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Effects of forage and grain legume-based silages supplemented with faba bean meal or rapeseed expeller on lactational performance, nitrogen utilization, and plasma amino acids in dairy cows

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ABSTRACT

The objective of this experiment was to investigate the effect of forage type [red clover (51%)-grass silage, i.e., RCG; vs. faba bean (66%)-grass silage, i.e., FBG] and concentrate type (faba bean, FB; vs. rapeseed expeller, RE) on lactational performance, milk composition and nitrogen (N) utilization in lactating dairy cows. Eight lactating multiparous Nordic Red cows were used in a replicated 4 × 4 Latin Square experiment, with 21-d periods, in a 2 × 2 factorial arrangement of treatments. The experimental treatments were as follows: (1) RCG with RE, (2) RCG with FB, (3) FBG with RE, and (4) FBG with FB. Inclusion rates of RE and FB were isonitrogenous. Crude protein contents of the experimental diets were 16.3, 15.9, 18.1, and 17.9% of dry matter, respectively. All diets included oats and barley and were fed ad libitum as total mixed rations with forage-to-concentrate ratio of 55:45. Dry matter intake and milk yield were recorded daily, and spot samples of urine, feces, and blood were collected at the end of each experimental period. Dry matter intake did not differ across diets, averaging 26.7 kg/d. Milk yield averaged 35.6 kg/d and was 1.1 kg/d greater for RCG versus FBG, and milk urea N concentration was lower for RCG compared with FBG. Milk yield was 2.2 kg/d and milk protein yield 66 g/d lower for FB versus RE. Nitrogen intake, urinary N, and urinary urea N excretions were lower, and milk N excretion tended to be lower for RCG compared with FBG. The proportion of the dietary N excreted as fecal N was larger in cows fed RCG than for those fed FBG, and the opposite was true for urinary N. We detected an interaction for milk N as percentage of N intake: it increased with RE compared with FB for RCG-based diet, but only a marginal increase was observed for FBG-based diet. Plasma concentration of His and Lys were lower for RCG than for FBG, whereas His tended to be greater and Lys lower for FB compared with RE. Further, plasma Met concentration was around 26% lower for FB than for RE. Of milk fatty acids, saturated fatty acids were decreased by RCG and increased by FB compared with FBG and RE, respectively, whereas monounsaturated fatty acids were increased by RCG versus FBG, and were lower for FB than for RE. In particular, 18:1n-9 concentration was lower for FB compared with RE. Polyunsaturated fatty acids, such as 18:2n-6 and 18:3n-3, were greater for RCG than for FBG, and 18:2n-6 was greater and 18:3n-3 was lower for FB versus RE. In addition, *cis*-9,*trans*-11 conjugated linoleic acid was lower for FB compared with RE. Faba bean whole-crop silage and faba bean meal have potential to be used as a part of dairy cow rations, but further research is needed to improve their N efficiency. Red clover-grass silage from a mixed sward, without inorganic N fertilizer input, combined with RE, resulted in the greatest N efficiency in the conditions of this experiment.
Key words: red clover, faba bean, nitrogen utilization, dairy cow

INTRODUCTION

In Nordic growing conditions, cultivation of grass for dairy cattle is an important part of utilization of agricultural land and resources, but requires large inputs of artificial, fossil fuel-based nitrogen (N) fertilizers. There has been a considerable increase in the cost of inorganic N fertilizers, which imposes a strain on the economic sustainability of dairy farms. In addition, manufacturing of N fertilizers is a significant source of greenhouse gas emissions, which contribute to the total greenhouse gas emissions output from the agricultural sector (Stoddard et al., 2009). Therefore, forage legumes, such as red clover (*Trifolium pratense*),

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have gained interest in grassland cultivation due to their ability to fix N and increase carbon sequestration in the soil (Stoddard et al., 2009; Shurpali et al., 2020). Grain legumes, such as faba bean (*Vicia faba*), can be harvested both as seed and as whole-crop silage. This offers a possibility not only to include faba bean as a locally grown protein source in dairy cow diets but also to use it as a forage after an unfavorable growing season (Palmio et al., 2022). Use of legume feeds decreases the reliance on fossil fuel-based N fertilizers and imported protein concentrates, improves carbon sequestration, and diversifies crop rotations, thereby improving the economics and sustainability of dairy farms (Stoddard et al., 2009; Watson et al., 2018).

Inclusion of red clover in dairy cow rations alone or as a part of a legume-grass silage has shown to increase DMI and milk yield (**MY**). However, due to the high CP content, N efficiency is generally lower for red clover-based diets (Vanhatalo et al., 2009; Moorby et al., 2016). Further, a low plasma Met concentration has been observed in cows fed red clover-based diets (Vanhatalo et al., 2009), which can limit milk protein synthesis. However, N emissions from red clover diets are usually lower because the high polyphenol oxidase content decreases RDP fraction of red clover silage protein and leads to a greater proportion of the CP being excreted in feces rather than in urine (Lee et al., 2019a). Milk fatty acid (**FA**) profiles differ in cows fed red clover silage compared with those fed grass silage. Indeed, diets with red clover silage increase concentrations of MUFA and PUFA, especially 18:2n-6 and 18:3n-3, in milk fat compared with grass silage (Dewhurst et al., 2003; Vanhatalo et al., 2007; Halmemies-Beauchet-Filleau et al., 2014). Further, these alterations in milk FA concentrations have been accompanied with reductions in 10:0 to 16:0 concentrations (Vanhatalo et al., 2007).

Data on the utilization of faba bean whole-crop silage in dairy cow rations is scarce. Only a few experiments have examined whole-crop faba bean silage compared with grass silage (McKnight and MacLeod, 1977; Ingalls et al., 1979; Palmio et al., 2022) or corn silage (Guevara-Oquendo et al., 2022) in lactating dairy cow diets. In recent studies, replacing grass silage (Palmio et al., 2022) or corn silage (Guevara-Oquendo et al., 2022) with whole-crop faba bean silage resulted in similar or increased DMI, MY, and ECM but decreased N efficiency.

Faba bean seed has previously been studied as an alternative for rapeseed meal, but data are scarce and inconsistent. Generally, faba bean meal, and especially untreated faba bean meal (no heat treatment or dehulling), has a lower RUP content compared with rapeseed meal (Puhakka et al., 2016) or soybean meal (Cherif et al., 2018) and thereby has generally resulted in lower milk and milk protein yields. The greater starch content of faba bean meal has been suggested to partly compensate for the lower RUP content of faba bean protein through an increased rumen microbial protein synthesis (a combined effect of RDP and readily fermentable energy; Puhakka et al., 2016). Further, Puhakka et al. (2016) reported an increased plasma glucose concentration when rapeseed meal was replaced with faba bean meal, due to the greater small intestinal digestion of starch when faba bean meal was fed. In addition, the amount and profile of digestible AA (**dAA**) in cows fed faba bean meal has been reported to differ from that of rapeseed and soybean meals (Puhakka et al., 2016; Hansen et al., 2021). In particular, the Met concentration in faba bean is lower, leading to lower plasma Met concentration, which in turn can potentially limit milk protein synthesis in faba bean-containing diets (Puhakka et al., 2016). Comparisons of faba bean meal and rapeseed meal have so far been conducted with grass- or corn silage-based diets (e.g., Hansen et al., 2021; Kuoppala et al., 2021), and data with legume silage-based diets is lacking. In addition, no comparison has been performed between faba bean meal and rapeseed expeller, which has a greater fat content, greater potential to alter the milk FA profile, and lower RUP fraction compared with rapeseed meal (Luke, 2022).

Therefore, the objective of this experiment was to investigate the effect of legume forage type with high DMI potential (red clover-grass silage vs. faba bean-rich silages) and protein concentrate type (faba bean meal, **FB**; vs. rapeseed expeller, **RE**) on lactational performance, plasma AA concentration, and N efficiency in lactating dairy cows. The hypothesis was that faba bean-rich silage would result in greater MY due to its greater starch content, whereas feeding RE would result in greater milk and milk protein yields and N efficiency, due to its greater RUP content.

MATERIALS AND METHODS

Animals, Housing, and Experimental Design

The experiment was conducted at the University of Helsinki research farm in Helsinki, Finland. All experimental procedures were approved by the National Animal Experiment Board in Finland, in accordance with the guidelines established by the European Union Directive 2010/63/EU and the current Finnish legislation on animal experimentation (Act on the Protection of Animals Used for Scientific or Educational Purposes 497/2013).

A total of 8 multiparous Nordic Red cows were assigned into 2 squares based on milk production level and DIM. Square 1 included 4 cows averaging (mean ± SD) 75 ± 21 DIM, 49.0 ± 0.82 kg/d MY, and 701 ± 39.5 kg of BW; and square 2 included 4 cows averaging 57 ± 18.8 DIM, 40.8 ± 2.06 kg/d MY, and 671 ± 77.4 kg of BW at the beginning of the experiment. The experiment was designed as a replicated 4 × 4 Latin square balanced for residual effects with a 2 × 2 factorial arrangement of treatments. Treatment sequences within a square were randomly assigned to each cow.

Each of the 4 experimental periods lasted for 3 wk, with 2 wk for adaptation and 1 wk for sample and data collections. The cows were housed in individual tiestalls equipped with Roughage Intake Control System (Insentec BV, Markenesse, the Netherlands), and had free access to drinking water. Cows were milked twice daily at 0600 and 1700 h, refusals were removed daily at 0800 h, and TMR was delivered 3 times a day at approximately 0830, 1400, and 2000 h.

