Effect of replacing calcium salts of palm oil with camelina seed at 2 dietary ether extract levels on digestion, ruminal fermentation, and nutrient flow in a dual-flow continuous culture system

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ABSTRACT

Camelina is a drought- and salt-tolerant oil seed, which in total ether extract (EE) contains up to 74% polyunsaturated fatty acids. The objective of this study was to assess the effects of replacing calcium salts of palm oil (Megalac, Church & Dwight Co. Inc., Princeton, NJ) with camelina seed (CS) on ruminal fermentation, digestion, and flows of fatty acids (FA) and AA in a dual-flow continuous culture system when supplemented at 5 or 8% dietary EE. Diets were randomly assigned to 8 fermentors in a 2×2 factorial arrangement of treatments in a replicated 4×4 Latin square design, with four 10-d experimental periods consisting of 7 d for diet adaptation and 3 d for sample collection. Treatments were (1) calcium salts of palm oil supplementation at 5% EE (MEG5); (2) calcium salts of palm oil supplementation at 8% EE (MEG8); (3) 7.7% CS supplementation at 5% EE (CS5); and (4) 17.7% CS supplementation at 8% EE (CS8). Diets contained 55% orchardgrass hay, and fermentors were fed 72 g of dry matter/d. On d 8, 9, and 10 of each period, digesta effluent samples were taken for ruminal NH_3 , volatile fatty acids, nitrogen metabolism analysis, and long-chain FA and AA flows. Statistical analysis was performed using the MIXED procedure (SAS Institute Inc., Cary, NC). We detected an interaction between FA source and dietary EE level for acetate, where MEG8 had the greatest molar proportion of acetate. Molar proportions of propionate were greater and total volatile fatty acids were lower on CS diets. Supplementation of CS decreased overall ruminal nutrient true digestibility, but dietary EE level did not affect it. Diets containing CS had greater biohydroge-

nation of 18:2 and 18:3; however, biohydrogenation of 18:1 was greater in MEG diets. Additionally, CS diets had greater ruminal concentrations of *trans*-10/11 18:1 and *cis*-9,*trans*-11 conjugated linoleic acid. Dietary EE level at 8% negatively affected flows of NH_3 -N (g/d), nonammonia N, and bacterial N as well as the overall AA outflow. However, treatments had minor effects on individual ruminal AA digestibility. The shift from acetate to propionate observed on diets containing CS may be advantageous from an energetic standpoint. Moreover, CS diets had greater ruminal outflow of *trans*-10/11 18:1 and *cis*-9,*trans*-11 conjugated linoleic acid than MEG diets, suggesting a better FA profile available for postruminal absorption. However, dietary EE at 8% was deleterious to overall N metabolism and AA outflow, indicating that CS can be fed at 5% EE without compromising N metabolism.

Key words: amino acid, biohydrogenation, fatty acid, in vitro fermentation

INTRODUCTION

Camelina sativa is an oil seed crop from the mustard family (Brassicaceae), which is salt- and drought-tolerant. It is adapted to a variety of climate and soil conditions (Zubr, 1997), including dry regions such as the western United States (Keske et al., 2013). Camelina seed (CS) contains, in total ether extract (EE), up to 74% PUFA, of which 46% is linolenic acid (Hurtaud and Peyraud, 2007). Another feature of CS is its high protein content and good AA profile (Zubr, 2003), primarily Arg (Miller et al., 1962; Zubr, 2003). Therefore, feeding CS may be advantageous because of its energy content, as well as its FA and AA profiles. However, CS contains antinutritional factors such as glucosinolates and erucic acid (Kramer et al., 1990; Mawson et al., 1994) that may affect digestion.

Although UFA (notably PUFA, when expressed in % of total FA) are predominant in commonly used rumi-

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nant feedstuffs, milk and milk products are relatively low in PUFA content. This is due to extensive biohydrogenation (**BH**) by the ruminal microbial population (Morimoto et al., 2005). Therefore, extent as well as type of BH determine the quantity and structures of FA leaving the rumen (Fievez et al., 2007) and ultimately found in milk. However, it is possible to increase PUFA concentration in milk through dietary PUFA supplementation (Theurer et al., 2009; Moate et al., 2013) using oil seeds such as CS. Yet diets excessively high in UFA may have deleterious effects on nutrient digestibility, microbial population, and digestion. Typically, UFA have greater negative effects on ruminal fermentation than calcium salts of palm oil, due to the calcium salts of palm oil partial protection from ruminal fermentation.

Thus, CS may be a promising source of UFA and capable of providing supplementary EAA as well. However, to our knowledge, the literature lacks reports on the effects of CS supplementation on ruminal fermentation as well as its effects on ruminal FA and AA outflows. The objective of this study was to assess the effects of replacing calcium salts of palm oil with CS on ruminal fermentation, digestion, and flows of FA and AA in a dual-flow continuous culture system when supplemented at 5 or 8% dietary EE. Therefore, our hypotheses were that (1) when replacing calcium salts of palm oil at a level of 5% dietary EE, CS would not negatively affect ruminal fermentation; and (2) CS and calcium salts of palm oil would have different ruminal fermentation patterns.

MATERIALS AND METHODS

Animal care and handling were approved by the University of Nevada–Reno Institutional Animal Care and Use Committee (IACUC protocol # 00588).

Experimental Design and Diets

This study was conducted in a replicated 4×4 Latin square design with four 10-d experimental periods, consisting of 7 d of diet adaptation followed by 3 d of sampling. Each fermentor unit was randomly assigned within Latin square to receive each diet once over the 4 periods. Treatments were arranged in a factorial 2×2 , where factor A consisted of CS as supplement versus calcium salts of palm oil supplement, and factor B consisted of 2 dietary EE levels (5 vs. 8%). Therefore, the treatments were (1) calcium salts of palm oil (Megalac, Church & Dwight Co. Inc., Princeton, NJ) supplementation at 5% EE (**MEG5**); (2) calcium salts of palm oil supplementation at 8% EE (**MEG8**); (3) 7.7% CS

supplementation at 5% EE (**CS5**); and (4) 17.7% CS supplementation at 8% EE (**CS8**).

Fermentors were manually fed 72 g of DM/d, equally distributed twice daily at 0800 and 2000 h. Experimental diets were fed on a DM basis and were formulated to meet or exceed NRC recommendations (NRC, 2001) for a Holstein dairy cow, with 660 kg of BW and producing 35 kg of milk/d, with 3.5% fat and 3.2% protein. Diets were formulated to have 16% CP and approximately 35% NDF (Table 1). Diets consisted of orchardgrass hay, ground corn, canola meal, and either ground CS or calcium salts of palm oil fatty acids. Treatments CS5 and CS8 contained 83.4 and 85.4% UFA, respectively, and MEG5 and MEG8 contained 42.4 and 47.7% SFA, respectively (Table 2).

Camelina seed, genotype Calena, was used in this experiment and contained 35.5% EE and 88.5% UFA; calcium salts of palm oil fatty acids contained 53% SFA. In addition, CS contained 29.4% CP, 19.8% NDF, and 9.4% ADF. All dietary ingredients were ground through a 2-mm screen in a Wiley mill (model #2, Arthur H. Thomas Co., Philadelphia, PA) and orchardgrass hay was pelleted. Dietary AA composition is presented in Table 3.

