Temporal changes in milk fatty acid composition during diet-induced milk fat depression in lactating cows

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ABSTRACT

Diet-induced milk fat depression (MFD) in lactating cows has been attributed to alterations in ruminal lipid metabolism leading to the formation of specific fatty acid (FA) biohydrogenation intermediates that directly inhibit milk fat synthesis. However, the mechanisms responsible for decreased lipid synthesis in the mammary gland over time are not well defined. The aim of this study was to evaluate the effect of diet on milk FA composition and milk fat production over time, especially during MFD, and explore the associations between MFD and FA biohydrogenation intermediates in omasal digesta and milk. Four lactating Finnish Ayrshire cows used in a 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments and 35-d experimental periods were fed diets formulated to cause differences in ruminal and mammary lipid metabolism. Treatments consisted of an iso-nitrogenous total mixed ration based on grass silage with a forage to concentrate ratio of 65:35 or 35:65 without added oil, or with sunflower oil at 50 g/kg of diet dry matter. The high-concentrate diet with sunflower oil (HSO) induced a 2-stage drop in milk fat synthesis that was accompanied by specific temporal changes in the milk FA composition. The MFD on HSO was associated especially with *trans*-10 18:1 and also with *trans*-9,*cis*-11 conjugated linoleic acid (CLA) in milk and omasal digesta across all diets and was accompanied by the appearance of *trans*-10,*cis*-15 18:2. *Trans*-10,*cis*-12 CLA was increased in HSO, but milk fat secretion was not associated with omasal or milk *trans*-10,*cis*-12 CLA. The temporal changes in milk fat content and yield and milk FA composition reflect the shift from the predominant ruminal biohydrogenation

pathway to an alternative pathway. The ambiguous role of *trans*-10,*cis*-12 CLA suggests that *trans*-10 18:1, *trans*-9,*cis*-11 CLA and *trans*-10,*cis*-15 18:2 or additional mechanisms contributed to the diet-induced MFD in lactating cows.

Key words: ruminant, dairy cow, plant oil, *trans* fatty acid

INTRODUCTION

Ruminant-derived foods are a significant source of saturated fat in the human diet (Kliem and Shingfield, 2016). Therefore there is considerable interest in altering the fatty acid (FA) composition of dairy products without losing the benefits of other nutrients present in milk. The diet of dairy cows is a central factor in regulating the concentration and composition of milk fat. Supplementation of dairy cow diets with oilseeds and plant oils lower the concentrations of nutritionally undesirable SFA such as 12:0, 14:0, and 16:0, and increase *cis*-9 18:1 and PUFA in milk (Kliem and Shingfield, 2016). However, diets supplemented with plant or marine oils, diets containing highly fermentable components, and low fiber-high concentrate diets are known to induce milk fat depression (MFD) in lactating cows (Bauman and Grinari, 2003). In MFD, milk fat yield is reduced without changes in milk yield or other milk components (Shingfield et al., 2010). Therefore, understanding the influence of nutrition on the regulation of milk fat synthesis is essential not only for enhancing the nutritional quality of milk fat for consumers but also for preventing MFD and using MFD in some cases to attenuate negative energy balance at early lactation as milk fat represents an important energy loss for lactating cows (Bauman and Grinari, 2003; Shingfield et al., 2010).

Milk fat depression is a result of an interaction between dietary nutrients, rumen microbiota, and the mammary gland (Rico and Harvatine, 2013). Nutritional studies over the past decades have made considerable progress in understanding the effects of rumen lipid metabolism and milk fat synthesis, but studies

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investigating the temporal changes in MFD are scarce. The time-dependent effects of a mixture of fish oil (FO) and sunflower oil (SO; Shingfield et al., 2006), SO (Roy et al., 2006), FO (Gama et al., 2008), and soy oil (Rico and Harvatine, 2013) incorporated in different basal diets on milk fat secretion and milk FA composition and the effects of SO and starch level on FA composition in the rumen digesta of dry cows over time (Zened et al., 2013) have been outlined in previous experiments. Several hypotheses have been proposed to explain diet-induced MFD in dairy cows. The most recent and most widely accepted is the biohydrogenation (BH) theory of diet-induced MFD (Bauman and Griinari, 2001), which suggests that the changes in ruminal lipid metabolism, mainly a shift from the *trans*-11 to *trans*-10 pathway, increase the formation of specific BH intermediates from dietary PUFA that inhibit milk fat synthesis (Bauman and Griinari, 2001, 2003). *Trans*-10,*cis*-12 CLA is the only intermediate that is known unequivocally to lower milk fat synthesis in lactating cows (Baumgard et al., 2000; Glasser et al., 2010; Harvatine and Bauman, 2011). However, the earlier findings suggest that other intermediates, such as *cis*-10,*trans*-12 CLA (Saebø et al., 2005) and *trans*-9,*cis*-11 CLA (Perfield et al., 2007) may exert a potential antilipogenic effect. In addition, other unidentified BH intermediates or mechanisms may also contribute to the regulation of fat synthesis in the bovine mammary gland during MFD.

In a companion paper (Ventto et al., 2017), the ruminal BH intermediates were characterized in cows fed a high or low concentrate diet containing no additional lipid or SO. The treatments were formulated to cause varying effects on ruminal lipid metabolism and milk fat synthesis. High-concentrate diet supplemented with SO decreased milk fat synthesis up to −31.9%, but ruminal *trans*-10,*cis*-12 CLA outflow did not explain the lowered milk fat synthesis. The present paper from the same experimental setup provides more information about the responses in the mammary gland. Data related to animal performance, ruminal methane emissions, omasal flow of FA (Bayat et al., 2017; Ventto et al., 2017), and ruminal microbial ecology (Tapio et al., 2017) were published earlier. The aim of this study was to test the hypothesis that ruminal synthesis of specific BH intermediates and end products with known or putative anti-lipolytic activity appear in milk fat and explain the decrease in milk fat synthesis on a diet causing MFD. The study describes the time-dependent changes in milk fat and FA composition in cows fed diets formulated to induce variable effects on FA metabolism and milk fat synthesis to understand the role of ruminal FA BH intermediates in MFD in lactating cows.

MATERIALS AND METHODS

Animals, Experimental Design, and Diets

All experimental procedures were approved by the National Ethics Committee (ESAVI/794/04.10.03/2011, Kuopio, Finland) in accordance with the guidelines established by the European Community Council Directive 86/609/EEC (European Council, 1986). Details on experimental animals, treatments, experimental design, sampling, and chemical analysis have been reported in a companion paper (Ventto et al., 2017). In brief, 4 multiparous Finnish Ayrshire cows (89 ± 11.8 d postpartum) fitted with rumen cannulas (i.d. 100 mm, Bar Diamond Inc., Parma, ID) were used in a 4×4 Latin square design with a 2×2 factorial arrangement of treatments. Each experimental period consisted 14-d diet adaptation, 12-d sample collection interval, and 9-d washout. Treatments consisted of iso-nitrogenous diets based on grass silage [forage to concentrate (FC) ratio 65:35 and 35:65 on a DM basis, respectively] containing either 0 (treatments **L** and **H**, respectively) or 50 g/kg of diet DM of SO (treatments **LSO** and **HSO**, respectively). Diets were formulated based on Finnish Feed Tables (Luke, 2018); offered ad libitum at 0600, 0900, 1630, and 1930 h; and fed as a TMR to avoid selection of dietary components and maintain the desired FC ratio. Cows were housed in individual tiestalls in a dedicated metabolism unit with free access to water and salt block, and milked at 0700 and 1645 h.

Sampling and Chemical Analysis

Intake of all cows was measured daily, and representative feed samples were collected daily from d 21 to 25 of each period, and analyzed for chemical composition (Shingfield et al., 2001, 2002; Ventto et al., 2017). Daily milk yields and milk composition of all cows were recorded throughout the experiment (Ventto et al., 2017). Unpreserved samples of milk at each milking were collected, composited according to yield, and stored at -20°C before submitting to FA analysis. The flow of digesta at the omasal canal was sampled and measured using the omasal sampling technique and Cr, Yb, and indigestible neutral detergent fiber as indigestible markers from d 22 to 24 as described by Ventto et al. (2017).

