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Use of protected fat sources to reduce fatty acid biohydrogenation and improve abomasal flow in dry dairy cows

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ABSTRACT

The objective of this study was to evaluate the effects of dietary fat supplementation on dry dairy cows feed intake, digestion, ruminal kinetics, biohydrogenation, and abomasal flow of fatty acids (FAs). Eight Holstein rumen and abomasum fistulated dry cows (average body weight of 614 ± 59 kg), were assigned to a replicated 4 × 4 Latin square design experiment, with 21-d periods. The experimental diets were: 1) control (CON): corn- and soybean meal-based diet, with no fat source; 2) soybean oil (SO) diet with 30 g/kg dry matter (DM) of soybean oil; 3) whole raw soybean (WS) diet with 160 g/kg DM of whole raw soybean grain; 4) calcium salts of fatty acids (CS) diet with 32 g/kg DM of calcium salts of unsaturated FA. Fat-supplemented diets increased ether extract intake and digestibility without affecting DM intake. However, these diets promoted a decrease in DM and neutral-detergent fiber (NDF) total tract apparent digestibility. Fat sources decreased ruminal acetate to propionate ratio (C2:C3). In addition, SO diet increased ruminal propionate concentration and decreased C2:C3 in relation to protected sources of FA (CS and WS). Furthermore, cows fed CS diet exhibited higher ruminal pH, NH_3-N and acetate concentration compared to those fed WS diet. Fatty acid supplementation did not alter serum glucose and urea concentration, but increased the serum cholesterol concentration. Although FA supplementation increased net energy intake of cows, energy and nitrogen balances, and microbial protein synthesis were not affected by the experimental diets. Fat supplementation had no effect on ruminal digestion neither on DM and NDF passage rates. Cows fed CS and WS diets presented higher DM and NDF ruminal digestion rates whether compared to SO one. Consequently, cows fed CS and WS had higher truly digestible NDF ruminal removal rate than those fed SO. Calcium salts of unsaturated FA increased DM and NDF rumen passage rate and decreased

Abbreviations: ADF, acid detergent fiber; C2:C3, acetate to propionate ratio; CON, control; CP, crude protein; CS, calcium salts of fatty acids; DM, dry matter; EE, ether extract; FA, fatty acids; iNDF, indigestible neutral detergent fiber; NDF, neutral detergent fiber; NH_3-N, ammonia nitrogen; VFA, volatile fatty acid; WS, whole raw soybean.

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NDF digestibility in relation to WS diet. Fat-supplemented diets increased the abomasal flows of C18:2, *trans* C18:1, C18:0, C16:0 and total FA. The CS supplementation resulted in a higher abomasal flow of DM, total FA, *trans* C18:1, C16:0 and C18:3 when compared to WS one. On the one hand, FA supplementation increased C18:2 and C18:1 biohydrogenation rates; on the other hand, it protected FA sources decreased C18:2 and C18:1 biohydrogenation rates. In conclusion, fat-protected sources were effective to prevent FA from ruminal biohydrogenation.

1. Introduction

Genetic selection for cows with high milk yield has improved dairy cattle performance over the last decades (VandeHaar and St-Pierre, 2006). However, the susceptibility of dairy cows to diseases has also increased at the same pace (Oltenacu and Broom, 2010). Even though nutritional strategies can meet high-yielding cow requirements, mitigating health and reproductive problems, such an approach is a major concern among dairy farmers.

Dietary lipid supplementation for lactating dairy cows provides energy and extra-caloric benefits from specific fatty acids (FA). In lactating cows, the unsaturated FAs benefit immunological system (Silvestre et al., 2011), improve reproductive performance (Ambrose et al., 2006), and increase the amount of unsaturated FA in the milk (Barletta et al., 2015; Venturelli et al., 2015). Nevertheless, there is limited knowledge regarding biohydrogenation processes and optimal FA diet during the dry period. Fat supplementation potentially optimizes liver peroxisomal activity, reducing hepatic triacylglycerol accumulation and its detrimental effects (Grum et al., 1996), in addition to enhancing the immunological condition of cows in the early post-partum period (Gandra et al., 2016).

Small intestine represents the main place of FA absorption. The ratio of absorbed unsaturated FA in the intestine depends on dietary FA profile and ruminal protection level of FA sources. Protected fat sources have lower ruminal biohydrogenation, improving transfer efficiency of dietary unsaturated FA to the milk (Sterk et al., 2012).

