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Effects of flaxseed and chia seed on ruminal fermentation, nutrient digestibility, and long-chain fatty acid flow in a dual-flow continuous culture system[1]

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ABSTRACT: Flaxseed (FS) and chia seed (CS) are oilseeds rich in omega-3 fatty acids, which may change meat and milk composition when added to ruminants' diets and may have health benefits for humans. Literature on the effects of CS supplementation on ruminal metabolism is nonexistent. A dual-flow continuous culture fermenter system consisting of 6 fermenters was used to assess the effect of FS and CS supplementation in an alfalfa hay–based diet on ruminal fermentation, nutrient digestibility, microbial protein synthesis, and long-chain fatty acid flow. Diets were randomly assigned to fermenters in a replicated 3 × 3 Latin square design, with 3 consecutive periods of 10 d each, consisting of 7 d for diet adaptation and 3 d for sample collection. Each fermenter was fed a total of 72 g of DM/d divided in 6 equal portions. Treatments were 1) alfalfa hay + calcium soaps of palm oil fatty acid (MEG; 69.3 g DM/d of alfalfa hay plus 2.7 g DM/d of calcium soaps of palm oil fatty acid), 2) alfalfa hay + FS (FLAX; 68.4 g DM/d of alfalfa hay plus 3.6 g DM/d of ground FS), and 3) alfalfa hay + CS (CHIA; 68.04 g DM/d of alfalfa hay plus 3.96 g DM/d of ground CS). Dietary treatments had similar amounts of total fat, and fat supplements were ground to 2-mm diameter. Effluents from the last 3 d of incubation were composited for analyses. Data were analyzed using the MIXED procedure of SAS. Ruminal apparent and true nutrient digestibility of all nutrients did not differ ($P > 0.05$) among treatments. Compared with MEG, FLAX and CHIA increased the flows of C18:3 *n*-3, C20:4 *n*-6, and total PUFA ($P < 0.01$). Both CHIA and FLAX treatments had greater ruminal concentrations of C18:0, indicating that both CS and FS fatty acids were extensively biohydrogenated in the rumen. The NH_3–N concentration, microbial N flow, and efficiency of microbial protein synthesis were not affected ($P > 0.05$) by treatments. Lastly, there were no differences ($P > 0.05$) among diets for total VFA concentration and molar proportions of individual VFA. Results from this study indicate that FS and CS supplementation did not impair ruminal fermentation, digestibility, microbial efficiency, and ruminal N metabolism. Overall, CS appears to be as effective as FS as a fat source when added to ruminants' diets using a dual-flow continuous culture system.

Key words: chia seed, dual-flow continuous culture, flaxseed, long-chain fatty acid

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INTRODUCTION

Salvia hispanica L., commonly known as chia, is an annual herbaceous plant, native to southern Mexico and northern Guatemala (Ixtaina et al., 2008), that grows well at up to 2,200 m of elevation across different ecosystems (Ayerza, 2009). Chia has recently been revived as a new crop due to its greater oil and omega-3 fatty acid content among productive oilseeds (Cahill, 2004). The seed has about 25 to 38% oil, and about 60% of its fatty acids can be α-linolenic acid (Ayerza, 1995); moreover, its protein content ranges

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from 19 to 23%, which is greater than other traditional cereals such as wheat, corn, rice, and oats (Coates and Ayerza, 1996). Because of its nutritional value and its ability to grow well in high elevations, chia is well suited to grow in parts of Nevada, notably in the northern region of the state. Alfalfa hay accounts for more than half of the total value crops yielded in Nevada (NDA, 2015) and is the leading cash crop of the state, totaling US$232,100,000 annually (Nevada Agriculture, 2013). Because of this, it is largely used in cattle operations (beef and dairy) and it can represent a large proportion of its diets.

There is an increasing recognition that foods can be contributing factors for the prevention and development of several human disease conditions. As a result, attention has been given to designing foods with enhanced compounds that have beneficial effects on human health (NRC, 1988). Supplementation of ruminants' diets with lipid sources rich in PUFA may be an effective method to improve meat and milk fat composition by reducing SFA and enhancing beneficial fatty acids, such as *n*-3 α-linolenic acid (omega-3) and CLA (Singh et al., 2011). Consumption of food products with more PUFA has a wide range of beneficial effects on human health, such as anticarcinogenic effects (Ip et al., 1994; McGuire et al., 1997), diminishing atherosclerosis (Vedtofte et al., 2011), preventing coronary heart disease (Lee et al., 1994), antidiabetic effects (Bauman et al., 2001), decreasing fat deposition (Park et al., 1997), and improving immune function (Miller et al., 1994).

Flaxseed (**FS**) and chia seed (**CS**) are 2 oilseeds that contain greater amounts of omega-3 fatty acids. Flaxseed has been used in supplementation of dairy cows' and egg-laying hens' diets (Neveu et al., 2014; Moallem et al., 2012). The use of this oilseed in dairy cows may increase milk production, reduce milk fat content, and increase omega-3 fatty acid content (Neveu et al., 2014) and increases omega-3 fatty acids in eggs and reduces cholesterol in egg yolk (Ayerza and Coates, 1999).

