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Dietary calcium salts of fatty acids and soybean oil effects on mid-lactation dairy cows performance

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Additional keywords

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SUMMARY

This study aimed to evaluate the effects fat supplementation with soybean oil (SO) and calcium salts of fatty acids (CSFA) on mid-lactation dairy cows on feed intake and nutrients digestibility, ruminal fermentation, milk yield and composition, and nitrogen balance. Sixteen multiparous Holstein cows (638 ± 73 kg of body weight and 116 ± 26 days in milk) were used in a 4 × 4 Latin square design for evaluate following diets: CONT) control, without additional fat source; SO) 30 g/kg of soybean oil; CSFA1) inclusion of 30 g/kg of calcium salts of long-chain fatty acids (MEGALAC-E®); and CSFA2) inclusion of 30 g/kg of calcium salts of long-chain fatty acids (LACTOPLUS®) on dry matter basis. Both evaluated CSFA were manufactured using soybean oil. Fat sources addition decreased dry matter intake (23.2 vs 21.7 kg/d) and increased (P<0.001) ether extract intake and digestibility (P<0.001). Ruminal pH, NH3-N, total volatile fatty acid, acetate, propionate and butyrate production were similar among treatments (P≥0.121). Fat supplementation had no effect on milk yield (32.0 kg/d) and composition. However, soybean oil decreased milk fat yield compared to CSFA diets. Dietary addition of fat sources decreased N intake (P < 0.001) without affect on N balance. Inclusion of fat source in the diet, either as free or rumen-protected decreased dry matter intake without affect the ruminal fermentation and performance of mid-lactation dairy cows.

Palavras chave adicionais

Digestibilidade.
Lipídios.
Ácido linoleico.
Produção de leite.
Balanço de nitrogênio.

Efeitos da suplementação de lipídeos no desempenho de vacas leiteiras

RESUMO

Este estudo teve como objetivo avaliar os efeitos de suplementação de gordura com óleo de soja (OS) e sais de cálcio de ácidos graxos (SC) em vacas leiteiras em meio de lactação no consumo e digestibilidade de nutrientes, fermentação ruminal, produção e composição do leite e balanço de nitrogênio. Dezesseis vacas holandesas multíparas (638 ± 73 kg de peso corporal e 116 ± 26 dias em leite) foram utilizadas em um delineamento quadrado latino 4 x 4 para avaliar as seguintes dietas: cont) controle, sem fonte de gordura adicional; OS) 30 g/kg de óleo de soja; SC1) inclusão de 30 g/kg de sais de cálcio de ácidos graxos de cadeia longa (MEGALAC-E ®); e SC2) inclusão de 30 g/kg de sais de cálcio de ácidos graxos de cadeia longa (LACTOPLUS ®) na base de matéria seca. Ambos avaliados SC foram fabricados com óleo de soja. FA adição de fontes de gordura reduziu ingestão de matéria seca (23,2 vs 21,7 kg/d) e aumentou (P<0,01) o consumo e digestibilidade do extrato etéreo (P<0.001). pH ruminal, NH_3-N, produção de ácido graxo volátil total, acetato, proprionato e butirato foram semelhantes entre os tratamentos (P≥0,121). A suplementação de gordura não teve efeito na produção de leite (32,0 kg/d) e composição. No entanto, o óleo de soja diminuiu o rendimento do leite em comparação com dietas SC. A adição dietética de fontes gordas diminuiu o consumo de N (P<0,001) sem afetar o balanço de nitrogênio. Inclusão de fonte de gordura na dieta, seja como livre ou protegida, diminuiu a da ingestão de matéria seca, sem afetar a fermentação ruminal e desempenho de vacas leiteiras em meio de lactação.

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INTRODUCTION

The use of fat supplement has been a common practice in the nutrition of high-producing dairy cows, especially to improve cow's energy status (Santos et al., 2009). Feeding fat sources to lactating dairy cows may increase energy intake without reduce diet fiber content, providing higher energy intake. In this sense, the effect of supplemental fat sources on the performance of lactating dairy cows may be evaluated by the replacement of concentrate for fat sources (Jenkins & Bridges, 2007).

