus served as its own reference. Eighteen Holstein or Red Holstein dairy cows (12 multiparous and six primiparous) were used for the experiment; eight of them were ruminally cannulated. The experimental cows were equally distributed into three groups of six cows (two groups had three ruminally cannulated cows and one group had two) based on body weight, energy-corrected milk (ECM) and days in milk. At the beginning of the experiment, the average number of lactations of the cows was 3.3 (standard deviation [SD] 2.2) and they were 122 (SD 45) days in milk. They had an average body weight of 607 (SD 53) kg and produced 28.2 (SD 5.2) kg of milk/day. During the experiment, all cows had access to pasture for approximately 17 h/day with an average herbage allowance of 16 kg DM/cow/day above eight rising plate meter units (RPMU). They were offered in-barn, after milking, either 10 kg DM/day of chopped whole plant corn silage, a mixed ration of 8.2 kg DM/day corn silage plus 1.8 kg DM/day of protein concentrate (CSP) or received no supplementation (NS) in the barn. The protein concentrate consisted of 60% expeller soybean meal, 25% corn gluten, 10% potato protein and 5% dried sugar beet pulp. Supplements were offered in weighing troughs (Insentec B.V.).

On pasture, all experimental cows were kept together in one paddock at a time (0.30 ha) from 0730 to 1500 h and from 1730 to 0445 h the following morning. The paddocks were rotationally grazed for 1–3 days each, and the number of meals per paddock depended on the remaining sward height, which was fixed at eight RPMU, measured with an electronic rising plate meter (Jenquip; 1 RPMU = 0.5 cm). The average pregrazing sward height was 15.5 (SD 3.9, $n = 26$) RPMU, corresponding to 1287 (SD 696) kg DM/ha above eight RPMU and the average postgrazing sward height was 7.6 (SD 0.9, $n = 26$) RPMU. The pastures were long established and composed predominantly of grasses (58, SD 10%; mainly *Lolium perenne* and *Poa pratensis*), herbs (29, SD 14%; mainly *Taraxacum officinale* and *Plantago lanceolata*) and legumes (13, SD 6%; mainly *Trifolium repens* and *T. pratense*). Coming from pasture, the cows were milked and weighed and samples were collected. Between milking and getting out on pasture, the cows were kept in a cubicle barn and had access to the supplements according to treatments. Throughout the experiment, cows had free access to water and mineral mix from licking buckets (UFA 999, UFA AG). The outdoor ambient temperature and amount of rainfall were recorded daily at a local meteorological station (Meteo-Schweiz). During the experiment, the average ambient outdoor temperature was 15°C (minimum 10°C, maximum 20°C) and on 27 out of 63 experimental days, rainfall occurred with an average daily precipitation of 6 mm (minimum 0.1 mm, maximum 11 mm).

2.2 | Measuring devices

The reticular pH was recorded using the commercially available smaXtec system with electronic boli (smaXtec; validated by Schori & Münger, 2022). Nine boli were administered evenly across the three experimental groups in the second adaptation week of the first period, and the pH value in the reticulum was measured continuously until the end of the experiment. The recorded pH values were averaged over intervals of 600 s by the smaXtec system. Data recorded by the pH boli were read out daily when the cows were in the catchment area of the smaXtec mobile reader. The recorded pH values were used to calculate the daily mean, nadir and maximum pH values, as well as durations when pH < 6.0. This value corresponds to a threshold of 5.8, suggested as defining the onset of an increasing risk of ruminal acidosis, corrected by a perceived offset of 0.2 units due to measurement in the reticulum instead of in the rumen (Falk et al., 2016).

The behavioural characteristics of the experimental cows were recorded and processed using the RumiWatch system (RWS), which consists of the RumiWatch halter (RWH) (Itin & Hoch GmbH) as a measuring system and the RumiWatch converter 0.7.3.11 (Con11; Itin & Hoch GmbH) as the evaluation software. The RWS was described in more detail by Rombach et al. (2018). The data recorded were time and number of chews attributable to eating, as well as time, number of chews and number of boli attributable to rumination.

2.3 | Sample collection and data recording

The milk yield was recorded twice daily at 0500 and 1600 h during milking (Pulsameter 2, SAC). The milk from each cow was sampled on days 2 and 5 of each experimental week. The subsamples of the morning and evening milk were pooled proportionally to the respective milk yields and then filled in two sample tubes. One tube contained a Broad-Spectrum Microtab II preserving agent (Gerber Instruments AG) and was stored at 8°C for later measurement of milk fat, protein and lactose content. The second tube was stored at −20°C for later analysis of the milk urea content.