Lipid Analysis

The FAME of lipid in feed, omasal digesta, and milk samples were prepared as described earlier (Shingfield et al., 2003; Ventto et al., 2017). The FAME were ana-

lyzed using a gas chromatograph (model 6890N, Agilent Technologies, Santa Clara, CA) fitted with a CP-Sil 88 column (100 m $\times$ 0.25 mm i.d., 0.2 μ m film thickness, Agilent Technologies) and flame ionization detector by using a temperature gradient program (Shingfield et al., 2003) and hydrogen as the carrier gas operated at constant pressure (206.8 kPa) and nominal initial flow rate of 2.1 mL/min. Individual isomers of 18:1 were further resolved in a separate analysis under isothermal conditions at 170°C (Shingfield et al., 2003) at a constant pressure (158.6 kPa) and a nominal initial flow rate of 0.9 mL/min. Under these conditions *trans*-10, *cis*-15 18:2 and *trans*-11, *cis*-15 18:2 eluted as a single peak. To resolve these isomers, GC analysis was repeated using a SLB-IL111 column (100 m $\times$ 0.25 mm i.d., 0.2 μ m film thickness, Sigma-Aldrich, St. Louis, MO; Ventto et al., 2017). The FAME not available as authentic standards were identified by GC-MS (6890 and 5973; Agilent Technologies) analysis of FAME and 4,4-dimethyloxazoline derivatives prepared from total FAME according to earlier reports (Halmemies-Beauchet-Filleau et al., 2011). Distribution of CLA isomers was determined by Ag⁺-HPLC (Halmemies-Beauchet-Filleau et al., 2011). The CLA isomers and *trans*-10, *cis*-15 18:2 were determined in d 0, 12, 18, 25, and 35 samples.

Statistical Analysis

Milk FA data recorded during d 22 to 25 of each experimental period were averaged before statistical analysis and analyzed by ANOVA for a 4 $\times$ 4 Latin square design with a 2 $\times$ 2 factorial arrangement of treatments with a statistical model that included the fixed effects of period, concentrate level, SO supplementation, and their interaction, and the random effect of cow using the Mixed procedure of SAS (version 9.4, SAS Institute, Cary, NC). Milk composition and milk FA composition data over time were analyzed in 5-d intervals (d 1–5, d 6–10, d 11–15, d 16–20, d 21–25, d 26–30, and d 31–35) by repeated measures ANOVA with a model that included the fixed effects of concentrate level, SO supplementation, their interaction (FC $\times$ SO), their interaction with time (FC $\times$ time, SO $\times$ time, FC $\times$ SO $\times$ time), time, and period, and the random effect of cow assuming an auto regressive order one covariance structure. Denominator degrees of freedom were calculated by the Kenward-Roger method. Relationship between flow of FA at the omasum, secretion of FA in milk and milk fat yield were evaluated by linear regression analysis using PROC REG procedure of SAS. Least squares means $\pm$ standard error are reported, and treatment effects were considered significant at $P \leq 0.05$ and probabilities at $0.05 < P \leq 0.10$ were considered as a trend.

RESULTS

Concentrate level by SO supplementation by time interaction over the 35-d experimental periods was significant ($P < 0.01$) for milk fat content and yield (Figure 1). The decrease in milk fat content of cows fed HSO occurred within 7 d, decreased further until d 17, and recovered by d 34 (Figure 1A), whereas decline in milk fat yield was evident after d 17 (Figure 1B). Concentrate level by SO supplementation by time interaction over the 35-d experimental periods was not significant ($P > 0.10$) for milk yield and protein content (Supplemental Figure S1A and S1B; <https://doi.org/10.3168/jds.2018-15860>). Milk yield increased on HSO and H after start of the treatments and reached maximum concentration on d 6 to 8.

Milk FA composition was affected by FC ratio and SO supplementation. In particular, high concentrate diets increased ($P < 0.05$) the proportions of 12:0, 14:0, 16:0, total *cis* 16:1, total 16:1, 20:3n-6, and 20:4n-6 in milk fat, and decreased ($P < 0.05$) 18:0, total *cis* 18:1, total 18:1, *cis*-9, *trans*-11, *cis*-15 18:3, 20:0, total *cis* 20:1, 20:4n-3, 20:5n-3, and 22:0 (Table 1). The SO supplementation lowered ($P < 0.05$) the proportion of 12:0, 14:0, 16:0, 18:3n-3, *cis*-9, *trans*-11, *cis*-15 18:3, 20:3n-6, 20:4n-3, 20:4n-6, 20:5n-3, 22:5n-3, 22:6n-3, and total SFA, but increased ($P < 0.01$) the proportion of total *trans* 16:1, 18:0, total *cis* 18:1, total *trans* 18:1, total 18:1, total MUFA, and total PUFA. Decreases in the proportions of 4:0, 6:0, 8:0, 10:0, *cis*-9 10:1, and 22:5n-6 in milk fat were greater when SO was included in the H than L treatment ($P < 0.05$ for FC $\times$ SO interaction). Increases in total CLA were greater when SO was included in the L than H treatment, whereas the reverse was true for nonconjugated 18:2 ($P < 0.05$ for FC $\times$ SO interaction). The SO increased *cis*-9 14:1 when included in the H treatment, but decreased the proportions of this FA in milk on the L treatment ($P < 0.05$ for FC $\times$ SO interaction; Table 1).

Irrespective of the FC ratio, SO supplementation increased ($P < 0.05$) the proportion of certain MUFA, such as *cis*-6 16:1 + *cis*-7 16:1 + *trans*-11 16:1, *cis*-9 18:1 + *trans*-14 18:1, *cis*-13 18:1, *cis*-16 18:1 + *cis*-8, *cis*-12 18:2, *trans* (Δ 4, 9, 12, 13, 15, 16) 18:1, and decreased *cis*-13 16:1 + *cis*-15 16:1 in milk fat (Table 2). Changes in response to a higher proportion of concentrates were characterized by increases ($P < 0.05$) in *cis*-13 16:1 + *cis*-15 16:1 and *cis*-11 18:1, and decline ($P < 0.05$) in *cis*-9 18:1 and *cis*-9 20:1. Amounts of specific MUFA induced by addition of SO were dependent on the dietary FC ratio. Increases in the proportions of *trans*-8 16:1 (β -oxidation product of *trans*-10 18:1), *cis*-13 18:1, *trans*-6 + 7 + 8 18:1, and *trans*-10 18:1 in milk fat were higher when SO was included in the H than L

treatment ($P < 0.05$ for FC $\times$ SO interaction), whereas the reverse was true for *trans*-9 16:1 (β -oxidation product of *trans*-11 18:1), *cis*-12 18:1, and *trans*-11 18:1 ($P < 0.05$ for FC $\times$ SO interaction). Furthermore, SO increased *cis*-9 16:1 when included in the H treatment, but decreased the proportion of this FA in milk on the L treatment ($P < 0.05$ for FC $\times$ SO interaction).

