Ruminal biohydrogenation and abomasal flow of fatty acids in lactating cows fed diets supplemented with soybean oil, whole soybeans, or calcium salts of fatty acids

J. E. Freitas Jr.,* C. S. Takiya,† T. A. Del Valle,† R. V. Barletta,† B. C. Venturelli,† T. H. A. Vendramini,† R. D. Mingoti,† G. D. Calomeni,† R. Gardinal,† J. R. Gandra,‡ V. P. Bettero,§ E. Ferreira de Jesus,§ M. D. S. Oliveira,§ and F. P. Rennó¹

*Department of Animal Science, Federal University of Bahia, Salvador, Brazil, 0170-110

†Department of Animal Nutrition and Animal Production, University of São Paulo, Pirassununga, Brazil, 13635-900

‡Department of Animal Science, Federal University of Grande Dourados, Dourados, Brazil, 79825-070

§Department of Animal Science, Sao Paulo State University, Jaboticabal, Brazil, 14884-900

ABSTRACT

Ruminants have a unique metabolism and digestion of unsaturated fatty acids (UFA). Unlike monogastric animals, the fatty acid (FA) profile ingested by ruminants is not the same as that reaching the small intestine. The objective of this study was to evaluate whole raw soybeans (WS) in diets as a replacer for calcium salts of fatty acids (CSFA) in terms of UFA profile in the abomasal digesta of early- to mid-lactation cows. Eight Holstein cows (80 ± 20 d in milk, 22.9 ± 0.69 kg/d of milk yield, and 580 ± 20 kg of body weight; mean $\pm$ standard deviation) with ruminal and abomasal cannulas were used in a 4×4 Latin square experiment with 22-d periods. The experiment evaluated different fat sources rich in linoleic acid on ruminal kinetics, ruminal fermentation, FA abomasal flow, and milk FA profile of cows assigned to treatment sequences containing a control (CON), with no fat source; soybean oil, added at 2.68% of diet dry matter (DM); WS, addition of WS at 14.3% of diet DM; and CSFA, addition of CSFA at 2.68% of diet DM. Dietary fat supplementation had no effect on nutrient intake and digestibility, with the exception of ether extract. Cows fed fat sources tended to have lower milk fat concentration than those fed CON. In general, diets containing fat sources tended to decrease ruminal neutral detergent fiber digestibility in relation to CON. Cows fed WS had lower ruminal digestibility of DM and higher abomasal flow of DM in comparison to cows fed CSFA. As expected, diets containing fat supplements increased FA abomasal flow of C18:0 and total FA. Cows fed WS tended to present a higher concentration of UFA in milk when compared

with those fed CSFA. This study suggests that under some circumstances, abomasal flow of UFA in early lactation cows can be increased by supplementing their diet with fat supplements rich in linoleic acid, regardless of rumen protection, with small effects on ruminal DM digestibility.

Key words: fat source, linoleic acid, milk fatty acid profile, ruminal digestibility

INTRODUCTION

Polyunsaturated fatty acids are an efficient source of energy and have been used as a tool to modulate the metabolism, reproduction, and immune system of dairy cows (Gandra et al., 2016a,b; Gardinal et al., 2018a,b). In addition, dietary supplementation of PUFA to ruminants has aimed to produce dairy products able to decrease the risk of cardiovascular diseases and obesity in humans (Bauman et al., 2006; Santos et al., 2017; Shokryzadan et al., 2017). However, ruminants are singular in terms of fatty acid (FA) digestion because, unlike monogastric animals, the FA profile ingested by ruminants is not the same as that reaching and absorbed from the small intestine. Rumen microorganisms hydrogenate double bonds within carbon chains to minimize toxic effects of PUFA on rumen bacteria (Oldick and Firkins, 2000), thus limiting the amount of PUFA absorbed by ruminants.

Providing dairy cow diets with rumen-protected fat sources such as calcium salts of fatty acids (CSFA) and oilseeds has increased the amount of PUFA reaching the small intestine. The protein complex surrounding the cotyledon in seeds protects their lipid content from enzymatic biohydrogenation (BH; Doreau et al., 2016). In addition, dietary supplementation of dairy cows with whole flaxseed or whole raw soybeans (WS) has increased milk content of PUFA (Chilliard et al.,

Received August 10, 2017.

Accepted April 13, 2018.

¹Corresponding author: francisco.renno@usp.br

2009; Venturelli et al., 2015). Furthermore, raw oilseeds might be more economically feasible than other PUFA sources, such as CSFA, because they do not undergo industrial processes, thus decreasing nutrient wastes and costs associated with electricity or fuel utilization.

Ruminal passage rate is one of the most important factors determining the extent of ruminal BH of UFA (Bettero et al., 2017), whereas the lipid FA profile determines the duodenal FA profile (Jenkins and Bridges, 2007). Our research group has published data regarding the ruminal outflow of FA in dry and mid- to late-lactation cows fed different sources rich in linoleic acid. Dietary supplementation of WS to dry cows reduced ruminal passage rate of DM in comparison with cows fed CSFA (2.38 vs. 2.91%/h, respectively) but did not alter the abomasal flow of C18:2 (Bettero et al., 2017). On the other hand, mid- to late-lactation cows fed WS and CSFA had similar ruminal passage rate of DM (3.1 vs. 3.0%/h), whereas cows fed WS tended to have a greater abomasal flow of C18:2 than those fed CSFA (Barletta et al., 2016). Early- to mid-lactation cows have a relatively high ruminal passage rate of DM, which would influence the response to dietary supplementation of WS and CSFA on abomasal flow of FA.

Gandra et al. (2016b) reported that transition cows fed WS had a higher percentage of neutrophils positive for phagocytosis of *Staphylococcus aureus* in comparison to those fed CSFA. The positive effects of WS supplementation on the immune system and milk FA profile of early-lactating cows (Gandra et al., 2016a,b; Gardinal et al., 2018a,b) could be supported by an increase in specific FA reaching the small intestine, such as linoleic acid and CLA. Although the ruminal outflow of FA was studied in lactating cows fed WS (Tice et al., 1993, 1994), the authors failed to describe DIM of cows. Thus, literature still lack data regarding the ruminal outflow of FA in early- to mid-lactation cows fed WS.