Experimental Diets and Treatments

The experimental feed ingredients and their chemical composition are presented in Table 1. The silages included a red clover-grass silage (**RCG**) with a high proportion of red clover (*Trifolium pratense* cultivar Saija), faba bean (*Vicia faba* cultivar Kontu) whole-crop silage, and grass silage. The red clover-grass sward included red clover (51%) and grass mixture [49% mix of timothy (*Phleum pratense*), meadow fescue (*Festuca pratensis*), and Italian ryegrass (*Lolium multiflorum*)], and was prepared from organically cultivated, secondary growth without any N fertilizer inputs. The proportion of red clover in the sward was estimated as described in Kuoppala et al. (2009). Shortly, samples of the sward were collected before harvest, and the plants were manually classified into red clover, grass species (i.e., timothy, meadow fescue, and Italian ryegrass), and other plant species (weeds). Proportions of each species were determined in the sample DM. The red clover-grass sward was harvested on September 2, 2020, in Humppila, Finland (60° N, 23° E), once red clover was at early to mid-flowering, wilted for 24 h, and ensiled into round bales using a McHale F550 (McHale, Ballinrobe, Ireland) round baler with a chopping length of around 46 mm. The whole-crop faba bean silage was prepared from green faba bean crop harvested on August 10, 2020, at the Viikki research farm in Helsinki, Finland (60° N, 25° E), when pods were mainly filled. It was wilted for 48 h and harvested into round bales using Lely Welger RPC 245 round baler (Lely International, Maassluis, the Netherlands) with a chopping length of around 80 mm. The grass silage was first-cut timothy-meadow fescue grass, harvested with a Krone XXL R/GL forage wagon (Krone GmbH, Germany) with a chopping length of around 60 mm, and stored in a bunker silo. All silages were preserved with a formic-acid additive AIV2 Plus Na (Taminco Finland Ltd., Oulu, Finland) targeted at 6 L/1,000 kg. During TMR preparation all silages were further chopped to 30- to 40-mm length in a feed mixer (CutMix, Pellon Group, Ylihärmä, Finland). The concentrate feeds included oats, barley, and milled untreated faba bean meal produced at the Viikki research farm as well as commercial cold-pressed RE (Hauhon Myllärit Ltd., Hämeenlinna, Finland), processed using a farm-scale screw-press (Reinartz, Neuss, Germany). Oats and barley were mixed in a ratio of 1:1, and 1% of propylene glycol was added to prevent dust. The final experimental TMR were mixed in a feeding wagon (TMR-SUK M2, Pellon Group, Ylihärmä, Finland).

The 4 factorially arranged experimental treatments were as follows: (1) RCG with RE, **RCG-RE** treatment; (2) RCG with FB, **RCG-FB** treatment; (3) faba bean-rich silage (**FBG**, 66% faba bean silage and 33% timothy-meadow fescue grass silage, respectively, of the forage portion of TMR) with RE, **FBG-RE** treatment; and (4) FBG with FB, **FBG-FB** treatment. Inclusion rate of RE and FB was isonitrogenous (based on percentage of protein feed CP in diet DM). All diets were fed as TMR, and feed was delivered 3 times daily at 0900, 1400, and 1930 h.

Sample Collections and Analysis

Feed and TMR Sampling. Amount fed and refusals were recorded daily, and feeding was adjusted to yield refusals at approximately 10%. Representative samples of the experimental forages (RCG, whole-crop faba bean, and grass silages) and concentrates (FB, RE, oats, and barley mix) were taken on d 17, 19, and 21 of each period. All feed samples were composited by period and dried at 50°C for 48 h for subsequent feed analysis. Subsamples of feed ingredients were dried at 103°C for 24 h, and DM was determined and used for calculation of DMI. The DM content of silages was further corrected for the loss of volatile compounds (lactic acid, VFA, NH_3-N, and ethanol) according to Huida et al. (1986). Separate samples of fresh silage were stored at −20°C for analysis of fermentation quality. Detailed description of sample processing and analyses are given in Puhakka et al. (2016). Briefly, lactic acid, water-soluble carbohydrates (**WSC**), and NH_3-N concentrations were analyzed using colorimetric methods, and ethanol concentration by an enzymatic kit (cat. no. 176290, Boehringer Mannheim, Mannheim, Germany). Volatile FA concentrations were determined by ultra-performance liquid chromatography (**UPLC**; Waters Acquity UPLC, Waters, Milford, MA) as described in

Table 1. Chemical composition (SD in parentheses) of feed ingredients used in the experimental diets[1]

Item	Faba bean silage[2]	Grass silage[2]	Red clover-grass silage[2,3]	Faba bean meal	Rapeseed expeller[4]	Barley and oats mix[5]
DM, %	27.8 (0.14)	28.4 (0.28)	32.0 (0.13)	85.5 (0.01)	89.8 (0.01)	86.3 (0.01)
Chemical composition, % of DM						
Ash	7.59 (0.07)	10.3 (0.09)	8.78 (0.02)	3.80 (0.002)	6.71 (0.003)	2.79 (0.01)
Acid-insoluble ash	1.05 (0.05)	2.51 (0.10)	1.00 (0.01)	0.020 (0.000)	0.128 (0.000)	0.803 (0.01)
NDF	43.7 (0.16)	51.6 (0.10)	42.2 (0.06)	14.2 (0.10)	26.2 (0.05)	22.1 (0.31)
Indigestible NDF	22.2 (0.18)	6.80 (0.10)	11.2 (0.11)	—	—	—
Starch	3.06	0.120	1.46	33.2	2.85	45.0
Total fat	0.861 (0.01)	2.49 (0.03)	2.70 (0.03)	1.35 (0.01)	12.3 (0.04)	3.68 (0.01)
OM	92.4	89.7	91.2	96.2	93.3	97.2
CP	16.9 (0.02)	16.5 (0.08)	13.5 (0.07)	31.8 (0.03)	34.5 (0.03)	13.7 (0.20)
MP[6]	7.51	8.51	8.18	12.5	16.7	9.65
ME,[6] MJ/DM	9.07	11.1	10.3	—	—	—
AA, % of CP						
Arg	3.20	2.27	3.5	8.24	5.58	4.87
Phe	3.44	4.29	4.33	3.75	3.84	4.62
His	1.42	1.55	1.57	2.25	2.50	1.86
Ile	3.36	3.80	3.89	3.60	3.73	3.23
Leu	5.34	6.58	6.73	6.59	6.57	6.39
Lys	3.92	4.50	4.28	5.36	5.38	3.40
Met	0.88	1.34	1.33	0.49	1.50	1.15
Tre	3.10	3.67	3.72	3.07	3.93	3.03
Try	0.76	0.87	1.02	0.69	1.02	0.87
Val	4.01	4.99	5.03	3.99	4.73	4.50
EAA	29.4	33.9	35.4	37.9	38.8	33.9
Branched-chain AA	12.7	15.4	15.7	14.1	15.0	14.1
NEAA	36.1	38.6	38.0	42.9	46.2	49.4
Total AA	65.6	72.5	73.4	80.8	85.0	83.3
Cornell N fractions,[7] % CP						
A	—	—	—	14.1	26.3	—
B_1	—	—	—	54.0	27.9	—
B_2	—	—	—	11.9	36.6	—
B_3	—	—	—	19.0	3.19	—
C	—	—	—	1.04	5.97	—
ED, %	—	—	—	78.1	76.4	—
Fatty acid composition, g/100 g fatty acid						
16:0	23.2	15.0	18.1	17.3	5.49	18.9
18:0	5.38	1.72	3.37	3.58	2.02	1.94
cis-9 18:1	3.66	2.29	5.70	21.9	52.2	29.8
cis-11 18:1	0.804	0.319	0.399	0.065	5.04	0.817
18:2n-6	32.3	14.7	20.9	49.3	22.5	42.7
18:3n-3	21.5	56.6	40.7	4.01	8.91	2.99
Other	11.7	8.98	9.48	3.93	2.24	2.08
Total fatty acids, % DM	0.652	1.42	0.880	1.16	11.2	2.33

[1]Chemical analyses were performed on 4 samples (1 composite sample per period) of each feed ingredient. For starch and AA analysis, one composite sample for the entire experiment was used.

[2]Fermentation quality of silages was as follows. Faba bean silage included in DM: lactic acid 3.23%, ethanol 0.16%, acetic acid 0.44%, water-soluble carbohydrates (WSC) 9.43%, NH_3-N 5.24% of total N, pH 4.08. Grass silage included in DM: lactic acid 5.58%, ethanol 0.21%, acetic acid 0.62%, propionate 0.018% WSC 6.28%, NH_3-N 3.91% of total N, pH 4.13. Red clover-grass silage included in DM: lactic acid 3.57%, ethanol 0.35%, acetic acid 0.56%, propionate 0.066% WSC 13.3%, NH_3-N 3.95% of total N, pH 4.82. D-values (proportion of digestible OM) were as follows: faba bean silage, 585 g/kg DM; grass silage, 693 g/kg DM, red clover silage, 642 g/kg DM. D-value is the amount of digestible OM in DM.

[3]Red clover-grass silage contained: red clover (51%) and grass mixture [49% mix of timothy (*Phleum pratense*), meadow fescue (*Festuca pratensis*), and Italian ryegrass (*Lolium multiflorum*)], and was prepared from organically cultivated, secondary growth without N fertilizer inputs. For details see Materials and Methods.

[4]Hauhon Mylläri Inc., Hämeenlinna, Finland.

[5]Milled oats and barley mixed in a ratio of 1:1.

[6]Calculated according to Luke (2022). For details see Materials and Methods.

[7]Calculated according to Licitra et al. (1996), where A = nonprotein N, B_1 = true protein soluble in mineral buffer, B_2 = true protein insoluble in mineral buffer but not soluble in neutral detergent, B_3 = true protein bound to NDF, C = protein bound to ADF. ED = effective degradability, calculated as $A + B_1 \times [k_{d\ B1} \div (k_{d\ B1} + k_p)] + B_2 \times [k_{d\ B2} \div (k_{d\ B2} + k_p)] + B_3 \times [k_{d\ B3} \div (k_{d\ B3} + k_p)]$, where degradation rates (k_d) for B_1, B_2, and B_3 were 2, 0.1, and 0.002 per hour, respectively, and passage rate (k_p) was 0.08/h (as described in Kuoppala et al., 2021). Fraction A was considered to be degraded instantly.