Proportions of certain 18:2 isomers in milk were affected by the FC ratio of the diet (Table 3). Changes in response to a higher proportion of concentrates were characterized by decreases ($P < 0.01$) in *trans*-11, *trans*-15 18:2, *trans*-12, *cis*-15 18:2, *trans*-11, *trans*-13 CLA, and *trans*-12, *trans*-14 CLA. The SO supple-

mentation increased the proportions of multiple 18:2 and CLA isomers in milk fat. The proportion of *cis*-9, *trans*-12 18:2, *cis*-9, *trans*-14 18:2 + *cis*-7, *cis*-12 18:2, *trans*-9, *cis*-12 18:2, *cis*-9, *cis*-11 CLA, *trans*-7, *cis*-9 CLA, *trans*-8, *cis*-10 CLA, *trans*-8, *trans*-10 CLA, and *trans*-10, *trans*-12 CLA were increased ($P < 0.05$) by SO. Including SO in the H treatment resulted in greater increases of *cis*-6, *cis*-12 18:2, *cis*-9, *cis*-12 18:2, *cis*-9, *trans*-13 18:2 + *cis*-10, *trans*-14 18:2, *trans*-9, *trans*-12 18:2, *trans*-9, *trans*-13 18:2, *trans*-9, *cis*-11 CLA, *trans*-10, *cis*-12 CLA proportions in milk fat compared with the supplementation of L treatment with SO ($P < 0.05$ for FC $\times$ SO interaction). In contrast, increases in *cis*-9, *trans*-11 CLA were higher when SO was included in the L treatment ($P < 0.05$ for FC $\times$ SO interaction). *Trans*-12, *cis*-14 CLA in milk fat increased when SO was included in the L treatment, but decreased when SO was included in the H treatment ($P < 0.05$ for FC $\times$ SO interaction). Decline in the proportions of *trans*-11, *cis*-13 CLA in milk fat were greater when SO was included in the L than H treatment ($P < 0.05$ for FC $\times$ SO interaction). The SO increased *trans*-11, *cis*-15 18:2 when included in the H treatment, but decreased the proportion of this FA in milk on the L treatment ($P < 0.05$ for FC $\times$ SO interaction). *Trans*-10, *cis*-15 18:2 was present only in milk from the HSO treatment. Both dietary FC ratio and SO supplement resulted in relatively minor changes in the proportions of odd- and branched-chain FA and FA containing oxo group in the milk fat (Supplemental Table S1; <https://doi.org/10.3168/jds.2018-15860>).

Concentrate level by SO supplementation by time interaction over the 35-d experimental periods was significant ($P < 0.05$) for 4:0 and *trans*-10, *cis*-12 CLA (Figure 2). Temporal changes in milk FA composition revealed that reductions in proportions of 4:0 on HSO treatment were evident within 6 d and continued to decrease until the end of dietary treatment period (Figure 2A). The proportion of 16:0 in milk decreased after 3 and 4 d on treatments containing SO (Figure 2B). Similar but less pronounced changes for SO supplementation were observed in the proportions of 12:0 and 14:0, whereas they increased on the H treatment compared with the L treatment (Supplemental Figure S2A and S2B; <https://doi.org/10.3168/jds.2018-15860>). The increase in 18:0 was more evident on the LSO than HSO treatment, starting after d 6 on HSO treatment (Figure 2C). The BH intermediates *trans*-10 18:1, *trans*-10, *cis*-12 CLA and *trans*-9, *cis*-11 CLA started to increase progressively on HSO treatment and reached maximum concentrations on d 17 to 19 (Figure 2D, 2E, and 2F). *Trans*-11 18:1 and *cis*-9, *trans*-11 CLA increased immediately after the addition of SO by d 3, but started to decline

Figure 1. Temporal changes in milk fat content (A) and milk fat yield (B) of lactating cows in response to dietary forage to concentrate ratio (FC) and sunflower oil (SO). Treatments consisted of diets based on grass silage (FC ratio 65:35 and 35:65 on a DM basis, respectively) containing either 0 (treatments L and H, respectively) or 50 g/kg of diet DM of SO (treatments LSO and HSO, respectively). Cows were fed the experimental diets for 26 d (indicated by the dashed line square) followed by 9 d of washout. Symbols indicate the significance of effects ($P \leq 0.1$) due to FC $\times$ time interaction (*), SO $\times$ time interaction (#), and FC $\times$ SO $\times$ time interaction (†) in 5-d intervals (d 1–5, d 6–10, d 11–15, d 16–20, d 21–25, d 26–30, and d 31–35). Significance of FC $\times$ time, SO $\times$ time, and FC $\times$ SO $\times$ time interaction is $P = 0.08$, $P < 0.01$, and $P < 0.01$, respectively, for milk fat content and $P = 0.42$, $P < 0.001$, and $P < 0.001$, respectively, for milk fat yield.

Table 1. Effect of dietary forage to concentrate ratio and sunflower oil on selected fatty acids in milk of lactating cows¹

Item	Treatment					<i>P</i> -value ²		
	L	LSO	H	HSO	SEM	FC	SO	FC × SO
Fatty acid, g/100 g of total fatty acid								
4:0	3.38	3.31	3.40	2.18	0.211	<0.05	<0.05	<0.05
6:0	2.13	1.61	2.35	1.17	0.089	0.25	<0.001	<0.01
8:0	1.29	0.82	1.55	0.68	0.039	0.16	<0.001	<0.01
10:0	2.96	1.61	3.90	1.67	0.112	<0.01	<0.001	<0.01
<i>cis</i> -9 10:1	0.32	0.17	0.41	0.16	0.020	<0.05	<0.001	<0.05
12:0	3.50	1.88	4.78	2.38	0.172	<0.01	<0.001	0.07
<i>cis</i> -9 12:1	0.087	0.038	0.140	0.063	0.0106	<0.01	<0.001	0.20
<i>trans</i> -9 12:1	0.081	0.060	0.118	0.066	0.0117	0.12	<0.05	0.23
14:0	12.3	7.94	13.3	9.68	0.497	<0.05	<0.001	0.37
<i>cis</i> -9 14:1	1.00	0.65	1.13	1.21	0.068	<0.01	0.06	<0.05
<i>cis</i> -11 14:1	0.036	0.012	0.069	0.021	0.0063	<0.05	<0.001	0.09
<i>trans</i> -9 14:1	0.012	0.0082	0.014	0.016	0.0011	<0.01	0.16	<0.05
16:0	29.9	18.9	31.1	22.2	0.79	<0.05	<0.001	0.15
Σ <i>cis</i> 16:1	1.45	1.13	1.55	1.65	0.103	<0.05	0.34	0.09
Σ <i>trans</i> 16:1	0.14	0.29	0.10	0.27	0.029	0.28	<0.001	0.64
Σ 16:1	1.58	1.42	1.65	1.92	0.117	<0.05	0.65	0.10
18:0	10.8	17.1	8.90	12.2	0.85	<0.01	<0.01	0.13
Σ <i>cis</i> 18:1	18.6	28.1	16.2	25.9	0.95	<0.05	<0.001	0.90
Σ <i>trans</i> 18:1	3.09	8.00	2.69	9.56	0.636	0.37	<0.001	0.15
Σ 18:1	21.7	36.1	18.9	35.5	0.88	<0.05	<0.001	0.14
Σ 18:2 ³	1.70	2.00	2.09	3.29	0.152	<0.01	<0.01	<0.05
Σ CLA	0.59	1.55	0.47	0.77	0.136	<0.01	<0.01	<0.05
18:3n-3 ⁴	0.50	0.33	0.45	0.38	0.025	0.87	<0.01	0.06
18:3n-6	0.019	0.019	0.022	0.020	0.0025	0.33	0.50	0.38
<i>cis</i> -9, <i>trans</i> -11, <i>cis</i> -15 18:3	0.036	0.022	0.027	0.013	0.0021	<0.01	<0.001	0.87
20:0 ⁵	0.20	0.19	0.15	0.14	0.008	<0.001	0.13	0.97
Σ <i>cis</i> 20:1	0.15	0.13	0.12	0.12	0.008	<0.05	0.26	0.16
Σ <i>trans</i> 20:1	0.039	0.056	0.067	0.063	0.0122	0.20	0.63	0.41
Σ 20:1	0.19	0.19	0.19	0.18	0.015	0.88	0.96	0.90
20:2n-6	0.031	0.020	0.029	0.026	0.0027	0.53	<0.05	0.20
20:3n-3 ⁶	0.019	0.015	0.028	0.018	0.0025	<0.05	<0.05	0.26
20:3n-6	0.060	0.043	0.086	0.069	0.0058	<0.001	<0.01	0.90
20:4n-3	0.039	0.023	0.029	0.019	0.0023	<0.01	<0.001	0.10
20:4n-6	0.075	0.047	0.100	0.060	0.0064	<0.01	<0.001	0.22
20:5n-3 ⁷	0.093	0.061	0.075	0.047	0.0033	<0.001	<0.001	0.38
22:0	0.071	0.074	0.046	0.051	0.0051	<0.01	0.33	0.78
<i>cis</i> -9 22:1	0.015	0.013	0.010	0.011	0.0021	<0.05	0.67	0.40
22:4n-6	0.0092	0.0051	0.0120	0.0095	0.00139	<0.01	<0.01	0.30
22:5n-3 ⁸	0.103	0.065	0.102	0.068	0.0080	0.84	<0.001	0.69
22:5n-6	0.0095	0.0038	0.0062	0.0049	0.00069	0.14	<0.01	<0.05
22:6n-3	0.0045	0.0025	0.0053	0.0036	0.00078	0.20	<0.05	0.81
<i>cis</i> -15 24:1	0.0125	0.0068	0.0086	0.0060	0.00166	0.11	<0.05	0.26
Σ Unidentified	0.126	0.100	0.085	0.068	0.0092	<0.01	0.06	0.65
Σ SFA	70.9	56.5	73.2	55.5	1.23	0.47	<0.001	0.09
Σ MUFA	25.7	39.2	23.2	39.6	1.04	0.19	<0.001	0.08
Σ PUFA	3.32	4.23	3.55	4.81	0.30	0.15	<0.01	0.51
Fatty acids, g/100 g of fat	94.2	94.5	94.2	94.6	0.02	<0.05	<0.001	<0.05
Concentration ratio								
<i>cis</i> -9 10:1/(<i>cis</i> -9 10:1 + 10:0)	0.099	0.095	0.094	0.087	0.0074	0.21	0.25	0.70
<i>cis</i> -9 12:1/(<i>cis</i> -9 12:1 + 12:0)	0.024	0.020	0.028	0.026	0.0021	<0.05	0.09	0.46
<i>cis</i> -9 14:1/(<i>cis</i> -9 14:1 + 14:0)	0.075	0.076	0.079	0.112	0.0076	<0.01	<0.05	<0.05
<i>cis</i> -9 16:1/(<i>cis</i> -9 16:1 + 16:0)	0.036	0.043	0.036	0.057	0.0035	0.09	<0.01	0.08
<i>cis</i> -9 18:1/(<i>cis</i> -9 18:1 + 18:0)	0.62	0.61	0.63	0.67	0.019	0.07	0.44	0.19
<i>trans</i> -7, <i>cis</i> -9 CLA/(<i>trans</i> -7, <i>cis</i> -9 CLA + <i>trans</i> -7 18:1)	0.12	0.14	0.14	0.14	0.009	0.20	<0.05	0.13
<i>cis</i> -9, <i>trans</i> -11 CLA/(<i>cis</i> -9, <i>trans</i> -11 CLA + <i>trans</i> -11 18:1)	0.34	0.29	0.35	0.31	0.025	0.44	0.09	0.91
<i>cis</i> -9 19:1/(<i>cis</i> -9 19:1 + 19:0)	0.56	0.54	0.61	0.56	0.036	0.36	0.29	0.67
<i>cis</i> -9 20:1/(<i>cis</i> -9 20:1 + 20:0)	0.41	0.41	0.43	0.46	0.017	0.06	0.58	0.28
<i>cis</i> -9 21:1/(<i>cis</i> -9 21:1 + 21:0)	0.37	0.40	0.28	0.24	0.044	<0.05	0.83	0.43