The *n*-alkane double indicator method described by Mayes et al. (1986) was used to estimate individual herbage intake. Six days before until the end of each collection week, the cows were dosed twice daily, at 0600 and 1600 h, with a gelatin capsule (HGK-17-60 sl, Capsula GmbH) containing 500 mg of dotriacontane (HC_{32}, Minakem Beuvry Production S.A.S.) on a carrier of 4.5 g dried fruit pomace. During the experimental week, the faeces of each cow were spot-sampled once a day after milking between 0600 and 0630 h for the analysis of *n*-alkane content. Samples were taken from spontaneous defecation or after mild stimulation; they were pooled for each cow and experimental week and stored at −20°C until freeze-drying (Delta, 1-24 LSC, Christ). Samples of the herbage eaten were collected twice daily, at 0800 and 1700 h, by clipping bunches with a battery grass shearer (Gardena, Husqvarna Schweiz AG) mimicking the observed grazing behaviour of the experimental cows. These samples were chopped and stored at −20°C for further analysis. The corn silage and protein concentrate supplements were sampled once per experimental week and stored at −20°C for further analysis.

Ruminal fluid samples were collected from the ruminally cannulated cows twice during the experimental weeks, on days 2 and 5, at 1530 h. Sampling was conducted using a 100 mL syringe equipped with a tube containing a terminal cone with a 1 mm sieve; the collected fluid was cooled directly on ice. Samples for analysis of volatile fatty acids (VFA) or ammonia were preserved by mixing 10 mL of ruminal fluid with 0.2 mL of 50% sulphuric acid (w/v) or 50% trichloroacetic acid (w/v) respectively.

2.4 | Laboratory analysis

Milk samples were analysed using Fourier-transformed mid-infrared spectrometry (Milkoscan FT+, Foss) for the contents of milk fat, protein and lactose. The milk urea concentration was analysed using a UreaFil test kit (MEA 549 EC Milk Urease, Eurochem).

Herbage and corn silage samples were lyophilised; the herbage, corn silage and protein concentrate samples were then milled through a 1.0 mm screen (Brabender mill with titanium blades, Anton Paar). The feed subsamples were dried for 3 h at 105°C to determine DM and subsequently incinerated at 550°C until a stable mass was reached to determine the ash content (AOAC, 1995; procedure 942.05). The contents of the *n*-alkanes HC_{32} and tritriacontane (HC_{33}) were analysed as described by Thanner et al. (2014). The nitrogen content was analysed using the Dumas method (International Organization for Standardization [ISO], 2008; method 16634-1) on a C/N analyser (Trumac CNS, Leco Instruments); these results were multiplied by 6.25 for CP content. The contents of acid detergent fibre (ADF, AOAC, 1995; procedure 973.18) and neutral detergent fibre (NDF, AOAC, 1995; procedure 2002.4) for herbage, corn silage and protein concentrate were analysed using a Gerhardt Fibertherm unit (Gerhardt GmbH & Co. KG). For analysis, heat-stable amylase and sodium sulphite were added. A correction for residual ash, obtained after a 2 h incineration at 550°C, was made for ADF (ADFom) and NDF (NDFom). Ethanol-soluble carbohydrates (ESC) and water-soluble carbohydrates (WSC) were analysed using the methods described by Hall et al. (1999). The ESC and WSC were detected using a Scalar Vis Spectrometer (Thermo Fisher Scientific) after a colourimetric orcinol–sulphuric acid reaction for ESC and an acid hydrolysis with sulphuric acid and colourimetric reaction with potassium ferricyanide for WSC. The starch content of the protein concentrate and corn silage fed during the experiment was determined using the polarimetric method (ISO International Organisation for Standardization, 2000; Method 6493).

The VFA and lactic acid in the ruminal fluid were analysed using high-performance liquid chromatography with a refractive index detector (Shodex RI-101; Denko K.K.) and a Nucleogel ION (300 OA 300 × 7.8 mm; Macherey-Nagel GmbH & Co. KG) column. The concentration of NH_3 in the ruminal fluid was measured by colourimetry using a commercial test kit (S 180, BioMerieux).

2.5 | Calculations and data analysis

The net energy for lactation (NEL) content of the herbage and corn silage was calculated according to Agroscope (2022, chapter: Formulas and regression equations). Feed efficiency was calculated as the ratio of ECM production to total DMI, and NUE was calculated as the ratio of milk protein nitrogen to total nitrogen intake. Herbage intake was estimated using HC_{32} as an external marker and HC_{33} as an internal marker, and the equations proposed by Mayes et al. (1986) were adapted to calculate intake.

The following equation was used to calculate the daily herbage DMI when cows were not supplemented in the barn:

$$\mathrm{DMI}_H = \frac{\frac{F_{33}}{F_{32}} \times A_{32}}{H_{33} - \frac{F_{33}}{F_{32}} \times H_{32}}. \tag{1}$$

The following equation was used to calculate the daily herbage DMI when cows were supplemented with corn silage in the barn.

$$\mathrm{DMI}_H = \frac{\frac{F_{33}}{F_{32}} \times ((A_{32} + (CS \times CS_{32})) - CS \times CS_{33})}{H_{33} - \frac{F_{33}}{F_{32}} \times H_{32}}. \tag{2}$$

The following equation was used to calculate the daily herbage DMI when cows were supplemented with corn silage and protein concentrate in the barn.