This study was designed to determine the influence of dietary supplementation of soybean oil (SO), WS, and CSFA on ruminal BH and FA profile reaching the small intestine in early- to mid-lactating cows. We hypothesized that cows fed SO (unprotected oil) would exhibit greater BH extent and lower UFA abomasal flow in comparison with cows fed either WS or CSFA (protected oils), whereas we expect similar BH extent and profile of FA in abomasum of cows fed protected oils.

MATERIALS AND METHODS

The experimental procedures were approved by the Ethics Committee of the School of Veterinary Medicine

and Animal Science of the University of São Paulo, São Paulo, Brazil (approval #1603/2009).

Animals, Experimental Design, and Diets

Eight multiparous Holstein cows (80 ± 20 DIM, 22.9 ± 0.69 kg/d of milk yield, 580 ± 20 kg of BW; mean $\pm$ SD), with ruminal and abomasal cannulas, were allocated to a replicated 4×4 Latin square experiment with 22-d periods, of which 10 d allowed for diet adaptation and 12 d for sampling. Dietary treatments were (1) control (CON), no dietary fat source; (2) SO, added at 2.68% (DM basis); (3) WS, added at 14.3%; and (4) CSFA (Megalac-E, Church & Dwight Co. Inc., Trenton, NJ), added at 2.68%. Diets were formulated based on NRC (2001) recommendations (Table 1), supplied twice daily (0600 and 1400 h) as TMR, and aiming refusals between 5 and 10% on an as-fed basis. Formulation of diets with fat sources (SO, WS, and CSFA) targeted a dietary FA content of 45 g/kg. Corn silage DM was assessed weekly for dietary adjustments when necessary. Treatment sequences were randomly distributed to cows.

Corn silage and concentrate ingredients were collected during each sampling period and pooled into a composite sample per period. Samples were dried in a forced-air oven (55°C during 72 h) and ground in a Wiley mill (1-mm screen). Samples were analyzed for NDF using heat-stable α -amylase (Van Soest et al., 1991), DM (method #930.15), ash (method #942.05), ether extract (EE; method #920.39), and CP (method #984.13) according to AOAC International (2000). Nonfiber carbohydrate content was estimated according to Hall (2000), as follows: $\text{NFC} = 1,000 - [(\text{CP} - \text{CP from urea} + \text{urea}) + \text{EE} + \text{ash} + \text{NDF}]$, all values expressed in grams per kilogram. To determine indigestible NDF (iNDF), samples were ground in a Wiley mill (2-mm screen), placed in nonwoven textile bags tissue (5×5 cm, pore size $50 \mu\text{m}$, 100 g/m^2). Bags with ground samples were incubated for 288 h (Huhtanen et al., 1994) in the rumen of 2 dry cows, previously adapted to the CON diet as described by Casali et al. (2008). Afterward, samples were removed from the rumen, washed in running tap water, and analyzed for NDF concentration as previously described.

Feed samples for FA profile analyses were lyophilized and ground (1-mm screen) with liquid nitrogen to avoid changes in FA profile. Lipid extraction was performed according to Folch et al. (1957) and methylated according to Kramer et al. (1997). Briefly, the lipids were extracted after homogenizing samples with a chloroform and methanol solution (2:1, vol/vol). Lipids were

collected after samples were mixed with NaCl solution (1.5%) and centrifuged for 20 min at $650 \times g$ at 4°C . Methyl esters from lipids were formed by adding 2 mL of C19:0 standard solution (as free FA at 1 mg/mL) and 2 mL of sodium methoxide (0.5 M) to lipids, then samples were placed in water bath (50°C) for 10 min. Samples were cooled and 3 mL of methanolic HCl solution (9:1 vol:vol, 3 mL) was added, and samples were vortexed and placed in a water bath (80°C) for 10 min. After samples reached room temperature, 1 mL of hexane was added, and content transferred to Falcon tubes containing 10 mL of potassium carbonate solution (6%). Falcon tubes were vortexed, centrifuged (5 min, at $250 \times g$), and the organic layer was transferred to test tubes containing 50 mg of sodium sulfide and activated charcoal. Test tubes were centrifuged for 5 min ($250 \times g$, 4°C), rested on ice for 30 min, and the supernatant pipetted into vials for GC analysis.

Methyl-FA were identified and quantified using a gas chromatograph (GC Shimadzu 2010 with automatic injection, Shimadzu Corporation, Kyoto, Japan) equipped with a SP-2560 capillary column (100 m $\times$ 0.25 mm i.d. with 0.02 μm film thickness; Supelco Sigma-Aldrich Group, Bellefonte, PA). The following standards were used to identify methyl-FA: C4–C24 (TM 37, Supelco Sigma-Aldrich Group); *trans*-11 C18:1 (V038-1G, Supelco Sigma-Aldrich Group); *trans*-10, *cis*-12 C18:2 (UC-61M 100 mg, Nu-Chek Prep Inc., Waterville, MN); and *cis*-9, *trans*-11 C18:2 (UC-60M 100 mg, Nu-Chek Prep Inc.).