Continued

Table 1 (Continued). Effect of dietary forage to concentrate ratio and sunflower oil on selected fatty acids in milk of lactating cows¹

Item	Treatment					P-value ²		
	L	LSO	H	HSO	SEM	FC	SO	FC × SO
<i>cis</i> -9 22:1/(<i>cis</i> -9 22:1 + 22:0)	0.17	0.15	0.18	0.17	0.013	0.13	0.06	0.42
Mean milk fat melting point, °C	38.3	36.1	38.0	37.7	0.46	0.17	<0.05	0.06

¹Values are LSM and pooled SEM, n = 16. Values represent the mean over d 22 to 25 of each experimental period. FC = forage to concentrate; L = low concentrate diet (FC ratio 65:35 on a DM basis) with no additional lipid; LSO = low concentrate diet (FC ratio 65:35) containing 50 g of sunflower oil/kg of diet DM; H = high concentrate diet (FC ratio 35:65) with no additional lipid; HSO = high concentrate diet (FC ratio 35:65) containing 50 g of sunflower oil/kg of diet DM; SO = sunflower oil.

²Significance of effects due to dietary forage to concentrate ratio (FC), supplements of sunflower oil (SO), and their interaction (FC × SO).

³Nonconjugated.

⁴Co-elutes with *trans*-15 20:1, *trans*-16 20:1, and *cis*-11 20:1.

⁵Co-elutes with unidentified fatty acid.

⁶Co-elutes with *cis*-13 22:1.

⁷Co-elutes with 24:0.

⁸Co-elutes with 26:0.

Table 2. Effect of dietary forage to concentrate ratio and sunflower oil on 16:1, 18:1, and 20:1 fatty acid composition in milk of lactating cows¹

Fatty acid, g/100 g of total fatty acid	Treatment					P-value ²		
	L	LSO	H	HSO	SEM	FC	SO	FC × SO
<i>cis</i> -6 + 7 16:1 ³	0.17	0.20	0.16	0.22	0.011	0.88	<0.01	0.29
<i>cis</i> -9 16:1	1.12	0.86	1.15	1.35	0.085	<0.05	0.71	<0.05
<i>cis</i> -10 16:1	0.014	0.024	0.016	0.022	0.0017	0.92	<0.01	0.24
<i>cis</i> -13 16:1 ⁴	0.145	0.043	0.227	0.069	0.0189	<0.05	<0.001	0.18
<i>trans</i> -6 + 7 16:1	0.033	0.038	0.027	0.039	0.0028	0.23	<0.01	0.11
<i>trans</i> -8 16:1	0.0081	0.0238	0.0066	0.1253	0.01957	<0.05	<0.05	<0.05
<i>trans</i> -9 16:1	0.055	0.183	0.035	0.062	0.0131	<0.001	<0.001	<0.01
<i>trans</i> -12 16:1	0.018	0.014	0.012	0.012	0.0010	<0.01	<0.05	<0.05
<i>trans</i> -13 16:1	0.015	0.023	0.014	0.024	0.0018	0.83	<0.001	0.17
<i>cis</i> -9 18:1 ⁵	17.7	26.7	15.2	24.4	0.93	<0.05	<0.001	0.91
<i>cis</i> -11 18:1	0.51	0.49	0.61	0.69	0.032	<0.01	0.35	0.15
<i>cis</i> -12 18:1	0.18	0.52	0.25	0.40	0.028	0.37	<0.001	<0.05
<i>cis</i> -13 18:1	0.076	0.132	0.084	0.209	0.0076	<0.01	<0.001	<0.01
<i>cis</i> -15 18:1	0.097	0.104	0.075	0.126	0.0100	0.99	<0.05	0.07
<i>cis</i> -16 18:1 ⁶	0.056	0.117	0.057	0.113	0.0041	0.67	<0.001	0.51
<i>trans</i> -4 18:1	0.026	0.065	0.027	0.070	0.0041	0.40	<0.001	0.61
<i>trans</i> -5 18:1	0.022	0.048	0.026	0.072	0.0040	<0.05	<0.001	<0.05
<i>trans</i> -6–8 18:1	0.23	0.60	0.21	0.73	0.028	0.08	<0.001	<0.05
<i>trans</i> -9 18:1	0.19	0.49	0.17	0.45	0.018	0.07	<0.001	0.71
<i>trans</i> -10 18:1	0.25	0.98	0.31	4.87	0.537	<0.01	<0.01	<0.05
<i>trans</i> -11 18:1	1.14	3.23	0.71	0.83	0.201	<0.001	<0.01	<0.01
<i>trans</i> -12 18:1	0.25	0.72	0.26	0.71	0.032	0.96	<0.001	0.58
<i>trans</i> -13 18:1	0.38	0.65	0.38	0.79	0.037	0.10	<0.001	0.11
<i>trans</i> -15 18:1	0.32	0.66	0.32	0.57	0.036	0.26	<0.001	0.28
<i>trans</i> -16 18:1 ⁷	0.27	0.56	0.27	0.46	0.026	0.06	<0.001	0.09
<i>cis</i> -9 20:1	0.14	0.13	0.11	0.11	0.008	<0.01	0.28	0.22
<i>trans</i> -9–12 20:1	0.028	0.041	0.053	0.052	0.0102	0.13	0.59	0.53
<i>trans</i> -13 20:1	0.011	0.015	0.015	0.015	0.0020	0.93	0.89	0.09