Nevertheless, literature on the effects of CS supplementation on ruminal metabolism is nonexistent. Therefore, the objective of this study was to assess the effect of FS and CS supplementation in an alfalfa hay–based diet on ruminal fermentation, nutrient digestibility, microbial protein synthesis, and long-chain fatty acid flow in dual-flow continuous culture fermentation. We hypothesize that CS would have effects similar to FS on ruminal fermentation.

MATERIALS AND METHODS

Care and handling of all experimental animals, including ruminal cannulation, were conducted under protocols approved by the University of Nevada, Reno, Institutional Animal Care and Use Committee (protocol number 00588).

Experimental Design and Diets

The study was conducted as a 3 × 3 Latin square design; each dual-flow continuous culture fermenter unit was randomly assigned to receive each diet once over the 3 periods. Each 10-d period consisted of a 7-d diet adaptation period followed by a 3-d sampling period.

Dietary treatments were formulated to meet NRC recommendations (NRC, 2001) and to contain similar amounts of total fat (3.1 to 4.8% DM basis), which included alfalfa hay and each tested fat source. Treatments were 1) alfalfa hay + calcium soaps of palm oil fatty acid (Megalac-R; Church & Dwight Co. Inc., Princeton, NJ; **MEG**; 69.3 g DM/d of alfalfa hay plus 2.7 g DM/d of calcium soaps of palm oil fatty acid), 2) alfalfa hay + FS (**FLAX**; 68.4 g DM/d of alfalfa hay plus 3.6 g DM/d of ground FS), and 3) alfalfa hay + CS (**CHIA**; 68.04 g DM/d of alfalfa hay plus 3.96 g DM/d of ground CS). Individual ingredients, chemical composition, and dietary chemical composition are presented in Tables 1 and 2. Ground alfalfa and supplements were combined each feeding time. Alfalfa hay, which is a typical forage used for dairy cows in Nevada, was prepared from the second cutting at 10% bloom and was harvested on June 30, 2013, and then ground through a 2-mm screen in a Wiley Mill (model number 2; Arthur H. Thomas Co., Philadelphia, PA). Megalac-R had a guaranteed minimum fat analysis of 82.5% and calcium range from 8.8 to 10.6%. Flaxseed was purchased from a local animal feed store, and CS was purchased from Bob's Red Mill (Milwaukie, OR; serial number 3997800339). Samples of FS and CS were ground in Hamilton Beach coffee grinders (80335 Fresh Grind; Hamilton Beach Brands, Inc., Glen Allen, VA) until particle size equaled approximately 2 mm. Samples were then separately weighed and stored in a properly sealed plastic bag and refrigerated.

Dual-Flow Continuous Culture System

For this study, a dual-flow continuous culture system (Omni-Culture Plus; Virtis Co. Inc., Gardiner, NY) originally developed by Hoover et al. (1976) and recently modified by Del Bianco Benedeti et al. (2015) was used.

Ruminal fluid was collected approximately 2 h after morning feeding from 2 ruminally cannulated Aberdeen Angus steers consuming strictly alfalfa hay diets. The ruminal digesta was manually collected from the ventral, central, and dorsal areas of the rumen and was strained through 4 layers of cheesecloth, and ap-

Table 1. Individual ingredients and chemical composition

Item	Ingredient			
	Megalac-R[1]	Flaxseed	Chia seed	Alfalfa
DM, %	97.4	94.4	94.5	93.0
OM, % of DM	74.5	95.2	95.3	89.2
CP, % of DM	ND[2]	19.3	23.0	19.2
NDF, % of DM	0.3	23.4	38.7	35.6
ADF, % of DM	0.3	11.8	22.5	28.7
NFC,[3] % of DM	ND	23.1	0.3	32.4
EE,[4] % of DM	82.5	29.4	37.3	1.7
Fatty acids, % of DM	82.5	28.4	36.3	0.7
Fatty acids, % of total of fatty acids				
C4:0	ND	ND	ND	ND
C6:0	ND	ND	ND	ND
C8:0	0.1	ND	ND	ND
C10:0	ND	ND	ND	ND
C11:0	ND	ND	ND	ND
C12:0	0.4	ND	ND	0.3
C13:0	ND	ND	ND	ND
C14:0	1.7	ND	ND	0.5
C14:1	0.1	ND	ND	0.5
C15:0	0.1	ND	ND	0.6
C15:1	ND	4.3	4.5	19.3
C16:0	28.2	2.4	2.6	10.4
C16:1	0.0	0.1	0.2	1.6
C17:0	0.4	0.1	0.1	0.8
C17:1	0.1	0.1	ND	ND
C18:0	6.6	2.1	1.7	2.0
C18:1 *n*-9*t*	ND	ND	ND	0.7
C18:1 *n*-9*c*	33.9	7.9	3.2	0.9
C18:2 *n*-6*t*	ND	ND	0.1	1.9
C18:2 *n*-6*c*	25.1	21.6	23.1	16.1
C18:2 *t*10, *c*12	ND	ND	ND	ND
C18:2 *c*9, *t*11 (CLA)	ND	3.2	3.4	1.5
C20:0	0.5	0.1	0.2	0.7
C18:3 *n*-6	ND	0.3	0.5	ND
C20:1	0.3	0.2	0.2	12.8
C18:3 *n*-3	0.9	55.8	57.8	24.6
C21:0	ND	0.0	2.8	2.0
C20:2	ND	0.1	0.1	ND
C22:0	0.1	0.1	0.1	0.8
C20:3 *n*-6	ND	ND	ND	ND
C22:1 *n*-9	ND	0.1	ND	ND
C20:3 *n*-3	0.3	0.1	0.1	ND
C20:4 *n*-6	0.1	0.1	0.1	1.0
C22:2	0.1	ND	ND	ND
C24:0	0.1	0.1	0.1	1.0
C20:5 *n*-3 (EPA[5])	0.2	ND	ND	ND
C24:1	ND	0.1	ND	ND
C22:6 *n*-3 (DHA[5])	ND	ND	ND	ND
n-3[6]	1.4	55.9	57.9	24.6
n-6[7]	25.2	22.0	23.8	19.0
n-6:*n*-3 ratio	17.9	0.4	0.4	0.8
Total SFA	38.2	4.9	7.6	19.1
Total MUFA	34.4	12.7	8.1	35.8
Total PUFA	26.6	81.2	85.1	45.2