Dual-Flow Continuous Culture System

Diets were randomly assigned to 8 dual-flow continuous culture fermentors, with volume ranging from 1,200 to 1,250 mL (Omni-Culture Plus; Virtis Co. Inc., Gardiner, NY) similar to that originally described by Hoover et al. (1976), and recently modified by Benedetti et al. (2015), Silva et al. (2016), and Paula et al. (2017). Briefly, this system consists of a glass fermentation vessel, in which rumen fluid from donor animals is maintained at constant temperature and agitation. It has a dual-effluent removal system consisting of separate liquid and solid flows. Artificial saliva is continuously infused and feed is provided through an orifice located in the fermentation vessel lid. Fermentor contents are continuously stirred by a central propeller apparatus driven by magnets at the rate of 155 rpm, and N_2 is infused to maintain an anaerobic environment.

Ruminal fluid from 2 rumen-cannulated steers (average BW: 910.5 ± 34.5 kg) was collected approximately 2 h after morning feeding. Donor steers were fed (DM basis) the same forage:concentrate ratio established for the experimental diets, containing 55% alfalfa hay, 45% concentrate, and ad libitum mineral mixture. The ruminal fluid was manually collected from the ventral, central, and dorsal areas of the rumen of the donor steers and strained through 4 layers of cheesecloth. Approximately 10 L of ruminal fluid was poured into a warmed, insulated bottle.

Rumen fluid from both steers was homogenized and then infused with N₂ to maintain an anaerobic environment, and the temperature was adjusted to 39°C by submerging a 5-L Erlenmeyer flask in a heated water bath. The rumen fluid was poured into each of the warmed fermentors until it cleared the effluent spout, and N₂ gas was continuously infused at a rate of 40 mL/min. Artificial saliva (Weller and Pilgrim, 1974) was continuously infused at 2.2 mL/min. Liquid and solid dilution rates were adjusted daily to 11.0 and 5.5%/h; respectively, by adjusting buffer input and liquid and solid removal. Individual pH controllers (model 5997-20, Cole-Parmer, Vernon Hills, IL) were used to monitor the pH of each fermentor.

Experimental Procedures and Sample Collections

On d 5, effluents (liquid and solid) were homogenized and samples were collected to determine the background ¹⁵N abundance (Calsamiglia et al., 1996). Subsequently, 0.077 g of 10.2% excess of (¹⁵NH₄)₂SO₄ (Sigma-Aldrich Co., St. Louis, MO) was infused into each fermentor to instantaneously label the NH₃-N pool. Saliva was reformulated and 0.077 g/L of enriched (¹⁵NH₄)₂SO₄

was infused in replacement of isonitrogenous quantities of urea to maintain a steady-state concentration of ¹⁵N enrichment inside the fermentors (Calsamiglia et al., 1996).

Liquid and solid effluents were collected separately in 4.3-L plastic containers. During the first 7 d of the adaptation period, the effluent containers were weighed once daily at 0800 h and their contents subsequently discarded. Twenty-four hours before the first collection and on d 7, 8, 9, and 10 of each period, liquid and solid effluent containers were immersed half way in a chilled water bath at 1°C to stop microbial activity.

On d 7, 8, and 9, pH was measured using an Accumet portable AP61 pH meter (Fisher Scientific, Atlanta, GA) at 7 time points: 0, 1, 2, 4, 6, 8, and 10 h after feeding. On d 8, 9, and 10, liquid and solid effluents from each fermentor were taken and mechanically homogenized for 1 min (T25 basics, IKA Works Inc., Wilmington, NC), and 500 mL was removed via vacuum system and stored at -20°C for further analysis of DM, OM, CP, NDF, ADF, EE, and ash. An additional 2 subsamples were filtered through 4 layers of cheesecloth for analysis of ruminal ammonia nitrogen (NH₃-N) and VFA. The subsamples used for NH₃-N analysis were

Table 1. Ingredient and chemical composition (% of DM, unless otherwise stated) of the experimental diets

Item	Diet ¹			
	MEG5	MEG8	CS5	CS8
Ingredient				
Orchardgrass hay	55.0	55.0	55.0	55.0
Ground corn	17.5	12.4	19.2	16.3
Canola meal	22.9	23.9	16.8	9.8
Camelina seed	—	—	7.7	17.7
Calcium salts of palm oil fatty acids ²	3.25	7.48	—	—
Minerals ³	1.25	1.24	1.25	1.25
Chemical composition				
DM, %	91.9	92.4	91.8	92.3
OM	92.3	91.4	93.0	93.1
CP	16.0	16.0	16.0	16.0
NDF	35.5	35.3	35.4	35.2
ADF	19.5	19.6	19.0	18.6
NFC ⁴	37.1	33.1	37.9	34.9
Ether extract	5.00	8.20	5.00	8.20
Ca ⁵	0.20	0.20	0.20	0.20
P ⁵	0.10	0.10	0.10	0.10
Glucosinolates, mg/g	0.34	0.36	1.18	2.27
NE _L , ⁶ Mcal/kg of DM	1.60	1.80	1.60	1.70
N intake, g/d	1.96	1.96	1.96	1.96

¹MEG5 = calcium salts of palm oil fatty acids at 5% dietary ether extract (EE); MEG8 = calcium salts of palm oil fatty acids at 8% dietary EE; CS5 = 7.7% camelina seed supplementation at 5% dietary EE; CS8 = 17.7% camelina seed supplementation at 8% dietary EE.

²Megalac (Church & Dwight Co. Inc., Princeton, NJ).

³Provided (per kg of DM): 955 g of NaCl, 3,500 mg of Zn, 2,000 mg of Fe, 1,800 mg of Mn, 280 mg of Cu, 100 mg of I, and 60 mg of Co.

⁴Estimated according to NRC (2001), using the following equation: NFC = 100 - (% NDF + % CP + % fat + % ash).

⁵Estimated according to NRC (2001).

⁶Estimated using the NRC (2001) model.

Table 2. Fatty acid (FA) composition of the experimental diets

FA, % of total	Diet ¹			
	MEG5	MEG8	CS5	CS8
14:0	0.88	1.02	0.19	0.14
15:0	0.11	0.08	0.09	0.07
16:0	36.3	41.5	10.6	8.70
16:1n-7	0.30	0.24	0.26	0.20
17:0	0.15	0.13	0.11	0.09
18:0	3.54	3.96	2.50	2.63
18:1n-9	31.7	34.3	17.7	16.6
18:1n-7	1.84	1.28	1.98	1.45
18:2n-6	16.4	12.1	25.8	24.2
18:3n-3	6.48	3.69	24.4	26.5
20:0	0.60	0.49	1.57	1.73
20:1n-9	0.21	0.18	9.03	11.4
21:0	0.04	0.03	0.03	0.02
22:0	0.30	0.19	0.53	0.48
22:1n-9	0.01	ND ²	2.02	2.58
23:0	0.26	0.15	0.27	0.17
24:0	0.30	0.18	0.41	0.33
24:1n-9	0.03	0.02	0.50	0.62
Unknown	0.64	0.36	2.01	2.10
SFA	42.4	47.7	16.3	14.4
UFA	57.2	52.1	83.4	85.4
MUFA	34.1	36.1	31.5	32.9
PUFA	23.2	16.1	51.9	52.6

¹MEG5 = calcium salts of palm oil fatty acids at 5% dietary ether extract (EE); MEG8 = calcium salts of palm oil fatty acids at 8% dietary EE; CS5 = 7.7% camelina seed supplementation at 5% dietary EE; CS8 = 17.7% camelina seed supplementation at 8% dietary EE.

²Not detected.