Puhakka et al. (2016). The dried feed samples were ground through a 1-mm screen and analyzed for DM, OM, NDF (reported on ash-free basis), indigestible NDF, CP, and acid-insoluble ash (**AIA**), starch, total fat, and AA. Briefly, NDF content was analyzed in the presence of sodium sulfite according to Van Soest et al. (1991) with a FiberTherm FT12 analyzer (Gerhardt, Königswinter, Germany). Indigestible NDF content was determined in the experimental silages by incubating nylon bags (pore size of 17 μm) in the rumen of 2 lactating rumen-cannulated cows fed a grass silage-based diet for 12 d according to Ahvenjärvi et al. (2000). Crude protein content was determined with the Kjeldahl method (AOAC International, 1995), AIA by acid hydrolysis (Van Keulen and Young, 1977), starch content by the amyloglucosidase method with a K-TSTA kit (Megazyme, Co. Wicklow, Ireland), total fat with petroleum ether extraction and hydrolysis with HCl (SoxCap 2047 Hydrolysis Unit, Foss Soxtec 8000; Foss Analytical, Hillerød, Denmark), and AA concentration by UPLC. Samples were hydrolyzed with 6 *M* HCl before AA analysis, and for analysis of sulfuric AA, samples were oxidized with performic acid. Feed FA composition was determined by gas chromatography (GC2010 Plus, Shimadzu, Kyoto, Japan) equipped with a flame-ionization detector and a J&W CP-Sil 88 capillary column (100 m × 0.25 mm i.d. × 0.2-μm film thickness; Agilent, Santa Clara, CA) as described in Lamminen et al. (2019).

The metabolizable energy content of experimental forages was calculated as 0.016 × D-value for RCG and grass silage, and 0.0155 × D-value for whole-crop faba bean silage. The D-value is the in vitro digestible OM in DM, determined according to Nousiainen et al. (2003).

Cornell N fractions were analyzed for FB and RE and calculated according to Licitra et al. (1996), and effective degradability of N was calculated as $A + B1 \times [k_d\ B1 \div (k_d\ B1 + k_p)] + B2 \times [k_d\ B2 \div (k_d\ B2 + k_p)] + B3 \times [k_d\ B3 \div (k_d\ B3 + k_p)]$, where degradation rates (k_d) for B1, B2, and B3 were 2, 0.1, and 0.002/h, respectively, passage rate (k_p) was 0.08/h (as described in Kuoppala et al., 2021), and A = NPN, B1 = true protein soluble in mineral buffer, B2 = true protein insoluble in mineral buffer but soluble in neutral detergent, B3 = true protein bound to NDF, and C = protein bound to ADF. Fraction A was considered to be degraded instantly.

The amounts of MP and dAA flowing to the small intestine were calculated based on the average DMI during the experiment, CP content of the experimental diets, analyzed AA composition of the feeds, AA concentration of microbial protein (Sok et al., 2017), and tabulated values and equations (Tuori et al., 1998; Luke, 2022).

The chemical composition of individual feed components (Table 1) was used to reconstitute the composition of each experimental diet (Table 2).

Fecal and Urine Samples. Spot fecal samples were collected at 0800 and 1500 h on d 15, 16, 17, and 18 of each period for a total of 8 samples/period. A total of approximately 500 g of sample was collected at each sampling time point from the rectum and stored frozen at −20°C. The fecal samples were thawed and composited by cow and period. The composite samples were dried at 70°C for 48 h, ground through a 1-mm sieve, and analyzed for chemical composition as described above for concentrate feed samples. The AIA content of the feeds and fecal samples was used as an internal marker for apparent total-tract digestibility of nutrients (Van Keulen and Young, 1977). Four spot urine samples were collected at 0530 and 1430 h and at 1000 and 1900 h on d 16 and 17 of each period, respectively. The urine was collected by stimulation of the vulva, and a total of 300 mL was filtered through cheesecloth and acidified with 15 mL of H_2SO_4 to decrease the pH below 3. Subsequently, a subsample was diluted 1:10 and stored at −20°C. Composited and diluted urine samples were analyzed for total N (Kjeldahl method), urinary urea N (**UUN**; colorimetric enzyme kit, Urea Liquicolor 10505, Human Gesellschaft, Wiesbaden, Germany), creatinine, uric acid, and allantoin using UPLC, as described in Puhakka et al. (2016). Creatinine concentration was used in estimation of total daily urine volume (Valadares et al., 1999), and a daily creatinine excretion rate of 22 mg/kg of BW was used, based on total urine collection data from our group (S. E. Räisänen, O. Pitkänen, Þ. H. Sigurðardóttir, A. Halmemies-Beauchet-Filleau, T. Kokkonen, A. Vanhatalo, Department of Agricultural Sciences, University of Helsinki, Finland, unpublished). Estimated daily urine volume was used for calculations of daily excretion of nitrogenous compounds and purine derivatives (**PD**; uric acid and allantoin). Total urinary PD excretion was used for calculation of microbial N flow from the rumen, according to Chen and Gomes (1992).

Blood Sampling. Blood samples were collected from the coccygeal vein or artery a total of 3 times at 0800, 1100, and 1400 h on the last day of each period. The samples were collected into 2 Vacutainer tubes (BD Medical, Franklin Lakes, NJ) containing EDTA and placed immediately on ice, and blood plasma was separated by centrifugation at 2,220 × *g* for 10 min, and the plasma was stored at −20°C. The plasma samples were thawed and analyzed for nonesterified fatty acid (**NEFA**), BHBA, and glucose concentrations with

Table 2. Ingredient and chemical composition and estimated protein supply of experimental diets

Item	Treatment[1]			
	RCG-FB	RCG-RE	FBG-FB	FBG-RE
Feed ingredients, % of DM				
Red clover-grass silage	56.7	56.9	—	—
Whole-crop faba bean silage	—	—	36.6	36.5
Grass silage	—	—	19.3	19.3
Rapeseed expeller	—	11.9	—	12.5
Faba bean meal	15.4	—	15.8	—
Barley-oats mix	27.0	30.4	27.6	30.8
Mineral mix	0.87	0.88	0.89	0.89
Chemical composition, % of DM				
OM	92.8	92.5	93.0	92.7
CP	16.3	15.9	18.1	17.9
NDF	32.1	33.8	34.3	36.0
Starch	18.1	14.8	18.8	15.3
Total fat	2.73	4.11	2.02	3.47
Ash	7.15	7.48	6.98	7.31
Acid-insoluble ash	0.788	0.829	1.09	1.13
MP[2]	9.17	9.56	9.01	9.44
ME, MJ/kg DM	11.2	11.5	10.9	11.3
MP, g/d				
Supply	2,461	2,484	2,358	2,470
Recommendation	2,407	2,407	2,407	2,407
Balance	54	77	−51	64
ME, MJ/d	295	306	290	308
Digestible AA supply,[3] g/d				
His	44.4	45.0	44.8	46.7
Lys	166	167	166	174
Met	45.0	46.7	46.7	49.0
Arg	113	111	108	113
Ile	121	123	121	127
Leu	177	179	179	187
Phe	120	122	121	127
Thr	113	115	114	120
Trp	25.7	26.4	26.2	27.4
Val	127	130	129	136
EAA	1,104	1,116	1,080	1,131
Total AA	1,978	2,004	1,951	2,044

[1]Experimental treatments were as follows: RCG-FB = red clover-grass silage with faba bean meal; RCG-RE = red clover-grass silage with rapeseed expeller; FBG-FB = faba bean-rich silage with faba bean meal; FBG-RE = faba bean-rich silage with rapeseed expeller.

[2]Based on chemical analysis described in Material and Methods. Includes microbial and feed protein, calculated according to Tuori et al. (1998) and based on analyzed CP content of the diets and tabulated values (Natural Resources Institute Finland; Luke, 2022) for rumen-degradable protein fractions.

[3]Calculated based on analyzed AA concentration of each feed ingredient, DMI of cows during the experiment, amount of digestible protein according to Tuori et al. (1998), and AA concentration of rumen microbial protein according to Sok et al. (2017).

enzymatic kits in Indiko Plus (Thermo Fisher Scientific Ltd., Vantaa, Finland); Randox FA115 (Randox Laboratories, Crumlin, UK) was used for NEFA; glucose GOD-POD kit (Thermo Fisher Scientific Ltd., Vantaa, Finland) for glucose; and a Ranbut Kit (Randox Laboratories, Crumlin, UK) for BHBA. Subsamples of plasma were composited by cow and period, and analyzed for AA and acetate concentrations via UPLC, and for insulin and glucagon concentrations using radioimmunoassay kits PI-12K and GL-32K (Millipore, St. Charles, MO), respectively.

Milk Yield and Composition. Daily MY was recorded throughout the experiment. Milk samples for determination of milk composition were collected from 4 consecutive milkings on d 16, 17, and 18 of each period. Samples were preserved with 2-bromo-2-nitropropane-1,3-diol (bronopol; Valio Ltd., Helsinki, Finland). The samples were analyzed for milk fat, CP, lactose, and urea (Milko Scan FT+ analyzer, Foss Electric A/S, Hillerød, Denmark) at a commercial laboratory (Valio Ltd., Seinäjoki, Finland). Milk urea N concentration was calculated as milk urea concentration multiplied by 0.47, and milk true protein (**TP**) concentration was calculated as milk CP% − MUN (mg/dL) × 6.38/1,000. Further, a separate unpreserved sample was taken from each milking, composited on milk yield-basis, stored at

−20°C, and processed and analyzed for milk FA composition by gas chromatography similarly to feed FA (Lamminen et al., 2019).