[1]Church & Dwight Co. Inc., Princeton, NJ.

[2]ND = not detected.

[3]NFC = nonfiber carbohydrates; NFC = 100 – (% NDF + % CP + % fat + % ash) + NDIN × 6.25, according to the NRC (2001).

[4]EE = ether extract.

[5]EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

[6]Sum of C18:3 *n*-3, C20:3 *n*-3, C20:5 *n*-3, and C22:6 *n*-3.

[7]Sum of C18:2 *n*-6*t*, C18:2 *n*-6*c*, C18:3 *n*-6, C20:3 *n*-6, and C20:4 *n*-6.

proximately 10 L of ruminal fluid was poured into the prewarmed insulated containers. The ruminal fluid was homogenized, bubbled with N_2 gas to maintain an anaerobic environment, and kept in water bath at 39°C. About 1,250 mL of liquid was then poured into each of the fermentation jars until it cleared the overflow spout.

The units also continuously received N2 gas at a rate of 40 mL/min and the temperature was maintained at 39°C. Fermenter contents were continuously stirred by a central propeller apparatus driven by magnets at the rate of 255 rpm. Artificial saliva was made as described by Weller and Pilgrim (1974); however, urea was not added. Saliva was continuously infused at 2 mL/min and saliva flow was measured twice daily for consistency. The solid mean retention time, solid dilution rate, and liquid dilution rate of fermenters were 24 h, 5%/h, and 10%/h, respectively, by regulating buffer input and filtrate removal. Individual pH controllers (model 5997-20; Cole-Parmer, Vernon Hills, IL) were used to monitor the pH of each fermenter, and the culture pH was maintained under constant control at 6.8 ± 0.05 using an automatic addition of 5 *N* NaOH or 3 *N* HCl when necessary.

Each fermenter was fed a total of 72 g DM/d divided in equal portions at 0200, 0600, 1000, 1400, 1800, and 2200 h to ensure proper functioning of the dual-flow continuous culture system.

Experimental Procedures and Sample Collections

Liquid and solid effluents were collected in 4-L plastic containers. During the first 7-d adaptation period, the weights of liquid and solid effluent output were recorded daily at 0600 h and discarded. After being emptied on Day 7 and during Days 8, 9, and 10, liquid and solid effluent containers were submerged approximately two-thirds of the way in a chilled (4°C) water bath, to prevent ruminal microbial fermentation. During the last 3 d of each period, liquid and solid effluent from each fermenter were combined and homogenized using a mixer (T25 basics; IKA Works, Inc., Wilmington,