$$\mathrm{DMI}_H = \frac{\frac{F_{33}}{F_{32}} \times ((A_{32} + (P \times P_{32}) + (CS \times CS_{32})) - (P \times P_{33} + CS \times CS_{33}))}{H_{33} - \frac{F_{33}}{F_{32}} \times H_{32}}. \tag{3}$$

DMI_H represents the daily herbage DMI (kg), and F_{33}, H_{33}, P_{33} and CS_{33} are the concentrations of HC_{33} (mg/kg DM) in faeces, herbage, protein concentrate and corn silage respectively. F_{32}, H_{32}, P_{32} and CS_{32} are the concentrations of HC_{32} (mg/kg DM) in faeces, herbage, protein concentrate and corn silage respectively. A_{32} is the daily dose of HC_{32} (mg/kg) administered via gelatin capsules. CS and P are the daily DMI of corn silage and protein concentrate respectively.

A total of 54 one-week files were recorded by the RWH for the detection of ingestion and rumination behaviours. Due to technical failures, 12 (22%) of the recorded files were lost and could not be evaluated further.

Overall, 48 (eight rumen cannulated cows × three periods × twice a week) rumen fluid samples were collected, and 189 (nine smaXtec boli × three periods × seven measuring days) pH day files were recorded during the data collection periods. Due to problems in data transmission and non-functional boli, 11 files (6%) of potentially collectable data files could not be used for further evaluation.

Statistical analyses were carried out using R (R Core Team, 2022; version 4.2.2). Data for milk yield, milk composition and ingestion and rumination behaviours, as well as pH values, VFA and ammonia nitrogen contents in the ruminal fluid, were averaged per cow and per data collection period. The weekly averages for these variables and the herbage intakes estimated by the *n*-alkane method were analysed using the following linear mixed model (lmerTest packages):

$$Y_{ijk} = \mu + \tau_i + \rho_j + (\tau\rho)_{ij} + K_k + \varepsilon_{ijk}, \quad (4)$$

where Y_{ijk} is the response, μ is the overall mean, τ_i is the fixed effect of the treatment *i* (*i* = CS, CSP, NS), ρ_j is the fixed effect of the period *j* (j = period 1, period 2, period 3), $(\tau\rho)_{ij}$ is the effect of the interaction between treatment *i* and period *j*, K_k is the random intercept of the cow *k* (1,…, 17; one cow was excluded from the evaluation, because of health problems) and ε_{ijk} is the random error.

If the residuals were not normally distributed, the data were transformed using logarithmic transformation. If no normal distribution of the residuals could be achieved by transformation, the data were analysed using the Kruskal–Wallis test.

Overall effects with a $p \le 0.05$ were considered statistically significant, whereas values between $0.05 < p < 0.10$ were considered as a trend.

3 | RESULTS

3.1 | Chemical composition of the ration components

Table 1 shows the chemical composition of the herbage, corn silage and protein concentrate fed during the experiment. The CP, ADFom, NDFom and calculated NEL content of the offered herbage varied depending on the period: 169–215, 191–261, 301–418 g and 6.0–6.5 MJ/kg DM respectively.

3.2 | Milk yield and milk composition

Nonsupplemented cows produced less milk, less ECM and had a higher milk urea content compared to the supplemented cows. Within the supplemented groups, a higher milk urea content, milk and ECM yield were found for the CSP cows compared to the CS cows. A lower milk protein content was determined for the NS and CS cows compared to the CSP cows, but no difference was evident between the NS and CS cows. Nonsupplemented cows produced less milk protein and fat per day compared to the CS and CSP cows, and the largest amount of milk protein and fat per day was produced by the CSP cows. The contents of milk lactose and fat did not differ among the feeding treatments. Detailed results on milk yield and

TABLE 1 Chemical composition of the offered herbage (*n* = 50), corn silage (*n* = 3) and protein concentrate (*n* = 3); (mean + standard deviation [SD]).

	Herbage						Corn silage		Protein con.		Corn + Protein[a]	
Item	Period 1	SD	Period 2	SD	Period 3	SD	Mean	SD	Mean	SD	Mean	SD
DM (g/kg)	149	25.7	152	28.1	157	26.6	398	33.8	882	23.9	485	32.0
Analysed nutrient composition (g/kg of DM)												
Organic matter	887	8.6	891	7.2	889	8.1	971	0.5	943	0.2	966	0.4
Crude protein	169	15.4	215	9.6	212	6.8	72	5.7	562	6.6	160	5.9
ADFom	261	18.7	206	11.1	191	5.7	194	29.3	76	3.0	173	24.6
NDFom	418	71.6	334	19.2	301	18.4	351	49.7	316	34.8	345	41.0
Starch	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	427	49.7	58	0.8	361	40.9
Crude fibre	187	19.8	180	18.8	192	21.1	163	23.8	34	0.1	140	19.5
WSC	158	32	131	23	183	32	22	2.9	98	2.0	36	2.7
ESC	95	28	78	15	101	28	17	8.1	81	4.5	29	7.5
Calculated energy content (MJ/kg DM)[b]												
Net energy lactation	6.0	0.2	6.5	0.1	6.5	0.1	6.9	-	8.5	-	7.2	-
Analysed *n*-alkane contents (mg/kg of DM)												
Dotriacontane	5.7	0.7	5.4	0.5	4.8	0.2	1.1	0.2	1.3	1.2	1.1	0.4
Tritriacontane	72.4	5.4	66.8	4.8	65.2	4.1	9.7	1.4	0.4	0.3	8.0	1.2

Abbreviations: ADFom, ash-free acid detergent fibre; DM, dry matter; ESC, ethanol-soluble carbohydrates; n.a., not analysed; NDFom, ash-free neutral detergent fibre; protein con., protein concentrate; WSC, water-soluble carbohydrates.