Low passage rates associated with a high dietary forage level, which is commonly offered during the dry period, potentially increases ruminal retention, exposing unsaturated FA to ruminal biohydrogenation (Allen, 2000). To our knowledge, there is no study evaluating the FA abomasal flow and the biohydrogenation rates in dry dairy cows fed diets supplemented with fat sources rich in linoleic acid. Since the FA absorption represents an important issue to animal health and nutrition, we evaluated the effects of linoleic acids sources on feed intake, digestion coefficients, ruminal kinetics, biohydrogenation, and intestinal flow of FA in dry Holstein cows. Our hypothesis was that rumen-protected fat sources (whole raw soybeans and FA calcium salts) could increase the abomasal flow of unsaturated FA and decrease ruminal biohydrogenation compared to an unprotected fat source (soybean oil).

2. Material and methods

Eight rumen and abomasum cannulated dry Holstein cows, with 614 ± 59.0 kg of body weight at the beginning of the trial, were housed in individual pens (17.5 m^2 of area), sand bedded, with forced ventilation, feed bunks and free access to water. The animals were allocated into two balanced Latin squares, receiving one of the four experimental diets (Table 1). The experimental diets were formulated according to the NRC (2001) recommendations, as follows: 1) control (CON) diet without fat source; 2) soybean oil diet (SO), with 30 g/kg of soybean oil; 3) whole raw soybean diet (WS), with 160 g/kg of whole raw soybean grain; and 4) calcium salts of fatty acids diet (CS), with 32 g/kg of calcium salts of unsaturated FA (Megalac-E®, Church & Dwight Company, Trenton, NJ).

Experimental procedures were approved by the Ethics Committee of the School of Veterinary Medicine and Animal Science of the University of São Paulo (approval number 1603/2009).

2.1. Data and sample collection

The experimental periods lasted 21 days, consisting of 14 days for diet adaptation, followed by seven days of sampling. Cows were fed daily at 8:00 a.m. and 1:00 p.m. Refusal rate were kept between 5 and 10% of feed offered. Samples of feeds and refusals were daily collected and frozen for future chemical analyses. Fecal samples (500 g) were collected every nine hours on days 15, 16, and 17 of each experimental period and gathered in one sample mount per cow for the entire experimental period.

Samples of diet ingredients, refusals, and feces were analyzed for DM (method 930.15), crude protein (CP, N × 6.25; method 984.13), ether extract (EE, method 920.39), and ash (method 942.05) according to AOAC (2000). Neutral detergent fiber (aNDFom), acid detergent fiber, and lignin (sa) were determined according to (Van Soest et al., 1991).

Daily DM fecal excretion was estimated using chromic oxide as an external marker. From day 9 to day 17 of each experimental period, 15 g/d of chromium oxide were placed into the rumen of each cow. Fecal chromium oxide concentration was

Table 1
Ingredients and nutritional composition of experimental diets.

Item	Experimental diets[a]			
	CON	SO	WS	CS
Ingredients, g/kg of dm				
Corn silage	800	800	801	800
ground corn	115	74.9	22.0	75.7
Soybean meal	60.1	70.1	–	70.1
soybean oil	–	30.0	–	–
Whole raw soybeans	–	–	160	–
calcium salts of fatty acids	–	–	–	32.0
Urea	11.3	11.3	3.2	11.3
Ammonium sulfate	1.6	1.6	1.6	1.6
salt	3.0	3.0	3.0	3.0
Dicalcium phosphate	2.5	2.5	2.5	2.5
limestone	3.0	3.0	3.0	–
Mineral[b]	2.5	2.5	2.5	2.5
Vitamin premix ADE	1.2	1.2	1.2	1.2
Nutrients, g/kg of dm				
Dry matter	404	407	408	406
organic matter	939	939	936	935
neutral detergent fiber (aNDF)	491	487	505	488
crude protein	128	130	127	130
Ether extract	24.0	52.2	52.6	49.9
Fatty acids (FA)	14.3	42.4	42.8	40.1
fatty acids, g/kg of FA				
C14:0	9.6	4.3	6.1	4.4
C16:0	201	137	146	166
C18:0	50.7	39.2	39.3	42.7
C18:1	259	267	199	210
C18:2	370	457	502	439
C18:3	38.1	49.3	65.8	39.7
Unsaturated FA	673	653	645	587

[a] cON: control; SO: soybean oil; WS: Whole raw soybeans; and CS: Calcium salts of fatty acids (Megalac-E®).
[b] Each kg contained: 125 mg of Co; 5625 mg of Cu; 9 mg of S; 312 mg of I; 5000 mg of Fe; 18,125 mg of Mn; 144 mg of Se; 23,750 mg of Zn; 2000 IU of Vitamin A; 500 IU of Vitamin D; and 12,500 IU of Vitamin E.

determined according to Williams et al. (1962) and readings were performed using a spectrophotometer (Biochrom Asys Expert, Holliston, MA).

