Effect of lipid supplementation on milk odd- and branched-chain fatty acids in dairy cows

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ABSTRACT

Eight ruminally fistulated, multiparous Holstein cows were arranged in a double 4 × 4 Latin square with 14-d periods to investigate the effects of lipid supplementation on performance, rumen parameters, the milk odd- and branched-chain fatty acid (OBCFA) profile, and the relationships between milk OBCFA and rumen parameters. Lipid supplementation is known to inhibit microbial growth in the rumen, decrease de novo microbial fatty acid synthesis, and increase the uptake of circulating fatty acids by the mammary gland; treatments were selected to isolate these effects on the milk OBCFA profile. The 4 treatments were (1) a lipid-free emulsion medium infused in the rumen (CTL), (2) soybean oil as a source of polyunsaturated fatty acids infused in the rumen (RSO), (3) saturated fatty acids (38% 16:0, 40% 18:0) infused in the rumen (RSF), and (4) saturated fatty acids infused in the abomasum (ASF). Fat supplements were provided continuously as emulsions at a rate of 450 g/d. Preplanned contrasts compared CTL to RSO, RSO to RSF, and RSF to ASF. Infusing RSO slightly decreased ruminal pH, but did not affect volatile fatty acids profile and milk fat concentration as compared with CTL. The yields of energy-corrected milk, fat, and protein were greater with RSF compared with RSO. The concentration of odd-chain fatty acids was decreased by RSO, whereas even-chain *iso* fatty acids were not affected. Milk fat concentration of 17:0 + *cis*-9 17:1 was higher for RSF than for RSO, due to the saturated fatty acids supplement containing 2% 17:0 + *cis*-9 17:1. Limited differences were observed in the milk OBCFA profile between RSF and ASF. A multiple regression analysis yielded the following equation for predicting rumen pH based on milk fatty acids: $\text{pH} = 6.24 - (0.56 \times 4:0) + (1.67 \times \text{iso } 14:0) + (4.22 \times \text{iso } 15:0) + (9.41 \times 22:0)$. Rumen propionate concentration was negatively correlated with milk fat concentration of *iso* 14:0 and positively correlated with milk 15:0, whereas the acetate-to-propionate ratio gave the op-

posite correlations with milk *iso* 14:0 and 15:0. Milk fat concentration of 17:0 + *cis*-9 17:1 was not related to rumen propionate or to acetate-to-propionate ratio, due to the presence of 17:0 and *cis*-9 17:1 in the saturated fatty acids supplement. The results suggest that although lipid supplementation can affect the profile of milk OBCFA, the promise remains of using these milk fatty acids to evaluate rumen function.

Key words: odd- and branched-chain fatty acids, soybean oil, prilled fat, rumen fermentation, milk fat

INTRODUCTION

Fat supplementation in the nutrition of lactating dairy cows is an approach used to increase the energy density of the ration. In early-lactation cows, this feeding practice limits the extent of the negative energy balance which may support milk yield (Palmquist and Jenkins, 1980). However, lipid supplementation may affect various metabolic processes which determine the fatty acid (FA) profile of milk. It is well documented that the inclusion of supplemental lipids in the diet can alter the rumen environment, affecting microbial fermentation and VFA production (Ferguson et al., 1990; Jenkins and Jenny, 1992; Harvatine and Allen, 2006b). Further, PUFA, such as *cis*-9, *cis*-12 18:2, a predominant FA in soybean oil (approximately 50%), have been shown to be toxic to rumen microbiota, particularly cellulolytic bacteria (Maia et al., 2007), and thereby, inclusion of such lipid supplements may decrease the levels of FA of microbial origin in milk (mechanism 1). Lipid supplementation may also change the milk FA profile by increasing uptake of dietary FA by rumen microbes, thereby decreasing the proportion of de novo synthesized microbial FA, as reported by Emmanuel (1978), and Weisbjerg et al. (1992; mechanism 2). Finally, lipid supplementation may also directly affect FA synthesis by the mammary gland, either by increasing uptake of preformed FA (Palmquist et al., 1993; mechanism 3), or by inhibiting mammary gland lipogenesis through rumen synthesis of the CLA isomer *trans*-10, *cis*-12 18:2 (Bauman and Griinari, 2001; mechanism 4).

Odd- and branched-chain FA (OBCFA) found in ruminant tissues are predominantly produced by ruminal

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Table 1. Feed and chemical composition of basal diet

Item	Content
Ingredient, % of DM	
Grass silage	39.7
Corn silage	24.1
Dry ground corn	25.8
Corn gluten meal	5.5
Soybean meal	2.4
Vitamin and mineral mix ¹	1.7
Sodium bicarbonate	0.8
Chemical composition	
DM, % as fed	44.8
OM, % of DM	92.8
NDF, % of DM	26.4
ADF, % of DM	20.5
CP, % of DM	18.0
Fatty acids, mg/g of DM	
16:0	4.7
18:0	1.1
<i>cis</i> -9 18:1	6.5
<i>cis</i> -11 18:1	0.3
<i>cis</i> -9, <i>cis</i> -12 18:2	14.1
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	4.4
Others ²	0.4
Total	31.5

¹Contained, on a DM basis, 18.00% Ca, 5.00% P, 6.00% Mg, 0.06% K, 9.50% Na, 15.00% Cl, 1.00% S, 3,620 mg/kg of Fe, 45 mg/kg of I, 600 mg/kg of Cu, 2,000 mg/kg of Mn, 3,000 mg/kg of Zn, 25 mg/kg of Se, 20 mg/kg of Co, 300 kIU of vitamin A, 100 kIU of vitamin D, and 1,500 IU of vitamin E.

²Other fatty acids included minor concentrations of 12:0, 14:0, 15:0, 16:1, and 17:0.

microorganisms (Keeney et al., 1962; Harfoot, 1981), although a small proportion could come from postruminal synthesis (Massart-Leën et al., 1986; Vlaeminck et al., 2015). Different groups of rumen microbial species have unique OBCFA profiles (Vlaeminck et al., 2006a). Cellulolytic bacteria synthesize more *iso* FA, whereas amylolytic bacteria produce elevated levels of *anteiso* and linear odd-chain FA and relatively low levels of *iso* FA (Fievez et al., 2012). The intestinal absorption of microbially produced OBCFA and their uptake by the mammary gland lead to the appearance of OBCFA in milk fat of lactating dairy cows. Given that lipid supplementation can affect the various steps which determine milk FA profile, it is of interest to evaluate how the OBCFA profile of milk is affected by the presence of supplementary lipids in the diet.

The review by Fievez et al. (2012) of milk OBCFA and their relationship to rumen function indicates that *iso* 14:0 and *iso* 16:0 in milk are positively related to rumen acetate production, whereas milk 15:0 and 17:0 are negatively related to rumen acetate production and positively related to propionate production. Therefore, the OBCFA profile of the milk could potentially be used as a noninvasive method to predict certain aspects of rumen function. The first objective of the present experiment was to investigate the effects of lipid supple-

mentation on the aforementioned mechanisms of milk fat synthesis as well as on animal performance, rumen parameters, and milk OBCFA. An additional objective was to estimate the effect of lipid supplementation on the relationship between milk OBCFA and rumen function.