Table 2. Ingredient and chemical composition of experimental diets

Item	Diet[1]		
	MEG	FLAX	CHIA
Ingredients, % DM			
Alfalfa hay	96.2	95.0	94.5
Fat supplement	3.8	5.0	5.5
Chemical composition			
DM, %	93.2	93.1	93.1
OM, % of DM	88.6	89.5	89.5
CP, % of DM	18.5	19.2	19.4
NDF, % of DM	37.1	37.9	38.7
ADF, % of DM	28.9	29.1	29.7
NFC,[2] % of DM	31.2	32.0	30.7
NDIN, % of DM	0.4	0.4	0.4
EE,[3] % of DM	4.8	3.1	3.7
Fatty acids, % of DM	3.8	2.1	2.7
ME,[4] Mcal/kg of DM	2.4	2.3	2.3
Fatty acids, % of total of fatty acids			
C4:0	ND[5]	ND	ND
C6:0	ND	ND	ND
C8:0	ND	ND	ND
C10:0	ND	ND	ND
C11:0	ND	ND	ND
C12:0	0.4	0.1	0.1
C13:0	ND	ND	ND
C14:0	1.5	0.2	0.1
C14:1	0.2	0.2	0.1
C15:0	0.2	0.2	0.2
C15:1	3.4	9.2	8.1
C16:0	25.2	5.0	4.5
C16:1	0.3	0.6	0.6
C17:0	0.5	0.3	0.2
C17:1	0.1	ND	ND
C18:0	5.8	2.1	1.8
C18:1 *n*-9*t*	0.1	0.2	0.2
C18:1 *n*-9*c*	28.1	5.7	2.6
C18:2 *n*-6*t*	0.4	0.6	0.5
C18:2 *n*-6*c*	23.6	20.0	21.2
C18:2 *t*10, *c*12	ND	ND	ND
C18:2 *c*9, *t*11 (CLA)	0.3	2.7	2.9
C20:0	0.5	0.3	0.3
C18:3 *n*-6	0.0	0.2	0.3
C20:1	2.5	4.2	3.3
C18:3 *n*-3	5.2	46.2	49.2
C21:0	0.4	0.7	2.6
C20:2	ND	0.1	0.1
C22:0	0.2	0.3	0.2
C20:3 *n*-6	ND	ND	ND
C22:1 *n*-9	ND	ND	ND
C20:3 *n*-3	0.3	0.1	0.1
C20:4 *n*-6	0.2	0.4	0.3
C22:2	ND	ND	ND
C24:0	0.2	0.4	0.3
C20:5 *n*-3 (EPA[6])	0.1	ND	ND
C24:1	ND	ND	ND
C22:6 *n*-3 (DHA[6])	ND	ND	ND
n-3[7]	5.5	46.3	49.2
n-6[8]	24.2	21.2	22.4
n-6:*n*-3 ratio	4.38	0.46	0.46
Total SFA	34.9	9.5	10.4
Total MUFA	34.8	20.1	14.9
Total PUFA	30.0	70.2	74.6

[1]MEG = alfalfa hay + calcium soaps of palm oil fatty acid (Megalac-R; Church & Dwight Co. Inc., Princeton, NJ); FLAX = alfalfa hay + flaxseed; CHIA = alfalfa hay + chia seed.

[2]NFC = nonfiber carbohydrates; NFC = 100 – (% NDF + % CP + % fat + % ash) + NDIN × 6.25, according to the NRC (2001).

[3]EE = ether extract.

[4]ME calculated according to the NRC (2001).

[5]ND = not detected.

[6]EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

[7]Sum of C18:3 *n*-3, C20:3 *n*-3, C20:5 *n*-3, and C22:6 *n*-3.

[8]Sum of C18:2 *n*-6*t*, C18:2 *n*-6*c*, C18:3 *n*-6, C20:3 *n*-6, and C20:4 *n*-6.

NC), for 30 s. After mixing, a 500-mL sample was collected via a vacuum system and stored at –20°C. An additional 20-mL effluent sample was squeezed through 2 layers of cheesecloth, and two 10-mL aliquots of fluid were preserved with 0.2 mL of 0.2 *N* sulfuric acid and swirled. Then, these samples were centrifuged for 10 min at 10,000 × *g* at 4°C, and the supernatant was frozen at –20°C for later analyses of NH_3–N and VFA.

The 500-mL effluent samples collected on each of the 3 collection days were composited by fermenter by period. The effluent composite (approximately 1,500 mL/fermenter per period) was handily mixed, and a 300-mL subsample was collected, freeze-dried, and ground using a pestle and mortar method. Then, the samples were placed in a plastic container for further analyses of DM, OM, ash, NDF, ADF, CP, ether extract, and total purines.

On the last day of each period, the entire fermenter content was used to harvest bacteria by mixing in a blender and straining through 2 layers of cheesecloth. Strained contents were centrifuged for 10 min at 1,000 × *g* at 4°C (Sorvall RC-5B Refrigerated Super speed Centrifuge; DuPont Instruments, Wilmington, DE) to remove feed particles. Bacteria were isolated by centrifuging for 20 min at 10,000 × *g* at 4°C (Bach et al., 2008) and prepared for analysis of purines by freeze-drying and grinding using a pestle and mortar method.

Chemical Analyses

Feed and effluent samples were analyzed for DM (method 934.01; AOAC, 1990), ash (method 938.08; AOAC, 1990), CP (method 984.13; AOAC, 1990),

ether extract (method 920.85; AOAC, 1990), and fatty acid content (O'Fallon et al., 2007). The OM was calculated as the difference between DM and ash contents. For NDF and ADF, samples were sequentially analyzed, being treated with thermostable α-amylase without sodium sulfite according to Van Soest et al. (1991) and adapted for the Ankom^{200} Fiber Analyzer (Ankom Technology, Macedon, NY).

The nonfiber carbohydrate (**NFC**) concentration of the alfalfa, Megalac-R, FS, and CS were calculated using the equation NFC = 100 – (% NDF + % CP + % fat + % ash) + NDIN × 6.25, according to the NRC (2001).

Samples of bacteria were analyzed for DM, CP, and ash as previously detailed for feed samples. Ruminal apparent and true digestibilities were calculated as described by Soder et al. (2013).