¹Values are LSM and pooled SEM, n = 16. Values represent the mean over d 22 to 25 of each experimental period. FC = forage to concentrate; L = low concentrate diet (FC ratio 65:35 on a DM basis) with no additional lipid; LSO = low concentrate diet (FC ratio 65:35) containing 50 g of sunflower oil/kg of diet DM; H = high concentrate diet (FC ratio 35:65) with no additional lipid; HSO = high concentrate diet (FC ratio 35:65) containing 50 g of sunflower oil/kg of diet DM; SO = sunflower oil.

²Significance of effects due to dietary forage to concentrate ratio (FC), supplements of sunflower oil (SO), and their interaction (FC × SO).

³Co-elutes with *trans*-11 16:1.

⁴Co-elutes with *cis*-15 16:1.

⁵Co-elutes with *trans*-14 18:1.

⁶Co-elutes with *cis*-8, *cis*-12 18:2.

⁷Co-elutes with *cis*-14 18:1.

rapidly on d 5 on HSO while staying higher on LSO (Figure 2G and 2H).

The SO supplementation increased ($P < 0.05$) the product/(product + substrate) concentration ratios *cis*-9 16:1/(*cis*-9 16:1 + 16:0) and *trans*-7,*cis*-9 CLA/(*trans*-7,*cis*-9 CLA + *trans*-7 18:1) in milk (Table 1). Changes in response to a higher proportion of concentrates were characterized by increases ($P < 0.05$) in *cis*-9 12:1/(*cis*-9 12:1 + 12:0) and decrease in *cis*-9 21:1/(*cis*-9 21:1 + 21:0). Increases in the *cis*-9 14:1/(*cis*-9 14:1 + 14:0) were greater when SO was included in the H treatment than L treatment ($P < 0.05$ for FC $\times$ SO interaction) and were close to significant for *cis*-9 16:1/(*cis*-9 16:1 + 16:0; $P = 0.08$).

Across all treatments the negative relationship between milk fat yield and secretion of *trans*-10 18:1 in milk ($P < 0.05$) was weaker than the negative relationship between milk fat yield and flow of *trans*-10 18:1 at the omasum ($P < 0.01$; Table 4). On the contrary, the negative association between milk fat yield and flow of *trans*-9,*cis*-11 CLA at the omasum ($P = 0.08$) was weaker than the relationship between milk fat yield and secretion of *trans*-9,*cis*-11 CLA in milk ($P < 0.05$). A close linear relationship existed between the flow at the omasum and secretion in milk for *trans*-10 18:1, *trans*-11 18:1, 18:2n-6, *trans*-11,*cis*-15 18:2 and *trans*-9,*cis*-11 CLA ($P < 0.05$), but not for *trans*-10,*cis*-12 CLA ($P = 0.09$; Table 4). Secretions of selected FA in

Table 3. Effect of dietary forage to concentrate ratio and sunflower oil on 18:2 fatty acid composition in milk of lactating cows¹

Fatty acid	Treatment				SEM	P-value ²		
	L	LSO	H	HSO		FC	SO	FC × SO
Nonconjugated 18:2, g/100 g of total fatty acid								
<i>cis</i> -6, <i>cis</i> -12 18:2	0.015	0.019	0.017	0.042	0.0031	<0.01	<0.01	<0.01
<i>cis</i> -9, <i>cis</i> -12 18:2 ³	1.05	1.09	1.58	2.24	0.118	<0.001	<0.05	<0.05
<i>cis</i> -12, <i>cis</i> -15 18:2 ⁴	0.028	0.028	0.044	0.030	0.0175	0.59	0.66	0.67
<i>cis</i> -9, <i>trans</i> -12 18:2	0.026	0.051	0.031	0.062	0.0047	0.11	<0.001	0.48
<i>cis</i> -9, <i>trans</i> -13 18:2 ⁵	0.15	0.32	0.14	0.38	0.020	0.10	<0.001	<0.05
<i>cis</i> -9, <i>trans</i> -14 18:2 ⁶	0.079	0.143	0.073	0.164	0.0092	0.26	<0.001	0.06
<i>trans</i> -9, <i>cis</i> -12 18:2	0.028	0.040	0.026	0.045	0.0021	0.47	<0.001	0.15
<i>trans</i> -10, <i>cis</i> -15 18:2	0.000	0.000	0.000	0.017	0.0045	0.11	0.11	0.11
<i>trans</i> -11, <i>cis</i> -15 18:2	0.176	0.116	0.067	0.087	0.0112	<0.001	0.13	<0.05
<i>trans</i> -12, <i>cis</i> -15 18:2	0.024	0.019	0.018	0.017	0.0015	<0.05	0.08	0.21
<i>trans</i> -9, <i>trans</i> -12 18:2	0.015	0.021	0.013	0.028	0.0014	<0.05	<0.001	<0.01
<i>trans</i> -9, <i>trans</i> -13 18:2	0.023	0.064	0.027	0.088	0.0016	<0.01	<0.001	<0.001
<i>trans</i> -10, <i>trans</i> -14 18:2	0.041	0.056	0.042	0.067	0.0104	0.62	0.10	0.66
<i>trans</i> -11, <i>trans</i> -15 18:2	0.043	0.044	0.017	0.019	0.0014	<0.001	0.37	0.96
Conjugated 18:2, mg/100 g of total fatty acid								
<i>cis</i> -9, <i>cis</i> -11 CLA	0.92	2.72	1.58	2.50	0.418	0.61	<0.05	0.33
<i>cis</i> -9, <i>trans</i> -11 CLA	468	1,332	360	494	133	<0.01	<0.01	<0.05
<i>cis</i> -11, <i>trans</i> -13 CLA	1.59	1.93	1.60	2.31	0.404	0.64	0.24	0.66
<i>cis</i> -12, <i>trans</i> -14 CLA	1.50	1.52	1.18	2.48	0.409	0.46	0.16	0.17
<i>trans</i> -6, <i>cis</i> -8 CLA	0.00	1.84	0.62	2.11	0.870	0.58	0.07	0.82
<i>trans</i> -7, <i>cis</i> -9 CLA	31.0	97.5	33.3	117.6	5.69	0.05	<0.001	0.11
<i>trans</i> -8, <i>cis</i> -10 CLA	2.43	6.48	2.20	4.89	1.369	0.52	<0.05	0.63
<i>trans</i> -9, <i>cis</i> -11 CLA	9.04	21.0	10.0	68.5	4.969	<0.01	<0.001	<0.01
<i>trans</i> -10, <i>cis</i> -12 CLA	3.29	7.82	3.96	25.3	2.69	<0.05	<0.01	<0.05
<i>trans</i> -11, <i>cis</i> -13 CLA	24.0	11.9	5.56	3.43	1.626	<0.001	<0.01	<0.05
<i>trans</i> -12, <i>cis</i> -14 CLA	4.61	4.73	4.55	1.95	0.432	<0.05	<0.05	<0.05
<i>trans</i> -7, <i>trans</i> -9 CLA	4.85	4.10	4.54	2.99	0.748	0.35	0.15	0.58
<i>trans</i> -8, <i>trans</i> -10 CLA	3.06	5.41	2.57	6.39	0.558	0.67	<0.01	0.23
<i>trans</i> -9, <i>trans</i> -11 CLA	12.3	18.4	13.2	9.51	2.188	0.10	0.57	0.05
<i>trans</i> -10, <i>trans</i> -12 CLA	3.94	11.7	5.22	11.4	1.112	0.62	<0.001	0.41
<i>trans</i> -11, <i>trans</i> -13 CLA	13.6	11.6	9.63	7.67	1.362	<0.01	0.09	0.98
<i>trans</i> -12, <i>trans</i> -14 CLA	8.87	8.22	5.63	4.53	0.907	<0.01	0.36	0.80
<i>trans</i> -13, <i>trans</i> -15 CLA	1.01	0.99	0.80	1.34	0.288	0.81	0.41	0.36