Ruminal fermentation was evaluated ruminal liquid, collected on the day 17 of each experimental period, every 2 h, from zero to 12 h after morning feeding. The pH was measured immediately after ruminal fluid collection using a digital pH-meter (MB-10, Marte Científica, Santa Rita do Sapucaí, Brazil). Ruminal volatile fatty acids (VFA) concentration was determined according to Shen et al. (2004), and NH_3-N ruminal concentration was analyzed using the phenol-hypochlorite method (Broderick and Kang, 1980).

On day 19 of each experimental period, before the morning feeding, blood samples were collected by coccygeal vessels puncture. The samples were centrifuged at $800 \times g$ for 10 min, and serum was transferred to plastic tubes for storage at −20 °C. Analyses were performed using colorimetric kits (glucose: cat. no. K-082; high-density lipoprotein cholesterol: cat no. K-015; urea: cat. no. k-056; total cholesterol: cat. no. K-083; Bioclin®, Belo Horizonte, Brazil). Readings were performed using a semi-automatic spectrophotometer (SBA 200, CELM®, São Caetano do Sul, Brazil).

Spot urine samples were collected on day 16 of each experimental period, four hours after morning feeding. Daily urinary volume was estimated based on creatinine concentration in spot samples and a predefined daily creatinine excretion rate. Creatinine concentration in urine samples was analyzed using a colorimetric kit (kinetic creatinine: cat. no. K-067, Bioclin®) and readings were performed in a semi-automatic spectrophotometer (SBA 200, CELM®). It was assumed a daily creatinine excretion rate of 24.05 mg/kg of body weight, in order to estimate urinary volume (Chizzotti et al., 2007). Urinary uric acid concentration (uric acid stable liquid: cat. no. K-052, Bioclin®) was evaluated in a semi-automatic spectrophotometer (SBA 200, CELM®) and urinary allantoin concentration was analyzed according to Fujihara and Yamaguchi (1978). The sum of uric acid and allantoin urinary excretions was considered as the total excretion of purine derivatives. Microbial protein synthesis was estimated according to Chen and Gomes (1992). Energy and protein balances were calculated according to the NRC (2001) equations.

Eight abomasal digesta samples (1000 g) were collected every nine hours from days 15 to 17 of each experimental period, being gathered in one sample per cow representing the entire experimental period. Abomasal samples were squeezed with a nylon cloth (1 mm mesh) to separate the liquid from solid contents, being subsequently refrozen. One subsample was pre-dried (60 °C for 72 h) and ground in a Wiley mill (1 and 2-mm screens). Another subsample was ground in a refrigerated Wiley mill and lyophilized to avoid FA lost. After ground, the samples were reconstituted at the original DM ratio.

Ruminal digesta was entirely evacuated on days 20 and 21 of each experimental period at 12h30 (4.5 h after the morning feeding) and 5h30 (2.5 h before the morning feeding), respectively (Harvatine and Allen, 2006a). During the evacuation, a 100 g/kg sample was separated from the digesta to improve the sampling. Ground samples of feed, refusals, ruminal and abomasal contents (2 mm) were packed in non-woven bags (100 g/m^2), being incubated for 288 h in the rumen of two Nellore steers (Casali et al., 2008). Afterward, the bags were analyzed for NDF (Van Soest et al., 1991) to assessing the indigestible neutral detergent fiber (iNDF), which was used as an internal marker. Ruminal and abomasal ground samples (1 mm) were also analyzed for NDF concentration.

Rumen turnover rate was calculated according to Oba and Allen (2003), as described below:

$$Turnover\ rate\left(\frac{1}{h}\right) = \frac{Component\ intake\left(\frac{kg}{d}\right)}{Component\ ruminal\ pool\,(kg) \times 24\left(\frac{h}{d}\right)}$$

$$Passage\ rate\left(\frac{1}{h}\right) = \frac{Component\ abomasal\ flow\left(\frac{kg}{d}\right)}{Component\ ruminal\ pool\,(kg) \times 24\left(\frac{h}{d}\right)}$$

Ruminal digestion rate (k_d) was calculated as the difference between turnover rate and passage rate (Allen and Mertens, 1988). Rumen apparent digestibility of NDF was taken as: $k_d/(k_d + k_p)$. Truly digestible NDF (tdNDF) concentration was calculated as the difference between NDF and iNDF (NRC, 2001).