Fat supplement to dairy cows could be provided as free-oils, oilseeds or by-product. The use of protected fat source in ruminant diets is advantageous since the lipid content is slowly released in the rumen, preventing the negative effects of unsaturated fatty acids (FA) on fibrolytic microorganisms (Coppock & Wilks, 1991; Palmquist & Conrad, 1991). Calcium salts of fatty acids (CSFA) is a form of rumen-protected fat that consist of FA source complexed with calcium ion making them insoluble. Normally, an unprocessed fat source (free oil) has ruminal fatty acid biohydrogenation among 80% to 90%, while CSFA have ruminal fatty acid biohydrogenation between 30 to 40% (Klusmeyer et al., 1991).

Different responses to supplemental fat sources in dairy cows diets are observed on dry matter intake (DMI), especially when different forms of fat supply are compared (NRC, 2001). Moreover, the negative effects of supplemental fat are more variable when diets are mostly or only based on corn silage as roughage source (Jenkins & Bridges, 2007). Our hypothesis was different protected fat source (CSFA) would affect release of fatty acids, ruminal fermentation and consequently milk fat production. Therefore, this study aimed to evaluate the effects of additional free (soybean oil) or rumen-protected fat source (CSFA) in the diet of dairy cows on intake, nutrient digestibility, ruminal fermentation, nitrogen balance, serum parameters, milk yield and composition.

MATERIAL AND METHODS

Animals, design and diets

Sixteen multiparous Holstein cows averaging 116 ± 26 days in milk, 638 ± 73 kg of body weight (BW) were used in a 4 × 4 Latin square design, lasted for 21 days, being 14 days of adaptation and the last seven days for sampling and data records. Throughout the experimental periods, cows were housed in a barn containing individual pens (17.5 m^2), sand bed, with forced ventilation and free access to water.

The animals were allocated into four balanced Latin squares, considering milk yield, body weight and days in milk. Within Latin square, cows were randomly assigned to receive one of four treatments sequences. Experimental diets (Table 1) were formulated accord-

Table I. Ingredients and chemical composition of diets (Ingredientes e composição química de dietas).

Item	Experimental diets[1]			
	CONT	SO	CSFA1	CSFA2
Ingredient (g/kg)				
Corn silage	500	501	501	501
Ground corn	263	230	239	239
Soybean meal	203	206	206	206
Soybean oil	-	29.9	-	-
Calcium salts of fatty acids	-	-	29.9	29.9
Urea	4.0	4.0	4.0	4.0
Ammonium sulfate	0.4	0.4	0.4	0.4
Sodium bicarbonate	7.9	7.9	7.9	7.9
Dicalcium phosphate	5.5	5.5	5.5	5.5
Magnesium oxide	0.9	0.9	0.9	0.9
Limestone	9.9	9.9	1.3	1.3
Mineral mix[2]	2.2	2.2	2.2	2.2
Salt	2.6	2.6	2.6	2.6
Chemical composition (g/kg of DM)				
Dry matter (g/kg as fed)	585	589	588	587
Organic matter	913	913	905	905
Non-fiber carbohydrate[3]	369	367	373	373
Neutral detergent fiber	314	312	313	313
Acid detergent fiber	201	200	200	200
Crude protein	178	176	177	177
Ash	87.4	86.6	95.0	94.9
Ether extract	31.2	60.0	55.4	54.9
NEL_{3x}[4] (Mcal/kg DM)	1.91	1.98	2.00	2.00

[1]Control (CO), Soybean oil (SO), Megalac - E® (CSFA1), and Lactoplus® (CSFA2); [2]Each kg contained: 42.7 g of Mg; 42.7 g of Zn; 10.4 g of Cu; 206 mg of S; 250 mg of Co; 625 mg of I; 316 mg of Se; 400,0000 UI of Vitamin A; 1,000,000 UI of Vitamin A; 18,750 UI of Vitamin E; [3]Non-fiber carbohydrate = 1000 – [(crude protein content – crude protein from urea + urea) + neutral detergent fiber + ether extract + ash] in which values were expressed as g/kg DM, from Hall (1998); [4]Estimated using the NRC (2001) model.

ing to NRC (2001), as follows: CONT) control diet, without additional fat source; SO) inclusion of 30 g/kg of soybean oil; CSFA1) inclusion of 30 g/kg of calcium salts of long-chain fatty acids (MEGALAC-E®, Química Geral do Nordeste and Arm & Hammer, Inc., Brazil); and CSFA2) inclusion of 30 g/kg of calcium salts of long-chain fatty acids (LACTOPLUS®, Dalquim-Nutriacid Nutrição e Ciência, Brazil) on diet dry matter (DM) basis.