Nutrient Digestibility

Refusals from each animal were daily weighed at 0600 h to determine feed intake. Samples of refusals were collected through the sampling period and composited to per period and cow for further chemical analyses. Refusals were analyzed for DM, NDF, iNDF, CP, EE, and FA, as previously described. Potentially digestible NDF (**pdNDF**) was calculated as the difference between NDF and iNDF values. Daily DM fecal excretion was estimated based on chromic oxide concentration in feces as described in Harvatine and Allen (2006a). Briefly, 5 g of chromic oxide (Cr_2O_3) was provided through ruminal cannula at 0700, 1500, and 2300 h from d 7 until d 14. Fecal samples were collected directly from the rectum of each cow every 9 h on d 12, 13, and 14 of each period and composited (on a wet basis) into one sample per cow per period. Feces were analyzed for DM and NDF content as described earlier. Chromic oxide was quantified by flame atomic absorption spectrometry (SpectraAA 220, Varian, Victoria, Australia) according to the manufacturer's recommendations. Dry matter

and NDF digestibility were calculated based on feed intake, total daily fecal excretion, and their concentration in feces, as described below:

$$\text{Fecal excretion (kg/d)} = \frac{\text{Chromic oxide dosed daily (g/d)}}{\text{Chromic oxide fecal concentration (g/kg)'}}$$

$$\text{Digestibility coefficient (g/kg)} = \frac{(\text{Intake (kg/d)} - \text{Fecal excretion (kg/d)})}{\text{Intake (kg/d)}} \times 1,000.$$

Table 1. Ingredients, chemical composition, and fatty acid profile of experimental diets

Item	Diet ¹			
	CON	SO	WS	CSFA
Ingredient (% DM)				
Corn silage ²	55.5	55.3	55.3	55.4
Ground corn	27.9	24.9	22.4	25.4
Soybean meal	13.4	13.9	5.19	13.9
Soybean oil		2.68		
Whole raw soybean			14.3	
Calcium salts of fatty acids ³				2.68
Sodium bicarbonate	0.80	0.80	0.80	0.80
Urea	0.62	0.63	0.18	0.62
Limestone	0.62	0.63	0.63	
Dicalcium phosphate	0.53	0.54	0.54	0.54
Salt	0.27	0.27	0.27	0.27
Vitamin and mineral mix ⁴	0.18	0.18	0.18	0.18
Ammonium sulfate	0.09	0.09	0.09	0.09
Magnesium oxide	0.09	0.09	0.09	0.09
Chemical (% DM unless otherwise stated)				
DM (g/kg)	49.7	49.8	49.9	49.8
OM	89.6	89.6	89.5	89.8
NFC ⁵	37.4	35.2	34.4	35.6
NDF	34.8	34.4	35.3	34.5
Indigestible NDF	10.7	10.6	10.6	10.6
CP	16.1	16.1	15.8	16.2
Ether extract	2.81	5.39	4.73	5.03
Fatty acid	2.45	4.50	4.43	4.53
Calcium	0.70	0.70	0.70	0.75
Fatty acid profile (%)				
C16:0	15.5	13.0	14.3	12.0
C18:0	3.24	6.30	9.32	3.68
<i>cis</i> -9 C18:1	12.8	17.1	13.5	17.3
<i>cis</i> -9, <i>cis</i> -12 C18:2	31.4	39.6	33.2	36.8
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	6.26	5.58	5.22	4.86

¹Diets: control (CON), whole raw soybean (WS), soybean oil (SO), and calcium salts of unsaturated fatty acids (CSFA).

²Corn silage average composition (% DM): 36.7 DM, 47.7 NDF, 8.02 CP, 10.7 indigestible NDF, and 7.40 ash.

³Megalac-E (Church & Dwight Co. Inc., Trenton, NJ): 11.5% of calcium.

⁴Contained per kilogram: 120 g of Ca, 73 g of P, 30 g of S, 44 g of Mg, 340 mg of Cu, 1,350 mg of Zn, 940 mg of Mn, 3 mg of Co, 16 mg of I, 10 mg of Se, 1,064 mg of Fe, 100,000 IU of vitamin A, 40,000 IU of vitamin D, and 60 IU of vitamin E.

⁵Nonfiber carbohydrate (% DM) = $100 - [(\text{CP} - \text{CP from urea} + \text{urea}) + \text{ether extract} + \text{ash} + \text{NDF}]$, from Hall (2000).

Milk Yield and Composition

Cows were milked twice daily (0600 and 1530 h), milk samples were collected from each cow on d 16, 17, and 18 of each experimental period and analyzed for fat and protein content by infrared methodology (Lactoscan, Entelbra, Sao Paulo, Brazil). Milk samples were deproteinized according to Broderick and Clayton (1997), analyzed for milk urea nitrogen by a colorimetric method through commercial kits (Bioclin, Belo Horizonte, Brazil) and absorbance measured in a semi-automatic biochemistry analyzer (SBA-200, CELM, Sao Caetano do Sul, Brazil). The FCM was estimated as follows: $3.5\% \text{ FCM} = (0.432 + 0.165 \times \text{milk fat}) \times \text{milk yield (kg/d)}$, as described by Sklan et al. (1992). Lipid extraction from milk samples was performed according to Feng et al. (2004) and methylated according to Kramer et al. (1997). Methyl-FA were quantified by GC, as previously described.

Ruminal Dynamics

Rumen was evacuated on d 20 and 21 at 1300 and 0700 h, respectively. Rumen digesta was weighted out and volume was measured (Harvatine and Allen, 2006a). During the evacuation, 10% aliquots were sampled to represent ruminal pool and frozen for further chemical analysis. Samples were analyzed for DM, NDF, and iNDF. Indigestible NDF was used as a marker to calculate abomasal DM flow, as follows:

$$\text{Abomasal DM flow (g/d)} = \frac{\text{iNDF intake (g/d)}}{\text{iNDF abomasal concentration (g/g)}}.$$

Samples of abomasal digesta (1,000 g) were collected through simple T-cannulas inserted 10 cm from the pylorus (Leão and Coelho da Silva, 1980) every 9 h from d 12 until d 14 (8 samples; 0600, 1500, and 2400 h on d 12; 0900 and 1800 h on d 13; and 0300, 1200, and 2100 h on d 14). Samples of abomasal digesta were composited into one sample per cow per period. Indigestible NDF concentration in abomasal digesta was assessed as previously described. Ruminal nutrient digestibility was calculated based on nutrient intake, abomasal flow, and concentration in abomasal digesta. Ruminal turnover and digestion and passage rates were calculated according to the following equations (Oba and Allen, 2003):

$$\text{Ruminal turnover rate (\%/h)} = \left(\frac{\text{Intake of component (kg/d)}}{\text{Ruminal pool of component (kg)}} \right) \times \left(\frac{100\%}{24 \text{ h/d}} \right),$$

$$\text{Passage rate (\%/h)} =$$

$$\frac{\text{Abomasal flow of component (kg/d)}}{\text{Ruminal pool of component (kg)}} \times \left(\frac{100\%}{24 \text{ h/d}} \right),$$

$$\text{Digestion rate (\%/h)} = \text{ruminal turnover rate (\%/h)} - \text{passage rate from rumen (\%/h)}.$$