¹Values are LSM and pooled SEM, n = 16. Values represent the mean over d 22 to 25 of each experimental period. FC = forage to concentrate; L = low concentrate diet (FC ratio 65:35 on a DM basis) with no additional lipid; LSO = low concentrate diet (FC ratio 65:35) containing 50 g of sunflower oil/kg of diet DM; H = high concentrate diet (FC ratio 35:65) with no additional lipid; HSO = high concentrate diet (FC ratio 35:65) containing 50 g of sunflower oil/kg of diet DM; SO = sunflower oil.

²Significance of effects due to dietary forage to concentrate ratio (FC), supplements of sunflower oil (SO), and their interaction (FC $\times$ SO).

³Contains *cis*-9,*cis*-15 18:2 as a minor component.

⁴Co-elutes with unidentified fatty acid.

⁵Co-elutes with *cis*-10,*trans*-14 18:2.

⁶Co-elutes with *cis*-7,*cis*-12 18:2.

Figure 2. Temporal changes in the proportion of 4:0 (A), 16:0 (B), 18:0 (C), *trans*-10 18:1 (D), *trans*-10, *cis*-12 CLA (E), *trans*-9, *cis*-11 CLA (F), *trans*-11 18:1 (G), and *cis*-9, *trans*-11 CLA (+ *trans*-7, *cis*-9 CLA and *trans*-8, *cis*-10 CLA as minor components; H) in milk of lactating cows in response to dietary forage to concentrate ratio (FC) and sunflower oil (SO). Treatments consisted of diets based on grass silage (FC ratio 65:35 and 35:65 on a DM basis, respectively) containing either 0 (treatments L and H, respectively) or 50 g/kg of diet DM of SO (treatments LSO and HSO, respectively). Cows were fed the experimental diets for 26 d (indicated by the dashed line square) followed by 9 d of washout. Symbols indicate the significance of effects ($P \leq 0.1$) due to FC × time interaction (*), SO × time interaction (#), and FC × SO × time interaction (†) in 5-d intervals (d 1–5, d 6–10, d 11–15, d 16–20, d 21–25, d 26–30, and d 31–35). Significance of FC × time, SO × time, and FC × SO × time interaction is $P < 0.001$, $P = 0.08$, and $P < 0.05$, respectively, for 4:0; $P = 0.94$, $P = 0.09$, and $P = 0.93$, respectively, for 16:0; $P = 0.90$, $P < 0.001$, and $P = 0.32$, respectively, for 18:0; $P = 0.22$, $P = 0.15$, and $P = 0.33$, respectively, for *trans*-10 18:1; $P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively, for *trans*-10, *cis*-12 CLA; $P = 0.23$, $P = 0.83$, and $P = 0.61$, respectively, for *trans*-9, *cis*-11 CLA; $P = 0.13$, $P = 0.87$, and $P = 0.15$, respectively, for *trans*-11 18:1; and $P = 0.23$, $P = 0.83$, and $P = 0.28$, respectively, for *cis*-9, *trans*-11 CLA. FA = fatty acids.

Table 4. Relationship between the flow of selected fatty acids at the omasum, the secretion of selected fatty acids in milk, and milk fat yield in lactating cows fed diets of different forage to concentrate ratio with or without sunflower oil¹

Independent variable (X)	Dependent variable (Y)	Intercept	SE	P-value	Slope	SE	r ² _{adj} ²	P-value
Omasal flow, g/d	Milk fat yield, g/d							
<i>trans</i> -10 18:1		1,147	55.2	<0.001	−2.44	0.764	0.38	<0.01
<i>trans</i> -9, <i>cis</i> -11 CLA		1,098	59.7	<0.001	−137	73.4	0.14	0.08
<i>trans</i> -10, <i>cis</i> -12 CLA		1,100	99.6	<0.001	−127	197	−0.04	0.53
<i>trans</i> -10, <i>cis</i> -15 18:2		1,040	188	<0.05	−87.5	62.80	0.24	0.30 ³
Secretion in milk, g/d	Milk fat yield, g/d							
<i>trans</i> -10 18:1		1,153	69.7	<0.001	−8.11	3.645	0.21	<0.05
<i>trans</i> -9, <i>cis</i> -11 CLA		1,216	83.9	<0.001	−731	294	0.26	<0.05
<i>trans</i> -10, <i>cis</i> -12 CLA		1,160	86.0	<0.001	−1,283	766	0.17	0.12
<i>trans</i> -10, <i>cis</i> -15 18:2		1,078	227	<0.05	−2,192	1,734	0.17	0.33 ³
Omasal flow, g/d	Secretion in milk, g/d unless otherwise indicated							
<i>trans</i> -10 18:1	<i>trans</i> -8 16:1, mg/d	85.4	30.30	<0.05	5.71	0.420	0.92	<0.001
<i>trans</i> -10 18:1	<i>trans</i> -10 18:1	4.09	1.865	<0.05	0.22	0.026	0.82	<0.001
<i>trans</i> -11 18:1	<i>trans</i> -11 18:1	1.91	2.103	0.38	0.27	0.035	0.80	<0.001
<i>cis</i> -9, <i>cis</i> -12 18:2	<i>cis</i> -9, <i>cis</i> -12 18:2	2.61	2.254	0.27	0.58	0.105	0.66	<0.001
<i>trans</i> -10, <i>cis</i> -15 18:2	<i>trans</i> -10, <i>cis</i> -15 18:2	0.024	0.0184	0.32	0.037	0.0061	0.95	<0.05 ³
<i>trans</i> -11, <i>cis</i> -15 18:2	<i>trans</i> -11, <i>cis</i> -15 18:2	0.19	0.296	0.53	0.30	0.094	0.38	<0.01
<i>trans</i> -9, <i>cis</i> -11 CLA	<i>trans</i> -9, <i>cis</i> -11 CLA	0.18	0.040	<0.001	0.14	0.049	0.38	<0.05
<i>trans</i> -10, <i>cis</i> -12 CLA	<i>trans</i> -10, <i>cis</i> -12 CLA	0.044	0.0289	0.15	0.10	0.057	0.19	0.09

¹Linear regressions derived using individual results, n = 16.²Adjusted coefficient of determination.³Only 4 observations from HSO treatment [HSO = high concentrate diet (forage to concentrate ratio 35:65) containing 50 g of sunflower oil/kg of diet dm]. No observations for other treatments.

milk are shown in Supplemental Table S2 (<https://doi.org/10.3168/jds.2018-15860>).

DISCUSSION

Diets containing both high proportion of concentrates and PUFA decrease milk fat content and yield, representing a typical response called diet-induced MFD in lactating cows (Bauman and Griinari, 2003; Shingfield and Griinari, 2007; Harvatine et al., 2009). Even though changes in milk fat composition and secretion on low-fiber, high-oil diets have been characterized (Griinari et al., 1998; Peterson et al., 2003; Roy et al., 2006; Rico and Harvatine, 2013), a direct cause and effect of diet-induced MFD has not been established and studies investigating the temporal changes in the onset and recovery from MFD are scarce.