The experimental procedures were approved by the Ethics Committee of the School of Veterinary Medicine and Animal Science of the University of Sao Paulo (approval number 2965/2013).

Data, sampling collection and chemical analyses

Cows were fed twice daily as total mixed ration at 0800 and 1300h to supply 105-110% of expected intake. Samples of feeds and orts were daily collected on the last seven days of each experimental period, and then composited into one sample per cow and period, and were frozen until analysis. On d 16 to 18 of each period, samples of feces were collected from cows twice daily after milking and composited into one sample per cow and period. Samples of feeds, orts and feces were dried in a 60°C forced-air oven for 72 h, ground to pass through a 1-mm screen (Wiley Mill, Arthur H. Thomas, Philadelphia, PA, USA), and then analyzed for DM (950.15), ash (942.05), ether extract (EE, 920.39), crude protein (CP, N × 6.25; 984.13), according to AOAC (2000). Neutral detergent fiber (NDF), and acid detergent fiber (ADF) were analyzed according to Van Soest et al. (1991). The NDF analysis was determined using α-amylase without addition of sodium sulfite to the detergent (Ankom Tech. Corp., Fairport, NY, USA).

Digestibility of nutrients was determined based on fecal excretion and nutrient concentration in feces. Total fecal excretion was estimated using indigestible acid detergent fiber (iADF) as marker. Samples of feeds, orts and feces were dried at 60°C forced-air oven for 72 h, ground to pass through a 2-mm screen (Wiley Mill, Arthur H. Thomas, Philadelphia, PA, USA), and placed in bags of non-woven textile (100 g/m^2) and incubated over 288 h in the rumen of two cows fed a similar diet used in this trial (Casali et al., 2008). After removal from the rumen, bags were analyzed for ADF concentration as previously described.

On days 16 of each period, spot urine samples were collected from each cow 4 hours after the morning feeding. Urine samples were filtered and 10 mL aliquots were diluted immediately with 40 mL of sulfuric acid (0.036 N), and stored at -20°C for analysis of uric acid and allantoin. Uric acid and creatinine concentrations were analyzed using commercial kits (Laborlab, Guarulhos, Brazil) in a semi-automatic spectrophotometer (SBA 200, São Caetano do Sul, Brazil). Allantoin in the urine and milk were determined by colorimetric method (Fijuhara & Yamagushi, 1978). Total nitrogen was analyzed according to method (984.13; AOAC, 2000). Daily urine volume was estimated from the daily creatinine excretion as 0.212 mMol/kg of BW (Chizzotti et al., 2008). Microbial protein synthesis was calculated from total excretion of purine derivatives (uric acid and allantoin) according to Chen & Gomes (1992), and nitrogen balance was performed according to NRC (2001).

Ruminal fermentation parameters

Rumen fluid samples were collected from each cow using esophageal gavage 3 h after the morning feeding on day 20 of each period. The ruminal pH value was determined immediately after each collection using a digital pH meter (MB-10, Marte Científica, Santa Rita do Sapucaí, Brazil). The ammonia nitrogen (NH_3-N) concentration was analyzed by the colorimetric phenol-hypochlorite method (Broderick & Kang, 1980). Ruminal volatile fatty acid (VFA) were measured with a gas chromatograph (GC-2014, Shimadzu, Tokyo, Japan) with split injector and dual flame ionization detector temperature at 250°C, and equipped with a capillary column (Stabilwax, Restek, Bellefonte, PA, USA) at 145°C, according to the method described by Erwin et al. (1961) and adapted by Getachew et al. (2002).

Serum parameters

Blood samples (10ml) were obtained on 19th day of each experimental period, before the morning feeding. Samples were centrifuged at 500 × g for 15 min. and serum was stored at -20°C for analysis of metabolites. It was accessed cholesterol (K-083), HDL-cholesterol (K-071), glucose (K-082), urea (K-056), AST (K-048) and GGT (K-080) levels, using commercial colorimetric kits (Laborlab) and readings were performed in a semi-automatic spectrophotometer (SBA 200).

Milk production and composition

Cows were mechanically milked twice daily at 0600 and 1600 h, and milk production was recorded electronically by an automatic milk meter (Alpro®, DeLaval – Tumba, Sweden). Fat-corrected milk was calculated according to Sklan et al. (1992). On days 16 to 18 of each period, fresh milk samples proportional to daily milking's were collected and analyzed for fat, protein, and lactose (Milkoscan, Foss Electric, Hillerod, Denmark).