Table 3. Amino acid composition of the experimental diets

AA, % of total	Diet ¹			
	MEG5	MEG8	CS5	CS8
Taurine	1.16	1.17	1.18	1.09
Hydroxyproline	0.49	0.42	0.54	0.55
Asp	8.49	7.73	8.84	8.51
Thr	4.31	3.88	4.42	4.36
Ser	3.84	3.78	4.01	3.98
Glu	14.1	14.5	14.6	15.1
Pro	6.50	6.58	6.63	6.70
Gly	5.21	4.85	5.39	5.30
Ala	6.34	6.26	6.55	6.34
Cys	1.69	1.64	1.74	1.84
Val	5.66	5.14	5.84	5.74
Met	1.96	1.91	2.01	2.02
Ile	4.44	4.02	4.56	4.49
Leu	8.88	9.08	9.17	9.07
Tyr	2.56	2.46	2.67	2.64
Phe	5.21	4.99	5.39	5.23
Hydroxylysine	0.99	0.79	0.98	0.89
Orn	0.07	0.05	0.08	0.07
Lys	5.33	4.53	5.42	5.37
His	2.31	2.25	2.37	2.43
Arg	4.99	4.63	5.37	5.33
Trp	0.96	0.93	0.99	1.01

¹MEG5 = calcium salts of palm oil fatty acids at 5% dietary ether extract (EE); MEG8 = calcium salts of palm oil fatty acids at 8% dietary EE; CS5 = 7.7% camelina seed supplementation at 5% dietary EE; CS8 = 17.7% camelina seed supplementation at 8% dietary EE.

preserved with 0.2 mL of 0.2 *N* sulfuric acid plus 10 mL of sample, and subsamples (8 mL) used for VFA were preserved with 2 mL of 25% metaphosphoric acid. Then, samples for NH₃-N and VFA were centrifuged at 1,000 × *g* for 15 min at 4°C, and the supernatant was separated, isolated, and stored at −20°C for subsequent analysis.

On the last day of each period, the entire fermentor content was blended for 30 s, squeezed through 4 layers of cheesecloth, and washed with 200 mL of saline solution (0.9% NaCl). Filtered samples were centrifuged for 10 min at 1,000 × *g* at 5°C, and the pellets were carefully discarded. The supernatant was centrifuged for 20 min at 11,250 × *g*, at 5°C (Avanti JXN-30 refrigerated high speed centrifuge; Beckman Coulter, Brea, CA). The supernatant was discarded and pellets were resuspended in 100 mL of McDougall's solution and the last centrifugation was performed at 16,250 × *g* for 20 min at 5°C. The bacterial isolation procedure was performed using a modification of Krizsan et al. (2010). The final supernatant was discarded and bacterial pellets were freeze-dried, ground using a mortar and pestle method, and stored for further ¹⁵N enrichment and total N and OM analyses (Bach et al., 2008).

Chemical Analysis

Ruminal digesta samples were freeze-dried for further chemical analysis, and samples contained on average 94% DM. Feed and effluent samples were analyzed for DM (method 934.01), ash (method 938.08), and EE (method 920.85) according to AOAC (1990). Crude protein content of feed and effluent samples was determined using a Leco combustion N analyzer (Leco CN628 Carbon/N Analyzer, Leco Instruments Inc., St. Joseph, MI; method 990.13, AOAC International, 2005). The OM was calculated as the difference between DM and ash contents, and NFC (% of DM) was calculated according to NRC (2001):

$$\text{NFC} = 100 - (\% \text{ NDF} + \% \text{ CP} + \% \text{ fat} + \% \text{ ash}).$$

For NDF and ADF, samples were sequentially analyzed, being treated with thermo-stable α-amylase, according to Van Soest et al. (1991) and adapted for the Ankom²⁰⁰ Fiber Analyzer (Ankom Technology, Macedon, NY). Effluent samples were analyzed for NH₃-N content according to Chaney and Marbach (1962). Volatile fatty acids concentration was determined using a gas chromatograph (Agilent 6890N, Agilent Technologies, Santa Clara, CA) equipped with a capillary column (10.0 m × 530 μm × 1.00 μm nominal). The flow rate of carrier gas (helium) was 27 mL/min. Oven temperature was

programmed at 90°C for 2 min, increasing from 90°C to 190°C at 15°/min, and holding at 190°C for 2 min.

Nitrogen metabolism was calculated as follows:

$$\text{NH}_3\text{-N (g/d)} = \text{mg/dL of effluent NH}_3\text{-N} \\ \times (\text{g of total effluent flow/100});$$

$$\text{NAN flow (g/d)} = \text{g of effluent N} \\ - \text{g of effluent NH}_3\text{-N};$$

$$\text{Bacterial N flow (g/d)} = \\ [\text{NAN flow} \times \text{atom percentage excess (ape) of } ^{15}\text{N} \\ \text{of effluent}]/(\text{ape of } ^{15}\text{N of bacteria});$$

$$\text{Dietary N flow (g/d)} = \text{g of effluent NAN} \\ - \text{g of effluent bacterial N};$$

$$\text{RUP-N} = \text{total N flow (g/d) on effluent} \\ - \text{effluent bacterial N flow (g/d)};$$

$$\text{RDP-N} = \text{total N intake (g/d)} - \text{RUP-N (g/d)};$$

$$\text{Bacterial efficiency} = \text{g of bacterial N flow/} \\ \text{kg of OM truly digested.}$$

Bacterial N flow and bacterial efficiency were calculated according to Calsamiglia et al. (1996); RUP-N and RDP-N were calculated according to Paula et al. (2017). Amino acids analysis was performed on feed and digesta samples according to method 999.13 of AOAC International (2005), using an amino acid analyzer (model 8900, Hitachi, Tokyo, Japan). Analyses were performed with post-ninhydrin detection and using norleucine as the internal standard. Fatty acids analysis was performed at the Agricultural Experiment Station Chemical Laboratories at the University of Missouri (Columbia). Fatty acids profile was determined according to AOAC International (2001; method 996.06), preparation of FAME was performed according to AOCS (2012; method Ce 2-66), n-3 FA analysis was performed according AOCS (2012; method Ce 1d-91), and CLA analysis was performed according to AOCS (2012; method Ce 1h-05) using a Supelco SP2560 (100 m × 0.25 mm × 0.2 μm film) column. The GC (Agilent 7890A with 7683B Autosampler; Agilent Technologies) settings were as follows: Oven temperature was set held for 5 min at 140°C, increased at 4°C/min to 200°C (15 min), increased at 2°C/min to 240°C (20 min), and held for 15 min (total: 55 min). The inlet temperature was

250°C, pressure was 35 psi, split ratio was 60:1, and injection volume was 2 μL. Individual FA are reported as percentage of total FA.

Glucosinolate samples were defatted, mixed with methanol, and filtered through a 0.45-μm filter into an autosampler vial. For quantification, we used a modification of an HPLC method developed by Betz and Fox (1993). The extract was run on a Shimadzu (Columbia, MD) HPLC system (2 LC 20AD pumps; SIL 20A auto-injector; DGU 20 As degasser; SPD-20A UV-vis detector; and a CBM-20A communication BUS module) running under the Shimadzu LC solutions software (version 1.25). The column was a C18 Inertsil reverse phase column (250 mm × 4.6 mm; RP C-18, ODS-3, 5u; GL Sciences, Torrance, CA). Glucosinolates were detected by monitoring at 237 nm.