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). The model included the fixed effects of treatment and square, and period within square. Cow within square was included as a random effect. Daily feed intake and milk yields were averaged over the last 7 d of each experimental period and used in statistical analysis. Milk composition data were weighted averages based on the milk production at each milking of milk sample collection. Data for MY and milk composition from one cow on RCG-FB treatment during period 3 was excluded due to mastitis. The normality model residuals were tested by the Shapiro-Wilk test, and heterogeneity was explored visually. For plasma metabolites (BHBA, NEFA, and glucose), the model also included the repeated term of sampling time with an autoregressive model of order one [AR(1)] covariance structure; additional fixed effects of sampling time, period × sampling time, and treatment × sampling time; and random effects of cow, cow × period × treatment, and cow × sampling time. We detected no treatment × sampling time interactions, and therefore all data are presented as treatment least squares means (**LSM**). The sums of squares of the treatment effects were separated into single degree of freedom comparisons using orthogonal contrasts to test the effect of silage type (RCG vs. FBG), protein concentrate type (FB vs. RE), and their interaction. Treatment effects were considered significant at $P \leq 0.05$, and a tendency was considered at $0.05 < P \leq 0.10$. All data are presented as LSM.

RESULTS

Feed Composition

The composition of experimental feeds is presented in Table 1. The NDF content of whole-crop faba bean and RCG silages were similar (43.7 and 42.2% of DM), and lower than that of grass silage (51.6% of DM). As expected, starch content was greatest for faba bean silage among forages. Crude protein content was similar between faba bean and grass silages and lowest for RCG silage. Of the protein concentrates, NDF and total fat contents were lower, whereas starch content was greater for FB versus RE. Crude protein content and estimated MP concentrations were 2.8 and 4.2 percentage units lower for FB compared with RE. The estimated Cornell N fractions differed between the 2 protein concentrates, the A and B_2 fractions being lower, and B_1 and B_3 fractions greater for FB than for RE. Finally, of feed FA, whole-crop faba bean silage had the greatest concentration of 18:2n-6, followed by RCG and grass silages. The opposite was true for 18:3n-3. Faba bean meal had a greater concentration of 16:0 and 18:2n-6 and lower concentrations of *cis*-9 18:1, *cis*-11 18:1, and 18:3n-3 compared with RE.

The recomposited chemical compositions of the experimental diets and estimated supplies of MP and dAA are presented in Table 2. Notably, CP and starch contents were greater for treatments with FBG compared with RCG, whereas starch content was greater and total fat content lower for diets containing FB versus RE. The estimated supplies of MP, EAA, and total AA (**TAA**) were around 5% lower for FBG-FB compared with the FBG-RE diet, but were similar between RCG-FB and RCG-RE diets.

Feed and Nutrient Intake and Apparent Total-Tract Digestibility

Nutrient intake and apparent total-tract digestibility of nutrients are presented in Table 3. We observed no difference in DMI across experimental diets, whereas OM intake tended to be lower for cows fed RCG versus FBG ($P = 0.07$). Further, CP, NDF, and starch intakes were 0.62, 0.77, and 0.25 kg/d lower ($P \leq 0.01$) and total fat intake 0.16 kg/d greater ($P = 0.001$) for RCG than for FBG. For protein concentrates, total fat, and NDF intakes were 0.39 and 0.63 kg/d lower ($P < 0.001$) and starch intake 0.82 kg/d greater ($P < 0.001$) for cows fed FB compared with RE. Apparent total-tract digestibility of nutrients was not different across treatments, except for 7 percentage units lower ($P = 0.001$) CP digestibility for RCG than for FBG, and 0.35 percentage units lower ($P = 0.03$) starch digestibility for FB than for RE. Intakes of TAA, EAA, and branched-chain AA (**BCAA**) were 9, 6, and 6% lower ($P < 0.001$) for RCG compared with FBG but similar between FB and RE, whereas of the individual EAA, intakes of Arg, His, and Lys were lower ($P < 0.001$) for RCG than for FBG, and that of Met, Thr, and Val was lower ($P \leq 0.006$) for FB versus RE. No interactions were detectable for any of the variables. Finally, the intake of total FA was 9% lower ($P < 0.001$) for RCG than for FBG, and 49% lower ($P < 0.001$) for FB compared with RE. Of the individual FA, intakes of 16:0, 18:0, *cis*-11 18:1, and 18:3n-3 were lower ($P \leq 0.01$) and that of 18:2n-6 greater ($P < 0.001$) for cows receiving RCG versus FBG. The intakes of all individual FA were greater ($P < 0.001$) for RE compared with FB.

Table 3. Nutrient intake and apparent total-tract digestibility of mid-lactation dairy cows fed forage and grain legume-based silages with faba bean meal or rapeseed expeller

	Treatment[1]					*P*-value[3]		
Item	RCG-FB	RCG-RE	FBG-FB	FBG-RE	SEM[2]	RCG vs. FBG	FB vs. RE	Interaction
Nutrient intake, kg/d								
DM	26.3	26.6	26.6	27.3	0.55	0.11	0.18	0.40
OM	24.4	24.5	24.7	25.3	0.52	0.07	0.27	0.39
CP	4.27	4.22	4.83	4.90	0.098	<0.01	0.89	0.35
Total fat	0.718	1.09	0.538	0.949	0.021	<0.001	<0.001	0.22
NDF	8.45	8.96	9.1	9.85	0.19	<0.001	<0.001	0.34
Starch	4.76	3.93	5.00	4.20	0.099	0.001	<0.001	0.79
Apparent total-tract digestibility, %								
DM	66.4	65.6	66.4	66.4	1.56	0.83	0.74	0.83
OM	67.8	66.8	67.7	67.7	1.49	0.85	0.69	0.71
CP	61.1	58.5	66.4	67.3	1.72	0.001	0.64	0.31
NDF	41.2	44.8	41.8	43.3	2.85	0.87	0.34	0.70
Starch	98.1	98.6	98.5	98.7	0.144	0.12	0.03	0.32
AA intake, g/d								
Arg	224	185	230	196	4.62	0.007	<0.001	0.42
Phe	180	181	189	194	3.83	<0.001	0.32	0.35
His	78.6	79.5	84.9	88.4	1.76	<0.001	0.10	0.31
Ile	156	155	168	171	3.4	<0.001	0.61	0.34
Leu	282	279	294	299	6.0	0.001	0.80	0.34
Lys	189	183	208	208	4.3	<0.001	0.33	0.28
Met	44.4	56.1	43.8	57.5	1.06	0.61	<0.001	0.20
Thr	144	152	153	166	3.2	<0.001	<0.001	0.28
Trp	38.0	41.5	37.7	42.5	0.83	0.53	<0.001	0.25
Val	197	203	206	219	4.27	<0.001	0.006	0.33
Branched-chain AA	635	637	668	688	13.7	<0.001	0.22	0.33
EAA	1,533	1,515	1,614	1,640	33.1	<0.001	0.85	0.32
NEAA	1,444	1,455	1,599	1,655	32.5	<0.001	0.13	0.31
Total AA	2,978	2,971	3,213	3,295	65.6	<0.001	0.38	0.31
Fatty acid intake, g/d								
16:0	63.2	78.7	66.3	84.5	1.49	<0.001	<0.001	0.19
18:0	9.32	15.2	9.71	16.3	0.271	0.002	<0.001	0.08
cis-9 18:1	67.1	247	65.9	263	4.51	0.11	<0.001	0.06
cis-11 18:1	1.91	19.7	2.17	21.7	0.393	0.01	<0.001	0.04
18:2n-6	128	202	121	187	3.39	<0.001	<0.001	0.12
18:3n-3	60.3	90.9	61.8	96.4	1.71	0.01	<0.001	0.12
Total fatty acids	344	671	373	739	12.1	<0.001	<0.001	0.07

[1]Experimental treatments were as follows: RCG-FB = red clover-rich silage with faba bean meal; RCG-RE = red clover-rich silage with rapeseed expeller; FBG-FB = faba bean-rich silage with faba bean meal; FBG-RE = faba bean-rich silage with rapeseed expeller.

[2]Largest SEM published in table; n = 32 (n represents the number of observations used in the statistical analysis). Data are presented as LSM.

[3]RCG vs. FBG = main effect of forage type (red clover-grass silage vs. faba bean-grass silage); FB vs. RE = main effect of protein concentrate type (faba bean meal vs. rapeseed expeller); Interaction = interaction between forage and protein concentrate type.

Milk Production and Composition

Milk yield and feed efficiency were 1.1 kg/d and 0.07 kg/kg greater ($P \leq 0.05$; Table 4) for RCG compared with FBG and 2.2 kg/d and 0.06 kg/kg lower ($P < 0.001$) for FB than for RE. Energy-corrected MY was 1.6 kg/d lower ($P = 0.01$) for FB compared with RE. Milk protein and milk TP concentrations were 0.21 and 0.17 percentage units lower ($P < 0.001$), fat concentration tended to be lower ($P = 0.09$), and lactose concentration was 0.07 percentage units greater ($P < 0.001$) for RCG compared with FBG. Milk protein yield tended to be lower ($P = 0.08$), and lactose yield was greater ($P = 0.01$) for RCG than for FBG, and lactose yield was lower ($P = 0.002$) for FB versus RE. Milk urea N concentration was 6.27 mg/dL lower ($P < 0.001$) for RCG than FBG and 2.67 mg/dL greater ($P < 0.001$) for FB compared with RE.

Nitrogen Utilization

Nitrogen utilization and urinary PD excretion are presented in Table 5. Nitrogen intake, urinary N, and UUN excretions were 12, 30, and 40% lower ($P < 0.001$), and milk N excretion tended ($P = 0.08$) to be lower for RCG compared with FBG. Milk N excretion was 5% lower ($P = 0.002$) and urinary N and UUN excretions tended ($P \leq 0.10$) to be greater for FB versus RE.