MATERIALS AND METHODS

Cows, Feeding, and Treatments

All procedures involving animals were conducted according to the regulations of the Canadian Council on Animal Care (1993), and were approved by the Université Laval Animal Care Committee. Eight ruminally fistulated, multiparous Holstein cows (BW: 698 ± 56 kg) in midlactation (101 ± 11 DIM) were housed in a tiestall facility at the Centre de Recherche en Sciences Animales de Deschambault (Deschambault, QC, Canada). Cows received a TMR (Table 1) formulated to meet predicted nutrient requirements (NRC, 2001). Cows were fed once per day at 1000 h, and always had free access to water. Samples of silages used in the TMR were taken once a week and oven-dried at 55°C for 48 h to determine DM content. The TMR was subsequently recalculated to maintain similar proportions of feed ingredients on a DM basis. Feed refused was weighed daily before feeding, and the amount of feed offered was adjusted to maintain 10% refusals.

Cows were arranged in a double 4 × 4 Latin square design with 14-d periods. For the duration of each period, cows received 1 of the following 4 treatments, which were all prepared as emulsions: (1) a ruminally infused lipid-free emulsion medium used as the control (**CTL**); (2) 450 g/d of a ruminally infused PUFA supplement [soybean oil, Soya Excel, Beloeil, QC, Canada, (**RSO**)] with putative effects on mechanisms 1 to 4; (3) 450 g/d of a ruminally infused SFA supplement [Energy Booster 100, Milk Specialties Global, Eden Prairie, MN, (**RSF**)] with putative effect on mechanisms 2 and 3; or (4) 450 g/d of an abomasally infused SFA supplement (**ASF**) with putative effect on mechanism 3. The FA profile of the experimental fat supplements is presented in Table 2. At 0900 h on d 14 of each period, 5 L of rumen content were transferred from cows who had received a specific treatment during the previous 14 d to cows that would receive that treatment for the next 14 d to decrease the time required for rumen adaptation to the new treatment.

Preparation of Emulsions and Infusion Procedure

Emulsions were prepared following the procedure described by Drackley et al. (1992) with modifications

Table 2. Fatty acid profile of experimental fat supplements

Fatty acids, % of total fatty acids	Soybean oil ¹	SFA ²
8:0	—	0.80
10:0	—	0.16
12:0	—	0.73
14:0	0.08	3.16
<i>iso</i> 15:0	—	0.08
<i>anteiso</i> 15:0	—	0.07
<i>cis</i> -9 14:1	—	0.11
15:0	—	0.56
<i>iso</i> 16:0	—	0.14
16:0	11.77	37.79
<i>trans</i> -9 16:1	—	0.07
<i>iso</i> 17:0	—	0.35
<i>cis</i> -9 16:1	0.09	0.33
<i>anteiso</i> 17:0	—	0.43
17:0	0.13	1.87
<i>iso</i> 18:0	—	0.16
<i>cis</i> -9 17:1	—	0.07
18:0	3.93	39.93
<i>trans</i> -6–8 18:1	—	0.50
<i>trans</i> -9 18:1	—	0.82
<i>trans</i> -10 18:1	—	1.80
<i>trans</i> -11 18:1	—	0.88
<i>trans</i> -12 18:1	—	0.29
<i>cis</i> -9 18:1	18.78	6.50
<i>trans</i> -15 18:1	—	0.19
<i>cis</i> -11 18:1	1.42	0.30
<i>cis</i> -12 18:1	—	0.10
<i>cis</i> -13 18:1	—	0.14
<i>trans</i> -16 18:1	—	0.23
19:0	—	0.20
<i>cis</i> -9, <i>cis</i> -12 18:2	56.52	0.66
20:0	0.34	0.49
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	6.35	0.06
<i>cis</i> -11 20:1	0.16	0.07

¹Soya Excel Inc., Beloeil, QC, Canada.²Energy Booster 100, Milk Specialties Global, Eden Prairie, MN.

(Dallaire et al., 2014). The emulsions were prepared on a weekly basis, and were stored at 4°C until utilization. The ingredient composition of experimental emulsions is presented in Table 3. Emulsions were ruminally infused using Nalgene tubing (4.8 mm i.d., 1.6-mm wall; Fisher Scientific, Ottawa, ON, Canada) that was passed through the rumen fistula (CTL, RSO, and RSF) or abomasally infused (ASF; Gressley et al., 2006). A peristaltic pump (Masterflex, Cole-Parmer Instrument Company, Montréal, QC, Canada) was used to continuously infuse the emulsions at a rate of 5.5 kg/d so that cows received 450 g/d of the lipid supplements (Table 3). Infusions started on d 0 of each period at 1000 h and ended on d 14 at 0900 h.

Experimental Measurements, Sampling, and Analyses

Cow Weights, DMI, and TMR. Cows were weighed at 1430 h on d 0, 1, and 2 of each period, and again for 3 consecutive days at the end of the ex-

periment. Feed offered and refused was weighed, and samples of the TMR were collected from d 9 to 13. Samples were dried at 55°C for 48 h to determine DM content, and then ground to 2 mm using a Wiley mill (model 4, Arthur H. Thomas Co., Philadelphia, PA). After being pooled by period, samples were ground to 1 mm using a Cyclotec Sample Mill (model 1093, Tecator Inc., Höganäs, Sweden). Pooled TMR samples were analyzed according to the following methods: residual moisture (method 934.01; AOAC International, 2005), ADF [Ankom Technology (Fairport, NY) method 5: ADF in feeds—filter bag technique for A200; solutions as in method 973.18, AOAC International (2005)], NDF [Ankom Technology method 6: NDF in feeds—filter bag technique for A200; solutions as in Van Soest et al. (1991) with the inclusion of heat-stable α -amylase], CP [$N \times 6.25$; method 968.06, AOAC International (2005)].

Dietary FA were directly transesterified, and FAME were extracted following the method described by Jenkins (2010) with modifications (Villeneuve et al., 2013). Fatty acid profile was determined with a gas chromatograph (Clarus 680; Perkin Elmer, Waltham, MA) equipped with a polar capillary column (HP-Innowax 30-m length, 0.320 mm i.d., 0.25 μ m film thickness; Agilent Technologies Canada Inc., Mississauga, ON, Canada) and a flame ionization detector. At the time of the sample injection (1 μ L) the column temperature was maintained at 185°C for 0.5 min, and then increased at 3°C/min to 220°C. Inlet and detector temperatures were at 240 and 250°C, respectively, and the split ratio was 50:1. The flow rate for hydrogen carrier gas was 1 mL/min.