Background samples and bacterial pellets were analyzed for DM, N, and ash as described previously for feed and effluent samples, and were analyzed for ¹⁵N enrichment (Werner et al., 1999). Isotope analyses were performed using a Eurovector model 3000 (Euro EA 3000, Eurovector s.p.a., Milano, Italy) elemental analyzer interfaced to a Micromass Isoprime (IsoPrime, Micromass UK Ltd., Manchester, UK) stable isotope ratio mass spectrometer. Bacterial N flow and bacterial efficiency were calculated as follows: Bacterial N flow (expressed in g/d) = (NAN flow × atom percentage excess of ¹⁵N of effluent)/(atom percentage excess of ¹⁵N of bacteria pellet), with ¹⁵N digesta effluents background subtracted from ¹⁵N enrichment. Bacterial efficiency = bacterial N flow (g)/OM truly digestible (kg) according to Calsamiglia et al. (1996). Ruminal true digestibilities were calculated as described by Soder et al. (2013) and Benedetti et al. (2015).

Biohydrogenation of PUFA was calculated according to Fievez et al. (2007), using the following equation:

$$\text{BH PUFA}_i = 100 \times \frac{\text{PUFA}_{\text{diet}} - \text{PUFA}_{\text{effluent}}}{\text{PUFA}_{\text{diet}}},$$

where $\text{PUFA}_{\text{diet}}$ = PUFA supplied through the diet (%) and $\text{PUFA}_{\text{effluent}}$ = PUFA present in ruminal effluent (%).

Completeness of BH was calculated according to Alves et al. (2017), using the following equation:

$$\text{Completeness (\%)} = \frac{18:0_{\text{rumen}}}{\text{Maximum } 18:0_{\text{rumen}}} \times 100,$$

where 18:0_{rumen} is the 18:0 in the rumen as a percentage of total C18 FA, and Maximum 18:0_{rumen} = (*cis*-9 18:1_{diet} - *cis*-9 18:1_{rumen}) + (18:2n-6_{diet} - 18:2n-6_{rumen}) + (18:3n-3_{diet} - 18:3n-3_{rumen}) + 18:0_{diet}.

Table 4. Effects of camelina seed supplementation at 2 dietary ether extract levels on ruminal true digestibility in a dual-flow continuous culture system

Digestibility, %	Diet ¹				SEM	P-value		
	MEG5	MEG8	CS5	CS8		Source	Level	Source × level ²
DM	56.1	54.9	50.2	48.1	4.32	0.02	0.51	0.84
OM	58.7	57.1	53.9	53.3	4.12	0.05	0.58	0.78
CP	55.5	56.9	49.2	51.5	27.9	0.03	0.49	0.85
NDF	56.1	57.6	47.0	44.8	15.5	0.01	0.91	0.52
ADF	49.1	52.4	40.1	36.7	9.04	0.01	0.98	0.44

¹MEG5 = calcium salts of palm oil fatty acids at 5% dietary ether extract (EE); MEG8 = calcium salts of palm oil fatty acids at 8% dietary EE; CS5 = 7.7% camelina seed supplementation at 5% dietary EE; CS8 = 17.7% camelina seed supplementation at 8% dietary EE.

²Interaction between dietary EE level (5 or 8%) and fat source (MEG, saturated, or CS, unsaturated).

Statistical Analysis

All data were subjected to least squares ANOVA using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) as a replicated 4 × 4 Latin square arrangement. The statistical model used was

$$Y_{ijklm} = \mu + S_i + L_j + (S \times L)_{ij} + LS_k + R_{(k)l} + P_m + \varepsilon_{ijklm},$$

where Y_{ijklm} = dependent variable; μ = overall mean; S_i = fixed effect of fat source; L_j = fixed effect of dietary EE level; $(S \times L)_{ij}$ = interaction between fat source and dietary EE level; LS_k = random effect of Latin square; $R_{(k)l}$ = random effect of fermentor within Latin square; P_m = random effect of period; and ε_{ijklm} = random error. The interaction between Latin square and treatment was included in the model; however, it was not significant and therefore it was removed from the model.

Data from different time points (pH) were included as repeated measures in the experimental model. The commonly used correlation structures were compared and autoregressive order 1 (AR1) provided the best fit for repeated measurements data, based on Akaike's information criterion. For all analysis, Tukey's test was used to provide multiple comparisons, and differences were declared when $P \leq 0.05$ and tendency when $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Overall Ruminal Metabolism Effects

Ruminal true digestibilities of DM, OM, CP, NDF, and ADF ($P < 0.05$; Table 4) were affected only by fat source: CS diets resulted in lower digestibility than did MEG diets. Feeds high in PUFA content, such as CS, can modify the ruminal environment and consequently

fermentation patterns toward decreasing ruminal nutrient digestibility, especially of fiber (Tice et al., 1993). Similar effects of reduced overall nutrient digestibility were reported previously when high EE diets were fed (Palmquist and Jenkins, 1980; Allen, 2000; Kazama et al., 2010). However, in our study, this response was associated with the CS FA profile and not to dietary EE level, indicating that FA source may be more determinant than total EE to nutrient digestibility. Moreover, when the MEG8 diet was fed, less dietary fermentable carbohydrates were available (Table 1); consequently, this diet had less energy readily available to ruminal microorganisms, which may have played a role in DM and OM digestion.

Previous studies have shown that UFA may reduce DMI and shift BH from complete to incomplete, increasing the ruminal concentration of BH intermediates (Shingfield et al., 2003; Alves et al., 2013). On the other hand, diets containing calcium salts of palm oil fatty acids may increase milk fat and have little effect on ruminal fermentation profile (Grummer, 1988; Chouinard et al., 1998; Theurer et al., 2009). The lack of consistency in ruminal fermentation response to fat supplementation may be due to differences in chemical forms, sources, and inclusion levels (Piantoni et al., 2015). Our results are likely associated with deleterious effects of PUFA on specific cellulolytic ruminal bacteria. In fact, in a companion study evaluating the effects of replacing calcium salts of palm oil with CS on ruminal microbial population, our research group (Dai et al., 2017) reported that CS changed the structure and composition of bacterial communities. A decrease was observed (Dai et al., 2017) in the relative abundance of *Fibrobacter* and *Ruminococcus*, which are genera associated with ruminal fiber digestion (Wu et al., 2012). Therefore, despite all diets having similar NDF content (35%; Table 1), the lower NDF and ADF digestibility observed when CS diets were fed (Table 4) may be explained by the decrease in relative abundance of cel-

lulolytic bacterial communities caused by high PUFA content of CS. We expected minor changes in ruminal digestibility in MEG diets because of the fat source used in these diets, which was relatively inert to ruminal degradation. This characteristic also explains why we observed predominantly an effect of fat source on digestibility.

Similar to our results for fiber digestibility, the molar proportion of acetate and the acetate:propionate ratio ($P < 0.05$; Table 5) were lower when CS diets were fed. In contrast, CS supplementation increased the molar proportion of propionate ($P = 0.01$). Our results are in agreement with those of Hurtaud and Peyraud (2007), who evaluated the effects of feeding CS and camelina meal to lactating dairy cows and reported an increase in propionate and decrease in acetate when cows were fed camelina compared with a control diet. Similar to OM and DM ruminal digestibility (Table 4), total VFA concentration (mM) was lower in CS diets ($P < 0.05$). Contrary to our results, Lawrence et al. (2016), did not observe an effect on total VFA concentration when evaluating the effects of feeding 10% camelina meal in the total ration of dairy heifers. However, Lawrence et al. (2016) used camelina meal with a lower EE content than the CS used in our study. From an animal production standpoint, this shift may be advantageous considering that diets containing CS had increased propionate concentration; propionate is the main precursor of glucose and ultimately of milk production in the mammary gland (Lemosquet et al., 2009). Furthermore, CS had higher molar proportions of valerate and isovalerate, and higher total branched-chain VFA ($P < 0.05$) concentration.