Table 4. Lactational performance of mid-lactation dairy cows fed forage and grain legume-based silages with faba bean meal or rapeseed expeller

Item	Treatments[1]				SEM[2]	P-value[3]		
	RCG-FB	RCG-RE	FBG-FB	FBG-RE		RCG vs. FBG	FB vs. RE	Interaction
Milk yield, kg/d	35.1	37.2	34.0	36.2	1.26	0.05	<0.001	0.97
Feed efficiency, kg/kg	1.33	1.40	1.28	1.32	0.045	0.007	0.008	0.49
ECM yield,[4] kg/d	37.0	38.8	37.3	38.7	1.22	0.86	0.01	0.66
Milk protein, %	3.51	3.53	3.78	3.68	0.076	<0.001	0.24	0.06
Milk true protein,[5] %	3.43	3.47	3.66	3.58	0.074	<0.001	0.54	0.06
Milk fat, %	4.44	4.36	4.69	4.46	0.213	0.09	0.12	0.45
Milk lactose, %	4.59	4.58	4.53	4.51	0.029	<0.001	0.26	0.72
Milk protein yield, kg/d	1.22	1.30	1.27	1.32	0.034	0.08	0.002	0.44
Milk true protein yield, kg/d	1.19	1.28	1.23	1.28	0.034	0.26	0.001	0.39
Milk fat yield, kg/d	1.54	1.60	1.57	1.61	0.074	0.61	0.17	0.76
Milk lactose yield, kg/d	1.61	1.70	1.54	1.63	0.056	0.01	0.002	0.91
MUN,[6] mg/dL	11.8	8.87	17.8	15.4	0.83	<0.001	<0.001	0.53
SCC, $\times 10^3$/mL	60.3	101	65.6	73.4	32.7	0.49	0.14	0.31
BCS[7]	3.16	3.07	3.16	3.19	0.139	0.06	0.30	0.09
BCS change[7]	0.01	−0.08	0.06	0.03	0.036	0.05	0.13	0.45

[1]Experimental treatments were as follows: RCG-FB = red clover-rich silage with faba bean meal; RCG-RE = red clover-rich silage with rapeseed expeller; FBG-FB = faba bean-rich silage with faba bean meal; FBG-RE = faba bean-rich silage with rapeseed expeller.

[2]Largest SEM published in table; n = 31 (n represents the number of observations used in the statistical analysis). Data are presented as LSM.

[3]RCG vs. FBG = main effect of forage type (red clover-grass silage vs. faba bean-grass silage); FB vs. RE = main effect of protein concentrate type (faba bean meal vs. rapeseed expeller); Interaction = interaction between forage and protein concentrate type.

[4]Calculated according to Sjaunja et al. (1990).

[5]Calculated as milk protein % − MUN (mg/dL) × 6.38/1,000.

[6]Calculated as milk urea concentration × 0.47.

[7]Based on the 5-point scale by Edmonson et al. (1989). Body condition score change was calculated as the difference in BCS between the periods.

Fecal N as percentage of N intake was 20% greater ($P = 0.002$) and urinary N as percentage of N intake was 21% lower ($P < 0.001$) for RCG versus FBG. Urinary N as percentage of N intake tended ($P = 0.07$) to be greater for FB than for RE. We detected an interaction ($P = 0.05$) for milk N as percentage of N intake: it increased by 2.5 percentage units with RE compared with FB for RCG-based diet, but only marginally, by 0.7 percentage units, for FBG-based diet. Further, uric acid excretion and estimated microbial N flow were 6.0 and 16 g/d lower ($P \leq 0.04$) for RCG than for FBG, whereas estimated microbial N flow was 16 g/d lower ($P = 0.05$) for FB versus RE.

Plasma Concentration of Amino Acids, Hormones, and Metabolites

Plasma concentration of EAA did not differ between RCG and FBG but was 12% lower ($P = 0.03$; Table 6) for FB compared with RE, as was the concentration of BCAA ($P = 0.03$). Of individual AA, His and Lys were 9 and 10% lower ($P \leq 0.03$) for RCG than for FBG, whereas His tended to be greater ($P = 0.07$) and Lys lower ($P = 0.10$) for FB versus RE. Further, Met concentration was around 26% lower ($P < 0.001$) for FB than for RE.

Plasma concentrations of acetic acid, glucagon, and insulin did not differ across treatments (Table 7). Both NEFA and BHBA concentrations were 17 and 16% greater ($P \leq 0.02$), respectively, for RCG versus FBG. Glucose concentration tended to be greater ($P = 0.07$) and NEFA concentration was 18% lower ($P = 0.001$) for FB versus RE.

Milk Fatty Acid Composition

Of the milk FA, no interactions occurred between silage and protein concentrate types except for 9:0, being greatest ($P = 0.03$; Table 8) for the FBG-FB diet and similar among the other 3 treatments. Overall, de novo FA and SFA concentration were 4 and 2% lower ($P \leq 0.002$), and MUFA and PUFA respectively 3 and 24% greater ($P \leq 0.04$) for RCG compared with FBG, whereas de novo FA and SFA were 5 and 8% greater ($P < 0.001$) and MUFA and PUFA 24 and 1% lower ($P < 0.001$) for FB versus RE. Further, 18:2n-6 and 18:3n-3 were greater ($P \leq 0.03$) and *trans*-11 18:1, *cis*-12 18:1, and *trans*-11,*cis*-15 18:2 lower ($P \leq 0.05$) for RCG vs. FBG. Most *trans* and *cis* FA, in particular *cis*-9 18:1, as well as 18:0, 18:3n-6, and *cis*-9,*trans*-11 CLA were lower ($P \leq 0.01$) for FB than for RE.

DISCUSSION

The overall aim of the study was to investigate whether locally cultivated legumes produced without

Table 5. Nitrogen (N) utilization and purine derivatives excretion of mid-lactation dairy cows fed forage and grain legume-based silages with faba bean meal or rapeseed expeller

Item	Treatment[1]				SEM[2]	*P*-value[3]		
	RCG-FB	RCG-RE	FBG-FB	FBG-RE		RSG vs. FBG	FB vs. RE	Interaction
N intake, g/d	692	676	772	783	16.66	<0.001	0.79	0.18
Milk N, g/d	191	204	199	207	5.39	0.08	0.002	0.44
Fecal N, g/d	261	281	259	255	15.8	0.36	0.60	0.42
Urinary N, g/d	235	203	317	311	12.3	<0.001	0.08	0.23
Urinary urea N, g/d	161	136	252	245	11.2	<0.001	0.10	0.34
Urinary urea N, % of urinary N	60.5	58.7	69.9	69.2	1.32	<0.001	0.33	0.65
Milk N, % intake	27.8	30.3	25.9	26.6	0.85	<0.001	0.003	0.05
Fecal N, % of intake	38.0	41.5	33.6	32.7	1.86	0.002	0.49	0.23
Urinary N, % of intake	33.9	30.2	41.1	39.9	1.62	<0.001	0.07	0.35
N balance, g/d	2.07	−12.1	−2.21	11.4	20.5	0.60	0.99	0.45
Urine volume,[4] L/d	27.0	26.7	25.3	24.8	1.68	0.20	0.79	0.92
Allantoin, mmol/d	481	492	491	516	24.9	0.07	0.06	0.44
Uric acid, mmol/d	59.4	63.4	68.3	66.5	4.01	0.005	0.54	0.13
Total urinary purine derivatives, mmol/d	544	559	560	581	31.4	0.23	0.26	0.82
Estimated microbial N,[5] g/d	390	403	403	422	20.1	0.04	0.05	0.66

[1]Experimental treatments were as follows: RCG-FB = red clover-rich silage with faba bean meal; RCG-RE = red clover-rich silage with rapeseed expeller; FBG-FB = faba bean-rich silage with faba bean meal; FBG-RE = faba bean-rich silage with rapeseed expeller.

[2]Largest SEM published in table; n = 31 (n represents the number of observations used in the statistical analysis). Data are presented as LSM.

[3]RCG vs. FBG = main effect of forage type (red clover-grass silage vs. faba bean-grass silage); FB vs. RE = main effect of protein concentrate type (faba bean meal vs. rapeseed expeller); Interaction = interaction between forage and protein concentrate type.

[4]Calculated based on assumed daily creatinine excretion of 22 mg/kg BW (S. E. Räisänen, O. Pitkänen, Þ. H. Sigurðardóttir, A. Halmemies-Beauchet-Filleau, T. Kokkonen, A. Vanhatalo, Department of Agricultural Sciences, University of Helsinki, Finland, unpublished data).

[5]Includes estimated purine derivative absorption into milk as well as urine (Chen and Gomes, 1992).

inorganic N fertilizers can support the production levels of high-yielding dairy cows. Both forage and grain legumes were used to maximize the use of home-grown feeds in the diet. The specific aim of this experiment was to investigate the effect of forage type (RCG vs. FBG) and protein concentrate type (FB vs. RE) on production parameters and N efficiency in lactating dairy cows. It is noted that due to the lower-than-expected CP content of the RCG silage, the experimental diets were not isonitrogenous as for the comparison between the forage types. This partly confounds the comparison of the forage type in regard to N utilization parameters. Overall, greater MY with lower CP intake resulted in greater N efficiency of cows fed diets based on RCG compared with FBG. In addition, cows receiving diets with RE had greater production and N efficiency compared with cows fed diets with FB as the protein concentrate.

Composition of Experimental Feeds

In the current experiment, all silages were restrictively fermented and well preserved, as indicated by the low concentrations of fermentation acids and NH_3-N, as well as by the high concentration of WSC. The low CP content of the RCG silage was likely caused by the absence of N fertilizer application during cultivation of the sward under organic conditions (Rinne and Nykänen, 2000). The whole-crop faba bean silage was harvested early, which led to lower CP, NDF, and starch contents compared with previous experiments (Lamminen et al., 2015; Palmio et al., 2022). In addition, the differences in nutrient content between faba bean silages in the current experiment and that of Palmio et al. (2022) can be partly explained by a presumably greater harvest loss of pods in the current experiment (mower conditioner vs. disk mower) as well as cultivar type (Kontu vs. Taifun).