Statistical analysis

Data were analyzed by PROC MIXED (9.3, SAS Institute Inc., Cary, NC, USA) according to the following model:

$$Y_{ijkl} = \mu + S_i + a_{j:i} + T_k + P_l + e_{ijkl}$$

with , , Y_{ijkl} where is the value of the dependent variable; μ is the overall mean; S_i is the fixed effect of the ith Latin Square (i = 1 to 4); $a_{j:i}$ is the random effect of the jth animal within the ith Latin square; T_k is the fixed effect of the kth treatment (k = 1 to 4); P_l is the fixed effect of the lth experimental period (l = 1 to 4); e_{ijkl} is the random residual error; N stands for the Normal distribution; is the variance due to animals; is the variance due to animals and is the residual variance. Degrees of freedom were corrected using Satterth option. The effect of treatments was decomposed into three orthogonal contrasts: (1) the 3 diets with fat source vs the control; (2) Soybeans oil vs diets containing CSFA; and (3) CSFA1 vs CSFA2. Difference was declared significant at ≤ 0.05. Tendency was considered when $0.05 < P \leq 0.10$.

Table II. Effects of different forms of fat supplementation on nutrient intake and digestibility of lactating dairy cows (Effects of different forms of fat supplementation on nutrient intake and digestibility of lactating dairy cows).

Item	Experimental diets[1]				SEM[2]	P-value[3]		
	CONT	SO	CSFA1	CSFA2		C1	C2	C3
Intake (kg/day)								
Dry matter	23.2	21.2	22.0	21.9	0.32	0.003	0.074	0.862
Organic matter	21.2	19.6	19.9	19.9	0.29	0.002	0.406	0.865
NFC[4]	9.35	8.26	8.22	8.26	0.13	<0.001	0.899	0.822
NDF[5]	6.74	6.11	6.36	6.36	0.10	0.001	0.080	0.984
Crude protein	4.31	3.93	4.08	4.07	0.05	0.003	0.047	0.871
Ether extract	0.72	1.32	1.26	1.23	0.03	<0.001	0.003	0.179
NEL_{3x}[6]	43.2	47.8	47.2	46.0	0.68	<0.001	0.172	0.199
Digestibility coeficients								
Dry matter	0.701	0.726	0.724	0.709	0.005	0.081	0.425	0.246
Organic matter	0.720	0.746	0.739	0.725	0.005	0.109	0.203	0.252
NDF[5]	0.546	0.539	0.580	0.555	0.007	0.379	0.063	0.151
Crude protein	0.757	0.776	0.776	0.764	0.004	0.088	0.540	0.281
Ether extract	0.809	0.887	0.881	0.871	0.007	<0.001	0.098	0.457

[1]Control (CONT), Soybean oil (SO), Megalac - E® (CSFA1), and Lactoplus® (CSFA2); [2]Standard error of the mean; [3]Probabilities: C1: control *vs*. fat sources [CONT *vs* (SO+CSFA1+CSFA2); C2: soybean oil *vs.* CSFA (CSFA1+CSFA2); and C3: CSFA1 *vs.* CSFA2; [4]Non-fiber carbohydrate; [5]Neutral detergent fiber; [6]Net energy of lactation, at three times maintenance intake level.

RESULTS

Intake and digestibility

Animals feeding diets containing fat sources showed lower DM, organic matter (OM),non-fibrous carbohydrates (NFC), NDF and CP and higher EE intake in relation to those fed with control diet (P≤0.003; Table 2). Additionally, fat addition increased (P<0.001) NEL_{3x} intake and EE digestibility and tended to increase (P≤0.088) DM and CP digestibility.

Soybean oil showed lower (P≤0.047) CP and higher EE intake than CSFA. Moreover, SO tended to decrease (P≤0.080) NDF digestibility and DM and NDF intake. However, animals fed with SO tended to have higher (P=0.098) EE digestibility, then those fed with CSFA. Evaluated CSFA's showed similar (P≥0.151) nutrients intake and digestibility.

Ruminal fermentation

There was no effect (P≥0.121; Table 3) of fat sources on ruminal pH and NH_3-N concentration. Similarly, dietary inclusion of fat sources did not affect (P≥0.152) the concentration of total VFA, acetate, propionate and butyrate, beyond acetate: propionate ratio.