Ruminal Fermentation

Ruminal fluid samples (straining digesta from 5 different sites of the rumen) were collected on d 18 of each experimental period, before (time 0), and 2, 4, 6, 8, 10, and 12 h relative to the morning feeding. After sampling, pH of ruminal fluid was measured using a digital pH meter (MB-10, Marte Cientifica, Santa Rita do Sapucaí, Brazil). Aliquots from ruminal fluid samples (1.6 mL) were mixed with methanoic acid (98–100% H_2CO_2 , 400 μL), centrifuged at $7,000 \times g$ for 15 min at 4°C , and the supernatant of each sample was frozen for subsequent VFA analysis. Other aliquots from ruminal fluid samples (2 mL) were mixed with 1 mL of sulfuric acid (0.5 M) and frozen for $\text{NH}_3\text{-N}$ analysis using the colorimetric phenol-hypochlorite method (Broderick and Kang, 1980). Ruminal concentration of VFA was determined according to Erwin et al. (1961) using a gas chromatograph (GC-2014, Shimadzu Corporation) equipped with a capillary column (Stabilwax, Restek, Bellefonte, PA). The gases used in the analyses were helium (8.01 mL/min flow) as the carrier gas, hydrogen (pressure of 60 kPa) as the fuel gas, and synthetic air (pressure of 40 kPa) as the oxidizer gas. The steamer temperature was set at 220°C , the ionization detector flames at 250°C , and the separation column at 145°C for 3 min, which was raised by $10^\circ\text{C}/\text{min}$ until it reached 200°C .

Biohydrogenation Extent

Samples of refusals, feces, ruminal digesta, and abomasal digesta were ground (1-mm screen) in a knives mill with liquid nitrogen and lyophilized, as previously described. Lipid extraction and methylation were performed according to Folch et al. (1957) and Kramer et al. (1997), respectively. Fatty acids were determined using a gas chromatograph (GC Shimadzu, Shimadzu Corporation), as previously described. Biohydrogenation extent (BE) was calculated according to Jenkins and Bridges (2007) as follows:

$$\text{BE (g/kg)} = \frac{\text{UFA intake (g/d)} - \text{UFA abomasal flow (g/d)}}{\text{UFA intake (kg/d)}}.$$

Statistical Analysis

Data were submitted to MIXED procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC) according to the following model:

$$Y_{ijkl} = \mu + S_i + c_j(S_i) + P_k + D_l + e_{ijkl}$$

where Y_{ijkl} is the dependent variable, μ is the overall mean, S_i is the fixed effect of square (cow group), $c_j(S_i)$ is the random effect of cow within square, P_k is the fixed effect of period, D_l is the fixed effect of diet, and e_{ijkl} is the residual error.

Data regarding ruminal fermentation were analyzed as repeated measures using the MIXED procedure of SAS adding the sampling time points (0, 2, 4, 6, 8, 10, or 12 h relative to the morning feeding) and their interaction (diet by time) as fixed effects to the previous statistical model. Covariance structures (compound symmetry, heterogeneous compound symmetry, unstructured, autoregressive 1, and heterogeneous autoregressive 1) were selected based on Akaike criteria. Differences among diets were analyzed through orthogonal contrasts: C1 (fat supplementation effect):

CON versus fat sources (SO, WS, and CSFA); C2 (effect of FA protection): SO versus protected fat sources (CSFA and WS); and C3 (effect of protected fat source type): WS versus CSFA. Significance level was set at 0.05 and trends were declared when $0.05 < P < 0.10$.

RESULTS

Dietary fat supplementation increased ($P \leq 0.01$) EE intake and total-tract digestibility and had no effect ($P \geq 0.15$) on intake of nutrients and nutrient digestibility (Table 2). Cows fed protected fat sources had lower ($P < 0.01$) EE intake than SO-fed cows, whereas cows fed CSFA exhibited higher ($P = 0.04$) EE intake in relation to those fed WS. Cows fed protected fat sources (WS and CSFA) and SO had similar ($P \geq 0.38$) nutrient total-tract digestibility. Although fat supplementation had no effect ($P \geq 0.18$) on milk yield, milk protein, and urea nitrogen concentration, a tendency toward a decrease ($P = 0.08$) in milk fat concentration in relation to CON was observed.

Diets had no effect ($P \geq 0.19$) on ruminal digesta volume and DM content (Table 3). Further, no differences were detected ($P \geq 0.37$) on ruminal pool of

Table 2. Effects of different fat supplements (oil, oilseed, and calcium salts) rich in linoleic acid on nutrient intake and digestibility, and milk yield and composition of early- to mid-lactation dairy cows

Item	Diet ¹				SEM	P-value ²		
	CON	SO	WS	CSFA		C1	C2	C3
Intake (kg/d)								
DM	18.6	18.4	18.2	17.9	0.42	0.49	0.46	0.65
OM	16.6	16.5	16.3	16.0	0.38	0.50	0.49	0.70
NDF	6.33	6.21	6.35	5.98	0.140	0.48	0.84	0.13
CP	3.10	3.07	2.93	2.98	0.069	0.25	0.22	0.60
pdNDF ³	4.50	4.38	4.49	4.21	0.099	0.37	0.88	0.12
iNDF ⁴	1.83	1.83	1.86	1.77	0.042	0.84	0.76	0.22
Ether extract	0.54	1.06	0.90	0.97	0.018	<0.01	<0.01	0.04
Total-tract digestibility (%)								
DM	68.6	66.2	65.4	66.9	0.71	0.15	0.99	0.45
OM	70.0	66.3	66.3	68.5	0.86	0.16	0.60	0.35
NDF	52.0	51.4	51.6	50.0	0.82	0.61	0.73	0.48
CP	73.7	70.4	70.8	73.5	0.81	0.28	0.38	0.24
Ether extract	82.7	89.4	87.7	89.5	0.72	<0.01	0.67	0.68
Production (kg/d)								
Milk	22.9	22.1	23.5	23.0	0.69	0.96	0.34	0.69
3.5% FCM ⁵	23.5	21.4	22.9	22.6	0.53	0.26	0.49	0.76
Fat	0.827	0.720	0.776	0.771	0.0290	0.31	0.89	0.98
Protein	0.693	0.663	0.685	0.690	0.0156	0.69	0.55	0.90
Composition								
Fat (%)	3.61	3.26	3.30	3.35	0.073	0.08	0.73	0.83
Protein (%)	3.02	3.0	2.98	3.01	0.016	0.40	0.63	0.35
MUN (mg/dL)	19.1	19.9	21.2	19.5	3.43	0.29	0.65	0.18