Milk. Cows were milked twice daily at 0700 and 1700 h. Milk yield was recorded and samples were taken at

Table 3. Amount of lipid emulsion ingredients and fat supplements infused on a daily basis

Ingredient, g	Treatment ¹		
	CTL	RSO	RSF/ASF
Tap water	5,431	4,981	4,981
Whey protein concentrate ²	48	48	48
Tween 80 ³	21	21	21
Fat supplement			
Soybean oil ⁴	—	450	—
Energy Booster 100 ⁵	—	—	450
Total emulsion	5,500	5,500	5,500

¹CTL = control; RSO = soybean oil as a source of PUFA infused into the rumen; RSF = SFA infused into the rumen; ASF = SFA infused into the abomasum.²AMP 80, American Meat Protein Corporation, Boone, IA.³Fisher Scientific, Fair Lawn, NJ.⁴Soya Excel Inc., Beloeil, QC, Canada.⁵Milk Specialties Global, Eden Prairie, MN.

each milking from d 9 to 13 of each period. A first subsample was stored at 4°C with bronopol as preservative before being analyzed for fat, protein, and lactose by Valacta (Dairy Production Centre of Expertise, Ste-Anne-de-Bellevue, QC, Canada) using an infrared procedure with a Foss MilkoScan FT 6000 instrument (Foss, Hillerød, Denmark).

A second subsample was stored at -20°C without preservative for analysis of FA composition. Before analysis, these samples were thawed and pooled by period in proportion to daily milk yield. Milk FA profiles were determined according to the procedure described by Boivin et al. (2013).

Rumen Fluid. Fluid was collected on d 11 and 12 (one square per day) of each period from the ventral sac of the rumen using a 50-mL syringe screwed to a stainless tube ending with a probe covered by a fine metal mesh (RT rumen fluid collection tube; Bar Diamond Inc., Parma, ID), as described by Benchaar et al. (2013). Samples were collected at 0, 1, 2, 4, 6, 8, 10, and 12 h relative to feeding time. The pH of harvested rumen fluid was immediately measured (pHTestr 30; Oakton Instruments, Vernon Hills, IL). A 10-mL sample was pipetted into a glass vial containing 0.2 mL of sulfuric acid (50%), and stored at -20°C.

For the analysis of NH₃-N and VFA, the samples of rumen fluid collected in the glass vials were thawed at room temperature, transferred to 15-mL conical tubes, centrifuged at 25,200 × *g* for 15 min at 4°C, and transferred to microtubes. Concentration of NH₃-N was determined as in Weatherburn (1967) using a Varioskan spectrophotometer (type 3001, Thermo Electron Corporation, Vantaa, Finland) at 625 nm. The profile of ruminal VFA was determined with a gas chromatograph (Agilent 6890N; Agilent Technologies Canada Inc.) equipped with a DB FFAP capillary column (30 m × 0.25 mm i.d. × 0.25 µm film; Agilent Technologies Canada Inc.), and a flame ionization detector. The split ratio was 25:1. At the time of the sample injection the column temperature was 80°C, maintained for 0.5 min, followed by a first increase to 180°C at 10°C/min, and a second increase to 220°C at 30°C/min. The temperature was then maintained at 220°C for 2 min. All ruminal fluid samples were assayed in triplicate.

Statistical Analysis

Data were analyzed with the MIXED procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). Because no square effect was observed it was removed from the model and data were analyzed as a duplicated 4 × 4 Latin square design according to the following model:

$$Y_{ijk} = \mu + T_i + P_j + (T \times P)_{ij} + C_k + \varepsilon_{ijk},$$

where Y_{ijk} = individual observation, μ = overall mean, T_i = fixed effect of treatment ($i = 1$ to 4), P_j = fixed effect of period ($j = 1$ to 4), $(T \times P)_{ij}$ = interaction between treatment and period, C_k = random effect of cow ($k = 1$ to 8), ε_{ijk} = residual error term, and where the subject of the repeated statement was cow. Interaction between treatment and period was removed from the model when not significant. The covariance structure selection was based on the Akaike's information criterion. Satterthwaite's option was used to calculate the denominator degrees of freedom. To discriminate the different effects of lipid supplementation on mammary lipogenesis and the resulting effect on milk OB-CFA concentrations and yields, preplanned contrasts were established as follows: PUFA supplementation in the rumen against the control (RSO vs. CTL), PUFA supplementation against SFA supplementation in the rumen (RSO vs. RSF), and SFA supplementation in the rumen against SFA supplementation in the abomasum (RSF vs. ASF). Correlations between individual milk FA and rumen VFA concentrations were analyzed using the REG procedure of SAS (SAS Institute Inc.).

For statistical evaluation of the relationship between milk FA profile and rumen pH, a correlation analysis was first performed to calculate the Pearson coefficients of correlation (CORR procedure of SAS) of each individual milk FA concentration. Milk FA for which a significant relationship with rumen pH was observed were included in a selection procedure for multiple regression using a stepwise procedure (GLMSELECT of SAS) with rumen pH being the dependent variable and stepwise selection of milk FA based on the Schwarz Bayesian information criterion. The selected model was then evaluated using the REG procedure of SAS in terms of multicollinearity (variance inflation factor >10), high influence and leverage observations by DF-FITS [$>2\sqrt{(p/n)}$, where p is the number of parameters estimated in the model and n the number of observations], Cook's distance ($>4/n$), and homoscedasticity by the normality of the residuals. The model was then fitted into a random regression using the MIXED procedure of SAS including cow as the subject of a random intercept, and of the repeated statement. The TYPE = VC (variance component) structure provided best fit for both the random and the repeated statements. Denominator degrees of freedom were calculated by the Satterthwaite equation. The associations between observed rumen pH and the values predicted by the model were evaluated by linear regression. In addition, prediction bias was assessed by regressing residuals against predicted values as described by Rico et al. (2016), and based on St-Pierre (2003). Results are reported as least

squares means, considered significant when $P \leq 0.05$, and reported as a tendency when $P < 0.10$.

RESULTS AND DISCUSSION

Cow Performance

Infusing RSO increased DMI as compared with CTL (Table 4). As summarized by Allen (2000), lipid supplementation has resulted in higher DMI in several studies; this author proposed that substitution of fat for grain can reduce the hypophagic effects of propionate by reducing its flux to the liver, a situation that does not apply to the current trial where soybean oil was infused into the rumen and where grain supplementation was not different between treatments. Allen (2000) also suggested that dietary FA have a lower heat increment than other energy sources. Inclusion of fat in diets would therefore allow for a greater DM consumption according to the concept of thermostatic control of feed intake (Bhattacharya and Warner, 1968).

Moreover, DMI tended to be lower in cows receiving ASF as compared with RSF (Table 4). Dietary fat is a potent stimulator of cholecystokinin release in the small intestine, and evidence exists that this hormone contributes to satiety (Allen, 2000). It could be hypothesized that abomasally infused fat (ASF) could have contributed to a greater stimulation of cholecystokinin secretion, potentially through a more intimate contact with duodenum epithelium, as compared with ruminally infused fat (RSF), a phenomenon that could not be assessed in the current experiment.