Nitrogen balance and microbial protein synthesis

Daily N intake and fecal N were decreased (P≤0.006; Table 4) by diets containing additional fat source compared to control. Furthermore, fat supplementation tended to increase (P=0.088) N usage efficiency and showed no effects (P≥0.578) on milk N, N balance and microbial protein. On the other hand, cows fed

Table III. Effects of different forms of fat supplementation on ruminal fermentation of lactating dairy cows (Efeito da suplementação de diferente fontes de gordura na fermentação ruminal de vacas em lactação).

Item	Experimental diets[1]				SEM[2]	P-value[3]		
	CONT	SO	CSFA1	CSFA2		C1	C2	C3
pH	6.58	6.61	6.67	6.64	0.03	0.410	0.520	0.767
NH_3-N (mg/L)	1.87	2.24	1.87	2.17	0.084	0.153	0.185	0.121
VFA[4] (mmol/L)								
Total	143	139	133	138	5.11	0.648	0.801	0.725
Acetate	95.9	95.9	90.8	91.9	3.37	0.716	0.598	0.918
Propionate	30.7	27.8	26.5	30.8	1.33	0.464	0.795	0.265
Butyrate	15.9	15.3	15.7	15.8	0.67	0.822	0.788	0.998
C2:C3[5]	3.37	3.48	3.54	3.14	0.10	0.926	0.389	0.152

[1]Control (CO), Soybean oil (SO), Megalac - E® (CSFA1), and Lactoplus® (CSFA2); [2]Standard error of mean; [3]Probabilities: C1: control *vs*. fat sources [CONT *vs* (SO+CSFA1+CSFA2); C2: soybean oil *vs.* CSFA (CSFA1+CSFA2); and C3: CSFA1 *vs.* CSFA2; [4] Volatily fatty acids; [5]Acetate to propionate ratio.

Table IV. Effects of different forms of fat supplementation on nitrogen balance and microbial protein synthesis of lactating dairy cows (Efeito da suplementação de diferente fontes de gordura no balanço de nitrogênio e síntese de proteína microbiana de vacas em lactação).

Item	Experimental diets[1]				SEM[2]	P-value[3]		
	CONT	SO	CSFA1	CSFA2		C1	C2	C3
Nitrogen (g/day)								
Intake	690	629	653	651	8.66	0.003	0.047	0.871
Fecal	185	160	164	176	6.14	0.006	0.159	0.145
Urinary	171	169	171	155	5.21	0.578	0.561	0.210
Milk	149	149	148	150	3.67	0.809	0.912	0.664
Balance	184	151	170	171	14.6	0.586	0.130	0.972
Efficiency[4]	0.216	0.237	0.227	0.230	0.065	0.088	0.101	0.618
Microbial protein (kg/d)	2.32	2.56	2.17	2.22	0.091	0.992	0.063	0.809
Microbial efficiency (g/kg TDN)	142	160	135	143	6.50	0.689	0.089	0.610

[1]Control (CO), Soybean oil (SO), Megalac - E® (CSFA1), and Lactoplus® (CSFA2); [2]Standard error of mean; [3]Probabilities: C1: control *vs*. fat sources [CONT *vs* (SO+CSFA1+CSFA2); C2: soybean oil *vs.* CSFA (CSFA1+CSFA2); and C3: CSFA1 *vs.* CSFA2; [4]Milk N and N intake ratio.

SO showed reduced (P=0.047) N intake and tended (P≤0.063) to have higher microbial protein and microbial efficiency than those fed CSFA diets. Diets containing CSFA showed similar results (P≥0.145) on nitrogen usage variables and microbial protein.

Serum parameters

Fat supplementation increased (P≤0.022; Table 5) total and HDL cholesterol, without major effects on serum parameters (P≥0.111). Animals fed SO showed lower (P=0.046) serum urea than those fed CSFA. There was no differences (P=0.150) between evaluated CSFA on serum parameters.

Milk yield and composition

In general, fat source dietary addition had no effect on milk yield and composition (P≥0.135; Table 6). However, SO diet decreased (P=0.036) fat production and tended to decrease (P≤0.083) milk yield and fat corrected milk. Milk fat, protein and lactose proportions were not affected by CSFA type.