As expected, fat source affected FA profile in the ruminal outflow of SFA, UFA, MUFA, and PUFA ($P < 0.01$; Table 6). Notably, CS supplementation had greater proportions of UFA, MUFA, and PUFA ruminal outflow. The MEG diets resulted in greater BH of 18:1n-9 ($P = 0.01$) and CS diets resulted in greater BH of 18:2n-6 ($P = 0.05$) and 18:3n-3 ($P = 0.01$). These results can be explained in part by the intake of these FA in each diet; that is, higher intake of 18:3n-3 and 18:2n-6 on CS diets and higher intake of 18:1n-9 on MEG diets. In a study evaluating the effects of intestinal flow of FA in dairy cows fed a high-concentrate diet supplemented with fish oil, sunflower oil, or linseed oil, Looor et al. (2005) observed BH values of 84.7% for 18:2n-6 and of 95.1% for 18:3n-3 in cows supplemented with linseed oil. In the present study, BH of 18:2n-6 was 73.8 and 74.6% on CS5 and CS8, respectively; and BH of 18:3n-3 was 83.8 and 80.9% on CS5 and CS8, respectively. However, in a dual-flow continuous culture system, BH values can be lower and our results are in agreement with those of AbuGhazaleh and Jacobson (2007), who observed BH ranging from 66.1 to 88.6% for 18:2n-6 and 18:3n-3 in a dual-flow continuous culture system.

In the present study, BH of 18:1n-9 was relatively low compared with detected in in vivo studies, ranging from 23 to 47.7% in the current study. One possible reason is that certain bacterial species seem to be preferably enriched in in vitro systems (Weimer et al., 2011), and those with important roles in hydrogenation of 18:1n-9 to 18:0 might not be present to a large degree in this study. Furthermore, our results are in agreement with a continuous culture fermentor study by Looor et al.

Table 5. Effects of camelina seed supplementation at 2 dietary ether extract levels on VFA concentrations in a dual-flow continuous culture system

Item	Diet ¹				SEM	P-value		
	MEG5	MEG8	CS5	CS8		Source	Level	Source × level ²
Total VFA, mM	89.9	89.1	85.1	79.4	2.47	<0.01	0.19	0.32
VFA, % of total VFA								
Acetate (A)	55.8 ^b	60.6 ^a	49.1 ^c	50.1 ^c	1.73	<0.01	<0.01	0.05
Propionate (P)	25.9	23.5	30.1	28.9	1.14	<0.01	0.13	0.62
Butyrate	14.6	12.9	14.9	14.9	1.44	0.26	0.40	0.42
Isobutyrate	0.44	0.51	0.42	0.57	0.05	0.61	0.02	0.37
Valerate	2.40	1.79	3.23	3.67	0.66	<0.01	0.83	0.19
Isovalerate	0.77	0.65	2.12	1.84	0.43	<0.01	0.45	0.77
A:P	2.21	2.60	1.64	1.77	0.14	<0.01	0.04	0.28
Total BCVFA, ³ mmol	1.09	1.02	2.20	1.92	0.40	<0.01	0.49	0.67

^{a-c}Treatment effects with different superscripts are significantly different ($P < 0.05$).

¹MEG5 = calcium salts of palm oil fatty acids at 5% dietary ether extract (EE); MEG8 = calcium salts of palm oil fatty acids at 8% dietary EE; CS5 = 7.7% camelina seed supplementation at 5% dietary EE; CS8 = 17.7% camelina seed supplementation at 8% dietary EE.

²Interaction between dietary EE level (5 or 8%) and fat source (MEG, saturated, or CS, unsaturated).

³Branched-chain VFA.

Table 6. Effects of camelina seed supplementation at 2 dietary ether extract levels on ruminal effluent fatty acid concentration and biohydrogenation in a dual-flow continuous culture system

Item, %	Diet ¹				SEM	P-value		
	MEG5	MEG8	CS5	CS8		Source	Level	Source × level ²
14:0	1.15 ^a	1.12 ^a	0.74 ^b	0.47 ^c	0.07	<0.01	<0.01	0.01
15:0	0.91	0.54	0.76	0.48	0.08	0.13	<0.01	0.49
16:0	27.0 ^a	35.3 ^b	11.9 ^c	9.88 ^c	1.04	<0.01	<0.01	<0.01
16:1n-7	0.36	0.29	0.38	0.31	0.03	0.52	0.03	0.89
17:0	0.40	0.28	0.38	0.23	0.02	0.13	<0.01	0.43
18:0	24.3	19.4	11.3	11.6	3.44	0.01	0.48	0.43
18:1n-9	16.5 ^b	21.0 ^a	13.5 ^b	11.9 ^b	0.98	<0.01	0.16	0.03
18:1 <i>trans</i>								
6/7/8/9	0.94	0.85	1.43	0.45	0.55	0.93	0.30	0.39
10/11 ³	7.20	5.53	16.12	16.03	2.02	<0.01	0.57	0.61
12/13 ⁴	1.44	1.06	2.13	1.59	0.56	0.21	0.34	0.88
18:1n-7	2.72	1.91	2.95	1.89	0.20	0.60	<0.01	0.52
9c12/t11c15/t10c15 18:2 ⁵	0.88	0.52	6.35	8.07	0.99	<0.01	0.34	0.15
18:2 conjugated								
<i>cis</i> -9, <i>trans</i> -11	1.31	0.90	1.98	2.49	0.49	0.01	0.89	0.21
<i>cis</i> -9, <i>cis</i> -11	0.08	0.06	0.12	0.14	0.01	<0.01	0.88	0.07
<i>trans</i> -9, <i>trans</i> -11	0.44	0.32	0.52	0.52	0.06	0.01	0.18	0.17
18:2n-6	4.68	3.90	6.72	6.27	0.59	<0.01	0.66	0.60
18:3n-3	1.47	0.98	3.92	5.00	0.44	<0.01	0.51	0.09
20:0	0.57	0.51	2.05	2.84	0.44	<0.01	0.26	0.19
20:1n-9	0.22 ^c	0.19 ^c	6.07 ^b	9.31 ^a	0.49	<0.01	<0.01	<0.01
22:0	0.46	0.33	0.61	0.58	0.02	<0.01	<0.01	0.19
22:1n-9	0.04 ^c	0.03 ^c	1.51 ^b	2.32 ^a	0.07	<0.01	<0.01	<0.01
23:0	0.40	0.26	0.39	0.29	0.39	0.15	<0.01	0.11
24:0	0.37 ^b	0.25 ^c	0.44 ^a	0.38 ^b	0.01	<0.01	<0.01	<0.01
24:1n-9	0.10 ^c	0.09 ^c	0.48 ^b	0.65 ^a	0.02	<0.01	<0.01	<0.01
Unknown	6.17	4.31	6.29	6.52	0.81	0.02	0.06	0.35
SFA	55.5	58.0	28.7	26.8	2.96	<0.01	0.92	0.50
UFA	38.7	38.1	66.9	70.1	3.66	<0.01	0.72	0.59
MUFA	29.6	31.0	45.9	45.3	2.40	<0.01	0.85	0.68
PUFA	9.18	7.13	21.1	24.8	1.64	<0.01	0.58	0.06
Biohydrogenation ⁶								
18:1	47.7	38.6	23.0	28.1	3.98	0.01	0.59	0.09
18:2	71.1	67.8	73.8	74.4	2.34	0.05	0.54	0.40
18:3	77.2	73.3	83.8	80.9	2.30	0.01	0.11	0.78
Completeness ⁷	42.5	45.2	17.6	17.8	3.92	0.01	0.64	0.69

^{a-c}Treatment effects with different superscripts are significantly different ($P < 0.05$).