Cows receiving RSO gained more weight as compared with CTL (Table 4). Although the length of the experimental periods was relatively short, the positive effect on BW gain could be explained by a greater DMI (+1.1 kg/d; Table 4), including the energy provided by soybean oil infusion (450 g/d). No differences were observed for actual milk yield in any of tested contrasts, but fat and protein yields were higher for RSO than for CTL, as were ECM (Table 4). The increase in ECM for RSO compared with CTL is explained by cows receiving RSO having numerically higher actual milk yield and fat percentage. Such a positive effect on the yield of ECM and milk constituents could be explained by a higher feed consumption combined with the additional energy provided by the ruminally infused soybean oil.

Fat percentage and yield, as well as protein yield, were higher for cows receiving RSF compared with cows receiving RSO. Likewise, ECM was also higher for RSF than for RSO. We noted no significant differences on fat and protein percentages and yields between RSF and ASF (Table 4).

Rabiee et al. (2012) reviewed the effects of feeding saturated versus unsaturated fat supplements on milk yield and composition. In accordance with the results of the current experiment, Jenkins and Jenny (1992) observed no effect on DMI, but reported an increase in actual milk or FCM yield, and greater milk fat content and yield in cows fed prilled hydrogenated animal FA as compared with canola oil. On the contrary, in a more recent trial using Energy Booster 100, Abdelqader et al. (2009) reported no effect on the yield of milk, ECM,

Table 4. Dry matter intake, BW change, milk yield, and milk composition of cows receiving different lipid supplements

Parameter	Treatment ¹					P-value		
	CTL	RSO	RSF	ASF	SEM	CTL vs. RSO	RSO vs. RSF	RSF vs. ASF
DMI, ² kg/d	29.3	31.4	31.2	29.1	1.44	0.05	0.80	0.06
BW change, ³ kg	-4.71	3.30	2.06	3.09	2.46	0.05	0.74	0.78
Milk yield, kg/d								
Actual	36.1	36.9	36.4	36.1	1.83	0.72	0.83	0.90
Energy-corrected ⁴	36.3	38.2	40.1	38.9	1.51	0.02	0.03	0.14
Milk fat								
Content, %	4.11	4.22	4.43	4.35	0.17	0.17	0.01	0.31
Yield, g/d	1,495	1,588	1,709	1,655	74	0.01	<0.01	0.13
Milk protein								
Content, %	3.23	3.19	3.21	3.19	0.07	0.16	0.42	0.31
Yield, g/d	1,170	1,206	1,244	1,216	43	<0.01	0.03	0.08
Lactose								
Content, %	4.71	4.73	4.71	4.69	0.03	0.30	0.23	0.28
Yield, g/d	1,717	1,806	1,824	1,776	79	0.03	0.63	0.21

¹CTL = control; RSO = soybean oil (Soya Excel Inc., Beloeil, QC, Canada) as a source of PUFA infused into the rumen; RSF = SFA (Energy Booster 100, Milk Specialties Global, Eden Prairie, MN) infused into the rumen; ASF = SFA infused into the abomasum.

²Including ruminally or abomasally infused fat supplements.

³Over a 14-d period.

⁴ $\{[23.8 \times (\text{protein yield in kg/d})] + [38.9 \times (\text{fat yield in kg/d})] + [16.3 \times (\text{lactose yield in kg/d})]\}/3.14$ (Madsen et al., 2008).

and fat, but an increase in milk fat concentration as compared with cows fed corn oil.

A decrease in milk fat content is expected when vegetable oils rich in *cis*-9,*cis*-12 18:2 (e.g., soybean or sunflower) are fed to dairy cows (Glasser et al., 2008). Such inhibition of milk fat synthesis has been attributed to a shift in PUFA biohydrogenation pathways occurring when rumen environment is altered (Bauman and Grinari, 2001), leading to the production of *trans*-10,*cis*-12 18:2. This CLA isomer has been identified as an inhibitor of milk fat synthesis in lactating dairy cows (Bauman and Grinari 2001). However, in the current trial, milk fat percentage was not statistically different between RSO and CTL, despite the soybean oil representing 1.5% of DMI. As no significant levels of *trans*-10,*cis*-12 18:2 were found in the milk from cows receiving RSO treatment, we hypothesized that the continuous nature of the emulsified soybean oil infusions, at the current inclusion level, prevented the shift in ruminal biohydrogenation (Lanier and Corl, 2015) and thereby avoided the milk fat depression.

Nonetheless, milk fat content was lower with RSO as compared with RSF. Accordingly, a consistent increase in milk fat content has been observed in previous trials when prilled saturated fat supplements were compared with different sources of PUFA, such as canola oil (Jenkins and Jenny, 1992), corn oil (Abdelqader et al., 2009), and calcium soaps of UFA (Harvatine and Allen, 2006a). An analysis of the saturated fat supplement used in this experiment (Table 2) showed that it contained nearly 40% of each 16:0 and 18:0. As stated by Loften et al. (2014), dietary sources of 16:0 usually increase milk fat production, and supplying a mixture of 16:0 and 18:0 could optimize their utilization for milk production and overall performance in dairy cattle.

Rumen Parameters

Rumen pH was significantly lower for the RSO treatment than for the CTL or RSF (tendency) treatments (Table 5). There was no difference in rumen pH between cows receiving RSF and ASF treatments. In beef heifers, Krysl et al. (1991) reported a decrease in ruminal pH following a ruminal infusion of soybean oil. In dairy cows, Bateman and Jenkins (1998) reported linear and quadratic effects on ruminal pH as they increased soybean oil from 0 to 8% of diet DM. Specifically relevant to the results of the current experiment was that rumen pH decreased from 6.73 to 6.67 when soybean oil was included at 2% of DM. Nonetheless, previous work has indicated changes in pH above 6.2 do not affect ruminal fermentation (Pitt et al., 1996).

Total VFA concentration, proportions of individual VFA, and the acetate-to-propionate ratio were not affected by treatments (Table 5). Huang et al. (2008) also found no significant effects on total VFA concentration, or on individual VFA proportions between a control diet and a diet supplemented with soybean oil at 5% DM. When looking at the effects of SFA, Jenkins and Jenny (1992) found that prilled fat had no effect on total VFA content, acetate and propionate proportions, and acetate-to-propionate ratio when supplemented at 5% DM. However, when prilled fat was incrementally replaced by canola oil, linear decreases in total VFA content, acetate proportion, and acetate-to-propionate ratio were observed (Jenkins and Jenny, 1992).