The most notable difference in the AA concentration of the feed ingredients was in Met concentration, being lowest for both the faba bean silage and FB compared with the other feed ingredients. Further, the BCAA concentration and TAA concentration in faba bean silage was lower compared with the other silages. These differences were reflected in the estimated dAA intake as well as plasma AA concentrations, as described below. In addition, the Cornell N fractions differed between the protein feeds. The N fractions for FB in the current experiment correspond well with values reported by Kuoppala et al. (2021). They also analyzed the N fractions for rapeseed meal, which had a greater B_2 (63.8 vs. 36.6%) and lower A (14.4 vs. 26.3%) and B_1 (10.1 vs. 27.9%) fractions compared with the cold-pressed RE used in the current experiment. The difference in the distribution of N between the A, B_1, and B_2 fractions of RE and rapeseed meal can be attributed to differences

Table 6. Plasma amino acid concentrations (μ*M*) in mid-lactation dairy cows fed forage and grain legume-based silages with faba bean meal or rapeseed expeller

	Treatment[1]					*P*-value[3]		
Item	RCG-FB	RCG-RE	FBG-FB	FBG-RE	SEM[2]	RCG vs. FBG	FB vs. RE	Interaction
Arg	79.4	85.5	88.4	86.3	3.96	0.16	0.55	0.23
His	58.2	56.4	64.5	61.3	3.41	<0.001	0.07	0.60
Ile	117	137	127	137	5.52	0.39	0.01	0.39
Leu	137	155	143	151	6.58	0.89	0.06	0.49
Lys	83.5	94.6	97.1	101	4.27	0.03	0.10	0.40
Met	15.2	21.2	16.6	21.6	0.61	0.13	<0.001	0.45
Phe	48.7	51.6	49.3	50.1	1.61	0.75	0.18	0.46
Thr	109	126	120	121	4.70	0.53	0.04	0.09
Trp	26.3	28.3	27.6	29.6	1.13	0.07	0.01	0.99
Val	247	286	276	288	13.1	0.16	0.03	0.20
Σ BCAA[4]	501	578	545	576	24.5	0.36	0.03	0.31
Σ EAA	921	1,042	1,008	1,047	33.9	0.20	0.03	0.25
Ala	201	227	206	211	11.4	0.35	0.01	0.07
Asn	62.3	63.4	70.0	67.5	2.49	0.01	0.73	0.38
Cys	18.1	19.9	19.0	19.8	0.66	0.57	0.05	0.39
Glu	85.9	85.8	80.4	78.5	3.88	0.003	0.57	0.62
Gln	203	194	227	217	8.31	<0.001	0.12	0.91
Pro	89.0	86.3	95.0	86.2	3.68	0.21	0.02	0.20
Ser	113	98.1	113	94.7	6.26	0.54	<0.001	0.51
Tyr	48.7	53.9	52.5	53.2	2.77	0.35	0.09	0.18
Σ NEAA	799	802	816	805	28.0	0.60	0.83	0.69
Σ TAA[5]	1,720	1,845	1,824	1,852	51.5	0.26	0.13	0.33
Beta-alanine	4.61	4.81	4.87	4.66	0.20	0.77	0.98	0.26
Cit	74.3	72.2	77.2	74.3	5.27	0.35	0.35	0.86
Cyst	1.48	1.84	1.63	2.00	0.09	0.04	<0.001	0.94
Orn	49.9	50.9	55.7	49.9	3.02	0.22	0.23	0.09
Tau	27.2	33.1	25.4	31.0	1.94	0.25	0.002	0.95
Carnosine	20.3	20.8	20.3	22.4	1.01	0.06	0.003	0.05
1-methyl-His	3.50	3.72	3.34	3.52	0.12	0.06	0.03	0.84
3-methyl-His	4.32	4.53	5.20	4.53	0.42	0.21	0.50	0.21

[1]Experimental treatments were as follows: RCG-FB = red clover-rich silage with faba bean meal; RCG-RE = red clover-rich silage with rapeseed expeller; FBG-FB = faba bean-rich silage with faba bean meal; FBG-RE = faba bean-rich silage with rapeseed expeller.

[2]Largest SEM published in table; n = 32 (n represents the number of observations used in the statistical analysis). Data are presented as LSM.

[3]RCG vs. FBG = main effect of forage type (red clover-grass silage vs. faba bean-grass silage); FB vs. RE = main effect of protein concentrate type (faba bean meal vs. rapeseed expeller); Interaction = interaction between forage and protein concentrate type.

[4]Branched-chain AA.

[5]Total AA.

Table 7. Plasma concentration of hormones and metabolites in mid-lactation dairy cows fed forage and grain legume-based silages with faba bean meal or rapeseed expeller

	Treatment[1]					*P*-value[3]		
Item	RCG-FB	RCG-RE	FBG-FB	FBG-RE	SEM[2]	RCG vs. FBG	FB vs. RE	Interaction
Acetic acid, m*M*	1.33	1.23	1.24	1.20	0.11	0.50	0.42	0.73
Glucagon, pg/mL	180	182	184	176	9.24	0.83	0.52	0.35
Insulin, μIU/mL	25.2	25.6	25.6	20.6	2.82	0.26	0.28	0.19
Glucose, m*M*	3.76	3.67	3.81	3.73	0.065	0.28	0.07	0.94
NEFA, m*M*	0.104	0.129	0.09	0.110	0.0084	0.02	0.001	0.71
BHBA, m*M*	0.800	0.790	0.660	0.715	0.0561	0.002	0.51	0.33

[1]Experimental treatments were as follows: RCG-FB = red clover-rich silage with faba bean meal; RCG-RE = red clover-rich silage with rapeseed expeller; FBG-FB = faba bean-rich silage with faba bean meal; FBG-RE = faba bean-rich silage with rapeseed expeller.

[2]Largest SEM published in table; n = 32 for glucagon and insulin, n = 96 for glucose, nonesterified fatty acids (NEFA), and BHBA (n represents the number of observations used in the statistical analysis). Glucose, NEFA, and BHBA were analyzed with the repeated term of sampling time. Data are presented as LSM.

[3]RCG vs. FBG = main effect of forage type (red clover-grass silage vs. faba bean-grass silage); FB vs. RE = main effect of protein concentrate type (faba bean meal vs. rapeseed expeller); Interaction = interaction between forage and protein concentrate type.

Table 8. Milk fatty acid composition (g/100 g of total milk fatty acids) in mid-lactation dairy cows fed forage and grain legume-based silages with faba bean meal or rapeseed expeller

Item	Treatment[1]				SEM[2]	*P*-value[3]		
	RCG-FB	RCG-RE	FBG-FB	FBG-RE		RCG vs. FBG	FB vs. RE	Interaction
4:0	3.02	3.15	3.08	3.14	0.099	0.68	0.10	0.48
5:0	0.035	0.033	0.036	0.035	0.0028	0.19	0.19	0.65
6:0	2.16	2.20	2.20	2.20	0.060	0.50	0.44	0.36
7:0	0.038	0.038	0.043	0.035	0.0038	0.52	0.07	0.07
8:0	1.42	1.44	1.46	1.45	0.028	0.16	0.75	0.51
9:0	0.049	0.049	0.058	0.049	0.0056	0.03	0.03	0.03
10:0	3.65	3.53	3.87	3.63	0.081	0.02	0.008	0.38
11:0	0.464	0.416	0.485	0.426	0.019	0.11	<0.001	0.55
12:0	4.57	4.13	4.95	4.34	0.121	0.002	<0.001	0.30
13:0	0.161	0.159	0.194	0.160	0.0177	0.14	0.11	0.17
anteiso 13:0	0.114	0.093	0.128	0.101	0.0048	0.001	<0.001	0.39
iso 13:0	0.019	0.015	0.021	0.019	0.0018	0.06	0.06	0.68
14:0	13.6	13.0	14.1	13.5	0.27	0.001	<0.001	0.68
iso 14:0	0.081	0.076	0.098	0.094	0.0068	<0.001	0.29	0.88
cis-9 14:1	1.20	0.990	1.22	1.04	0.069	0.19	<0.001	0.58
15:0	1.36	1.20	1.45	1.28	0.085	0.002	<0.001	0.86
anteiso 15:0	0.388	0.380	0.391	0.389	0.0129	0.44	0.54	0.76
iso 15:0	0.173	0.173	0.221	0.215	0.0068	<0.001	0.52	0.52
16:0	37.3	29.9	37.7	30.1	0.67	0.25	<0.001	0.80
iso 16:0	0.216	0.190	0.231	0.234	0.0180	0.08	0.46	0.38
trans-6 + 8 16:1	0.025	0.029	0.024	0.029	0.0021	0.68	0.01	0.68
trans-9 16:1 + *iso* 17:0	0.219	0.224	0.211	0.220	0.0207	0.79	0.74	0.93
trans-10 16:1	0.009	0.013	0.038	0.046	0.022	0.18	0.78	0.91
trans-11 16:1	0.015	0.024	0.009	0.024	0.0017	0.03	<0.001	0.03
trans-12 16:1	0.115	0.111	0.109	0.113	0.0056	0.29	1.0	0.12
trans-13 16:1	0.013	0.020	0.013	0.020	0.0016	1.0	<0.001	1.0
17:0	0.525	0.476	0.515	0.465	0.0170	0.13	<0.001	0.93
cis-9 17:1	0.160	0.133	0.158	0.131	0.0052	0.50	<0.001	0.82
trans-9 16:1+ *iso* 17:0	0.219	0.224	0.211	0.220	0.0207	0.79	0.74	0.93
anteiso 17:0 + *cis*-9 16:1	1.65	1.24	1.64	1.30	0.051	0.64	<0.001	0.40
18:0	6.74	10.5	6.41	10.2	0.241	0.17	<0.001	0.81
iso 18:0	0.028	0.024	0.021	0.025	0.0030	0.42	1.0	0.23
cis-9 18:1 + *trans*-13–15	13.8	17.8	13.1	17.6	0.44	0.07	<0.001	0.29
cis-11 18:1	0.370	0.476	0.364	0.475	0.0305	0.70	<0.001	0.87
cis-12 18:1	0.154	0.304	0.189	0.331	0.0186	0.05	<0.001	0.80
cis-13 18:1	0.045	0.056	0.048	0.090	0.0172	0.28	0.12	0.35
cis-15 18:1	0.034	0.066	0.025	0.061	0.0127	0.58	0.01	0.88
trans-9 *cis*-18:1	0.090	0.173	0.084	0.175	0.0064	0.43	<0.001	0.17
trans-4 18:1	0.009	0.024	0.008	0.024	0.0018	0.73	<0.001	0.73
trans-5 18:1	0.008	0.016	0.006	0.018	0.0017	1.0	<0.001	0.44
trans-6–8 18:1	0.140	0.288	0.138	0.270	0.0088	0.10	<0.001	0.21
trans-9 18:1	0.090	0.173	0.084	0.175	0.0064	0.43	<0.001	0.17
trans-10 18:1	0.106	0.219	0.118	0.225	0.0111	0.10	<0.001	0.80
trans-11 18:1	0.436	0.695	0.496	0.766	0.0387	0.01	<0.001	0.82
trans-12 18:1	0.178	0.353	0.185	0.345	0.0149	1.0	<0.001	0.58
trans-16 + *cis*-14 18:1	0.194	0.348	0.218	0.406	0.0185	0.03	<0.001	0.31
18:2n-6	1.51	1.47	1.36	1.26	0.059	<0.001	0.02	0.25
cis-9,*trans*-11 18:2 (CLA)	0.268	0.366	0.251	0.346	0.0210	0.12	<0.001	0.87
trans-11,*cis*-15 18:2	0.104	0.111	0.125	0.135	0.0162	0.02	0.33	0.89
18:3n-3	0.719	0.769	0.396	0.403	0.0250	<0.001	0.05	0.11
18:3n-6	0.024	0.013	0.038	0.016	0.0087	0.33	0.08	0.57
19:0	0.095	0.135	0.070	0.176	0.0200	0.69	0.002	0.12
20:0	0.136	0.179	0.124	0.194	0.0141	0.93	0.001	0.33
3S,7R,11R,15-tetramethyl-16:0	0.018	0.049	0.040	0.041	0.0140	0.61	0.28	0.31
3R,7R,11R,15-tetramethyl-16:0	0.030	0.021	0.033	0.025	0.0021	0.06	<0.001	0.68
cis-9 20:1	0.068	0.098	0.084	0.088	0.0135	0.81	0.21	0.33
cis-11 20:1	0.016	0.035	0.019	0.039	0.0049	0.80	0.008	0.45
20:2n-6	0.020	0.016	0.020	0.023	0.0036	0.40	0.87	0.40
20:3n-6	0.083	0.083	0.083	0.080	0.0068	0.76	0.76	0.76
20:3n-3	0.005	0.006	0.010	0.006	0.0022	0.24	0.55	0.24
20:4n-6	0.105	0.080	0.109	0.091	0.0075	0.18	0.001	0.49
20:5n-3	0.040	0.025	0.019	0.036	0.0099	0.62	0.90	0.12
21:0	0.009	0.011	0.009	0.013	0.0023	0.77	0.15	0.77