The effluent samples were analyzed for NH_3–N content (Chaney and Marbach, 1962), and VFA concentrations were determined using gas chromatography (Varian model 3800; Varian, Inc., Walnut Creek, CA; equipped with a glass column [180 cm by 4 mm i.d.]) packed with GP 10% SP-1200/1% H3PO4 on 80/100 Chromosorb WAW [Supelco Inc., Bellefonte, PA]), with N2 used as a carrier gas at a flow rate of 85 mL/min. The oven, injection port, and detector port temperatures were 125, 175, and 180°C, respectively.

Concentrations of total purines (Zinn and Owens, 1986) in effluent samples and isolated bacteria pellets were used to partition overflow N flow into bacterial and nonbacterial fractions and to calculate true DM and OM digestibilities and flows.

Extraction of fatty acids in samples of feed and effluent was performed using the direct fatty acid methyl ester (**FAME**) synthesis (O'Fallon et al., 2007). The internal standard used was nonadecanoic acid (C19:0; Sigma-Aldrich, Inc., St. Louis, MO). Composition of FAME was determined by capillary gas chromatography (Agilent Technologies 7890A GC system with Agilent Technologies 7693 Auto sampler and Agilent Technologies 5975C inert XL EI/CI MSD with Triple-Axis detector; Agilent Technologies, Inc., Santa Clara, CA). The capillary column is a SP-2560, 100 m by 0.25 mm by 0.20 μm film thickness (Supelco Inc.). The carrier gas was helium, and the flow rate was 20 cm/s. The oven temperature was programmed from 140°C for 5 min to 240°C at 4°C/min and then held at 240°C for 20 min. The source and analyzer temperature of the mass spectrophotometer was set at 260°C. Peaks were identified by comparison of retention times with FAME standards (FAME Mix 18919-1AMP [Supelco Inc.] and CLA standard, UC-60-M, and UC-61-M [Nu-Chek-Prep Inc., Elysian, MN]). Fatty acids are reported as percent of total fatty acids.

Table 3. Effect of fat supplementation in alfalfa hay–based diets on nutrient digestibility in dual-flow continuous culture

	Diet[1]				
Item	MEG	FLAX	CHIA	SEM	*P*-value
Ruminal apparent digestibility, %					
DM	27.8	29.2	27.1	2.6	0.80
OM	29.7	30.6	27.6	2.3	0.58
CP	30.1	32.1	26.3	3.1	0.33
NDF	42.8	41.1	35.4	4.4	0.21
ADF	46.6	45.6	41.0	4.1	0.35
Ruminal true digestibility, %					
DM	35.9	37.1	36.9	2.3	0.94
OM	37.5	37.7	36.9	2.7	0.96
CP	51.4	51.8	49.4	3.9	0.89

[1]MEG = alfalfa hay + calcium soaps of palm oil fatty acid (Megalac-R; Church & Dwight Co. Inc., Princeton, NJ); FLAX = alfalfa hay + flaxseed; CHIA = alfalfa hay + chia seed.

Statistical Analysis

Data were analyzed as a replicated 3 × 3 Latin square arrangement, using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC). The following model was used:

$$Y_{ijkl} = \mu + S_i + F(S)_{ij} + P_k + T_l + \varepsilon_{ijkl},$$

in which μ is the overall mean, S_i is the square, $F(S)_{ij}$ is the fermenter (F) within square, P_k is the period, T_l is the treatment, and ε_{ijkl} is the residual error associated with the i_{jkl}th observation. Fermenters within square were random effects whereas all other factors were fixed. Least squares means and SEM are reported for all data with a significance declared at $P \leq 0.05$.

RESULTS AND DISCUSSION

Diet Composition and Nutrient Digestibility

The MEG diet contained 3.8% (DM basis) Megalac-R as a ruminally inert fat source and had no inclusion of FS or CS. Diets were formulated to contain similar amounts of fat and also similar amounts of CP, NDF, NFC, and energy; this ensured that the observed effects were due to only different dietary fatty acids. Ruminal digestibilities of all nutrients did not differ ($P > 0.05$) among treatments (Table 3). Soder et al. (2013) observed a reduction in ruminal apparent digestibility of OM and NDF when using 10% FS in a forage-based diet in a continuous culture system. Typically, dietary lipid content of about 5% should not cause any harmful imbalance to the rumen environment and is not likely to negatively affect digestibility (Moran, 2005). In the present study, up to 4.8% total fat did not negatively

Table 4. Effect of fat supplementation in alfalfa hay–based diets on ruminal effluent fatty acid concentrations in dual-flow continuous culture