Milk FA

Infusing RSO decreased the proportion of short- and medium-chain FA and increased milk fat content of most long-chain 18-carbon FA in milk fat (Table 6). These

Table 5. Ruminal pH, total VFA concentration, and VFA molar proportions of rumen cannulated cows receiving different lipid supplements

Variable	Treatment ¹					P-value		
	CTL	RSO	RSF	ASF	SEM	CTL vs. RSO	RSO vs. RSF	RSF vs. ASF
pH	6.34	6.23	6.33	6.38	0.08	0.05	0.09	0.40
Total VFA, mmol/L	79.4	91.7	90.5	80.2	4.87	0.08	0.85	0.14
Individual VFA, mol/100 mol								
Acetate	63.38	65.07	65.68	65.53	1.77	0.50	0.81	0.95
Propionate	19.76	18.94	18.63	17.97	0.95	0.54	0.82	0.62
Isobutyrate	1.16	1.09	1.07	1.16	0.09	0.60	0.85	0.47
Butyrate	11.85	11.00	10.92	11.26	0.63	0.32	0.93	0.69
Isovalerate	1.81	1.80	1.69	1.81	0.15	0.97	0.61	0.56
Valerate	1.39	1.36	1.32	1.40	0.09	0.81	0.82	0.61
Caproate	0.66	0.74	0.68	0.87	0.10	0.55	0.64	0.16
Acetate:propionate	3.35	3.56	3.63	3.73	0.24	0.52	0.84	0.74

¹CTL = control; RSO = soybean oil (Soya Excel Inc., Beloeil, QC, Canada) as a source of PUFA infused into the rumen; RSF = SFA (Energy Booster 100, Milk Specialties Global, Eden Prairie, MN) infused into the rumen; ASF = SFA infused into the abomasum.

effects are consistent with the reduction in de novo FA synthesis due to feeding unsaturated oils, which occurs as a result of greater uptake and secretion of dietary-derived FA (Palmquist et al., 1993). More specifically, infusion of RSO increased the proportions of 18:0, most of the *cis* and *trans* isomers of 18:1, as well as major biohydrogenation intermediates, such as *trans*-11,*cis*-15 18:2 and *cis*-9,*trans*-11 18:2, as compared with CTL and RSF (Table 6). These FA have been identified as the final product (18:0) or intermediates of ruminal biohydrogenation of *cis*-9,*cis*-12 18:2 and *cis*-9,*cis*-12,*cis*-15 18:3 (Shingfield and Wallace, 2014), the 2 major PUFA found in soybean oil (Table 2). In addition, infusion of RSF increased milk fat concentration of 16:0 as compared with RSO, which is consistent with the supply of this FA from Energy Booster 100 infusion.

We observed numerous treatment effects on individual milk OBCFA. Compared with CTL, RSO decreased the concentrations (Table 7) and yields (Table 8) of milk *iso* 13:0 and *iso* 15:0; *anteiso* 17:0 and the sum of *anteiso* FA; as well as 11:0, 13:0, 15:0, 17:0, and the sum of odd-chain FA (OCFA). Moreover, the concentration of *iso* 17:0 and the yield of *iso* 14:0 were increased by RSO, whereas no differences were observed for the concentrations and yields of *iso* 16:0 and *iso* 18:0 in milk between CTL and RSO (Tables 7 and 8).

Rumen amyolytic bacteria are enriched in *anteiso* FA and OCFA, whereas cellulolytic bacteria have higher levels of *iso* FA, as stated by Fievez et al. (2012). Maia et al. (2007) reported that rumen bacteria, particularly cellulolytic bacteria, are negatively affected by PUFA. However, as the concentrations and yields of odd *iso* and even *iso* FA in milk were not affected by RSO, it seems unlikely that RSO, at the current level of supplementation, had toxic effects on rumen bacteria. This is further evidence that the continuous infusion of the emulsified soybean oil helped diminish the negative effects normally associated with feeding PUFA. Whereas the reason for the decrease in proportions of *anteiso* FA and OCFA with RSO is not immediately clear, one could hypothesize that the increase in DMI with RSO could be effecting rumen bacteria and, thus, the milk OBCFA concentrations and yields. Alternatively, it has been established that rumen bacteria take up dietary FA and that lipid supplementation increases this uptake and decreases OBCFA synthesis by rumen bacteria (Bauchart et al., 1990; Weisbjerg et al., 1992). These findings corroborate with the current results in milk FA profile, where the proportion of OBCFA in relationship to total FA decreased for RSO. One possibility to explain why RSO decreased milk proportions of *anteiso* FA and OCFA compared with CTL, but did not affect even *iso* FA, may be that amyolytic bacteria are more adept replacing de novo FA synthesis with uptake

of dietary FA than cellulolytic bacteria. Although this idea requires further research, it could be supported by the observation that amyolytic bacteria are less sensitive to exogenous FA than cellulolytic strains (Doreau and Ferlay, 1995).

The RSF treatment significantly increased the concentrations and yields of milk *anteiso* 13:0, *anteiso* 17:0, and the sum of *anteiso* FA; 17:0, *cis*-9 17:1, and the sum of OCFA; *iso* 18:0; and total OBCFA, compared with RSO (Tables 7 and 8). The proportions of *anteiso* 13:0 and *iso* 18:0 were very low, so drawing any conclusions about the biological meanings of their variations in milk fat is difficult. The increase in concentration of 17:0 and *cis*-9 17:1 with RSF as compared with RSO is due to the SFA supplement used in the current experiment which contained 2% of 17:0 + *cis*-9 17:1 (Table 2). It is important to consider *cis*-9 17:1 together with 17:0 when looking at OBCFA in milk, as it has been shown that the Δ^9 -desaturase activity in the mammary gland converts biologically important quantities of 17:0 to *cis*-9 17:1 (Fievez et al., 2003). Infusing RSF at the rate of 450 g/d therefore provided a total supply of 9 g/d of 17:0 + *cis*-9 17:1, which were transferred in milk fat with an apparent efficiency of 46% for RSF and 42% for ASF, as calculated as recovery above the CTL treatment.

The increased proportion of *anteiso* 17:0 in the milk of cows receiving RSF compared with RSO, and the fact that this proportion numerically resembled the proportion from cows on CTL and ASF, gives rise to speculation that the PUFA in the soybean oil influenced this result. Whereas protozoa were found to contain lower levels of most OBCFA compared with rumen bacteria, they did have higher levels of *anteiso* 17:0 and UFA (Or-Rashid et al., 2007), which have been implicated with maintaining microbial membrane fluidity (Hauser et al., 1979). Based on this information, hypotheses to explain the depressed level of milk *anteiso* 17:0 for RSO would be that (1) soybean oil PUFA inhibited the growth of rumen protozoa, as reported by Doreau and Ferlay (1995), or (2) rumen protozoa were able to use some of the UFA from the soybean oil in place of de novo-synthesized *anteiso* 17:0 to maintain membrane fluidity.