[a]Calculated based on the individual analysis results of corn silage and protein concentrate.

[b]Calculated according to Agroscope (2022, chapter: Formulae and regression equations).

composition, including differences between the measurement periods, are presented in Table 2.

3.3 | Feed and Nutrient Intake

Intakes of total DM, ADFom, NDFom, WSC plus starch and energy were lower for the NS cows than for the CS and CSP cows, whereas no differences were found between the CS and CSP cows for intakes of total DMI, ADFom, NDFom and energy. A higher WSC plus starch intake was measured for the CS cows than for the CSP cows. Compared to the CS and CSP cows, the NS cows had a higher herbage DMI; however, the CS and CSP cows showed an equal herbage DMI. A higher CP intake occurred for the CSP cows compared to the CS and NS cows. The energy intake was lower for the NS cows than for the CS and CSP cows. Overall, only the CP intake per unit of NEL intake and DMI showed significant treatment × period interactions. Detailed results on feed and nutrient intake, including differences between the measurement periods, are shown in Table 3.

3.4 | Efficiency

The NS cows showed higher energy efficiency when compared to the supplemented cows, whereas no differences occurred between the CS and CSP cows. Further, a higher feed conversion efficiency (kg ECM/kg total DMI) was found for the NS and CSP cows than for cows supplemented with CS. The highest NUE was calculated for the CS cows in comparison to the NS and CSP cows, with the lowest NUE for the NS cows. Detailed results on feed efficiency traits, including differences between the measurement periods, are shown in Table 3.

3.5 | Ingestion and rumination behaviour

When compared to the supplemented cows, the NS cows spent more time eating, performed more mastication chews during eating, spent less time ruminating, performed fewer mastication chews during rumination and performed less mastication per bolus. No differences were noted between the CS and CSP cows for these characteristics. The NS cows also showed a lower mastication rate during rumination when compared to the CS cows, but the CSP cows exhibited no differences when compared to the other two treatments. Compared to the NS and CS cows, the CSP cows showed a trend toward a lower mastication rate when eating on pasture. No treatment effects were observed for the mastication rate during eating or the number of rumination boli. Detailed results on ingestion and rumination behaviour, including differences between the measurement periods, are shown in Table 4.

3.6 | Ruminal fermentation and reticular pH

No effect of treatment was found on the daily mean pH, nadir pH, maximum pH, duration of pH < 6.0 and the area under the curve delimited by pH < 6.0. The concentration of total VFA in the ruminal fluid was lower for the NS cows than for the CS and CSP cows. For the NS cows, the proportions of acetic and isobutyric acid, as well as the acetic to propionic acid ratio, were higher and the proportions of propionic, *n*-butyric, *n*-valeric and isovaleric acid in the ruminal fluid were lower compared to CS and CSP cows. No differences were observed between the CS and CSP cows for the proportion of acetic, propionic, *n*-butyric and isovaleric acid or for the acetic-to-propionic acid ratio. A comparison between the supplemented treatments revealed lower proportions of isobutyric and *n*-valeric acid in the CS cows. The ammonia nitrogen concentration in the ruminal fluid was lower for the NS and CS cows than for the CSP cows, whereas the NS

TABLE 2 Effect of supplementation on milk production and milk composition (least square means and standard error of the mean [SEM]).

	Treatment			Period				*p* Value		
Item	NS	CS	CSP	1	2	3	SEM	Treatment	Period	TxP
Milk (kg/day)	20.9^{c}	23.9^{b}	25.1^{a}	23.2^{ab}	24.2^{a}	22.4^{b}	1.04	<0.001	0.005	0.95
ECM (kg/day)	21.3^{c}	23.8^{b}	25.8^{a}	23.8^{a}	24.8^{a}	22.4^{b}	0.92	<0.001	0.002	0.93
Fat (%)	4.18	4.05	4.24	4.04	4.13	4.30	0.15	0.30	0.09	0.74
Fat (g/day)	864^{c}	960^{b}	1051^{a}	930	997	949	38.9	<0.001	0.06	0.70
Protein (%)	3.27^{b}	3.28^{b}	3.40^{a}	3.08^{c}	3.35^{b}	3.52^{a}	0.06	<0.001	<0.001	0.83
Protein (g/day)	685^{c}	781^{b}	846^{a}	711^{b}	811^{a}	790^{a}	31.8	<0.001	<0.001	0.94
Lactose (%)	4.50	4.55	4.52	4.63^{a}	4.46^{b}	4.49^{b}	0.053	0.43	<0.001	0.91
Urea (mg/L)	413^{a}	286^{c}	350^{b}	339^{b}	378^{a}	332^{b}	7.7	<0.001	<0.001	0.50

Abbreviations: CS, supplementation with corn silage in the barn; CSP, supplementation with corn silage and additional protein concentrate in the barn; ECM, energy-corrected milk; NS, no supplementation in the barn; TxP, interaction between treatment and period.