Ruminal fluid turnover was estimated using 4000 molecular weight Polyethylene glycol (PEG) as a marker of liquid passage. On day 18 of each experimental period, 100 g of PEG (Synth, Diadema, Brazil) were diluted in 400 mL of water and placed in the rumen of cows. Samples were collected 0, 1, 2, 4, 8 and 24 h after PEG infusion. Polyethylene glycol concentration was analyzed according to Hyden (1956) methodology. Liquid passage rates were obtained by linear natural logarithmic regression as a function of time.

Diet ingredients, refusals, ruminal and abomasal digesta samples were ground in a 5 mm sieve Wiley mill, using liquid N at −192 °C to avoid FA oxidation (Harvatine and Allen, 2006b). Lipid extraction was performed according to the method proposed by Folch et al. (1957) and methylation performed according to Kramer et al. (1997). Fatty acids were quantified using a gas chromatograph (GC 2010 with automatic injection, Shimadzu, Tokyo, Japan) equipped with a capillary column SP-2560 (100 m × 0.25 mm i.d. with 0.02 mm of film thickness, Supelco, Bellefonte, PA). The initial temperature was set at 70 °C and kept for four minutes. Temperature was increased by 13 °C every min until reaching 175 °C, remaining stable for 27 min. Afterwards, an increase of 4 °C per min was set up until temperature reaches 215 °C, being then maintained for 31 min. Hydrogen was used as the carrier gas flowing 40 cm/s. Four standards were used to identify FA profile: C4-C24 FA (Supelco® TM 37, Sigma-Aldrich, St. Louis, MO, USA), C18:1 trans-11 FA (V038-1G, Sigma-Aldrich), C18:2 trans-10, cis-12 FA (UC–61 M 100MG, NU-CHEK-PREP Inc., Elysian, MN, USA), and C18:2 cis-9, trans-11 FA (UC–60 M 100MG, NU-CHEK-PREP) identifying FA formation during the biohydrogenation.

The biohydrogenation rates were calculated according to Jenkins and Bridges (2007) model, using the following equation:

$$BRPUFA\,(\%) = 100 \times \frac{(PUFAaf - PUFAi)}{PUFAi}$$

Wherein *BRPUFA* was the biohydrogenation rate of polyunsaturated fatty acid, *PUFAaf* was the polyunsaturated fatty acid abomasal flow, and *PUFAi* was the polyunsaturated fatty acid intake.

2.2. Statistical analyses

Data were analyzed with PROC MIXED (Statistical Analysis System for Windows 9.3, SAS Institute Inc., Cary, USA), according to the following statistical model:

$$Y_{ijklm} = \mu + D_i + P_j + S_k + a_l(s_k) + e_{ijklm}$$

Wherein: Y_{ijklm} was the observed value in the animal l, from k square, in the jth period, which received the i^{th} experimental diet; μ was the overall mean; D_i was the diet fixed effect; P_j was the experimental period fixed effect; S_k was the Latin square fixed effect; $a_l(s_k)$ was the random effect of the animal within Latin square; e_{ijklm} was the random residual error.

Diet effects were studied by three orthogonal contrasts: C1: fat source effect (CON vs. SO + WS + CS), C2: protected fat source effect (SO *vs.* WS + CS), and C3: protection type effect (WS *vs.* CS). Degrees of freedom correction was performed according to Kenward and Roger (1997), and means were adjusted by LSMEANS.

The ruminal fermentation variables (pH, NH_3-N, acetate, propionate, butyrate, and total VFA) were analyzed as repeated measures using the PROC MIXED of SAS 9.3. For that, we considered the effects of animal, period, and experimental diet, besides the effects of time and its interaction with experimental diet effect. Akaike methodology was used to determine the covariance matrix. The significance level was set at 0.05 and tendency was considered when $0.05 < P \leq 0.10$.

Table 2
Nutrient intake and total apparent digestibility of dry dairy cows fed different fatty acid sources.

Item	Experimental diets[a]				SEM[b]	*P*[c]		
	CON	SO	WS	CS		C1	C2	C3
Intake, kg/d								
Dry matter	11.9	11.7	11.5	12.2	0.61	0.76	0.31	0.34
Neutral detergent fiber	5.59	5.39	5.56	5.60	0.14	0.71	0.36	0.87
$_{i}$NDF[d]	1.77	1.70	1.71	1.77	0.05	0.47	0.52	0.48
Crude protein	1.64	1.67	1.59	1.73	0.08	0.74	0.11	0.40
Ether extract	0.30	0.72	0.72	0.71	0.04	<0.01	0.83	0.65
Coefficients of digestibility								
Dry matter	0.706	0.678	0.689	0.679	0.006	<0.01	0.16	0.98
Neutral detergent fiber	0.619	0.577	0.591	0.580	0.009	<0.01	0.29	0.80
Crude protein	0.736	0.730	0.730	0.733	0.005	0.53	0.89	0.74
Ether extract	0.849	0.871	0.871	0.866	0.002	<0.01	0.61	0.26

[a] CON: Control; SO: Soybean oil; WS: Whole raw soybeans; and CS: Calcium salts of fatty acids (Megalac-E®).
[b] Standard error of mean.
[c] C1 = control vs fatty acid sources (SO, WS and CS); C2 = SO vs WS and CS; C3 = WS vs CS.
[d] NDF: indigestible neutral detergent fiber.