¹MEG5 = calcium salts of palm oil fatty acids at 5% dietary ether extract (EE); MEG8 = calcium salts of palm oil fatty acids at 8% dietary EE; CS5 = 7.7% camelina seed supplementation at 5% dietary EE; CS8 = 17.7% camelina seed supplementation at 8% dietary EE.

²Interaction between dietary EE level (5 or 8%) and fat source (MEG, saturated, or CS, unsaturated).

³Sum of *trans*-10 18:1 and *trans*-11 18:1.

⁴Sum of *trans*-12 18:1 and *trans*-13 18:1.

⁵Sum of *trans*-9,*cis*-12 + *trans*-11,*cis*-15 + *trans*-10,*cis*-15 18:2.

⁶Biohydrogenation (BH) calculated according to Fievez et al. (2007), using the following equation: BH of PUFA_i = 100 × (PUFA_{0h} - PUFA_{1h}) / (PUFA_{0h}), where PUFA_{0h} = PUFA supply (%) and PUFA_{1h} = PUFA (%) present in ruminal effluent.

⁷Completeness of BH was calculated according to Alves et al. (2017), using the following equation: Completeness = 18:0_{rumen} / Maximum 18:0_{rumen} × 100, where Maximum 18:0_{rumen} = (*cis*-9 18:1_{diet} - *cis*-9 18:1_{rumen}) + (18:2n-6_{diet} - 18:2n-6_{rumen}) + (18:3n-3_{diet} - 18:3n-3_{rumen}) + 18:0_{diet}.

(2003), in which BH of 18:1n-9 ranged from 21.2 to 85.6%. An incomplete BH results in lower accumulation of 18:0 and greater accumulation of BH intermediates, notably *trans*-18:1 isomers. Therefore, supplementation of CS has the potential to reduce the last step of BH as verified by the low values of the estimated completeness of BH (Table 6), increased concentration of *trans*-10/11 18:1, and lower concentration of 18:0 observed on CS diets. Additionally, the accumulation of 18:1 observed in CS diets may be related to inhibition of reductase

enzyme in the ruminal bacteria related to the terminal hydrogenation of 18:1 to 18:0 (AbuGhazaleh and Jacobson, 2007). Our results are in agreement with Halmemies-Beauchet-Filleau et al. (2011), who studied different plant oils, including camelina oil, and reported increased concentrations of BH intermediates in milk fat when camelina oil was fed, suggesting that camelina may favor incomplete BH.

Camelina diets had lower concentrations of 18:1n-9 in the ruminal outflow and greater ($P < 0.01$) concentra-

tion of *trans*-10/11 18:1 (Table 6), which is consistent with the literature that describes higher inputs of UFA results in accumulation of *trans*-18:1 (Loor et al., 2005). Vaccenic acid (*trans*-11 18:1) is a desirable FA flowing out of rumen because it can be used as substrate to produce *cis*-9,*trans*-11 CLA in animal tissue via Δ^9 -desaturase (Grinari et al., 2000). In addition, this FA has been shown to have beneficial implications for human health. (Kritchevsky, 2000). In Hurtaud and Peyraud (2007), a higher concentration of *trans*-10 18:1, rather than *trans*-11 18:1, was reported in milk FA of cows fed diets containing camelina, which could explain our results indicating that CS supplementation potentially increases ruminal outflow of these FA. However, we were not able to detect *trans*-11 18:1 and *trans*-10 18:1 individually and, although the sum of these 2 FA was increased in CS diets, it is not clear whether the amount of *trans*-11 18:1 was greater than that of *trans*-10 18:1.

Three CLA were identified in the ruminal effluent (Table 6). Ruminal concentrations of 18:2n-6, the 3 detected CLA, and 18:3n-3 were higher when CS diets were fed ($P < 0.01$). In particular, supplementing CS resulted in a greater flow of *cis*-9,*trans*-11 CLA. Previous studies have shown that feeding lipid sources can modulate the concentration of *cis*-9,*trans*-11 CLA in milk fat (Shingfield et al., 2003), as this isomer is associated with anticarcinogenic properties (Williams, 2000) and immune response (Kritchevsky, 2000). In a study evaluating the effects of feeding camelina meal or seed to lactating dairy cows, Hurtaud and Peyraud (2007) observed an increased concentration of *cis*-9,*trans*-11 CLA in milk lipid of cows fed diets containing camelina compared with a control diet. Similarly, Bayat et al. (2015) studied the effects of supplementing 60 g of camelina oil/kg of dietary DM on milk profile and observed an increase in concentration of *cis*-9,*trans*-11 CLA on milk fat when cows were fed camelina oil. Additionally, in the same study, the authors observed an increased concentration of 18:1n-9, 18:2n-6, and 18:3n-3 in the milk of cows fed camelina oil compared with control diets. Although we did observe an increase in *cis*-9,*trans*-11 CLA in the ruminal effluent, the major source of *cis*-9,*trans*-11 CLA in milk comes from endogenous desaturation of *trans*-11 18:1 in the mammary gland (Grinari et al., 2000; Corl et al., 2001).

We detected an interaction between fat source and dietary EE level for 16:0 ($P < 0.01$; Table 6), and treatment MEG8 had the greatest concentration (35.3%) compared with MEG5, CS5, and CS8 (27.0, 11.9, and 9.88%; respectively); MEG supplementation also increased the concentration of 14:0 in the ruminal effluent ($P = 0.01$; Table 6). These results are likely associated with the greater concentration of these FA in the MEG

diets, and consequently greater intake. Hurtaud and Peyraud (2007) observed lower concentration of 14:0 and 16:0 in milk of cows fed diets containing camelina meal or seed compared with control diets. However, their control diets did not contain calcium salts of palm oil.

Camelina contains antinutritional factors such as erucic acid and glucosinolates. Therefore, the percentage of erucic acid (C22:1n-9) was higher in the CS diet ($P < 0.01$; Table 6), as was the concentration of glucosinolate (Table 1). This was expected because MEG diets had no detectable erucic acid. Diets CS5 and CS8 had 2.02 and 2.58% erucic acid, respectively. The US Food and Drug Administration (FDA) has established a limit of 2% erucic acid in total dietary FA; however, in Europe, the limit is 5% of erucic acid in total dietary FA (EFSA, 2016). Therefore, the CS5 diet was below the FDA limit and both CS5 and CS8 diets were below the European limit. These limits were established because of the increased risk of myocardial lipidosis in monogastrics (Kramer et al., 1990; Guil et al., 1997). The concentration of glucosinolates ranged from 1.18 to 2.27 mg/g in CS5 and CS8, and from 0.36 to 0.34 mg/g in MEG5 and MEG8 (Table 1). Glucosinolates could affect thyroid function; however, in a study evaluating performance of dairy heifers feeding camelina meal, linseed meal, or distilled dried grains, Lawrence et al. (2016) did not find negative effects on T_3 (triiodothyronine) and T_4 (thyroxine) concentration in heifers fed camelina meal containing 1.24 mg/g of glucosinolates. Likewise, Cappellozza et al. (2012) reported no effect on thyroid-stimulating hormone or on T_3 and T_4 , when camelina meal was fed to beef heifers.