[a,b,c]Least squares means of the same row with different superscripts within treatment or period, respectively, differ significantly ($p < 0.05$).

TABLE 3 Effect of supplementation feed and nutrient intake, as well as on efficiency traits (least square means and standard error of the mean [SEM]).

Item	Treatment			Period			SEM	*p* Value		
	NS	CS	CSP	1	2	3		Treatment	Period	TxP
Crude protein density of the ration										
CP per DM (g/kg)	199[a]	147[c]	179[b]	153[c]	188[a]	183[b]	0.6	<0.001	<0.001	<0.001
CP per NEL (g/MJ)	31.0[a]	22.2[c]	26.7[b]	23.3[c]	28.7[a]	27.9[b]	0.11	<0.001	<0.001	<0.001
Intake (kg/day)										
Herbage dry matter	13.3[a]	9.9[b]	9.6[b]	10.2[b]	10.8[ab]	11.8[a]	0.48	<0.001	0.009	0.82
Total dry matter	13.3[b]	16.7[a]	16.6[a]	14.4[c]	15.4[b]	16.9[a]	0.49	<0.001	<0.001	0.83
Total ADFom	2.89[b]	3.47[a]	3.29[a]	3.50[a]	3.14[b]	3.00[b]	0.102	<0.001	<0.001	0.29
Total NDFom	4.62[b]	5.60[a]	5.29[a]	5.62[a]	5.03[b]	4.86[b]	0.164	<0.001	<0.001	0.51
Total WSC + Starch	2.06[c]	5.12[a]	4.67[b]	3.61[c]	3.88[b]	4.36[a]	0.089	<0.001	<0.001	0.06
Total crude protein	2.65[b]	2.46[b]	2.99[a]	2.18[b]	2.86[a]	3.06[a]	0.099	<0.001	<0.001	0.90
Intake rate (g/min)										
Intake rate barn[†]	-	92	111	81[b]	97[b]	133[a]	1.1	0.051	0.001	0.87
Intake rate pasture[†]	20.9	19.0	19.3	18.2[b]	19.3[b]	22.0[a]	1.08	0.29	0.03	0.97
Energy (MJ NEL/day)										
NEL intake	85[b]	110[a]	112[a]	95[c]	101[b]	112[a]	3.14	<0.001	<0.001	0.81
NEL output[‡]	104[c]	112[b]	118[a]	111[ab]	115[a]	108[b]	3.28	<0.001	0.002	0.92
Efficiency										
NEL balance (MJ/MJ)	1.25[a]	1.02[b]	1.07[b]	1.19[a]	1.16[a]	0.98[b]	0.028	<0.001	<0.001	0.72
FCE (kg ECM/kg TDMI)	1.63[a]	1.43[b]	1.56[a]	1.66[a]	1.63[a]	1.34[b]	0.046	0.002	<0.001	0.66
NUE (g milk N/g N intake)	0.26[c]	0.31[a]	0.28[b]	0.32[a]	0.28[b]	0.25[c]	0.008	<0.001	<0.001	0.76

Abbreviations: ADFom, ash-free acid detergent fibre; CP, crude protein; CS, supplementation with corn silage in the barn; CSP, supplementation with corn silage and additional protein concentrate in the barn; DM, dry matter; ECM, energy-corrected milk; FCE, feed conversion efficiency; NDFom, ash-free neutral detergent fibre; N, nitrogen; NEL, net energy for lactation; NEL balance, MJ output/MJ input; NS, no supplementation in the barn; NUE nitrogen use efficiency; TDMI, total dry matter intake; TxP, interaction between treatment and period; WSC, water soluble carbohydrates.

[a,b,c]Least squares means of the same row with different superscripts within treatment or period, respectively, differ significantly ($p < 0.05$).

[†]Log transformed for statistical analyses, least square means and SEM back-transformed.

[‡]Net energy of lactation output = 0.293 × (body weight$^{0.75}$) + 3.14 × energy-corrected milk.

and CS cows showed no differences. Detailed results on reticular pH and other ruminal fermentation traits, including differences between the measurement periods, are shown in Table 5.