Fatty acids, % of total fatty acids	Diet[1]			SEM	*P*-value
	MEG	FLAX	CHIA		
C4:0	13.0[c]	20.5[a]	18.1[b]	0.71	<0.01
C6:0	1.40	2.10	2.00	0.22	0.12
C8:0	0.03[a]	0.01[b]	0.01[b]	0.01	0.04
C10:0	0.04	0.06	0.05	0.01	0.49
C11:0	0.13	0.16	0.16	0.01	0.06
C12:0	0.20	0.22	0.19	0.01	0.08
C13:0	0.18[c]	0.30[a]	0.25[b]	0.01	<0.01
C14:0	0.77[a]	0.64[b]	0.58[c]	0.01	<0.01
C14:1	0.94[c]	1.55[a]	1.42[b]	0.03	<0.01
C15:0	1.34[c]	2.14[a]	1.94[b]	0.05	<0.01
C15:1	28.8[a]	13.6[b]	14.0[b]	0.37	<0.01
C16:0	16.1[a]	7.40[b]	7.55[b]	0.20	<0.01
C16:1	0.37[b]	0.51[a]	0.51[a]	0.02	<0.01
C17:0	0.68[b]	1.02[a]	1.00[a]	0.01	<0.01
C17:1	ND[3]	ND	ND		
C18:0	15.1[c]	29.9[b]	35.7[a]	0.81	<0.01
C18:1 *n*-9*t*	0.38[b]	0.50[a]	0.54[a]	0.04	0.05
C18:1 *n*-9*c*	9.19[a]	2.32[b]	0.85[c]	0.29	<0.01
C18:2 *n*-6*t*	1.07[a]	0.62[b]	0.54[b]	0.03	<0.01
C18:2 *n*-6*c*	4.34[a]	4.00[ab]	3.57[b]	0.22	0.03
C18:2 *t*10, *c*12	ND	ND	ND		
C18:2, *c*9, *t*11 (CLA)	0.03	0.04	0.05	0.01	0.11
C20:0	0.50[a]	0.67[b]	0.68[b]	0.01	<0.01
C18:3 *n*-6	ND	ND	ND		
C20:1	1.17[b]	2.83[a]	1.45[b]	0.21	<0.01
C18:3 *n*-3	1.70[b]	4.72[a]	4.13[a]	0.27	<0.01
C21:0	0.41[c]	0.77[a]	0.59[b]	0.04	<0.01
C20:2	0.57[b]	0.72[a]	0.60[b]	0.03	0.02
C22:0	0.44[c]	0.73[a]	0.68[b]	0.01	<0.01
C20:3 *n*-6	ND	0.12[b]	0.67[a]	0.07	<0.01
C22:1 *n*-9	0.04[c]	0.26[b]	0.62[a]	0.08	<0.01
C20:3 *n*-3	ND	ND	ND		
C20:4 *n*-6	0.50[c]	0.80[a]	0.72[b]	0.02	<0.01
C22:2	ND	ND	ND		
C24:0	0.56[c]	0.91[a]	0.80[b]	0.02	<0.01
C20:5 *n*-3 (EPA[2])	ND	ND	ND		
C24:1	ND	ND	0.3	0.07	0.02
C22:6 *n*-3 (DHA[2])	ND	ND	ND		
n-3[4]	1.70[b]	4.71[a]	4.12[a]	0.27	<0.01
n-6[5]	5.90	5.53	5.49	0.09	0.22
n-6:*n*-3 ratio	3.50[b]	1.17[a]	1.35[a]	0.14	<0.01
Total SFA	50.9[c]	67.4[b]	70.2[a]	0.90	<0.01
Total MUFA	40.9[a]	21.5[b]	19.7[c]	0.57	<0.01
Total PUFA	8.21[b]	10.3[a]	10.3[a]	0.48	<0.01

[a–c]Least squares means within the same row with different superscripts differ ($P \leq 0.05$).

[1]MEG = alfalfa hay + calcium soaps of palm oil fatty acid (Megalac-R; Church & Dwight Co. Inc., Princeton, NJ); FLAX = alfalfa hay + flaxseed; CHIA = alfalfa hay + chia seed.

[2]EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

[3]ND = not detected.

[4]Sum of C18:3 *n*-3, C20:3 *n*-3, C20:5 *n*-3, and C22:6 *n*-3.

[5]Sum of C18:2 *n*-6*t*, C18:2 *n*-6*c*, C18:3 *n*-6, C20:3 *n*-6, and C20:4 *n*-6.

affect digestibilities of DM, OM, CP, NDF, and ADF. This indicates that both FS and CS can potentially be fed to ruminants at 5.0 and 5.5%, respectively, as long as the total diet does not contain more than 5% total fat.

Effects of Treatments on Individual Fatty Acid Concentration

Concentrations of fatty acids in the ruminal effluent are presented in Table 4. As expected, supplementation with FS and CS profoundly changed ruminal fatty acid concentrations when compared with the MEG diet.

Concentration of C16:0 was greater for MEG when compared with FLAX and CHIA. This was most likely due to the greater percentage of C16:0 in the MEG diet (Table 2). After fermentation, MEG continued to have greater quantities of C16:0 when compared with FLAX and CHIA, which was expected. According to the World Health Organization (2003), there is evidence that consumption of C16:0 increases low-density lipoprotein cholesterol in humans and is associated with cardiovascular problems in humans (Noakes et al., 1996); therefore, alternative fat sources, such as FS and CS, containing less C16:0, could be healthier fat choices. Literature on the effects of CS on fatty acid ruminal metabolism is nonexistent and this may be the first study to report that.