We noted limited differences in the milk OBCFA profile between RSF and ASF. Among few exceptions, concentrations and yields of *iso* 14:0, *iso* 15:0, and *iso* 16:0 increased in milk from cows receiving ASF as compared with RSF (Tables 7 and 8). These FA are higher in cellulolytic bacteria, so the results indicate that infusing the emulsified SFA supplement directly into the rumen may have affected fiber digestion, or the growth of cellulolytic bacteria and their de novo FA synthesis. Similarly, Harvatine and Allen (2006b)

Table 6. Milk fat composition for cows receiving different lipid supplements¹

Item, % by weight	Treatment ²					P-value		
	CTL	RSO	RSF	ASF	SEM	CTL vs. RSO	RSO vs. RSF	RSF vs. ASF
4:0	2.09	2.21	2.14	2.14	0.06	0.06	0.24	0.91
6:0	1.61	1.64	1.55	1.53	0.04	0.50	0.05	0.58
8:0	0.99	0.96	0.90	0.89	0.03	0.42	0.03	0.54
10:0	2.52	2.26	2.15	2.12	0.12	<0.01	0.15	0.67
10:1	0.195	0.173	0.163	0.158	0.009	0.02	0.29	0.56
12:0	3.56	2.96	2.92	2.89	0.17	<0.01	0.77	0.81
12:1	0.095	0.071	0.079	0.077	0.005	<0.01	0.10	0.66
14:0	11.53	10.50	10.69	10.76	0.29	<0.01	0.29	0.72
<i>cis</i> -9 14:1	0.35	0.31	0.31	0.32	0.02	0.06	0.79	0.97
<i>cis</i> -11 14:1	0.053	0.045	0.045	0.046	0.004	0.14	0.88	0.86
16:0	32.32	27.26	31.81	32.03	0.56	<0.01	<0.01	0.67
<i>trans</i> -9 16:1	0.058	0.062	0.059	0.059	0.004	0.28	0.34	0.88
<i>cis</i> -9 16:1	1.14	0.94	1.06	1.14	0.08	<0.01	0.02	0.13
<i>cis</i> -11 16:1	0.065	0.078	0.075	0.061	0.007	0.07	0.66	0.07
<i>cis</i> -13 16:1	0.232	0.178	0.187	0.185	0.012	<0.01	0.29	0.85
18:0	8.50	11.45	10.07	9.49	0.45	<0.01	<0.01	0.02
<i>trans</i> -4 18:1	0.028	0.030	0.026	0.024	0.003	0.26	0.12	0.31
<i>trans</i> -5 18:1	0.016	0.021	0.023	0.019	0.002	0.20	0.59	0.28
<i>trans</i> -6–8 18:1	0.192	0.308	0.216	0.236	0.005	<0.01	<0.01	<0.01
<i>trans</i> -9 18:1	0.209	0.313	0.256	0.311	0.012	<0.01	<0.01	<0.01
<i>trans</i> -10 18:1	0.239	0.398	0.317	0.413	0.008	<0.01	<0.01	<0.01
<i>trans</i> -11 18:1	0.61	0.76	0.53	0.60	0.04	<0.01	<0.01	<0.01
<i>trans</i> -12 18:1	0.222	0.472	0.217	0.220	0.011	<0.01	<0.01	0.79
<i>trans</i> -13–14 18:1	0.41	0.85	0.40	0.37	0.02	<0.01	<0.01	0.08
<i>trans</i> -15 18:1	0.272	0.470	0.278	0.251	0.010	<0.01	<0.01	0.01
<i>trans</i> -16 18:1	0.250	0.436	0.250	0.225	0.013	<0.01	<0.01	0.06
<i>cis</i> -6–8 18:1	0.096	0.104	0.120	0.123	0.009	0.45	0.10	0.71
<i>cis</i> -9 18:1	12.43	14.94	13.76	13.97	0.47	<0.01	<0.01	0.48
<i>cis</i> -11 18:1	0.320	0.318	0.300	0.303	0.014	0.73	0.02	0.65
<i>cis</i> -12 18:1	0.231	0.479	0.230	0.226	0.012	<0.01	<0.01	0.71
<i>cis</i> -13 18:1	0.039	0.050	0.049	0.051	0.005	0.08	0.78	0.72
<i>cis</i> -14 18:1	0.028	0.035	0.026	0.023	0.003	0.01	<0.01	0.33
<i>cis</i> -15 18:1	0.036	0.038	0.047	0.044	0.004	0.54	0.08	0.53
<i>trans</i> -9, <i>trans</i> -12 18:2	0.014	0.014	0.021	0.016	0.003	0.97	0.16	0.32
<i>cis</i> -9, <i>trans</i> -12 18:2	0.141	0.121	0.124	0.109	0.007	0.02	0.71	0.05
<i>cis</i> -9, <i>trans</i> -13 18:2	0.183	0.268	0.194	0.170	0.008	<0.01	<0.01	0.03
<i>trans</i> -9, <i>cis</i> -12 18:2	0.075	0.104	0.080	0.071	0.005	<0.01	<0.01	0.22
<i>trans</i> -8, <i>cis</i> -13 18:2	0.077	0.122	0.081	0.071	0.005	<0.01	<0.01	0.09
<i>trans</i> -11, <i>cis</i> -15 18:2	0.085	0.105	0.086	0.086	0.007	0.02	0.04	0.99
<i>cis</i> -9, <i>cis</i> -12 18:2	1.44	1.53	1.30	1.34	0.08	0.09	<0.01	0.40
<i>cis</i> -6, <i>cis</i> -9, <i>cis</i> -12 18:3	0.035	0.035	0.043	0.036	0.003	0.97	0.04	0.08
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	0.190	0.196	0.169	0.173	0.013	0.59	0.02	0.73
<i>cis</i> -9, <i>trans</i> -11 18:2	0.20	0.24	0.17	0.18	0.03	0.04	<0.01	0.41
19:0	0.054	0.093	0.071	0.058	0.004	<0.01	<0.01	0.05
20:0	0.094	0.108	0.105	0.092	0.007	0.11	0.76	0.15
<i>cis</i> -9 20:1	0.056	0.067	0.066	0.056	0.004	0.04	0.84	0.05
<i>cis</i> -11 20:1	0.021	0.021	0.023	0.024	0.002	0.90	0.31	0.71
<i>cis</i> -5, <i>cis</i> -8, <i>cis</i> -11, <i>cis</i> -14 20:4	0.051	0.058	0.044	0.045	0.006	0.34	0.06	0.88
22:0	0.027	0.034	0.030	0.028	0.004	0.03	0.05	0.24
Others ³	0.71	0.69	0.69	0.75	0.04	0.65	0.98	0.30
Glycerol	11.43	11.34	11.35	11.34	0.03	<0.01	0.67	0.81
Sum								
De novo fatty acids ⁴	20.78	18.99	18.80	18.86	0.62	<0.01	0.56	0.86
C16 fatty acids	33.81	28.51	33.19	33.48	0.60	<0.01	<0.01	0.61
Preformed fatty acids ⁵	31.63	38.91	34.36	34.16	0.66	<0.01	<0.01	0.70

¹Excluding odd- and branched-chain fatty acids.²CTL = control; RSO = soybean oil (Soya Excel Inc., Beloeil, QC, Canada) as a source of PUFA infused into the rumen; RSF = SFA (Energy Booster 100, Milk Specialties Global, Eden Prairie, MN) infused into the rumen; ASF = SFA infused into the abomasum.³Represent unidentified chromatogram peaks.⁴Sum of straight even-chain fatty acids from C6 to C14.⁵Sum of odd- and branched-chain fatty acids (Table 7), and all fatty acids with a carbon chain length of 17 or more.