Ruminal Nitrogen Metabolism and Amino Acids

We detected no differences among treatments on ruminal pH and $\text{NH}_3\text{-N}$ (mg/dL, Table 7). Ruminal pH was analyzed as repeated measures and because there was no treatment effect, it is reported as the daily mean (Table 7). These results are in agreement with Halme-mies-Beauchet-Filleau et al. (2017), who fed diets with increasing camelina oil levels to lactating dairy cows, and with Martin et al. (2016), who studied the effects of increased linseed oil inclusion in dairy cow diets. Overall, feeding oilseeds or cakes do not seem to affect ruminal pH (Pires et al., 1997; Reveneau et al., 2005).

Treatments did not affect flows (g/d) of total N, dietary N, bacterial efficiency, or RUP-N, or RDP-N supply ($P > 0.05$; Table 7). However, dietary EE level affected $\text{NH}_3\text{-N}$ (g/d), NAN, and bacterial-N flows ($P < 0.05$; Table 7). Diets with 8% dietary EE had lower bacterial-N and greater $\text{NH}_3\text{-N}$ flow, indicating reduced bacterial-N synthesis. Even though RDP-N supply and

RUP-N flow were not affected by treatments, dietary EE level affected $\text{NH}_3\text{-N}$ and bacterial-N. We believe these results could be explained by 2 factors: (1) the deleterious effect on overall N metabolism observed when the CS8 diet was fed may be due to the inclusion of 17.7% CS and its negative effects on ruminal microbial population as previously described; and (2) when the MEG8 diet was fed, less dietary fermentable carbohydrates were available (Table 1); consequently, this diet had less energy readily available to produce bacterial-N. Similar effects on reduced bacterial-N observed when saturated fat sources were fed were observed by Dunkley et al. (1977), Canale et al. (1990), and Klusmeyer et al. (1991).

The AA flow was affected by treatments in similar manner as N metabolism; overall dietary EE level negatively affected outflow of individual AA (Table 8) except for taurine and ornithine. A dietary EE level of 8% resulted in lesser outflow of most AA, which resulted in lower outflow of EAA. These results may be associated with bacterial-N outflow, because the high EE diets decreased bacterial-N and increased NH_3 (g/d) outflow ($P < 0.05$; Table 7). The high EE level reduced the abundance of *Fibrobacter* and *Ruminococcus*, which

are generally associated with ammonia metabolism and fiber digestion; consequently, these diets had deleterious effects on AA profile flowing out of the fermentors. The greater ruminal escape of EAA may represent a better AA profile available for postruminal absorption and utilized for milk production.

The MEG diets had only canola meal as a protein source, whereas the CS diets contained canola meal plus CS. Diet CS5 had similar results as MEG5 in terms of AA outflow, indicating that partial replacement of canola meal by CS can produce similar ruminal AA outflows. High-producing dairy cows rely more on the AA profile that escapes ruminal degradation (RUP) than low-producing cows, which means that high-producing cows need good quality protein supplements such as canola and possibly camelina. In North America, canola meal is widely used as a protein source in dairy diets (Mulrooney et al., 2009), with positive responses on milk production and DMI, and reduced MUN when replacing different protein sources (Martineau et al., 2013, Broderick et al., 2015). Overall, CS5 had similar results as the diet containing exclusively canola meal (MEG5) for N metabolism and AA outflow, indicating that CS may be able to partially replace canola without

Table 7. Effects of camelina seed supplementation at 2 dietary ether extract levels on ruminal pH and nitrogen metabolism in a dual-flow continuous culture system

Item	Diet ¹					P-value		
	MEG5	MEG8	CS5	CS8	SEM	Source	Level	Source × level ²
pH	6.52	6.50	6.44	6.51	0.07	0.71	0.65	0.55
$\text{NH}_3\text{-N}$, mg/dL	12.1	10.8	12.9	11.8	0.14	0.29	0.18	0.92
N flows, g/d								
Total N	1.81	1.78	1.92	1.81	0.19	0.42	0.39	0.72
$\text{NH}_3\text{-N}$ ³	0.27	0.42	0.24	0.45	0.03	0.49	0.01	0.08
NAN ⁴	1.53	1.35	1.67	1.35	0.16	0.53	0.04	0.59
Bacterial-N ⁵	0.82	0.72	0.84	0.73	0.07	0.73	0.04	0.97
Dietary N ⁶	0.71	0.63	0.83	0.62	0.11	0.47	0.07	0.44
RUP-N ⁷	0.99	1.04	1.07	1.07	0.12	0.31	0.67	0.57
RDP-N ⁸ supply	0.97	0.92	0.89	0.89	0.12	0.20	0.84	0.72
Bacterial efficiency ⁹	21.2	19.7	23.7	20.2	2.27	0.61	0.23	0.74

¹MEG5 = calcium salts of palm oil fatty acids at 5% dietary ether extract (EE); MEG8 = calcium salts of palm oil fatty acids at 8% dietary EE; CS5 = 7.7% camelina seed supplementation at 5% dietary EE; CS8 = 17.7% camelina seed supplementation at 8% dietary EE.

²Interaction between dietary EE level (5 or 8%) and fat source (MEG, saturated, or CS, unsaturated).

³ $\text{NH}_3\text{-N}$ (g/d) = mg/dL of effluent $\text{NH}_3\text{-N}$ × (g of total effluent flow/100).

⁴NAN was calculated as following: NAN flow (g/d) = g of effluent N – g of effluent $\text{NH}_3\text{-N}$.

⁵Bacterial-N flow was calculated according to Calsamiglia et al. (1996) using the following equation: bacterial N flow (g/d) = (NAN flow × atom percentage excess of ^{15}N of effluent)/(atom percentage excess of ^{15}N of bacteria).

⁶Dietary N flow (g/d) = g of effluent NAN – g of effluent bacterial N.

⁷RUP-N = rumen undegraded protein nitrogen: RUP-N = total N flow (g/d) on effluent – effluent bacterial N flow (g/d). Estimated according to Paula et al. (2017).

⁸RDP-N = rumen degraded protein nitrogen: RDP-N = total N intake (g/d) – RUP-N (g/d), according to Paula et al. (2017).

⁹Bacterial efficiency was calculated according to Calsamiglia et al. (1996) using the following equation: bacterial efficiency = g of bacterial N flow/kg of OM truly digested.

compromising AA flow to the small intestine; this is likely the most important observation of the present study.

The Arg outflow was affected by fat source and EE level ($P = 0.04$ and $P = 0.03$, respectively; Table 8), and diets containing CS had greater Arg outflow. This may be because Arg is one of the most abundant AA in camelina seed (Zubr, 2003). Even though dairy cows are capable of synthesizing Arg through de novo synthesis (NRC, 2001), Arg is an EAA because the amount produced by de novo synthesis is not sufficient to meet the requirements of high-producing dairy cows (NRC, 2001). Mammary gland uptake of Arg can be 1 to 3 times the amount of this AA found in milk (Doepel and Lapierre, 2011), demonstrating the importance of ensuring the Arg supply for mammary gland.