¹Control (CON); soybean oil (SO), 2.68% of SO inclusion; whole raw soybeans (WS), 14.3% of WS addition; and calcium salts of fatty acids (CSFA, Megalac-E, Church & Dwight Co. Inc., Trenton, NJ), 2.68% of CSFA supply.

²Orthogonal contrasts: C1 = CON vs. fat-supplemented diets (SO, WS, and CSFA); C2 = SO vs. WS + CSFA; and C3 = WS vs. CSFA.

³pdNDF: potentially digestible neutral detergent fiber = NDF – indigestible NDF.

⁴iNDF: indigestible NDF (288 h – in situ incubation).

⁵3.5% FCM: according to Sklan et al. (1992).

Table 3. Effects of different fat supplements (oil, oilseed, and calcium salts) rich in linoleic acid on rumen pool, rumen kinetics, ruminal digestibility, and abomasal flow of early- to mid-lactation dairy cows

Item	Diet ¹				SEM	P-value ²		
	CON	SO	WS	CSFA		C1	C2	C3
Rumen digesta								
Volume (L)	76.3	78.6	75.9	73.9	1.62	0.94	0.19	0.53
DM (g/kg)	126	122	128	126	1.6	0.94	0.29	0.70
Rumen pool (kg)								
DM	9.57	9.71	9.68	9.31	0.283	0.99	0.69	0.54
OM	8.82	8.96	8.89	8.59	0.271	0.99	0.67	0.62
NDF	6.84	6.88	6.73	6.64	0.203	0.84	0.64	0.85
iNDF ³	3.36	3.49	3.47	3.34	0.084	0.37	0.62	0.54
Turnover rate (%/h)								
DM	8.35	8.03	7.84	8.16	0.187	0.36	0.94	0.46
NDF	4.02	3.82	3.94	3.82	0.085	0.41	0.74	0.61
iNDF	2.36	2.23	2.31	2.25	0.066	0.47	0.71	0.69
pdNDF ⁴	5.64	5.58	5.77	5.44	0.155	0.91	0.94	0.45
Passage rate (%/h)								
DM	3.90	4.04	3.97	3.61	0.127	0.91	0.31	0.22
NDF	2.04	2.20	2.27	2.17	0.084	0.42	0.93	0.67
pdNDF	1.70	2.20	2.24	2.14	0.152	0.20	0.99	0.81
Digestion rate (%/h)								
DM	4.45	3.99	3.87	4.55	0.116	0.17	0.32	0.02
NDF	2.00	1.62	1.67	1.66	0.074	0.06	0.80	0.96
pdNDF	3.96	3.38	3.53	3.30	0.169	0.16	0.94	0.62
Ruminal digestibility (%)								
DM	53.8	49.4	49.9	56.0	1.02	0.33	0.08	0.01
NDF	50.7	42.1	42.7	43.7	1.64	0.06	0.79	0.84
pdNDF	71.7	60.1	60.6	62.1	2.34	0.07	0.82	0.82
Abomasal flow (kg/d)								
DM	8.59	9.42	9.20	7.89	0.325	0.62	0.09	0.03
NDF	3.16	3.64	3.67	3.35	0.141	0.22	0.68	0.38

¹Control (CON); soybean oil (SO), 2.68% of SO inclusion; whole raw soybeans (WS), 14.3% of WS addition; and calcium salts of fatty acids (CSFA, Megalac-E, Church & Dwight Co. Inc., Trenton, NJ), 2.68% of CSFA supply.

²Orthogonal contrasts: C1 = CON vs. fat-supplemented diets (SO, WS, and CSFA); C2 = SO vs. WS + CSFA; and C3 = WS vs. CSFA.

³iNDF: indigestible neutral detergent fiber (288 h – in situ incubation).

⁴pdNDF: potentially digestible neutral detergent fiber = neutral detergent fiber – indigestible neutral detergent fiber.

DM, OM, NDF, and iNDF. Fat supplements tended to decrease the NDF digestion rate ($P = 0.06$) and ruminal digestibility of NDF ($P = 0.06$) and pdNDF ($P = 0.07$). Regarding rumen-protected fat sources, cows fed WS exhibited greater ($P = 0.03$) abomasal flow of DM, and smaller ($P \leq 0.02$) DM digestion rate and ruminal digestibility than those fed CSFA.

Diets had no effect ($P \geq 0.17$) on all ruminal parameters assessed (pH and VFA proportions) with the exception of ruminal concentration of $\text{NH}_3\text{-N}$, which was decreased ($P = 0.04$) by dietary fat supplementation (Table 4). As expected, dietary fat supplementation increased ($P < 0.01$) FA intake (including C16:0, C18:0, *cis*-9 C18:1, *cis*-9,*cis*-12 C18:2, and *cis*-9,*cis*-12,*cis*-15 C18:3) of cows (Table 5). Interestingly, cows fed SO had greater ($P \leq 0.022$) intake of C18:0, *cis*-9 C18:1, *cis*-9,*cis*-12 C18:2, and *cis*-9,*cis*-12,*cis*-15 C18:3 compared with those fed rumen-protected fat sources.