Table 3
Ruminal fermentation in dry dairy cows fed different fatty acid sources.

Item	Experimental diets[a]				SEM[b]		*P*[c]				
	CON	SO	WS	CS		Diet	Time	D*T	C1	C2	C3
pH	6.15	6.14	6.08	6.25	0.03	0.04	<0.01	0.42	0.90	0.55	<0.01
NH_3-N, *mg/dL*	20.4	17.8	15.3	19.4	0.51	0.02	<0.01	<0.01	0.03	0.77	0.02
Total VFA[d], *m*Mol/L	160	157	155	160	1.88	0.46	<0.01	0.74	0.32	0.99	0.21
Acetate (C2), *m*Mol/L	117	111	111	117	1.43	0.05	<0.01	0.88	0.10	0.23	0.05
Propionate (C3), *m*Mol/L	28.4	32.2	29.2	28.8	0.38	0.02	<0.01	0.21	0.10	0.01	0.75
Butyrate, *m*Mol/L	14.9	13.8	14.2	14.4	0.20	0.39	<0.01	0.77	0.16	0.36	0.68
C2:C3	4.13	3.51	3.81	4.10	0.04	<0.01	<0.01	0.12	<0.01	<0.01	0.01

[a] CON: Control; SO: Soybean oil; WS: Whole raw soybeans; and CS: Calcium salts of fatty acids (Megalac-E®).
[b] Standard error of mean.
[c] *Probability: D*T = diet by time interaction effect*; C1 = control vs fatty acid sources (SO, WS and CS); C2 = SO vs WS and CS; C3 = WS vs CS.
[d] Volatile fatty acids.

3. Results

3.1. Feed intake and digestibility

Diets supplemented with fat sources provided approximately 50 g/kg of EE per kg of DM (52.2, 52.6, and 49.9 g/kg of EE in SO, WS, and CS diets, respectively, Table 1). Supplemental FA had no effect ($P > 0.05$) on DM, CP, iNDF and NDF intake of cows (Table 2). As expected, cows fed fat-supplemented diets had higher ($P < 0.01$) EE intake than those fed CON. Fat-supplemented diets decreased ($P < 0.01$) DM and NDF total tract apparent digestibility and increased ($P < 0.01$) EE total tract apparent digestibility.

3.2. Ruminal fermentation

Dietary addition of fat sources decreased ($P \leq 0.03$) ruminal NH_3-N concentration and C2:C3 ratio of cows (Table 3). In addition, cows fed SO diet exhibited higher ($P = 0.01$) ruminal propionate concentration, and lower ($P < 0.01$) C2:C3 compared to those fed WS. Furthermore, CS increased ($P \leq 0.05$) ruminal acetate concentration, C2:C3 ratio, pH, and NH_3-N concentration in relation to WS.

3.3. Serum metabolites, energy and nitrogen balances, and microbial protein synthesis

Fat supplementation increased ($P < 0.02$) serum high-density lipoprotein and total cholesterol concentration, but showed a trend to decrease ($P = 0.10$) serum glucose concentration (Table 4). Animals fed SO had lower ($P = 0.05$) serum total cholesterol concentration than those fed protected fat sources, notably by the high total cholesterol concentration observed in cows fed with CS. In addition, cows fed CS tended to exhibit higher ($P = 0.09$) serum cholesterol levels than those fed WS. Although fat supplementation increased ($P = 0.01$) net energy intake, fat-supplemented diets had no effect on energy and nitrogen balances, as well as microbial protein synthesis of cows.

Table 4
Serum metabolites, energy and nitrogen balances of dry dairy cows fed different fatty acid sources.