DISCUSSION

Although supplemental fat sources reduced dry matter intake (DMI), it did not affect the performance of cows. In the current study, the dietary inclusion of fat sources reduced 1.5 kg/day the DMI and consequently decreased OM, NFC, NDF and CP intake. Similarly, previous studies reported that inclusion of fat sources in diets reduced DMI of lactating dairy cows from 0.1 to 2.1 kg/day (Weld & Armentano, 2017). Normally, the use of fat source such as oil or hydrogenated FA had negative effects on DMI in diets containing 50 to 60 g/kg of EE, and CSFA strongly diminished the DMI (NRC, 2001), but it was not observed in this study. According to Allen (2000), the major reasons fat source could inhibit DMI in ruminants includes the negative effect of fat on rumen fermentation, acceptability of diets, intestinal motility, release of gut hormones, and limited capacity of FA biohydrogenation; however, the exact mechanism involved in the regulation of consumption is unclear. On the other hand, the replacement of carbohydrate by fat source can increase dietary energy density, requiring less feed to meet energy re-

Table V. Effect of different forms of fat supplementation on serum parameters of lactating dairy cows (Efeito da suplementação de diferente fontes de gordura em parâmetros metabólicos de vacas em lactação).

Item	Experimental diets[1]				SEM[2]	P-value[3]		
	CONT	SO	CSFA1	CSFA2		C1	C2	C3
Serum parameters (mg/L)								
Cholesterol	17.6	19.3	20.0	21.9	0.54	0.008	0.140	0.150
C-HDL[4]	4.10	4.67	4.54	4.75	0.126	0.022	0.908	0.452
Glucose	6.52	6.95	6.60	6.82	0.110	0.111	0.188	0.278
Urea	2.67	2.58	2.77	2.74	0.078	0.738	0.046	0.750
Hepatic enzymes (UI/L)								
AST	54.7	52.0	53.7	52.2	1.95	0.620	0.830	0.760
GGT	25.7	26.8	27.5	26.0	1.84	0.434	0.467	0.658

[1]Control (CO), Soybean oil (SO), Megalac - E® (CSFA1), and Lactoplus® (CSFA2); [2]Standard error of mean; [3]Probabilities: C1: control *vs*. fat sources [CONT *vs* (SO+CSFA1+CSFA2); C2: soybean oil *vs.* CSFA (CSFA1+CSFA2); and C3: CSFA1 *vs.* CSFA2; [4]High-density lipoprotein cholesterol; [5]Aspartate transaminase; [6]Gamma-glutamyl transferase.

Table VI. Effect of different forms of fat supplementation on milk production and composition of lactating dairy cows (Efeito de diferentes formas de suplementação de gordura na produção de leite e composição de vacas leiteiras em lactação).

Item	Experimental diets[1]				SEM[2]	P-value[3]		
	CONT	SO	CSFA1	CSFA2		C1	C2	C3
Production (kg/day)								
Milk yield	31.8	31.3	32.7	32.2	1.64	0.680	0.075	0.553
3.5% FCM[4]	32.2	31.2	32.9	32.5	1.56	0.592	0.083	0.438
Fat	1.13	1.09	1.15	1.14	0.06	0.460	0.036	0.283
Protein	0.95	0.95	0.96	0.96	0.05	0.814	0.803	0.734
Milk composition (g/kg)								
Lactose	45.4	45.4	4.51	4.52	0.60	0.413	0.159	0.351
Fat	35.6	34.7	3.52	3.53	0.22	0.135	0.182	0.869
Protein	30.2	30.4	3.01	2.98	0.40	0.575	0.113	0.270

[1]Control (CO), Soybean oil (SO), Megalac - E® (CSFA1), and Lactoplus® (CSFA2); [2]Standard error of mean; [3]Probabilities: C1: control vs. fat sources [CONT vs (SO+CSFA1+CSFA2); C2: soybean oil vs. CSFA (CSFA1+CSFA2); and C3: CSFA1 vs. CSFA2; [4]3.5% FCM = (0.432 + 0.165 × milk fat percentage) × kg of milk yield, from Sklan et al. (1992).

quirement of dairy cows since fat has 2.25 times more energy than carbohydrate.