In general, CS supplementation affected fatty acid ruminal metabolism similarly to FS supplementation. There was an increase in C18:0 ruminal concentration ($P < 0.01$) when FS and CS were supplemented in the diet compared with Megalac-R. These findings are consistent with ruminal biohydrogenation of dietary PUFA. According to Wilde and Dawson (1966), the initial step of the metabolism of C18:3 *n*-3 to C18:0 is the isomerization of the *cis*-12 bond to either the D11 or D13 position. Thereafter, one of the bonds is hydrogenated to leave a C18:2 followed by hydrogenation of another bond producing a C18:1 intermediate. Hydrogenation of the C18:1 yields C18:0 as the final product. Megalac-R supplementation increased ruminal concentration of C18:1 *n*-9*c*, C18:2 *n*-6*t*, and C18:2 *n*-6*c* compared with FS and CS supplementation ($P < 0.01$). The greater ruminal concentration of C18:1 *n*-9*c* in fermenters fed MEG was expected because this fatty acid had greater concentration in this diet. However, the greater ruminal concentration of C18:2 *n*-6*t* ($P < 0.01$) and C18:2 *n*-6*c* ($P = 0.03$) in the MEG diet may indicate that fatty acids contained in Megalac-R may be partially protected from rumen biohydrogenation.

Compared with the control diet, ruminal concentration of C18:3*n*-3 increased when both FS and CS were fed ($P < 0.01$). Caroprese et al. (2010) fed cows

whole FS at 6.5% of the diet and observed an increase in C18:0 and C18:3 *n*-3 and a decrease in C16:0 in milk. Petit (2003) reported that C18:3 *n*-3 in milk was greater for cows fed diets based on whole FS (9.7% of dietary DM) compared with cows consuming diets with Ca salts of palm oil, whole sunflower seed, or no fat supplement. These results are in agreement with the present study, indicating that both CS and FS supplementation improve dietary fatty acid composition and may positively affect milk fatty acid profile, considering that ruminal escape of C18:3 *n*-3 may also increase the proportion of this fatty acid in milk. Consumption of PUFA has been shown to possess several human health benefits; in particular, they have been shown to decrease the incidence of cardiovascular diseases, hypertension, and arthritis (Simopoulos, 2002); therefore, the increase in C18:3 *n*-3, C20:4 *n*-6, and total PUFA concentrations ($P < 0.01$) observed in the present study when both CS and FS were fed may have health benefits for humans. To our knowledge, this is the first time that CS fatty acid ruminal metabolism data is presented.

According to Mann and Truswell (2007), linoleic acid (C18:2) and α-linolenic acid (**ALA**; C18:3) are referred to as the essential fatty acids. They can be precursors of important fatty acids such as eicosapentaenoic acid (**EPA**; C20:5 *n*-3) and docosahexaenoic acid (**DHA**; C22:6 *n*-3). Both EPA and DHA have been shown to be beneficial to humans (Adkins and Kelley, 2010). In the body, linoleic acid can be converted to arachidonic acid (C20:4 *n*-6), whereas α-linolenic acid can be metabolized to yield EPA, which further undergoes elongation, desaturation, and α oxidation to produce DHA (Adkins and Kelley, 2010). Both EPA and DHA ruminal concentration were not detected in our study, which was expected because these fatty acids are found mainly in fish oil and edible seaweed; however, both CS and FS supplementation increased ($P < 0.01$) C20:4 *n*-6 concentration in the rumen, which may be beneficial to human health. Moreover, according to Sim (1998), a *n*-6:*n*-3 fatty acid ratio less than 4:1 is the ideal ratio to reduce potential risk of coronary heart diseases and improve human health; in the present study, both FS and CS reduced ($P < 0.01$) the *n*-6:*n*-3 fatty acid ratio and both FS and CS increased the *n*-3 fatty acid concentration in the rumen.

Total SFA concentration was lower ($P < 0.01$) in the control group, indicating that both CS and FS fatty acids were subjected to extensive biohydrogenation in the rumen; C18:0 accounted for the majority of the SFA, which is consistent with the literature (da Silva-Kazama et al., 2011). Total MUFA concentration was greater ($P < 0.01$) in the control group and this was largely influenced by C15:1 and by C18:1 *n*-9*c*, which is an intermediate of C18:2 biohydrogenation. Compared with the control, FS and CS supplementation increased ($P < 0.01$) total PUFA concentration in the rumen, indicating that both FS and CS dietary supplementation may improve the ruminal fatty acid profile.

Table 5. Effect of fat supplementation in alfalfa hay–based diets on nitrogen metabolism in dual-flow continuous culture

	Diet[1]				
Item	MEG	FLAX	CHIA	SEM	*P*-value
NH_3–N, mg/dL	17.2	15.9	18.8	1.2	0.17
N flows, g/d					
Total N	1.50	1.50	1.66	0.1	0.16
NH_3–N	0.43	0.40	0.47	0.0	0.16
Non-NH_3–N	1.05	1.10	1.17	0.1	0.37
Microbial N	0.45	0.43	0.51	0.1	0.71
Dietary N	0.61	0.66	0.66	0.1	0.88
EMPS[2]	18.9	17.9	20.9	2.7	0.70

[1]MEG = alfalfa hay + calcium soaps of palm oil fatty acid (Megalac-R; Church & Dwight Co. Inc., Princeton, NJ); FLAX = alfalfa hay + flaxseed; CHIA = alfalfa hay + chia seed.