4 | DISCUSSION

4.1 | Intake and milk production

One objective of supplementing grazing dairy cows, besides better meeting their actual requirements and increasing feed efficiency, is to increase total DMI, energy intake and animal performance relative to pasture alone (Peyraud & Delaby, 2001). These effects were also evident in our study. The effect of corn silage supplementation on the intake and milk production of grazing dairy cows depends, among other factors, on pasture characteristics, the quality and quantity of the supplemental feeds and the herbage allowance (Miguel et al., 2023). The pasture herbage and corn silage had nutrient concentrations similar to the corresponding feeds in Miguel et al. (2023), and the quality can consequently be judged as good. Although more supplemented feeds were consumed in our trial, the cows substituted significantly less pasture herbage (around 0.5 kg/kg) induced by eating corn silage, with or without protein mixed in, compared to the average substitution rate over all treatments in the study of Miguel et al. (2023; 0.63–1.23 kg/kg). The herbage allowance seems to play a decisive role here. With a similar herbage allowance in Miguel et al. (2023), similar substitution rates were obtained as in our study. Similar substitution rates as in our study were also found by Burke et al. (2008). Due to the low substitution rates, the supplemented cows consumed more in total and produced more milk. Burke et al. (2008) and Miguel et al. (2023) found similar results, but only for restricted herbage allowances. In both cited studies, the restricted

TABLE 4 Effect of supplementation on ingestive and rumination behaviour (least square means and standard error of the mean [SEM]).

Item	Treatment			Period			SEM	*p* Value		
	NS	CS	CSP	1	2	3		Treatment	Period	TxP
Ingestive behaviour over 24 h										
Time (min)	663[a]	577[b]	570[b]	603[ab]	627[a]	580[b]	17.8	<0.001	0.03	0.90
Mastication (*n*/day)	48,245[a]	42,016[b]	41,252[b]	44,274	45,438	41,801	1571	0.001	0.06	1.00
Mastication rate (*n*/min)	73	73	72	73	72	72	0.94	0.89	0.46	0.71
Mast. rate pasture (*n*/min)[†]	74[a]	74[a]	72[b]	74	73	72	0.98	0.02	0.22	0.29
Rumination over 24 h										
Time (min)	402[b]	476[a]	470[a]	457	448	442	16.9	<0.001	0.70	0.70
Mastication (*n*/day)	24,119[b]	29,843[a]	28,910[a]	28,363	27,398	27,112	1226	<0.001	0.59	0.64
Mastication rate (*n*/min)	68.3[b]	70.4[a]	69.1[ab]	70.1	68.9	68.9	1.12	0.03	0.26	0.19
Rumination boli (*n*/day)	499	522	509	529	497	505	20.6	0.48	0.29	0.47
Chews per bolus (*n*/bolus)	47.5[b]	57.3[a]	56.8[a]	53.3	55.2	53.1	1.48	<0.001	0.07	0.67

Abbreviations: CS, supplementation with corn silage in the barn; CSP, supplementation with corn silage and additional protein concentrate in the barn; NS, no supplementation in the barn; TxP, interaction between treatment and period.

[a,b,c]Least squares means of the same row with different superscripts within treatment or period, respectively, differ significantly ($p < 0.05$).

[†]Mastication rate performed on pasture exclusively.

TABLE 5 Effect of supplementation on rumen fermentation traits (least square means and standard error of the mean [SEM]).

	Treatment			Period			SEM	*p* Value		
	NS	CS	CSP	1	2	3		Treatment	Period	TxP
Mean pH	6.12	6.10	6.11	6.11	6.15	6.08	0.051	0.88	0.12	0.82
Nadir pH[†]	5.78	5.72	5.79	5.74	5.81	5.73	0.059	0.51	0.21	-
Maximum pH	6.42	6.41	6.40	6.40	6.44	6.38	0.054	0.89	0.33	0.93
Duration, pH < 6.0 (min/day)	426	456	438	461	312	545	130	0.95	0.08	0.97
Area, pH < 6.0 (pH × min/day)[‡]	76.2	67.6	51.9	81.1	28.8	85.7	24.9	0.67	0.10	0.99
Total VFA (mmol/L)	102[b]	111[a]	115[a]	100[b]	111[a]	117[a]	4.87	0.023	0.005	0.09
VFA (molar %)										
Acetic acid	67.1[a]	59.8[b]	60.6[b]	64.1[a]	61.8[b]	61.6[b]	0.73	<0.001	0.01	0.67
Propionic acid	17.2[b]	19.6[a]	19.4[a]	18.5	18.9	18.8	0.35	<0.001	0.60	0.43
Acetic:Propionic acid	3.92[a]	3.05[b]	3.13[b]	3.50[a]	3.31[b]	3.30[b]	0.066	<0.001	0.02	0.25
n-Butyric acid	12.0[b]	14.7[a]	14.5[a]	12.5[b]	14.3[a]	14.4[a]	0.32	<0.001	<0.001	0.20
Isobutyric acid	1.13[a]	0.93[c]	1.03[b]	0.99	1.04	1.06	0.020	<0.001	0.06	0.28
n-Valeric acid	1.51[c]	1.85[b]	1.96[a]	1.65[b]	1.84[a]	1.83[a]	0.092	<0.001	0.005	0.66
Isovaleric acid	1.11[b]	1.67[a]	1.78[a]	1.36	1.52	1.69	0.145	<0.001	0.06	0.86
Ammonia N (mmol/L)	10.7[b]	10.2[b]	13.1[a]	10.2[b]	12.1[a]	11.7[a]	0.64	<0.001	0.02	0.16

Abbreviations: CS, supplementation with corn silage in the barn; CSP, supplementation with corn silage and additional protein concentrate in the barn; NS, no supplementation in the barn; VFA, volatile fatty acids; TxP, interaction between treatment and period.