Table 7. Milk fat content of odd- and branched-chain fatty acids for cows receiving different lipid supplements

Item, % by weight	Treatment ¹				SEM	P-value		
	CTL	RSO	RSF	ASF		CTL vs. RSO	RSO vs. RSF	RSF vs. ASF
Odd <i>iso</i> fatty acids								
<i>iso</i> 13:0	0.031	0.026	0.024	0.025	0.001	0.01	0.23	0.79
<i>iso</i> 15:0	0.190	0.166	0.168	0.179	0.006	<0.01	0.58	0.02
<i>iso</i> 17:0	0.112	0.127	0.124	0.130	0.005	0.04	0.64	0.34
Sum of odd <i>iso</i> fatty acids	0.333	0.316	0.323	0.331	0.010	0.15	0.56	0.47
Even <i>iso</i> fatty acids								
<i>iso</i> 14:0	0.136	0.147	0.140	0.174	0.011	0.20	0.40	<0.01
<i>iso</i> 16:0	0.351	0.344	0.345	0.426	0.031	0.82	0.96	0.02
<i>iso</i> 18:0	0.017	0.017	0.024	0.016	0.002	0.74	<0.01	<0.01
Sum of even <i>iso</i> fatty acids	0.504	0.507	0.508	0.616	0.039	0.93	0.97	0.01
Anteiso fatty acids								
<i>anteiso</i> 13:0	0.022	0.016	0.022	0.015	0.002	0.05	0.02	0.01
<i>anteiso</i> 15:0	0.817	0.750	0.748	0.792	0.056	0.05	0.95	0.19
<i>anteiso</i> 17:0	0.334	0.285	0.349	0.353	0.010	<0.01	<0.01	0.71
Sum of <i>anteiso</i> fatty acids	1.173	1.051	1.119	1.161	0.058	<0.01	0.01	0.11
Odd-chain fatty acids								
11:0	0.080	0.061	0.063	0.055	0.005	<0.01	0.67	0.11
13:0	0.124	0.104	0.105	0.100	0.007	<0.01	0.79	0.35
15:0	1.221	1.024	1.074	1.060	0.041	<0.01	0.18	0.69
17:0	0.480	0.428	0.621	0.618	0.012	<0.01		0.81
<i>cis</i> -7 17:1	0.036	0.041	0.059	0.060	0.004	0.23	<0.01	0.86
<i>cis</i> -8 17:1	0.017	0.019	0.018	0.018	0.002	0.10	0.56	0.64
<i>cis</i> -9 17:1	0.146	0.132	0.171	0.174	0.007	0.01	<0.01	0.46
Sum of odd-chain fatty acids	2.106	1.809	2.107	2.086	0.057	<0.01	<0.01	0.72
Sum of odd- and branched-chain fatty acids	4.116	3.683	4.057	4.194	0.104	<0.01	<0.01	0.05

¹CTL = control; RSO = soybean oil (Soya Excel Inc., Beloeil, QC, Canada) as a source of PUFA infused into the rumen; RSF = SFA (Energy Booster 100, Milk Specialties Global, Eden Prairie, MN) infused into the rumen; ASF = SFA infused into the abomasum.

Table 8. Yield of milk odd- and branched-chain fatty acids for cows receiving different lipid supplements

Item, g/d	Treatment ¹				SEM	P-value		
	CTL	RSO	RSF	ASF		CTL vs. RSO	RSO vs. RSF	RSF vs. ASF
Odd <i>iso</i> fatty acids								
<i>iso</i> 13:0	0.45	0.41	0.39	0.43	0.02	0.03	0.21	0.06
<i>iso</i> 15:0	2.84	2.56	2.99	2.84	0.09	<0.01	<0.01	0.01
<i>iso</i> 17:0	1.69	1.97	2.22	2.09	0.15	0.13	0.16	0.46
Sum of odd <i>iso</i> fatty acids	4.91	4.97	5.56	5.46	0.24	0.84	0.03	0.70
Even <i>iso</i> fatty acids								
<i>iso</i> 14:0	1.89	2.29	2.39	2.96	0.18	0.01	0.48	<0.01
<i>iso</i> 16:0	5.15	5.33	5.86	7.02	0.43	0.75	0.36	0.05
<i>iso</i> 18:0	0.26	0.26	0.40	0.27	0.03	0.97	<0.01	<0.01
Sum of even <i>iso</i> fatty acids	7.29	7.86	8.67	10.28	0.49	0.38	0.21	0.02
Anteiso fatty acids								
<i>anteiso</i> 13:0	0.31	0.26	0.38	0.26	0.04	0.29	0.01	0.02
<i>anteiso</i> 15:0	12.18	11.85	12.74	12.79	0.95	0.47	0.07	0.90
<i>anteiso</i> 17:0	5.10	4.54	5.92	5.77	0.31	0.05	<0.01	0.56
Sum of <i>anteiso</i> fatty acids	17.40	16.66	19.10	18.95	1.08	0.10	<0.01	0.74
Odd-chain fatty acids								
11:0	1.21	0.97	1.12	0.90	0.10	<0.01	0.05	0.01
13:0	1.89	1.65	1.79	1.64	0.15	0.05	0.23	0.18
15:0	18.59	16.32	18.22	17.40	1.15	0.02	0.04	0.35
17:0	7.38	6.80	10.38	10.03	0.18	0.08	<0.01	0.28
<i>cis</i> -7 17:1	0.52	0.62	1.04	0.99	0.06	0.16	<0.01	0.48
<i>cis</i> -8 17:1	0.26	0.30	0.26	0.34	0.02	0.01	0.01	<0.01
<i>cis</i> -9 17:1	2.17	2.11	2.87	2.94	0.18	0.60	<0.01	0.58
Sum of odd-chain fatty acids	32.07	28.79	35.67	34.17	1.85	0.03	<0.01	0.30
Sum of odd- and branched-chain fatty acids	60.79	58.26	69.69	69.07	2.85	0.22	<0.01	0.76

¹CTL = control; RSO = soybean oil (Soya Excel Inc., Beloeil, QC, Canada) as a source of PUFA infused into the rumen; RSF = SFA (Energy Booster 100, Milk Specialties Global, Eden Prairie, MN) infused into the rumen; ASF = SFA infused into the abomasum.

reported reduced NDF digestibility even when a highly saturated fat supplement, such as Energy Booster 100, was fed, and speculated this could be due to increased rumination, which in turn may have increased passage rate of digestible NDF from the rumen. However, it is not possible to confirm their hypothesis based on the current results, as rumination activity was not measured for our experiment. Furthermore, Harvatine and Allen (2006b) noted that fat supplementation decreased total rumen VFA, the proportion of acetate and the acetate-to-propionate ratio; these effects were not observed in the current experiment.