Inclusion of SO in the high-concentrate diet induced a decline in milk fat content by 7 d, but decreased fat secretion by 17 d. The explanation to this discrepancy is the rapid increase in milk yield immediately after the start of high-concentrate treatments resulting in unchanged fat secretion. The increased milk yield may also have induced a dilution effect on milk fat content on HSO and H and on protein content on HSO by d 7 (Supplemental Figure S1B; <https://doi.org/10.3168/jds.2018-15860>). However, the decline in milk fat content on HSO after d 7 is most likely an effect of

MFD, because the protein content on HSO was normalized and then increased and the decrease in milk fat content became more pronounced on HSO than H. Overall, HSO treatment lowered milk fat synthesis from −20.2 to −31.9% relative to other treatments without negative effects on total DMI (19.0, 18.6, 23.3, and 20.7 kg/d for L, LSO, H, and HSO, respectively; Ventto et al., 2017). The magnitude of MFD on the HSO treatment was within the expected range reported earlier for lactating cows (Bauman and Griinari, 2003; Shingfield and Griinari, 2007; Harvatine et al., 2009).

The milk fat lowering response on HSO occurred in 2 stages. On d 5 to 7 a turning point occurred in which milk yield and proportions of *trans*-11 18:1 and *cis*-9,*trans*-11 CLA in milk fat on HSO reached the highest values and after that started to decrease. Milk yield on HSO and H followed a similar temporal trend, but contrary to HSO, *trans*-11 18:1 and *cis*-9,*trans*-11 CLA continued to increase on LSO. At the same time point (d 5 to 7), milk fat content and proportion of 4:0 reached lower values. In addition, the proportion of 18:0 in milk fat on HSO started to increase after that time point. A second turning point on HSO was observed on d 15 to 17 in which another drop in milk fat content was observed and milk fat yield reached lower values. After this, the response in milk fat content and yield reached a plateau. Furthermore, at this time point the proportions of *trans*-10 18:1, *trans*-9,*cis*-11 CLA, and *trans*-

10,*cis*-12 CLA in milk fat on HSO reached the highest values, whereas the reverse was true for 4:0. After this time point a gradual increasing trend was observed for 16:0, and the proportion of 18:0 reached a plateau after a decrease from the highest values observed in d 13.

The BH theory of diet-induced MFD (Bauman and Griinari, 2001) attributes the inhibition of milk fat synthesis to changes in ruminal lipid metabolism and increased formation of specific BH intermediates. *Trans*-10,*cis*-12 CLA is the only intermediate that is known unequivocally to lower milk fat synthesis in lactating cows (Baumgard et al., 2000; Bauman and Griinari, 2003), with evidence that *cis*-10,*trans*-12 CLA (Saebø et al., 2005) and *trans*-9,*cis*-11 CLA (Perfield et al., 2007) may also act as a possible inhibitor. Under the conditions of this experiment, an increase in the omasal flow of *trans*-10,*cis*-12 CLA was observed on both H and HSO treatments, implying that other BH intermediates or mechanisms were contributing to the MFD (Ventto et al., 2017). The omasal flow of *trans*-9,*cis*-11 CLA on HSO was numerically higher but not statistically significantly affected by treatments (Ventto et al., 2017). Contrary to these observations, MFD on the HSO treatment was accompanied by an increase in both *trans*-10,*cis*-12 CLA and *trans*-9,*cis*-11 CLA in milk fat. The discrepancies in omasal and milk FA composition observed for *trans*-10,*cis*-12 CLA indicate that the mechanisms driving the progress of diet-induced MFD cannot be studied only by measuring specific FA BH intermediates in milk, but the omasal or duodenal flow of these FA available for absorption should also be taken into account and quantified. The unexpected difference between omasal flow and secretion in milk, especially in the case of *trans*-10,*cis*-12 CLA, could result from unique transfer pathways to milk. Urrutia et al. (2018) showed that *trans*-10,*cis*-12 CLA was transferred to milk fat at a lower rate than *cis*-9,*trans*-11 CLA when both CLA isomers were administered as equal abomasal bolus infusions to dairy cows. Compartmental modeling indicated that *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA are processed differently and that *trans*-10,*cis*-12 CLA is partly transferred to a transiently unavailable pool in extravascular tissues (primarily mammary gland) and slowly recycles to the available pool for milk fat synthesis (Urrutia et al., 2018). Whether this kind of transiently unavailable pool exists for other BH intermediates such as *trans*-9,*cis*-11 CLA is not known. Because *trans*-10,*cis*-12 CLA at the omasum was increased also in non-MFD causing H treatment in the present study, we speculate that other FA or other contributing factors occurring due to the treatments might affect the absorption rate from the intestine, transport and metabolism, mammary uptake, bioactive properties, or transfer of *trans*-10,*cis*-12 CLA (or other

MFD causing BH intermediates) to milk. However, this speculation remains unresolved.

Even though inclusion of SO on H diet resulted in increased secretion of *trans*-10,*cis*-12 CLA in milk (Supplemental Table S2; <https://doi.org/10.3168/jds.2018-15860>), no association between milk fat yield and omasal flow or secretion of *trans*-10,*cis*-12 CLA in milk was observed. However, 26% of variation in milk fat yield can be explained by *trans*-9,*cis*-11 CLA secretion in milk, whereas for omasal flow of *trans*-9,*cis*-11 CLA the coefficient of determination was 14% ($P = 0.08$). This could be considered evidence that *trans*-9,*cis*-11 CLA but not *trans*-10,*cis*-12 CLA is involved in the onset and progress of MFD in the present study. The previously reported postruminal flows of *trans*-10,*cis*-12 CLA and concentrations of *trans*-10,*cis*-12 CLA in milk fat are lower than expected in diet-induced MFD when compared with studies where MFD was caused by *trans*-10,*cis*-12 CLA supplementation (Bauman and Griinari, 2003; Shingfield and Griinari, 2007; Glasser et al., 2010). Furthermore, FO-induced MFD has been shown not to be associated with increases in milk *trans*-10,*cis*-12 CLA (Lor et al., 2005a; Kairenius et al., 2015) or the amounts of *trans*-9,*cis*-11 CLA and *trans*-10,*cis*-12 CLA at the omasum (Kairenius et al., 2015). Because of the limited number of milk samples over time with detailed CLA profile analyses in the present study, we were not able to exactly identify the onset in the increase of *trans*-10,*cis*-12 CLA proportion or the time point for peak concentration. However, *trans*-10,*cis*-12 CLA in milk fat seems to follow the same temporal pattern observed for *trans*-10 18:1 and *trans*-9,*cis*-11 CLA albeit a more rapid increase in *trans*-10,*cis*-12 CLA.

Under normal conditions *cis*-9,*trans*-11 CLA is an intermediate product of the major BH pathway in the rumen (Shingfield et al., 2010). Even though *cis*-9,*trans*-11 CLA and *trans*-11 18:1 in milk fat were increased during SO supplementation until d 5, their amount was thereafter rapidly decreased when SO was included in the H but not L treatment, reflecting their flow at the omasum on d 22 to 24 (Ventto et al., 2017). *Cis*-9,*trans*-11 CLA is the main CLA isomer formed in the BH of 18:2n-6 in the rumen and it is also produced in the Δ^9 desaturation of *trans*-11 18:1 in the mammary gland (Shingfield et al., 2010), which explains the identical responses in the proportion of *cis*-9,*trans*-11 CLA and *trans*-11 18:1 in milk fat. Previous studies have reported similar temporal shift from *cis*-9,*trans*-11 CLA and *trans*-11 18:1 formation to *trans*-10 18:1, *trans*-10,*cis*-12 CLA (Rico and Harvatine, 2013) and *trans*-9,*cis*-11 CLA formation (Roy et al., 2006; Shingfield et al., 2006) in milk fat on diet-induced MFD. In the present study, SO also induced an increase in

cis-9,*cis*-11 CLA, *trans*-8,*cis*-10 CLA, *trans*-8,*trans*-10 CLA, and *trans*-10,*trans*-12 CLA in milk fat regardless of the amount of concentrate in the diet.