[a,b,c]Least squares means of the same row with different superscripts within treatment or period, respectively, differ significantly ($p < 0.05$).

[†]Nonparametric test (Kruskal–Wallis) for the statistical analysis, interaction was not tested, mean values for treatment and period.

[‡]Area between pH 6.0 and the pH values < pH 6.0 of the cows.

herbage allowance was 15 kg herbage DM per cow and day compared to the overall herbage allowance of 16 kg in our study.

No further substitution of pasture intake was apparent using a balanced supplement CSP above what was effected by CS. Therefore, it is surprising that the milk and ECM yield in the CSP increased in comparison to the CS supplementation. Increasing the CP content in the supplemented rations, from (CS) 147 to (CSP) 179 g/kg DM had a positive effect on milk yield, although total DMI remains the same. In barn-fed Holstein dairy cows, Law et al. (2009) also found higher milk yields when the CP concentration in the ration was increased from 144 to 173 g/kg of DM. According to Bargo et al. (2003), increased rumen-undegradable protein intake of dairy cows in pasture-based rations had a significant, but highly variable, positive effect on milk yield. By contrast, Burke et al. (2008) found no significant differences in total DMI and milk yield when dairy cows grazed protein-rich herbage (22%–23% CP per DM) and were supplemented with a concentrate with 19% CP compared to maize silage supplementation. With protein-rich grazed herbage, neither the quantity nor the profile of amino acids available for absorption seems to be first-limiting in milk production, which can also be attributed to the highly degradable protein of grazed herbage (Kolver, 2003). Since the milk protein content was the same for the CS and NS treatments, and the energy intake was not significantly different between the CS and CSP treatments, energy supplementation may not be the primary reason for the higher milk protein content and the higher milk yield of the CSP cows. It may be more likely that the higher protein intake and improved amino acid composition led to increased milk protein content and higher milk yields in the CSP treatments (Haque et al., 2012).

4.2 | Ingestive and rumination behaviour

The total DMI was around 3.3 kg/day lower for the NS cows compared to the supplemented cows, even though the eating time was, on average, 1.5 h/day longer for the NS cows. The reduced total DMI and the concomitantly longer eating time are related to a much lower intake rate of grazed herbage. Similar to the results of Pérez-Ramírez et al. (2008), herbage intake rate was about 5.5 times lower compared to the offered supplements. The reasons for a lower intake rate of grazed herbage are the smaller bite size and extra time needed for searching and selecting feed. Kaufmann et al. (2011) mentioned a lower bite size as the reason for the extended eating time of grazing dairy cows when compared to cows fed the same feed in the barn. In addition, an upper limit of eating time is determined by the time required for other activities, such as ruminating (Rook, 2000); a negative correlation between rumination and eating time has been identified (Schirmann et al., 2012). The shorter eating time of the supplemented cows in our study agrees with previous reports (Bargo et al., 2003; Miguel et al., 2023). Opposed to the decrease in eating time, our study showed an increase in the rumination time of over 7 min/day/kg DM supplement. This increase agrees with the findings of Graf et al. (2005), who detected an increase in the rumination time of 7.6 min/day/kg DM corn silage. The longer rumination time and greater number of rumination chews suggest the possibility of better buffering for supplemented cows, as quantitatively significant saliva production is related to rumination activity (Storm et al., 2013). Therefore, a higher amount of starch eaten by the supplemented cows could be balanced by a higher amount of bicarbonate entering the rumen. This might explain the lack of differences in the pH characteristics in terms of mean, extremes and times below the threshold values between the groups.

4.3 | Ruminal fermentation

Another reason for the absence of differences in reticular pH, besides the longer rumination of supplemented cows, might be that pH was measured through pH boli residing predominantly in the reticulum. Due to the direct inflow of saliva via the oesophagus in the reticulum and the probable dilution effects from freshly eaten feed, the pH in the reticulum is less variable and overall higher compared to the rumen content (Falk et al., 2016; Sato et al., 2012). Although the total VFA concentrations in the ruminal fluid were higher in the supplemented cows, no differences occurred in reticular pH among the treatments. Increased concentrations of VFA may reduce pH in the ruminal fluid (Owens et al., 1998), but in our study, increased rumination activity of supplemented cows as well as the ammonia concentration of CSP cows may have counterbalanced the VFA effect on pH. The differences in individual VFA proportions were more pronounced between the supplemented and NS cows than between the CS and CSP treatments. In particular, a moderate shift from acetic to propionic and *n*-butyric acid production, as shown by Bargo et al. (2002), was observed in the present study when grazing dairy cows were supplemented with additional starch from corn silage. In contrast to the initial hypothesis, the extent of the detected fermentation pattern shifts did not suggest an obvious or systematically insufficient buffering or an overload of the VFA absorption capacity of the animals, as evidenced by the absence of pH differences. The balanced supplementation in the CSP treatment resulted in no relevant differences in the VFA pattern, apart from an increased proportion of isobutyric acid, when compared to the CS cows.