Item	Experimental diets[a]				SEM[b]	P[c]		
	CON	SO	WS	CS		C1	C2	C3
Serum metabolites, mg/dL								
Glucose	71.6	68.6	67.4	67.0	3.28	0.10	0.86	0.57
Total cholesterol	117	143	135	161	16.4	<0.01	0.05	0.09
High-density lipoprotein	72.4	83.0	82.9	96.7	7.22	0.02	0.28	0.07
Urea	34.0	31.7	29.6	27.9	2.81	0.20	0.96	0.34
Energy balance, Mcal/d								
Net energy intake	20.0	21.5	21.3	22.3	1.07	0.01	0.43	0.29
Energy balance	10.2	10.2	10.0	10.1	0.40	0.40	0.64	0.13
Nitrogen balance, g/d								
N intake	265	259	261	293	15.1	0.74	0.42	0.15
N balance	98.4	102	102	95.8	16.4	0.64	0.27	0.79
Microbial protein synthesis	610	850	638	703	56.3	0.27	0.24	0.28
Microbial efficiency, g/kg	73.1	98.9	77.9	83.0	6.90	0.32	0.36	0.33

[a] CON: Control; SO: Soybean oil; WS: Whole raw soybeans; and CS: Calcium salts of fatty acids (Megalac-E®).
[b] Standard error of mean.
[c] *Probability: D*T = diet by time interaction*; C1 = control vs fatty acid sources (SO, WS and CS); C2 = SO vs WS and CS; C3 = WS vs CS.

Table 5
Ruminal kinectis in dry dairy cows fed different fatty acid sources.

Item	Experimental diets[a]				SEM[b]	P[c]		
	CON	SO	WS	CS		C1	C2	C3
Rumen digesta, kg								
Fresh matter	71.9	73.7	74.3	69.8	2.05	0.72	0.46	0.10
Dry matter	8.47	9.01	8.97	8.73	0.32	0.04	0.45	0.32
Neutral detergente fiber (NDF)	6.67	7.08	6.85	6.60	0.22	0.21	0.03	0.16
Indigestible NDF (iNDF)	3.81	4.00	3.96	3.95	0.14	0.27	0.77	0.93
Ruminal removal rate, %/h								
Dry matter	5.94	5.49	5.43	5.89	0.13	0.03	0.27	0.02
NDF	3.53	3.20	3.42	3.56	0.07	0.20	0.01	0.29
Trully digestible NDF (tdNDF)	5.64	5.06	5.64	6.05	0.12	0.79	<0.01	0.13
iNDF	1.97	1.79	1.84	1.90	0.05	0.09	0.31	0.46
Digestion rate, %/h								
Dry matter	3.02	2.82	3.05	2.98	0.10	0.56	0.01	0.59
NDF	1.60	1.44	1.77	1.60	0.06	0.93	0.01	0.11
Passage rate, %/h								
Dry matter	2.93	2.68	2.38	2.91	0.08	0.08	0.84	<0.01
NDF	1.94	1.76	1.65	1.96	0.05	0.08	0.59	<0.01
NDF								
Intake, kg/d	5.59	5.39	5.56	5.60	0.14	0.71	0.36	0.88
Ruminal digestibility								
Absolute, *kg/d*	2.52	2.44	2.83	2.48	0.08	0.58	0.09	<0.01
Relative, *g/kg*	450	454	514	445	13.7	0.27	0.19	<0.01
$_{td}$*NDF*								
Ruminal digestibility								
Absolute, *kg/d*	2.52	2.44	2.83	2.48	0.08	0.59	0.09	0.02
Relative, *g/kg*	660	662	743	650	19.6	0.32	0.20	0.01
Liquid ruminal turnover, 1/d	1.97	2.00	2.01	2.04	0.03	0.41	0.64	0.61

[a] CON: Control; SO: Soybean oil; WS: Whole raw soybeans; and CS: Calcium salts of fatty acids (Megalac-E®).
[b] Standard error of mean.
[c] *Probability: D*T = diet by time interaction*; C1 = control vs fatty acid sources (SO, WS and CS); C2 = SO vs WS and CS; C3 = WS vs CS.

3.4. Ruminal kinetics

In general, dietary fat addition had no effect on DM and NDF ruminal digestion rate ($P \geq 0.56$). However, animals fed CON diet showed lower DM ruminal pool ($P = 0.04$) and DM removal rate ($P = 0.03$) than those fed fat supplemented diets. Additionally, fat supplementation tended to decrease DM and NDF passage rate ($P = 0.08$; Table 5). Animals fed protected fat sources had higher ($P = 0.01$) DM and NDF digestion rate and lower ($P = 0.03$) NDF ruminal pool. Hence, fat protected sources showed higher ($P \leq 0.01$) NDF and tdNDF ruminal removal rate than SO. Cows fed CS had higher ($P \leq 0.05$) DM and NDF

Table 6
Fatty acids intake, abomasal flow and ruminal biohydrogenation rate of dry dairy cows fed with different fatty acid sources.