Feeding WS increased ($P \leq 0.04$) the intake of C16:0, C18:0, and *cis*-9,*cis*-12,*cis*-15 C18:3 but decreased ($P \leq 0.02$) the intake of *cis*-9 C18:1 and *cis*-9,*cis*-12 C18:2 compared with when cows were fed CSFA. Although FA intake was greatly affected by experimental diets, fat supplementation only increased ($P \leq 0.05$) the abomasal flow of C16:0 and C18:0. Soybean oil and rumen-protected fat supplementation resulted in similar FA abomasal flows and extents of BH.

In general, cows fed fat supplements had greater ($P = 0.05$) C13:0 milk concentration in relation to CON (Table 6). Effects of fat supplementation on milk FA profile were dependent on fat source supplied. Rumen protected fat sources tended to increase ($P = 0.09$) C6:0 content and decreased ($P < 0.01$) C15:0 content in milk compared with SO. Regarding the rumen-protected fat supplements, CSFA increased ($P \leq 0.01$) C4:0 and C6:0 contents in milk compared with WS. Furthermore, cows

Table 4. Effects of different fat supplements (oil, oilseed, and calcium salts) rich in linoleic acid on ruminal fermentation of early- to mid-lactation dairy cows

Item	Diet ¹				SEM	P-value ²			
	CON	SO	WS	CSFA		INT	C1	C2	C3
pH	5.76	5.88	5.78	5.76	0.034	0.66	0.52	0.18	0.84
NH ₃ -N (mg/dL)	20.1	17.0	17.3	15.5	0.79	0.90	0.04	0.73	0.41
Total VFA (mM)	124	119	123	122	2.0	0.88	0.53	0.50	0.79
Acetate (% of VFA)	63.8	64.1	63.9	64.8	0.40	0.17	0.38	0.71	0.21
Propionate (% of VFA)	22.6	23.6	23.7	22.7	0.45	0.63	0.44	0.70	0.45
Butyrate (% of VFA)	13.6	12.2	12.4	12.4	0.42	0.96	0.17	0.84	0.98
Acetate:propionate	2.87	2.79	2.75	2.88	0.064	0.57	0.59	0.81	0.38

¹Control (CON); soybean oil (SO), 2.68% of SO inclusion; whole raw soybeans (WS), 14.3% of WS addition; and calcium salts of fatty acids (CSFA, Megalac-E, Church & Dwight Co. Inc., Trenton, NJ), 2.68% of CSFA supply.

²Orthogonal contrasts: C1 = CON vs. fat-supplemented diets (SO, WS, and CSFA); C2 = SO vs. WS + CSFA; and C3 = WS vs. CSFA. INT = diet by time interaction. Diet had no effect ($P \geq 0.172$), and time effect ($P < 0.0001$) was observed for all parameters.

fed CSFA tended to exhibit greater ($P \leq 0.08$) C8:0 and total SFA, and lower *cis*-9 C18:1 and total UFA content in milk in comparison to cows fed SO.

DISCUSSION

In agreement with the hypothesis, cows fed either CSFA or WS demonstrated similar FA profiles in abomasal flows as well as similar extents of BH. Surprisingly, this study demonstrated that SO had similar abomasal flow of FA in comparison with rumen-protected fat sources. Although it was expected that SO would decrease the PUFA in profile of FA in abomasum, cows

fed SO had greater intake of C18:2 in comparison to WS and CSFA. The greater the intake of a specific FA the greater the odds of increasing its amount in the abomasal/intestinal digesta. For instance, incrementing doses of SO linearly increased the total UFA in duodenal flow of FA in lambs (Kucuk et al., 2004). In addition, CSFA-fed cows had a greater intake of C18:2 than those fed WS. These effects might be related either to the lower EE content of WS in relation to CSFA (4.73% vs. 5.03%, respectively) or cows sorting against WS. In the current experiment, SO, WS, and CSFA had 48.9, 50.0, and 42.8 g/100 g of FA of C18:2 (data not shown). The sorting against WS is supported by the similar DMI and lower EE intake of cows fed WS

Table 5. Effects of different fat supplements (oil, oilseed, and calcium salts) rich in linoleic acid on fatty acid (FA) intake, abomasal flow, and biohydrogenation extent of early- to mid-lactation dairy cows

Item	Diet ¹				SEM	P-value ²		
	CON	SO	WS	CSFA		C1	C2	C3
Intake of FA (g/d)								
C16:0	58.8	97.5	105	86.7	2.09	<0.01	0.60	<0.01
C18:0	15.2	29.8	28.8	23.6	0.59	<0.01	<0.01	<0.01
<i>cis</i> -9 C18:1	96.1	180	147	178	3.5	<0.01	0.02	<0.01
<i>cis</i> -9, <i>cis</i> -12 C18:2	188	377	315	345	7.2	<0.01	<0.01	0.02
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	22.5	40.8	36.7	33.9	0.79	<0.01	<0.01	0.04
Total	450	833	809	813	17.2	<0.01	0.37	0.90
Abomasal flow of FA (g/d)								
C16:0	67.4	138	119	95.0	9.75	0.05	0.24	0.43
C18:0	276	585	548	443	46.6	0.05	0.48	0.48
<i>cis</i> -9 C18:1	34.1	52.1	59.7	44.7	4.09	0.12	0.99	0.25
<i>trans</i> -11 C18:1	7.52	15.0	10.6	11.9	1.78	0.27	0.67	0.80
<i>cis</i> -9, <i>cis</i> -12 C18:2	25.7	40.6	47.8	37.5	3.87	0.11	0.85	0.39
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	1.76	2.46	3.90	3.44	0.433	0.18	0.30	0.73
Total	461	927	868	721	68.9	0.04	0.47	0.49
Biohydrogenation extent (% of FA intake)								
<i>cis</i> -9 C18:1	61.6	62.7	55.0	67.3	4.02	0.99	0.87	0.25
<i>cis</i> -9, <i>cis</i> -12 C18:2	87.6	89.6	85.7	88.9	1.16	0.62	0.42	0.35
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	92.4	94.2	90.2	89.6	1.18	0.69	0.15	0.85

¹Control (CON); soybean oil (SO), 2.68% of SO inclusion; whole raw soybeans (WS), 14.3% of WS addition; and calcium salts of fatty acids (CSFA, Megalac-E, Church & Dwight Co. Inc., Trenton, NJ), 2.68% of CSFA supply.