Digestibility of nutrients was similar among treatments, except for EE digestibility, which was increased by dietary addition of fat. However, this response contrasts with the theory that unsaturated fat sources reduce the nutrient digestibility, especially NDF. Ben Salem et al. (1993) reported a decrease on NDF digestibility with addition of 7% of rapeseed oil in the diet of dairy cows, especially when corn silage was the forage source. Weld & Armentano (2017), in a metanalystic study found 8% of total tract NDF digestibility decrease with vegetable oil supplementation, while CSFA had a small positive effect on this variable. According to Jenkins (1993), fiber digestibility could be reduced when supplementing FA because fat may form films that cover feed particles impairing the microbial attachment or unsaturated FA have a toxic effect on the cellulolytic bacteria. Furthermore, in the current study the lack of effect of fat source on nutrient digestibility may be related to the ruminal microorganism adaptation that decreases adverse effects of lipids on ruminal fermentation (Coppock & Wilks, 1991; Palmquist & Conrad, 1991; Bettero et al., 2017).

In the present study, the dietary inclusion of fat sources either in free or rumen-protected forms did not affect ruminal fermentation. Several factors could contribute to changing ruminal fermentation when supplying fat sources to dairy cows, such as physical form (whole, ground, cake, oil), their ability to stimulate rumination, and DMI. In this sense, Rennó et al. (2014) reported no effects on ruminal pH and NH_3-N when cows were fed SO or CSFA compared to control. However, the concentration of propionate was increased and butyrate decreased when dairy cows were fed fat sources. This result probably occurs because rumen digestion of structural carbohydrates could be impaired when fat source is added to dairy cow diets, but NDF digestibility was not affected in the current study. Furthermore, the lack of effect of fat source on the ruminal fermentation even decreased NFC intake in the diets supplemented with fat sources in this study is probably related to microorganism adaptation to fat and better utilization of the energy diet in the rumen, since microbial protein synthesis and energy balance were not affected by fat source (Barletta et al., 2016). On the other hand, differences in diet composition, roughage source, amounts of fat added to diet, and FA profile of fat source may be related with different results from the studies.

The lower N intake and fecal N are related to the reduction of DM and CP intake in dairy cows fed diets with fat sources, since nutrient digestibility was not impaired. However, these effects did not change N balance and efficiency of N utilization. Similarly, Freitas Jr. et al. (2013) reported that the addition of fat source in ration of lactating dairy cows reduced N intake without affecting the N balance and efficiency of N utilization. Also, other previous studies that evaluated the effects of fat source on N did not show differences on N balance (Barletta et al., 2016; Gandra et al., 2016). According to Palmquist et al. (1993), microbial protein synthesis can be impaired by reducing the availability of rapidly fermentable carbohydrate with the dietary addition of fat source. However, in this study microbial protein synthesis was not impaired by FA supplementation. In agreement with these findings, studies did not show effects of fat sources on microbial protein synthesis (Barletta et al., 2016; Bettero et al., 2017). Thus, our results suggest that additional fat sources slightly affect ruminal microorganism growth and protein metabolism in mid-lactating cows.

The increase on total and HDL cholesterol was already expected due to higher serum lipid in cows fed supplemental fat (Bauman & Lock, 2006). Nevertheless the lower urea current in cows supplemented with SO, can be associated with higher EE intake in SO, whose ruminal fatty acid biohydrogenation is higher than rumen-protected sources (Klusmeyer et al., 1991), requiring greater use of nitrogen by microbes into the rumen.

Milk yield and composition did not differ among treatments, despite the reduced DMI in dairy cows fed supplemental fat. These results agree with other stud-

ies that reported no differences on milk yield when supplementing either free-oil or CSFA to dairy cows (Freitas Júnior et al., 2013; Rennó et al., 2014; Barletta et al., 2016). These findings suggest that the nutrient supply to dairy cows, especially to mammary gland, was not impaired by dietary fat addition. However, inclusion of SO showed lower fat yield compared to CSFA. According to Jenkins and Bridges (2007), free FA in the rumen are rapidly and extensively biohydrogenated due to faster activity of lipases that lead to increase conjugated linoleic acid (CLA) isomer production. Furthermore, when incomplete ruminal biohydrogenation of unsaturated FA occurs, duodenal flow of CLA trans-10, cis-12 increases, and this isomer has an inhibitory effect on synthesis de novo in mammary gland (Bauman & Griinari, 2003; Peterson et al., 2003).

Additional fat source in the diet of lactating dairy cows reduced DMI. However, fat source either as free or rumen-protected forms did not affect ruminal fermentation and performance of mid-lactating dairy cows.

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