Item	Experimental diets[a]				SEM[b]	*P*[c]		
	CON	SO	WS	CS		C1	C2	C3
Intake, g/d								
Total FA	170	497	493	488	27.7	<0.01	0.74	0.82
C16:0	34.1	68.1	72.3	81.2	3.70	<0.01	0.01	0.01
C18:0	8.62	19.5	19.4	20.9	1.01	<0.01	0.40	0.10
C18:1	44.1	133	98.4	102	6.27	<0.01	<0.01	0.35
C18:2	63.0	227.0	248	214	14.2	<0.01	0.63	<0.01
C18:3	6.48	24.50	32.5	19.4	1.80	<0.01	0.16	<0.01
Abomasal flow, g/d								
Dry matter	5899	5696	5157	6049	226	0.45	0.80	0.05
Total FA	302	536	500	602	31.0	<0.01	0.76	0.09
C16:0	56.5	75.6	67.5	105	5.1	<0.01	0.16	<0.01
C18:0	208	404	359	418	22.3	<0.01	0.66	0.15
C18:1 *trans*	7.9	26.9	4.0	34.9	2.9	<0.01	0.08	<0.01
C18:1 *cis*	9.0	8.7	10.0	11.8	0.7	0.38	0.12	0.27
C18:2	4.5	4.4	6.3	7.9	0.53	0.04	0.01	0.14
C18:3	0.17	0.23	0.27	0.52	0.05	0.09	0.11	0.05
Biohydrogenation rate, % of FA intake								
C18:1 *cis*	79.4	93.6	89.8	88.6	1.13	<0.01	<0.01	0.42
C18:2	92.8	98.1	97.5	96.4	0.42	<0.01	0.03	0.06
C18:3	97.3	99.1	99.2	97.5	0.39	0.10	0.37	0.08

[a] CON: Control; SO: Soybean oil; WS: Whole raw soybeans; CS: Calcium salts of fatty acids (Megalac-E®).
[b] Standard error of mean.
[c] *Probability: D*T = diet by time interaction*; C1 = control vs fatty acid sources (SO, WS and CS); C2 = SO vs WS and CS; C3 = WS vs CS.

ruminal passage rate and DM ruminal removal rate in relation to those cows fed WS. Furthermore, WS increased ($P \leq 0.02$) NDF and tdNDF ruminal digestibility compared to CS.

3.5. Fatty acid abomasal flow and ruminal biohydrogenation

Dietary fat sources increased the intake of all FA identified ($P < 0.01$, Table 6). Cows fed protected fat sources (WS and CS) had a higher ($P = 0.01$) intake of C16:0 FA and lower ($P < 0.01$) intake of C18:1 FA compared to those fed SO. Finally, cows fed CS had a higher ($P = 0.01$) intake of C16:0 FA and lower ($P < 0.01$) intake of C18:2 FA and C18:3 FA compared to those fed WS.

Fat-supplemented diets increased ($P \leq 0.04$) the abomasal flow of total FA, C16:0, C18:0, C18:1 trans and C18:2 FA. In addition, FA supplementation tended to increase ($P = 0.09$) the abomasal flow of C18:3 FA. Cows fed protected fat sources had higher ($P = 0.01$) abomasal flow of C18:2 FA than cows fed SO. Animals fed CS exhibited higher ($P \leq 0.05$) abomasal flow of DM, C16:0, C18:1 trans, and C18:3 FA compared to those fed WS.

Fatty acid supplementation increased ($P < 0.01$) the biohydrogenation rate of C18:1 and C18:2 FA, and also tended to increase ($P = 0.10$) that of C18:3 FA. Protected fat sources promoted lower ($P \leq 0.03$) biohydrogenation rates for C18:1 and C18:2 FA than that of SO. Calcium salts of fatty acids tended to decrease ($P \leq 0.08$) the biohydrogenation rate of C18:2 and C18:3 compared to WS.

4. Discussion

Post-ruminal absorption of polyunsaturated FA may enhance dairy cow health during the dry period, improving animal performance over the subsequent lactation. We evaluated ruminal kinetics and FA abomasal flow of dry cows fed with different fat sources. Protected fat sources decreased linoleic acid biohydrogenation rates in the rumen. Regarding the protected fat sources, WS increased ruminal digestion and tended to increase C18:2 and C18:3 FA biohydrogenation rates in relation to CS.

Fat sources decreased the apparent total tract digestibility of NDF and DM, without affecting DM intake. These responses could be related to the deleterious effects of unsaturated FA on cellulolytic bacteria, decreasing ruminal fiber digestibility (Oldick and Firkins, 2000). However, we found no differences in NDF ruminal digestibility when comparing CON and fat-supplemented diets. This fat supplementation effect may be attributed to the notable increase in ruminal NDF digestibility observed in WS-fed animals, which elevates the average digestibility in animals fed fat-supplemented diets. Animals fed WS had lower rumen passage rates, increasing rumen retention and digestibility of NDF and DM.