4.4 | NUE and feed efficiency

Besides converting nonedible feed sources into high-quality protein, keeping animal welfare and health intact and rewarding labour and investments, sustainable future milk production systems should use resources as efficiently as possible and reduce negative environmental impacts. Methane emissions legitimately receive a lot of attention (www.globalmethanepledge.org), but according to the United Nations Sustainable Development Goals (Sutton et al., 2021), nitrogen waste must also be halved by 2030.

The milk urea contents were high in our study, over 285 mg/L for all treatments, varied considerably among the treatments and were related to the CP content or the CP:NEL ratio of the ration. Burke et al. (2008) and Miguel et al. (2023) measured lower milk urea contents than our study. Dalley et al. (2020) observed very large differences in milk urea content between morning and evening milk, probably due to the timing of access to feeds. Averaged over the day, milk urea content in the study of Dalley et al. (2020) were in a similar range to our study, as was the case in Dickhoefer et al. (2022) with a starch-rich concentrate supplement. Nousiainen et al. (2004) showed a strong positive relationship (R^2 = 0.92) between milk urea nitrogen and dietary CP content in their meta-analysis. Furthermore, milk urea nitrogen content is positively associated with urinary nitrogen excretion and negatively associated with NUE (Huhtanen et al., 2015; Nousiainen et al., 2004). The relationship between milk urea concentration and NUE is also evident in our results, and the advantages of corn silage supplementation to protein-rich herbage in terms of nitrogen excretion can thus be anticipated. The CSP treatment took an intermediate position between NS and CS with regard to milk urea content and NUE, which was expected based on the CP content or the CP:NEL ratio of the rations. Based on the nitrogen metabolism of dairy cows, it can be inferred that ruminal ammonia concentration is related to milk urea, as Broderick and Clayton (1997) and Huhtanen et al. (2015) demonstrated. In general, ruminal ammonia N concentrations are high in our study (Huhtanen et al., 2015; McCarthy et al., 2023), which was expected based on milk urea contents. However, our results for ruminal ammonia N concentration do not show a clear relationship with dietary CP content, milk urea content or NUE. In particular, the ammonia N content with the pure herbage ration is out of line; it theoretically should be higher than in the supplementation treatments. The reasons for these inconsistent ruminal ammonia nitrogen concentration results may be complex and hypothetical. Tentative explanations might be that the ammonia nitrogen concentration was measured only in a part of the experimental animals. Further, daily patterns of ammonia nitrogen concentration (Reis & Combs, 2000), time of supplementary feed uptake, feeding behaviour and sampling procedure may have had an effect.

In terms of feed conversion efficiency (ECM per DMI), cows in the NS and CSP treatments performed better than those in the CS treatment. Average feed conversion efficiencies over the lactation of about 1.4 are good (Beever & Doyle, 2007; Coffey et al., 2017). Of course, higher feed conversion efficiencies can be achieved (Thorup et al., 2023), but this often occurs in short-term measurements and with the mobilisation of body reserves, especially at the beginning of lactation. Based on the calculated energy balance, the cows in the NS treatment would have had to draw on a substantial amount of mobilisable body energy to achieve increased feed conversion efficiency, which was not the case to the same extent in the CSP treatment. Unfortunately, the changes in body weight or BCS cannot be reliably determined over short measurement periods, as in the present experiment, although the loss and build-up of body mass play an important role in many respects with regard to feed efficiency (Ledinek et al., 2022). The CS and CSP cows had similar energy balance, and the cows in the CSP performed better than in the CS treatment in terms of feed conversion efficiency. The reason for this difference could be the higher protein intake and/or a better protein quality in terms of reduced protein degradability or improved amino acid profile of the ration.

5 | CONCLUSIONS

Under restricted grazing conditions, feeding corn silage, a ration-balancing supplementation, to dairy cows grazing a protein-rich herbage increased production and/or improved energy supply. Productive dairy cows under restricted grazing conditions had higher total DMI when supplemented with corn silage, although pasture herbage intake was lower due to the substitution. With an intrinsically balanced supplementation, a mix of corn silage and protein concentrate, to pasture herbage, the cows consumed the same amount of total DM but produced more milk, which positively affected feed conversion efficiency. However, corn silage-only supplementation performed better in terms of NUE. Results on intake, performance, reticular pH and efficiency traits may vary depending on pasture management, forage qualities and quantities and dairy cows' milk production potential. For the choice of a supplementation option, pasture offer and cost of supplement will have to be considered, but aspects of feed–food competition as well as animal welfare should not be ignored.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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