[2]EMPS = efficiency of microbial protein synthesis (g of bacterial N/kg of OM truly digested).

Effect of Treatments on N Metabolism

There were no differences ($P > 0.05$) among treatments for NH_3–N concentration; efficiency of microbial protein synthesis; and flows of total N, non-NH_3–N, microbial N, and dietary N (Table 5). The NH3–N concentration found in this study is similar to that in previously reported studies. Soder et al. (2013) reported 21.3 mg/dL when FS was fed at 10% of the diet (DM basis) in continuous culture. In an in vivo experiment, Neveu et al. (2014) reported 16.0 mg/dL when an extruded FS product consisted of 75% FS and 25% ground alfalfa meal was fed at 10% of the diet (DM basis) of dairy cows. Beauchemin et al. (2009) reported 17.6 mg/dL when FS was supplemented at 9.32% of the diet (DM basis) of lactating dairy cows.

Earlier studies (Satter and Slyter, 1974; Kang-Meznarich and Broderick, 1980; Brito et al., 2006) suggested that ruminal NH3–N concentration below 8.5 mg of N/dL could potentially depress microbial N synthesis in the rumen. The mean NH3–N concentration observed (17.3 mg of N/dL) across treatments in this study indicates that NH3–N availability did not impair microbial growth.

Table 6. Effect of fat supplementation in alfalfa hay–based diets on VFA concentration in dual-flow continuous culture

	Diet[1]				
Item	MEG	FLAX	CHIA	SEM	*P*-value
Total VFA, m*M*	125.6	119.1	116.4	5.3	0.48
Individual, % of total VFA					
Acetate	79.6	79.3	78.6	0.4	0.27
Propionate	12.6	12.7	13.2	0.2	0.17
Butyrate	4.8	5.1	5.2	0.2	0.55
Isobutyrate	0.7	0.6	0.7	0.1	0.32
Valerate	1.4	1.4	1.5	0.1	0.16
Isovalerate	0.9	0.9	0.8	0.1	0.94
Acetate:propionate ratio	6.4	6.3	6.0	0.1	0.13
Total BCVFA,[2] m*M*	3.5	3.6	3.9	0.2	0.52

[1]MEG = alfalfa hay + calcium soaps of palm oil fatty acid (Megalac-R; Church & Dwight Co. Inc., Princeton, NJ); FLAX = alfalfa hay + flaxseed; CHIA = alfalfa hay + chia seed.

[2]BCVFA = branched-chain VFA.

Effect of Treatments on VFA Concentration

Total VFA concentration (average 120.3 m*M*) of the present experiment was similar to previous published studies. Qiu et al. (2004) reported an average of 121.5 m*M* when evaluating the effects of different solid dilution rates, pH, and linoleic acid concentration in a continuous culture system. In an in vivo experiment, Neveu et al. (2014) reported an average of 122.0 m*M* when an extruded FS product consisted of 75% FS and 25% ground alfalfa meal was fed at 10% of the diet of dairy cows.

Treatments did not affect ($P > 0.05$) total VFA concentration and molar proportions of acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, and branched-chain VFA as well as the acetate:propionate ratio (Table 6). The results of the present study are consistent with the findings from Côrtes et al. (2010), which showed that feeding FS at 4.1% of the diet had no effect on total VFA concentration and molar proportions of individual VFA. However, Gonthier et al. (2004) reported that feeding FS at a greater level (12.5% of the diet) reduced molar proportions of acetate and increased molar proportions of propionate. In agreement with that, Soder et al. (2013) reported a decrease in the acetate:propionate ratio when 10% FS was supplemented in a forage-based diet in a continuous culture system and Neveu et al. (2014) reported a decrease in the acetate:propionate ratio when extruded FS was fed at 12.5% of the diet to dairy cows. Therefore, it seems that dietary oilseed inclusion levels need to be greater than 10% to promote the changes in the fermentation pattern observed in other studies.

Conclusions

In conclusion, literature on the effects of CS supplementation on ruminal metabolism is nonexistent and this may be the first study to report such findings. Supplementation of FS and CS in an alfalfa hay–based diet did not impair ruminal fermentation characteristics, digestibility, microbial efficiency, and ruminal N metabolism. Supplementation of FS at 5% or CS at 5.5% of the diet increased the concentrations of C18:3*n*-3, C20:4 *n*-6, and total PUFA when compared with the control treatment. Concentration of C18:2 *c*9, *t*11 (CLA) was not different among treatments. Both CS and FS treatments had greater ruminal concentrations of C18:0, indicating that both CS and FS fatty acids were extensively biohydrogenated in the rumen. Results from this study suggest that CS appears to be as effective as FS as a fat source when added to ruminants' diets using a dual-flow continuous culture system.

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