Continued

Table 8 (Continued). Milk fatty acid composition (g/100 g of total milk fatty acids) in mid-lactation dairy cows fed forage and grain legume-based silages with faba bean meal or rapeseed expeller

Item	Treatment[1]				SEM[2]	*P*-value[3]		
	RCG-FB	RCG-RE	FBG-FB	FBG-RE		RCG vs. FBG	FB vs. RE	Interaction
22:0	0.058	0.070	0.061	0.068	0.0025	0.80	0.001	0.21
cis-13 22:1	0.006	0.008	0.009	0.010	0.0026	0.33	0.62	1.00
22:5n-3	0.058	0.058	0.066	0.065	0.0037	0.02	0.85	0.85
24:0	0.048	0.050	0.065	0.070	0.0057	0.002	0.47	0.81
cis-15 24:1	0.011	0.006	0.013	0.005	0.0047	1.0	0.21	0.80
26:0	0.018	0.021	0.020	0.016	0.0031	0.64	1.0	0.18
Σ de novo[4]	29.5	28.3	30.8	29.4	0.411	0.002	<0.001	0.55
Σ SFA	78.1	72.7	79.6	73.8	0.66	<0.001	<0.001	0.54
Σ MUFA	17.7	22.9	16.8	22.5	0.53	0.04	<0.001	0.34
Σ PUFA	2.96	3.06	2.44	2.41	0.120	<0.001	0.42	0.10
Σ unidentified[5]	1.25	1.36	1.12	1.22	0.056	0.006	0.02	0.99

[1]Experimental treatments were as follows: RCG-FB = red clover-rich silage with faba bean meal; RCG-RE = red clover-rich silage with rapeseed expeller; FBG-FB = faba bean-rich silage with faba bean meal; FBG-RE = faba bean-rich silage with rapeseed expeller.

[2]Largest SEM published in table; n = 32 (n represents the number of observations used in the statistical analysis). Data are presented as LSM.

[3]RCG vs. FBG = main effect of forage type (red clover-grass silage vs. faba bean-grass silage); FB vs. RE = main effect of protein concentrate type (faba bean meal vs. rapeseed expeller); Interaction = interaction between forage and protein concentrate type.

[4]Sum of de novo fatty acids (≤C14).

[5]Sum of unidentified fatty acids.

in the processing methods (i.e., no additional heat in the processing of cold-pressed expeller).

Overall, the differences in the chemical composition of the individual feed ingredients were also reflected in the chemical and nutritional compositions of the experimental diets. Most notably, the 2 diets containing RCG had lower CP contents compared with FBG-containing diets (16.3 and 16.0 vs. 18.1 and 17.9%, respectively), which should be kept in mind when interpreting the results from this experiment. The greater CP in the FBG-containing diets led to similar estimated MP and dAA supplies from the experimental diets despite greater rumen degradability compared with RCG-containing diets. As expected, diets containing FB had greater starch contents compared with diets containing RE, whereas the opposite was true for total fat content.

Nutrient Intake and Apparent Total-Tract Digestibility of Nutrients

Overall, the DMI of the cows in the current experiment was relatively high regardless of dietary treatment, reflecting high intake potential of legume and whole-crop silages, especially when fed mixed with grass silage (Huhtanen et al., 2007). The greater NDF content of both whole-crop faba bean and grass silages compared with the RCG silage, which could have limited DMI, was most likely compensated by the fact that whole-crop silages and legume silages (i.e., faba bean silage) have greater intake capacity compared with grass silages with similar digestibility (Huhtanen et al., 2007; Johansen et al., 2018). To our knowledge no published data has compared the nutrient intake and lactational performance of dairy cows receiving whole-crop faba bean silage or RCG silage.

Only a few experiments have examined whole-crop faba bean silage compared with grass silage (McKnight and MacLeod, 1977; Ingalls et al., 1979; Palmio et al., 2022), or corn silage (Guevara-Oquendo et al., 2022) in lactating dairy cow diets, and none of these experiments reported difference in DMI between the 2 diets. Partial replacement of grass silage with red clover silage in the diets of lactating dairy cows has increased DMI compared with pure grass or pure red clover silage (Dewhurst et al., 2003; Kuoppala et al., 2009; Halmemies-Beauchet-Filleau et al., 2014). In light of the current and previous data, both RCG and FBG in the current experiment were highly palatable. This was most likely driven by the good fermentation quality, relatively high DM content for legume-containing silages, and high sugar concentration as well as inclusion of several plant species in the silages. The high DMI across dietary treatments in the current experiment may have led to the overall low total-tract digestibility values, but likely did not affect the differences, or lack thereof, between treatments.

The intake of nutrients, including CP, total fat, NDF, and starch, from the experimental diets was reflective of the differences in the nutrient compositions of the diets. However, the apparent total-tract digestibility of all nutrients did not differ between the diets, except for a lower CP digestibility of RCG diets compared with FBG diets and a slightly lower starch digestibility of FB compared with RE. The greater digestibility of CP in the faba bean silage compared with the RCG silage can be expected due to the generally lower digestibility of CP in red clover, reported previously (Halmemies-Beauchet-Filleau et al., 2014; Lee et al., 2019a). Starch in the diets containing FB originated partly from the protein feed, whereas barley and oats mixture was the main source of starch in the diets containing RE. Importantly, starch digestibility of ground faba bean has been reported to be lower than that of ground cereals such as barley or oat (Larsen et al., 2009). The combined effects of greater starch intake from diets containing FB and the generally lower starch digestibility of faba bean compared with barley and oats likely contributed to the marginally lower starch digestibility of FB compared with RE.

The differences in intake of total AA between the 2 forage types were due to the greater CP intake from FBG diets. However, the estimated dAA intakes did not differ as drastically, due to the greater RDP portion in faba bean silage compared with RCG silage. Similarly, intakes of AA from FB versus RE were reflective of the differences in the AA compositions of these 2 protein concentrates, most notable being the lower Met intake from FB. The differences in AA supply between the treatments were reflected in the plasma AA concentrations and may partly explain the observed differences in lactational performance, as discussed further below.

Similarly, the intake of total FA and individual FA corresponded with the differences in FA content of the experimental feed ingredients. Forage species resulted in small but statistically significant differences in FA intake. All individual FA intakes were greater for cows fed diets containing RE, most notable being the greater intakes of *cis*-9 18:1 (255 vs. 66.5 g/d) and *cis*-11 18:1 (20.7 vs. 2.04 g/d), respectively.

Lactational Performance

The 1.1 kg/d greater MY and improved feed efficiency of cows fed RCG diets compared with FBG diets may have been a result of a greater NDF content of FBG-based diets. However, ECM yield was not affected by forage type. In previous experiments, where grass silage has been partially replaced with red clover silage, MY and ECM yield were increased (Dewhurst et al., 2003; Halmemies-Beauchet-Filleau et al., 2014) or similar (Vanhatalo et al., 2009) between a red clover-grass and grass silage. Interestingly, MY was similar but ECM yield 1.0 kg/d greater for cows receiving a diet containing grass silage and rapeseed meal compared with cows fed a whole-crop faba bean silage (Palmio et al., 2022). In contrast, replacing corn silage and barley with whole-crop faba bean silage increased both MY and ECM yield in high-producing cows (Guevara-Oquendo et al., 2022). The greater milk protein concentration of cows fed FBG in the current experiment was mostly a result of the greater MUN concentration. Hence, much of the dietary CP was degraded in the rumen, converted to urea N in the liver, and excreted as excess N in the milk and urine (Nousiainen et al., 2004). This was also evident in the tendency for greater milk protein yield (1.30 vs. 1.26 kg/d) for FBG compared with RCG, but a lack of difference (1.26 vs. 1.24 kg/d) in the calculated milk TP yield, in which the N contribution from MUN is subtracted.