Interestingly, variations of milk branched-chain FA have been observed despite the fact that relative proportions of their precursors in the rumen (i.e., isobutyrate and isovalerate) were not different between treatments (Table 5). This observation suggests that VFA substrates are not limiting in the rumen, and that synthesis of branched-chain FA is more associated with the metabolic preference of some species of bacteria for those FA (Bas et al., 2003).

Milk FA and Rumen Parameters

Fievez et al. (2012) suggested the use of a combination of specific milk FA and a monitoring of variations in milk FA profile over an extended period of time to evaluate rumen acidosis. In the current experiment, a multiple regression analysis allowed the estimation of rumen pH using milk FA concentrations, after correction for the random effect of cow ($P < 0.001$, $R^2 = 0.81$; Figure 1):

$$\text{pH} = 6.24 - (0.56 \times 4:0) + (1.67 \times \text{iso } 14:0) \\ + (4.22 \times \text{iso } 15:0) + (9.41 \times 22:0).$$

The positive relationship between *iso* FA and rumen pH is to be expected, given that these FA are, as previously discussed, related to cellulolytic bacteria, which are negatively affected by low rumen pH (Russell and Wilson, 1996). Limited information was found on 22:0 in relation to rumen pH. Among the data available, mixed rumen bacteria harvested from dairy cows have been shown to contain more than 1% of this long-chain SFA (Or-Rashid et al., 2007). Moreover, Bas et al. (2003) reported that the concentration of 22:0 decreased significantly in microbial cell mass of duodenal digesta when the level of concentrate increased in the diet of dairy goats. Ruminal pH was not reported by Bas et al. (2003), but their data suggest that relative proportion of 22:0 could be affected by ruminal fermentation and consequently on microbial synthesis of this FA. Consistent with these observations, Boivin et

al. (2013) observed a concomitant decrease in rumen pH and milk fat concentration of 22:0 when dairy cows received a diet higher in starch (whole corn silage vs. earless corn silage).

Very few data were found regarding milk 4:0 and rumen pH. Butyrate is produced during fermentation in the rumen, and its relative proportions in VFA profile have been shown to increase with a decrease in ruminal pH (Esdale and Satter, 1972). DeFrain et al. (2004) reported that during absorption, butyrate is converted to BHB, mainly in the rumen wall before its release into circulation. The same authors have shown that dietary treatments increasing the proportion of butyrate among VFA in the rumen also raises the concentration of BHB in blood plasma. In the mammary gland,

Figure 1. Associations between observed and predicted rumen pH accounting for cow and period variation obtained from cows receiving different lipid supplements: ● = control; ▲ = soybean oil (Soya Excel Inc., Beloeil, QC, Canada) as a source of PUFA infused into the rumen; ■ = SFA (Energy Booster 100, Milk Specialties Global, Eden Prairie, MN) infused into the rumen; ◆ = SFA infused into the abomasum. (a) The association between model predicted and observed values. (b) The association between model predicted values and residuals. The equation in (b) was obtained from the association between predicted rumen pH centered around the mean of predicted values and residuals. The mean ($-3.125E-07$) and linear (0.029) biases were not different from 0 ($P > 0.75$). RMSPE = root mean square prediction error.

Figure 2. Associations between rumen propionate concentration and milk fat concentration of (a) *iso* 14:0; (b) 15:0; (c) 17:0 + *cis*-9 17:1, and between rumen acetate-to-propionate ratio and milk fat concentration of (d) *iso* 14:0; (e) 15:0; (f) 17:0 + *cis*-9 17:1 obtained from cows receiving different lipid supplements: ● = control; ▲ = soybean oil (Soya Excel Inc., Beloeil, QC, Canada) as a source of PUFA infused into the rumen; ■ = SFA (Energy Booster 100, Milk Specialties Global, Eden Prairie, MN) infused into the rumen; ◆ = SFA infused into the abomasum.

about half of 4:0 arises directly from BHB (Palmquist et al., 1993). In the current experiment, no differences were observed for ruminal butyrate concentration in any of tested contrasts (Table 5). However, this series of metabolic events could explain the observed inverse relationship between rumen pH and milk fat content of 4:0 on an individual cow basis.

An analysis of the relationship between individual rumen VFA and milk OBCFA was also performed, regardless of lipid treatments. The molar percentage of propionate was inversely related to milk *iso* 14:0 (Figure 2a), whereas it was positively correlated with milk 15:0 (Figure 2b). The acetate-to-propionate ratio tended to be positively correlated with *iso* 14:0 (Figure 2d) and was negatively correlated with 15:0 (Figure 2e). These relationships are as expected given the association between *iso* 14:0 and cellulolytic bacteria, which produce acetate, and between 15:0 and amylolytic bacteria, which produce propionate (Fievez et al., 2012). However, we found no significant correlation between 17:0 + *cis*-9 17:1 and the molar percentage of propionate (Figure 2c) or the acetate-to-propionate ratio (Figure 2f). This lack of relationship is in contrast with Vlaeminck et al. (2006b), who examined data from 10 feeding experiments and found that 17:0 + *cis*-9 17:1 was negatively correlated with acetate and positively

correlated with propionate. The lack of a significant relationship in the current experiment between the sum of these 2 milk FA and rumen VFA was likely due to the presence of 17:0 and *cis*-9 17:1 in SFA supplement, which underlines the effect that the FA profile of lipid supplements can have on the attempt to establish robust relationships between the milk OBCFA profile and rumen parameters.

CONCLUSIONS

This experiment evaluated the effect that lipid supplementation may have on cow performance, rumen parameters, the milk OBCFA profile, and the relationships between milk OBCFA and rumen parameters. Infusing RSO increased the production of milk fat as compared with CTL treatment. The continuous nature of the infusion and the emulsified form of soybean oil seemed to have alleviated the negative effects PUFA can have on rumen fermentation and milk fat synthesis in the mammary gland. When ruminally infused, saturated fat compared with PUFA increased the yield of ECM as well as milk fat and protein. Lipid supply had limited effects on rumen parameters in this experiment, though the RSO treatment did decrease mean rumen pH. Numerous effects of lipid supplementation

were observed on the milk OBCFA profile, notably an increase in milk 17:0 + *cis*-9 17:1 when cows received the RSF and ASF treatments, presumably due to the presence of 17:0 in Energy Booster 100, indicating that the FA profile of lipid supplements given to lactating dairy cows can affect the milk OBCFA profile. The presence of this 17:0 in the supplement also affected the relationships between milk OBCFA and rumen VFA. Milk fat concentrations of 4:0, *iso* 14:0, *iso* 15:0, and 22:0 can be useful to predict rumen pH, though the regression remains to be verified using an independent data set. Analyzing the milk OBCFA profile continues to be a promising, noninvasive method for evaluating rumen function, though the FA profile of dietary lipid supplements when used in the diet must be considered in the development of a diagnostic tool to evaluate rumen parameters based on the milk OBCFA profile.

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