Supplementation of SO increased the proportion of total 18:1 isomers in milk fat. The HSO treatment caused a shift in BH pathways resulting in the formation of *trans*-10 18:1 in the rumen instead of *trans*-11 18:1, which was the major BH intermediate on LSO (Ventto et al., 2017). This was reflected in milk fat with increased proportions of *trans*-10 18:1 and *trans*-11 18:1 on HSO and LSO, respectively. The increased synthesis of *trans*-10 18:1 in the rumen is known to increase *trans*-10 18:1 in milk from cows fed diets causing MFD (Bauman and Griinari, 2003; Loor et al., 2005b; Roy et al., 2006; Rico and Harvatine, 2013). The evidence that *trans*-10 18:1 inhibits milk fat synthesis is inconsistent (Lock et al., 2007; Kadegowda et al., 2008), but the negative association between milk fat secretion and *trans*-10 18:1 flow at the omasum and secretion in milk in the present study support the theory that *trans*-10 18:1 has a role in the regulation of milk fat synthesis. In addition, the association between the flow at the omasum and secretion in milk for *trans*-10 18:1 was stronger than for *trans*-9,*cis*-11 CLA.

Decreased milk fat fluidity and increased milk fat melting point has been suggested to contribute to MFD, especially when cows are fed FO supplements, due to the increased amounts of *trans* 18:1 and decreased amounts of *cis*-9 18:1 in milk (Loor et al., 2005b; Gama et al., 2008). In the present experiment, SO lowered the calculated mean milk fat melting points, which could theoretically indicate increased milk fat fluidity. In addition, the concentration ratios *cis*-9 14:1/(*cis*-9 14:1 + 14:0) and *cis*-9 16:1/(*cis*-9 16:1 + 16:0) indicating stearoyl-CoA desaturase activity were inversely related to milk fat, confirming earlier reports (Gama et al., 2008; Rico and Harvatine, 2013). Even a considerable decrease in the Δ^9 desaturation of FA in the mammary gland and an increase in calculated milk fat melting point caused by Co-EDTA did not affect milk fat content or output in lactating cows (Leskinen et al., 2016). Altogether these findings support the conclusion that a decrease in the availability of stearoyl-CoA desaturase products for milk fat synthesis and increasing milk fat melting point are not likely driving mechanisms in MFD (Shingfield and Griinari, 2007; Harvatine et al., 2009).

The content and yield of de novo synthesized FA (<16 carbons) in milk decrease during MFD (Peterson et al., 2003; Shingfield et al., 2006; Rico and Harvatine, 2013). In addition, inclusion of plant oils typically lowers the concentrations of short- and medium-chain FA (≤ 16 carbons) in milk without the occurrence of MFD (Kliem and Shingfield, 2016). The proportion (g/100 g

of FA) of de novo FA, 10:0, 12:0, and 14:0, and 16:0 in milk fat were decreased on oil-supplemented diets, but milk fat on the HSO contained proportionally more of these FA than milk fat on LSO (see also Supplemental Figure S2; <https://doi.org/10.3168/jds.2018-15860>), and the outputs (g/d; Supplemental Table S2; <https://doi.org/10.3168/jds.2018-15860>) of these FA in milk on d 22 to 25 were not different between LSO and HSO. The inclusion of SO in the diets induced a rapid decline in 16:0, indicating a quick response in de novo FA synthesis, but contrary to LSO, the proportion of 16:0 on HSO had an increasing trend after d 15. Contrary to the expectations based on ruminal flow of 18:0 (Ventto et al., 2017), the proportion of 18:0 in milk fat did not increase in a similar manner on HSO as on LSO. On HSO, a lag of 5 d was observed before the gradual increase in 18:0, and a peak concentration in 18:0 abundance occurred on d 13, 2 d before the rapid drop in milk fat content and yield (d 15). This indicates that a reduction in the de novo synthesis has not been a key factor in MFD in the present study. A similar increasing-decreasing trend in the proportion of 18:0 was observed in an earlier study with high-concentrate diets supplemented with SO (Roy et al., 2006).

Supplementation of SO in the high-concentrate diet decreased the proportions of short-chain FA (4:0, 6:0, and 8:0) and their secretion in milk was lower on the HSO treatment compared with LSO (Supplemental Table S2; <https://doi.org/10.3168/jds.2018-15860>). The lowered incorporation of 4:0 in milk fat following the same temporal pattern as milk fat content may be a result of reduced utilization of 4:0 and BHB in TG synthesis or reduced synthesis of 4:0 from acetate in the mammary gland. The rumen VFA concentration and composition were not changed on HSO (Bayat et al., 2017) and cannot explain the differences observed in the proportions of short-chain FA in milk fat. Plasma metabolites were not analyzed in the present experiment, but earlier studies have reported changes in the mammary uptake (jugular-mammary venous differences) for BHB and acetate in cows fed FO (Loor et al., 2005a) and high-concentrate diets (Loor et al., 2005b). However, earlier reports suggest that the possible shifts in the rumen production of acetate and butyrate, the plasma acetate and BHB concentrations, mammary uptake of these metabolites, and reductions in de novo FA synthesis cannot fully explain diet-induced MFD (Bauman and Griinari, 2003; Loor et al., 2005b).

Flow of 18:2n-6 at the omasum was increased on H treatments compared with L treatments as a result of decreased ruminal BH (Ventto et al., 2017). Furthermore, the proportion of 18:2n-6 in milk fat and the output of 18:2n-6 in milk (Supplemental Table S2; <https://doi.org/10.3168/jds.2018-15860>) was higher on

H treatments compared with L treatments, and a close linear relationship was observed between the flow at the omasum and secretion in milk for 18:2n-6. Intake of 18:3n-3 and flow of BH products *cis*-9,*trans*-11,*cis*-15 18:3, *trans*-11,*cis*-15 18:2, and *trans*-11,*trans*-15 18:2 at the omasum were higher on L than H treatments (Ventto et al., 2017) and this was reflected in milk fat resulting lower amounts of these BH products on H and HSO. Biohydrogenation product *trans*-10,*cis*-15 18:2 was only found in milk fat on HSO treatment providing more support to the existence of alternative *trans*-10 pathway of 18:3n-3 metabolism in the rumen (Alves and Bessa, 2014) that is pronounced in cows fed diets causing MFD.

CONCLUSIONS

The temporal changes in milk fat content, milk fat yield, and the proportions of MFD-related BH intermediates showed that the recovery from MFD was more rapid than the onset of MFD. Inclusion of SO in a high-concentrate diet induced a 2-stage drop in milk fat synthesis accompanied by specific changes in the milk FA composition over time. Biohydrogenation intermediates *trans*-10 18:1, *trans*-10,*cis*-12 CLA, and *trans*-9,*cis*-11 CLA in milk increased gradually during the progress of MFD, whereas *cis*-9,*trans*-11 CLA and *trans*-11 18:1 increased rapidly but declined subsequently at the onset of MFD, reflecting the shift from the predominant ruminal BH pathway to another pathway. Especially the flow of *trans*-10 18:1 at the omasum was associated with milk fat secretion across all treatments. In addition, increased proportions of *trans*-10,*cis*-15 18:2 in omasal digesta and milk fat were observed in MFD. The association of MFD with omasal and milk *trans*-10,*cis*-12 CLA, an intermediate known to inhibit milk fat synthesis in the mammary gland, was ambiguous, suggesting that other ruminal BH intermediates or additional mechanisms contributed to the diet-induced MFD in dairy cows. To identify the effects of certain FA BH intermediates on MFD, FA infusion studies are needed to remove the effect of other confounding factors causing MFD.

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