²Orthogonal contrasts: C1 = CON vs. fat-supplemented diets (SO, WS and CSFA); C2 = SO vs. WS + CSFA; and C3 = WS vs. CSFA.

in comparison with those fed SO or CSFA. However, neither Barletta et al. (2016) nor Bettero et al. (2017) reported any sorting effects of WS. Furthermore, the current experiment did not report differences in ruminal passage rate of DM, which affects the time that fat supplements are exposed to microbial BH.

Although high levels of dietary fat can decrease DMI in dairy cows (Allen, 2000), the moderate level of dietary fat used in the current study (almost 4.5% of FA) was not associated with decreases of feed intake and nutrient digestibility, regardless of fat source added to the diets. Feed intake may be affected by gastrointestinal tract distension, as well as by a high availability of oxidizable fuels in the liver (Allen, 2000). Because dietary effects were neither detected on ruminal passage rate of DM nor on ruminal fermentation in the current study, it is reasonable to find no differences in feed intake of cows. Researchers evaluated the effects of increasing dietary levels of UFA (2.49, 4.29, 5.11, and 5.77% FA) and reported no differences in DMI and digestibility of early- to mid-lactation cows (Benchaar et al., 2012).

Rumen passage rate is determined by physiological status of animals, feed intake level, and ruminal diges-

tion rate (particle breakdown processes and microbial activity; Forbes, 1995). This experiment did not show differences either in feed intake or ruminal digestion rate when comparing SO and protected fat sources. However, CSFA-fed cows had greater digestion rate in comparison with those fed WS, which can be related to the numerically smaller NDF intake of cows fed CSFA. Furthermore, ruminal digestibility and total-tract digestibility of DM were similar between cows fed CON and CSFA. According to Jenkins and Bridges (2007), one of the goals of protecting fat is to decrease the negative effects of FA on ruminal fermentation and nutrient degradation. Few studies have compared the effects of oilseeds and CSFA on NDF ruminal digestibility. Barletta et al. (2016) did not report significant differences in ruminal NDF digestibility between lactating cows fed WS or CSFA. On the other hand, Bettero et al. (2017) found greater ruminal NDF digestibility in dry cows fed WS than those fed CSFA. Differences among these studies might be related to the DM passage rate. For instance, the DM passage rate observed in cows was 3.22 and 2.73%/h by Barletta et al. (2016) and Bettero et al. (2017), respectively. Cows in our study exhibited an average DM passage rate of 3.88%/h.

Table 6. Effects of different fat supplements (oil, oilseed, and calcium salts) rich in linoleic acid on milk fatty acid (FA) profile of early- to mid-lactation dairy cows

Item	Diet ¹				SEM	P-value ²		
	CON	SO	WS	CSFA		C1	C2	C3
FA (% of FA)								
C4:0	1.07	0.98	0.96	1.23	0.036	0.85	0.23	0.02
C6:0	1.44	1.22	1.22	1.57	0.041	0.28	0.09	0.01
C8:0	1.07	0.89	0.88	1.08	0.039	0.20	0.34	0.09
C10:0	2.70	2.18	2.22	2.58	0.125	0.21	0.49	0.34
C12:0	3.48	2.81	3.02	3.12	0.152	0.18	0.50	0.83
C13:0	0.20	0.14	0.16	0.11	0.013	0.05	0.83	0.21
C14:0	11.4	10.0	10.7	10.7	0.26	0.14	0.32	0.98
<i>cis</i> -9 C14:1	0.33	0.27	0.36	0.24	0.025	0.48	0.67	0.11
C15:0	0.22	0.34	0.18	0.21	0.016	0.56	<0.01	0.50
C16:0	32.5	30.1	29.9	31.6	0.62	0.16	0.80	0.54
C17:0	0.37	0.33	0.30	0.33	0.013	0.13	0.70	0.47
C18:0	11.6	13.2	13.0	14.7	0.58	0.21	0.66	0.33
<i>cis</i> -9 C18:1	23.5	27.7	26.8	23.5	0.65	0.11	0.13	0.09
<i>trans</i> -11 C18:1	0.27	0.35	0.66	0.36	0.051	0.08	0.12	0.20
<i>cis</i> -9, <i>cis</i> -12 C18:2	2.51	2.65	2.64	2.44	0.076	0.75	0.54	0.37
<i>cis</i> -9, <i>trans</i> -11 C18:2	0.50	0.45	0.64	0.53	0.051	0.76	0.32	0.45
<i>trans</i> -10, <i>cis</i> -12 C18:2	0.02	0.03	0.03	0.03	0.004	0.38	0.92	0.76
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	0.15	0.14	0.17	0.16	0.010	0.91	0.33	0.73
C20:0	0.11	0.11	0.11	0.14	0.006	0.57	0.42	0.12
<i>cis</i> -13 C20:1	0.04	0.04	0.05	0.04	0.003	0.32	0.14	0.49
C20:2	0.05	0.05	0.04	0.05	0.009	0.79	0.83	0.35
C21:0	0.02	0.02	0.02	0.02	0.001	0.99	0.93	0.71
C22:0	0.09	0.04	0.07	0.07	0.013	0.26	0.32	0.91
Unsaturated	29.1	33.9	33.0	28.8	0.73	0.12	0.12	0.07
Saturated	66.9	63.9	63.0	67.1	0.71	0.20	0.56	0.07
Saturated:unsaturated	2.34	2.00	1.97	2.37	0.075	0.25	0.45	0.10

¹Control (CON); soybean oil (SO), 2.68% of SO inclusion; whole raw soybeans (WS), 14.3% of WS addition; and calcium salts of fatty acids (CSFA, Megalac-E, Church & Dwight Co. Inc., Trenton, NJ), 2.68% of CSFA supply.