Despite the slight difference in Lys and Met concentrations across diets, ruminal outflow of these AA was only affected by dietary EE level. Diets containing 8% dietary EE had reduced ($P < 0.05$; Table 8) ruminal outflow. Lysine and Met are often considered first-limiting EAA for dairy cows (NRC, 2001), and milk production and composition can respond to supplemental

Lys and Met (under low-protein diets) as long as DMI and other AA such as His are not limiting (Sinclair et al., 2014). Histidine ruminal outflow was only affected by dietary EE level ($P = 0.03$; Table 8), such that 5% EE resulted in greater His ruminal outflow. Compared with other EAA, bacterial production of His is relatively low, around 4% of total bacterial EAA (NRC, 2001), whereas milk and lean tissue have 6.3 and 5.5% of total EAA as His, indicating the importance of His supplementation in diets with low MP, especially if most of the MP comes from microbial protein (Lee et al., 2012). These results suggest that CS supplementation at 5% dietary EE may positively influence milk yield and composition.

Treatments had little to no effect on ruminal digestibility of individual AA (Table 9). Dietary EE level tended to increase ruminal digestibility of Glu, Pro, Cys, Leu, and Orn ($P < 0.10$; Table 9). Diets containing CS had decreased digestibility of taurine, Pro, and Leu ($P < 0.05$; Table 9) and tended to decrease digestibilities of Ser and Ala ($P = 0.08$ and 0.06 , respectively; Table 9). Despite the negative effect of CS supplementation on CP digestibility (Table 4), it did not affect overall

Table 8. Effects of camelina seed supplementation at 2 dietary ether extract levels on AA ruminal effluent outflow in a dual-flow continuous culture system

AA flow, g/d	Diet ¹				SEM	P-value		
	MEG5	MEG8	CS5	CS8		Source	Level	Source × level ²
Taurine	0.021	0.019	0.023	0.024	0.006	0.092	0.739	0.349
Hydroxyproline	0.031	0.026	0.036	0.034	0.008	0.011	0.103	0.534
Asp	0.835	0.685	0.896	0.781	0.144	0.137	0.017	0.727
Thr	0.426	0.350	0.447	0.383	0.075	0.302	0.015	0.828
Ser	0.329	0.272	0.356	0.316	0.057	0.09	0.024	0.687
Glu	1.146	0.940	1.238	1.095	0.213	0.095	0.023	0.660
Pro	0.427	0.360	0.471	0.417	0.078	0.066	0.030	0.810
Gly	0.482	0.394	0.512	0.449	0.088	0.163	0.018	0.663
Ala	0.567	0.461	0.599	0.537	0.105	0.145	0.028	0.535
Cys	0.114	0.095	0.122	0.107	0.023	0.166	0.029	0.743
Val	0.549	0.449	0.573	0.489	0.098	0.332	0.010	0.808
Met	0.170	0.144	0.185	0.165	0.033	0.128	0.054	0.814
Ile	0.458	0.377	0.478	0.412	0.081	0.333	0.017	0.796
Leu	0.710	0.582	0.761	0.668	0.131	0.132	0.020	0.686
Tyr	0.336	0.276	0.356	0.299	0.056	0.303	0.010	0.929
Phe	0.448	0.369	0.482	0.419	0.081	0.129	0.016	0.764
Hydroxylysine	0.021	0.016	0.022	0.019	0.004	0.148	0.043	0.744
Orn	0.013	0.009	0.012	0.014	0.02	0.125	0.701	0.110
Lys	0.466	0.385	0.479	0.431	0.086	0.358	0.056	0.611
His	0.160	0.132	0.172	0.148	0.032	0.216	0.029	0.816
Arg	0.411	0.337	0.454	0.421	0.081	0.037	0.037	0.553
Trp	0.086	0.076	0.094	0.082	0.015	0.250	0.090	0.871
EAA ³	3.797	3.123	4.030	3.525	0.702	0.188	0.021	0.720

¹MEG5 = calcium salts of palm oil fatty acids at 5% dietary ether extract (EE); MEG8 = calcium salts of palm oil fatty acids at 8% dietary EE; CS5 = 7.7% camelina seed supplementation at 5% dietary EE; CS8 = 17.7% camelina seed supplementation at 8% dietary EE.

²Interaction between dietary EE level (5 or 8%) and fat source (MEG, saturated, or CS, unsaturated).

³EAA = Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val.

Table 9. Effects of camelina seed supplementation at 2 dietary ether extract levels on AA digestibility in dual-flow continuous culture system

AA digestibility, %	Diet ¹					P-value		
	MEG5	MEG8	CS5	CS8	SEM	Source	Level	Source × level ²
Taurine	87.6	88.9	86.6	84.5	3.80	0.04	0.72	0.19
Asp	37.5	39.0	29.9	35.5	37.5	0.21	0.42	0.64
Thr	36.9	38.0	30.0	38.3	29.0	0.46	0.30	0.42
Ser	45.4	50.4	38.6	44.2	9.30	0.08	0.16	0.93
Glu	48.1	53.8	41.2	49.1	9.30	0.12	0.07	0.76
Pro	57.9	62.3	50.8	56.3	7.40	0.03	0.10	0.86
Gly	40.8	41.7	34.3	40.4	10.8	0.36	0.41	0.53
Ala	43.2	49.4	36.7	40.5	10.4	0.06	0.22	0.76
Cys	56.8	60.2	51.7	58.9	8.60	0.27	0.08	0.51
Val	38.1	39.9	32.9	40.2	11.1	0.49	0.24	0.46
Met	44.7	47.0	36.2	42.7	10.8	0.14	0.30	0.62
Ile	34.5	35.4	27.4	35.5	12.0	0.45	0.34	0.45
Leu	49.3	55.9	42.6	48.2	9.10	0.05	0.09	0.90
Tyr	16.7	19.7	10.1	20.4	13.2	0.47	0.18	0.40
Phe	45.1	48.4	38.2	43.6	10.0	0.12	0.25	0.77
Orn	-6.20	-15.8	-10.2	-45.8	15.5	0.16	0.07	0.46
Lys	44.4	41.6	38.8	43.6	13.0	0.70	0.83	0.42
His	55.8	59.7	50.1	57.2	9.00	0.24	0.12	0.65
Arg	47.0	49.8	41.6	45.8	10.1	0.22	0.37	0.86
Trp	43.1	37.1	34.7	42.6	23.1	0.75	0.84	0.14

¹MEG5 = calcium salts of palm oil fatty acids at 5% dietary ether extract (EE); MEG8 = calcium salts of palm oil fatty acids at 8% dietary EE; CS5 = 7.7% camelina seed supplementation at 5% dietary EE; CS8 = 17.7% camelina seed supplementation at 8% dietary EE.

²Interaction between dietary EE level (5 or 8%) and fat source (MEG, saturated, or CS, unsaturated).

digestibility of individual AA, which could be another indication of possible partial replacement of canola meal by CS without compromising protein metabolism.

CONCLUSIONS

Supplementation of CS resulted in a greater proportion of BH intermediates 18:3n-3 and 18:2n-6 in ruminal effluent compared with diets containing calcium salts of palm oil (MEG). Greater ruminal escape of beneficial FA might translate into better milk FA profile; however, this needs to be confirmed in vivo. In addition, CS supplementation resulted in lower acetate:propionate ratio than supplementation with MEG. However, ruminal true digestibilities of DM, OM, NDF, ADF, and CP were lower in CS diets. We observed no differences between CS and MEG in RUP-N and RDP-N when added at 5% dietary EE. However, 8% dietary EE caused negative effects on ruminal N metabolism and AA outflow regardless of CS inclusion, increasing NAN and bacterial N. This suggests CS can be used up to 5% dietary EE without compromising N metabolism and AA outflow and AA digestibility.

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