An even greater difference of 2.2 kg/d in MY and 1.6 kg/d greater ECM yield was observed for cows receiving RE versus FB, which is not surprising, due to the greater dAA and energy supply from RE. The more optimal AA profile of RE was also reflected in the greater milk protein yield and lower MUN concentration compared with FB, indicating more efficient utilization of dietary N for milk and milk protein synthesis. Similar to our data, Puhakka et al. (2016) reported greater MY, ECM, and milk protein yields for cows on a grass silage-based diet receiving rapeseed meal compared with cows fed faba bean as the protein concentrate. They attributed these results to the greater supply of RUP and dAA from rapeseed meal, as well as suggesting that Met supply may limit milk protein synthesis in diets containing faba bean (Puhakka et al., 2016), in accordance with the current experiment. Interestingly, in a recent experiment no difference was found in MY or ECM yield or MUN concentration when rapeseed meal or faba bean was fed to lactating cows, but milk protein yield was 43 g/d greater for cows receiving rapeseed meal (Kuoppala et al., 2021). Further, in another experiment with corn silage and grass-clover silage-based diet replacing rapeseed meal (64%) + wheat (36%) with faba bean increasing MY and ECM yield by 0.9 and 0.8 kg/d, respectively, but, expectedly, decreased milk protein yield and increased MUN concentration (Hansen et al., 2021).

Nitrogen Utilization and Purine Derivative Excretion

First, it is noted that the frequency of urine spot sampling (4 time points) was not optimal in the current experiment and may have underestimated the total urine output, as diurnal variation in urine volume may affect urinary creatinine concentration (Lee et al., 2019b). This may have affected the absolute values of estimated urinary excretion of nitrogenous compounds. However, comparisons between treatments within the current experiment are still meaningful (Lee et al., 2019b).

Reflective of the greater CP content of FBG diets, N intake of the cows fed these diets was greater. As expected from the greater CP intake and RDP fraction of FBG diets compared with RCG diets, both urinary N and UUN concentrations were greater. This also translated into lower utilization of dietary N for milk N of the cows receiving FBG diets compared with RCG diets, and lack of difference in N efficiency between the 2 protein concentrates in FBG-based diets. Because the forage inclusion rates were not balanced for N content, the comparisons between FBG and RCG diets in terms of N efficiency may be partly confounded. Legume silages in general result in lower N efficiency compared with grass silages (Dewhurst, 2013), but differences exist between legume types. A greater N efficiency of 21.0% was reported for red clover silage compared with both white clover (20.5%) and alfalfa silage (18.2%) when fed to lactating cows, whereas a red clover-grass silage and a white clover-grass silage resulted in even greater N efficiency compared with pure legume silages (i.e., 24.5 and 22.2%, respectively; Dewhurst et al., 2003). Similarly, Broderick (2018) reported greater N efficiency for lactating cows receiving a diet containing red clover silage (28.5%) compared with alfalfa silage (26.9%). It is worth noting that RCG diets had a greater proportion of dietary N excreted in feces in the current experiment. This is in agreement with previous experiments (Vanhatalo et al., 2009; Moorby et al., 2009; Halmemies-Beauchet-Filleau et al., 2014), and can be attributed to the lower digestibility of N in red clover silage, due to the high polyphenol oxidase content. The greater N efficiency, combined with the greater proportion of N being excreted in feces instead of urine, results in lower N emissions from cows receiving red clover in their diets (Lee et al., 2019a). Combined with the absence of N fertilizer input in the sward, utilization of mixed red clover-grass swards has great potential in decreasing N emissions in dairy systems.

The estimated microbial N flow was greater for FBG than for RCG was most likely due to the above-discussed greater RDP fractions and starch contents of FBG diets. Indeed, greater supplies of RDP and readily fermentable energy increase microbial protein synthesis in the rumen (Clark et al., 1992). In contrast and unexpectedly, FB had a lower estimated microbial N flow, even though the supply of available N (i.e., RDP), AA, and starch for rumen microbes were expected to be greater for FB compared with RE. This discrepancy might have partly been a result of greater milk protein yield of cows receiving RE diet, and as a result the estimated PD secretion into milk was also greater. Hence, the estimated microbial N thereby would seem to be greater for cows fed RE versus FB. Further, the AA profile of RDP in RE may have been more optimal for rumen microbial protein synthesis. Indeed, certain AA have been shown to stimulate microbial growth in vitro, including the BCAA and Trp, Tyr, Glu, and Met (Kajikawa et al., 2002), all of which were supplied at greater rates from RE compared with FB.

Plasma Amino Acid, Hormone, and Metabolite Concentrations

Plasma AA concentrations are a useful indicator of dAA supply and possible deficiencies in the diet (Broderick et al., 1974). In the current experiment, the forage type did not affect the overall EAA or TAA concentrations, but some differences were detectable in the plasma concentrations of individual AA, most notably lower His and Lys concentrations for RCG versus FBG silage. However, these differences did not affect the overall performance of the cows between forage types, and most likely the overall dAA supply was at or above requirements for both experimental forages. Interestingly, plasma concentration of 1-methyl-His tended to be greater for RCG- and RE-containing diets, both of which also resulted in greater MY and mobilization of adipose tissue, as indicated by increased NEFA concentration. This His-containing metabolite is hydrolyzed from anserine in the muscle tissue, but the exact mechanism and regulation of the release of 1-methyl-His in cattle is currently unknown (Houweling et al., 2012; Lage et al., 2021). For the 2 protein concentrates the plasma concentrations of BCAA and EAA were lower for FB compared with RE. This is to be expected, as the RUP fraction is lower for FB than for RE. Most notably of the individual AA, Met concentration was 26% lower for FB compared with RE and may have been the limiting factor for milk protein synthesis for diets containing FB in the current experiment (Puhakka et al., 2016).

The greater plasma concentrations of NEFA and BHBA for RCG diets point to a greater mobilization of adipose tissue (Grummer, 1995), also supported by the BCS change data, although BCS data in a crossover experiment should be interpreted with caution. The greater plasma NEFA and BHBA concentrations might have been a combined effect of lower starch intake in combination with a greater milk production of cows fed RCG diet. Moorby et al. (2009) observed an increased mobilization of body energy reserves in cows receiving a red clover-grass silage compared with cows fed grass silage. The mechanism behind this remains unclear but was said to lead to a greater MY per kilogram of digested OM (Moorby et al., 2009). The greater plasma NEFA and tendency for lower glucose concentration of cows fed RE-containing diets might similarly be an indication of a lower supply of starch from this diet, and a mobilization of some adipose tissue to support the greater MY of cows receiving the diets containing RE. Insulin concentration, however, was not different between the treatments, and therefore, based on insulin and NEFA concentrations, cows in the post-peak stage of lactation were likely not in a severe negative energy balance.

Milk Fatty Acid Composition

Milk FA composition of cows fed the RCG diet in the current experiment agrees with previous literature with red clover silages (Moorby et al., 2009; Halmemies-Beauchet-Filleau et al., 2014; Jaakamo et al., 2019). Despite small or opposite changes in PUFA intake, a much greater increase in the concentration of 18:2n-6 and 18:3n-3 in milk fat is commonly observed in red clover-based silages; Halmemies-Beauchet-Filleau et al. (2014) reported a linear increase in the concentrations of 18:2n-6 and 18:3n-3 as they increased the proportion of red clover silage from 0 to 100% of forage DM. This response has been attributed to lower ruminal biohydrogenation of red clover lipids and consequent increase in post-ruminal supply of PUFA with red clover silage-based diets (Halmemies-Beauchet-Filleau et al., 2013; Jaakamo et al., 2019). To the best of our knowledge this is the first data reporting the FA composition in milk of cows fed a whole-crop faba bean silage. Overall, many of the differences between the RCG and FBG diets in the current experiment discussed here were similar to experiments comparing red clover silage-based diets with grass silage-based diets (Moorby et al., 2009; Halmemies-Beauchet-Filleau et al., 2014; Jaakamo et al., 2019). The high level of WSC in both forage types in the present study may have increased butyrate formation in the rumen (Huhtanen et al., 2013) and thereby enhanced the de novo FA synthesis in the mammary gland, resulting in relatively high SFA in milk regardless of the forage type used in the diet.

The differences in milk FA composition of RE and FB were reflective of the differences in the fat contents of the 2 protein concentrates and the subsequent differences in FA intake. Fat-rich RE would supply the mammary gland with more unsaturated FA relative to FB, most notably *cis*-9 18:1 and its ruminal metabolites (Shingfield et al., 2010). Similar changes in milk FA profile have been observed in experiments where canola oil has been included in dairy cow diets (Halmemies-Beauchet-Filleau et al., 2011; Lage et al., 2021). Faba bean is low in fat, and thus most FA would originate from de novo synthesis at the mammary gland, reflected also in the greater concentration of SFA and de novo FA for FB than for RE.

CONCLUSIONS

In this experiment, both legume forages supported high DMI and production levels. Greater milk yield but a lower CP intake resulted in a greater N efficiency of cows fed diets with red clover-rich silage than with whole-crop faba bean-rich silage. Lower urinary N excretion with greater N efficiency of cows receiving red-clover-grass silage cultivated without N fertilizer inputs, demonstrate the potential for decreased animal level N emissions with dietary inclusion of red clover. As expected, rapeseed expeller resulted in greater energy-corrected milk yield and milk protein yield, and greater N efficiency compared with faba bean meal. Further, plasma Met concentrations were lower when legume-based forages were combined with faba bean, a legume protein concentrate. Overall, whole-crop faba bean silage and faba bean meal can be suitable ingredients in dairy cow rations, but more research is needed to improve N efficiency and optimal inclusion rate and combinations in different dietary situations.

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