²Orthogonal contrasts: C1 = CON vs. fat-supplemented diets (SO, WS, and CSFA); C2 = SO vs. WS + CSFA; and C3 = WS vs. CSFA.

Dietary fat supplementation increased the abomasal flow of total FA without affecting abomasal flow of UFA identified in this study. Dietary FA are isomerized and biohydrogenated in the rumen by microorganisms (Jenkins, 1993). Harvatine and Allen (2006b) reported an increase in UFA abomasal flow with fat supplementation. However, these authors found 28.9% of ruminal DM digestion, which differs from our results (52.3%). We recruited cows with lower milk yield and ruminal passage rate than those used by Harvatine and Allen (2006b) as these parameters affect abomasal FA flow. It was expected that cows fed SO would exhibit lower UFA abomasal flow in comparison with those fed CSFA and WS, due to the ruminal BH rate increase when FA as free oil is supplied (Jenkins, 1993; Barletta et al., 2016). Another evidence of this phenomenon is the greater abomasal flow of C18:0, in relation to its intake. According to Jenkins and Bridges (2007), the clearance of C18:1, C18:2, and C18:3 FA in the rumen varies according to the composition of diet, ruminal conditions, feeding levels, marker methods, and geographic location. In this study, in contrast to the findings of Barletta et al. (2016) and Bettero et al. (2017), experimental diets had no effect on ruminal pH, and cows exhibited lower ruminal passage rate compared with passage rates reported by Harvatine and Allen (2006b). We need to highlight that in the present study a single marker technique based on iNDF was used to estimate ruminal outflow, and this technique may not represent the outflow of the fluid phase and small particles, which can be evaluated using the triple marker technique (France and Siddons, 1986).

According to Doreau and Ferlay (1994), the disappearance of C18:3 and C18:2 in the rumen averages 93 and 85%, respectively, which were very similar to values found in the present study. However, diets had no influence on BH extent of C18:1, C18:2, and C18:3. Barletta et al. (2016) reported a decrease on C18:2 BH extent in cows fed protected fat sources, but these authors also reported decreases in ruminal DM passage and NDF digestibility when feeding protected fat sources. Opposing to what has been frequently reported in the literature, results from this experiment suggest that CSFA poorly protect their lipid content from microbial BH under certain circumstances. Van Nevel and Demeyer (1996) have shown that calcium salts incubated *in vitro* in ruminal fluid partially protect PUFA content at pH 6.9 but not at pH 5.9. In the current experiment, ruminal fluid pH averaged 5.8. In addition, the dissociation of calcium salts of SO has been positively correlated with decreases in pH value with an estimated pK_a value of 5.6 (Sukhija and Palmquist, 1990). Palmquist (1984) also suggested that CSFA were dissociated in rumen fluid and biohydrogenated with pH less than 6.0 based

on the relatively low milk fat content and low proportion of linoleic acid in milk observed in cows fed CSFA. Lundy et al. (2004) reported C18:2 BH extents averaging 92.2 and 89.1% for mid-lactation cows fed SO and CSFA, respectively, whereas we observed average C18:2 BH extents of 89.6 and 88.9% for cows fed SO and CSFA, respectively. Even though cows used in Lundy et al. (2004) had greater intake of C18:2 FA than cows exhibited in this experiment. In agreement with the current experiment, Jenkins and Bridges (2007) reported that all supplemental fats (either protected or unprotected) are biohydrogenated to a similar extent (C18:1, 86%; C18:2, 82%; and C18:3, 86%). Furthermore, feeding cows with increasing amounts of unprotected fats will increase UFA intestinal flow (Jenkins and Bridges, 2007).

Fatty acids trapped in vegetable cells, the concentrate proportion (Jenkins and Bridges, 2007), and the dietary starch level (Rico et al., 2015) are some of the factors causing variation in BH extent. Low pH limits the rate of lipolysis and impairs the isomerization and the reduction of the second double bound in linoleic acid (Chilliard et al., 2007). Low values of ruminal pH change microbial population, altering usual ruminal BH routes and formation of key intermediates of linoleic acid BH (Bauman and Griinari, 2003; Shingfield et al., 2010), resulting in increased concentration of conjugated linoleic acid isomers, which may affect milk fat synthesis (Fuentes et al., 2009).

Dietary fat supplementation tended to decrease milk fat concentration, without major effects on milk yield and composition, and solids concentration. The decrease in milk fat concentration was not expected as we did not observe differences in NDF digestibility and acetate-to-propionate ratio in ruminal fluid when comparing cows fed FA sources with those fed the CON diet. In the current study, CSFA showed a milk FA profile very similar to CON, whereas SO and WS tended to increase milk UFA concentration. Many reports are available in the scientific literature of changes in milk FA profile when cows were fed PUFA (Boerman and Lock, 2014; Gandra et al., 2016a). On the other hand, feeding calcium salts rich in linoleic acid or rich in linolenic acid to lactating cows had minor effects on the proportion of these FA in milk fat (Chouinard et al., 1998).

CONCLUSIONS

In this study, supplementing the diet of early- to mid-lactation cows with WS resulted in similar abomasal flow of UFA to that in cows fed CSFA, but the abomasal flow of UFA also depends on the level of intake of UFA. Contrary to what has consistently been reported

in the scientific literature, in this study, both unprotected (SO) and protected fat sources (WS and CSFA) presented similar abomasal flows of UFA in early- to mid-lactation cows. The reason for this is likely related to dissociation of calcium salts in relatively low ruminal pH. Biohydrogenation extent was neither affected by fat supplementation nor whether the fat supplement was protected or not. Finally, this study suggests that under some circumstances, abomasal flow of UFA in early-lactation cows can be increased by supplementing their diet with fat supplements rich in linoleic acid, regardless of rumen protection, with small effects on ruminal DM digestibility.

ACKNOWLEDGMENTS

The authors acknowledge the University of Sao Paulo (USP) and Dairy Cattle Research Laboratory staff. Authors express gratitude to K. Harvatine (Penn State U.) for support on methods and calculations. Authors are grateful to the São Paulo Research Foundation (Brazil) for partial financial support (grant #2010/00690-9).

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