Fat-supplemented diets decreased ruminal NH_3-N concentration and C2:C3 ratio. The SO diet increased ruminal propionate concentration and decreased C2:C3 ratio compared to those with protected fat sources. Similarly, Bateman and Jenkins (1998) reported that soybean oil could alter ruminal VFA profile in cows fed high forage diets. Ruminal fat, especially free fat, acts on the outer membrane of gram-positive bacteria, leading to bacterial death, consequently modulating ruminal fermen-

tation (Maia et al., 2007; Nagaraja et al., 1997). Moreover, these bacteria are primarily responsible for protein degradation (Doreau and Ferlay, 1995); therefore, their growth inhibition results in decreased ruminal NH_3-N concentration.

Previous studies showed no effects of CS supplementation on ruminal pH (Fiorentini et al., 2012). Low ruminal pH inhibits cellulolytic bacteria, which produces great amounts of acetate (Boken et al., 2005). Ruminal pH of animals fed CS was higher than WS-fed animals. Thereby, higher ruminal pH found on animals fed CS was related to increasing ruminal acetate concentrations and, consequently, a higher C2:C3 ratio in the rumen.

Protected fat sources increased ruminal DM and NDF digestion rates and tended to increase the ruminal digestibility of NDF (kg/d). Tamminga and Doreau (1991) associated such results with a negative free-fat effect on the activity of ruminal cellulolytic bacteria. The clearance of rumen contents occurs through digestion and passage (Nocek, 1988). Fat protected sources increased ruminal digestion rate, without changing passage rates. Furthermore, if compared to the use of protected fat sources, SO increased NDF ruminal pool. Moreover, animals fed WS had higher absolute and relative NDF ruminal digestion related to those fed CS. According to Waybright and Varga (1991), larger feed particle sizes reduce the rumen-reticulum flow and improve ruminal digestion. The WS diet contained 16.9 g/kg more NDF than the CS diet, and WS had higher particle size than ground concentrates used in this study. Thus, elevated NDF content and particle size decreased NDF and DM passage rates and DM abomasal flow of animals fed WS compared to those fed with CS.

Fat addition increased C16:0, C18:0, trans C18:1 and C18:2, total FA abomasal flows, besides tending to increase C18:3 FA abomasal flow. The rise of the FA abomasal flow and serum cholesterol may be related to the increasing FA intake of fat-supplemented animals. According to Noble (1981), intestine is the major site of cholesterol synthesis in ruminants. Therefore, there is a correlation between the abomasal flow of FA and serum cholesterol. Biohydrogenation rates of C18:1 and C18:2 FA were increased with supplemental fat. According to Harvatine and Allen (2006b), the biohydrogenation extent is increased with PUFA dietary addition and it depends on the fat source.

Protected fat sources decreased C18:1 and C18:2 biohydrogenation rates. The protein matrix surrounding the lipids fraction of WS or association of cations and FAs decreases the access of ruminal microorganisms (Palmquist and Jenkins, 1980). While the biohydrogenation decreased, the C18:2 duodenal flow increased.

According to Sukhija and Palmquist (1990), FA binding to metal partially depends on the rumen pH. These authors showed that pka is 5.6 for soybean oil calcium salts. This fact can explain why some studies reported increased biohydrogenation rate for calcium salts. Animals fed CS had higher passage rates and lower ruminal retention than animals fed with WS diet, being less susceptible to ruminal fermentation (Harvatine and Allen, 2006a). Calcium salts of FA supplementation tended to decrease C18:2 and C18:3 FA biohydrogenation, which seems to be linked with higher ruminal passage rate in comparison with WS. We need to highlight that biohidrogenation rate was calculated in relation to FA intake, which is affected by basal diet FA and explain these results and trends. Ruminal biohydrogenation may be described as a function of the available FA pool size, ruminal retention time, and bacterial hydrogenation capacity (Harvatine and Allen, 2006b). Low passage rates for cows fed high-forage diets have been reported (NRC, 2001). However, we could not find similar studies evaluating the abomasal FA flow using high-forage diets during the dry period, hindering comparisons that are more realistic with our results.

Fat-protect sources were effective to prevent FA from ruminal biohydrogenation. In addition, oilseed improved the ruminal digestion if compared to calcium salts, which tended to decrease FA biohydrogenation rates. In conclusion, FA biohydrogenation is highly associated with ruminal kinetics in dairy cows.

Conflict of interests

The authors declare that are no conflicts of interest to the current manuscript.

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