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Effect of subacute ruminal acidosis on milk fat concentration, yield and fatty acid profile of dairy cows receiving soybean oil

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The objective of this study was to investigate the effect of ruminal infusion of soybean oil (SBO) with either a moderate- or high-forage diet on fat concentration, yield and composition in milk from dairy cows. Six rumen-fistulated Holstein dairy cows (639±51 kg body weight, 140±59 days in milk) were used in the study. Cows were randomly assigned to one of two dietary treatments, a high forage:concentrate (HFC, 74:26) or a moderate forage:concentrate (MFC, 56:44) total mixed ration. Cows were fed at 08.00 and 13.00 h and pulse-dosed ruminally at 13.00 h over a 10-min duration with 2% of diet dry matter of SBO. Ruminal pH was recorded continuously. Cows receiving the MFC treatment had lower daily mean ruminal pH and ruminal pH was below 6·0 for a longer duration compared with the HFC treatment (640 vs. 262 min/d, $P<0·05$). Cows receiving the MFC treatment had a greater reduction (diet by week interaction, $P<0·05$) in milk fat concentration and yield than cows receiving the HFC treatment (42 vs. 22% and 45 vs. 21%, respectively). Additionally, cows receiving the MFC diet had a greater reduction in milk fat concentration (g/100 g FA) of FA <C16 (14 vs. 8%), and a greater increase in concentration of FA >C16 (17 vs. 9%), *trans*-10 18:1 (159 vs. 21%) and *trans*-9, *cis*-11 conjugated linoleic acid (121 vs. 55%) ($P<0·05$) compared with cows receiving the HFC diet. This study demonstrated that cows fed the MFC diet had lower ruminal pH and showed a greater rate of milk fat depression when infused with SBO.

Keywords: dietary fibre, ruminal pH, milk fat depression, soybean oil, fatty acid, biohydrogenation.

The effect of dietary physically effective fibre (peNDF) on ruminal pH in dairy cows has been extensively studied. In a meta-analysis, Zebeli et al. (2008) summarized data from 45 published studies and demonstrated that ruminal pH was increased by increasing dietary peNDF (Mertens, 1997) up to 31% (DM basis), beyond which ruminal pH reached a plateau (daily mean ruminal pH 6·27). Nonetheless, peNDF in that study was able to explain only 50% of the variation in ruminal pH. Additional dietary factors such as ruminal degradable starch from grain and DMI were also shown to affect ruminal pH despite the presence of apparently adequate levels of peNDF. For example, Zebeli et al. (2008) demonstrated that at a fixed level of peNDF (31% of DM), increasing the dietary ruminal degradable starch from 14 to 22% and DMI from 20 to 25 kg/d increased the duration that ruminal pH was below 5·8.

The effect of ruminal pH on milk fat (MF) is inconsistent in the literature. Some studies reported a reduction in MF concentration with low ruminal pH (Gentile et al. 1986; Stone, 1999), whilst others showed no effect of pH on MF concentration (Rustomo et al. 2006a, b). Additionally, Allen (1997) summarized the association between ruminal pH and MF concentration from 23 studies and concluded that ruminal pH explained 39% of the variation in MF percentage.

Davis & Brown (1970) defined two conditions for milk fat depression (MFD) to occur. The first was altering microbial processes (i.e., by low fibre diets) and the second was the presence of polyunsaturated fatty acids (PUFA) in the diet. Bauman & Griinari (2001, 2003) proposed the biohydrogenation theory which states that: intermediates resulting from altered ruminal biohydrogenation (BH) under specific dietary conditions act on the mammary gland, thus inhibiting *de novo* synthesis of FA. The role of *trans*-10, *cis*-12 conjugated linoleic acid (CLA) (Baumgard et al. 2000) and *cis*-10, *trans*-12 CLA (Sæbø et al. 2005) as potent inhibitors of FA synthesis in the mammary gland have been confirmed. Additional BH intermediates such as *trans*-9, *cis*-11 CLA (Perfield et al. 2007) and *trans*-10 18:1

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Table 1. Ingredient composition and chemical analyses of high forage:concentrate (HFC) and moderate forage:concentrate (MFC) total mixed rations (TMR)

Ingredient/TMR	HFC	MFC
	%, DM	
Corn silage	40·4	30·6
Alfalfa silage	26·9	20·4
Mixed hay	6·9	5·2
Corn (ground)	4·1	0·0
Wheat bran	4·2	0·0
Wheat (ground)	0·0	18·9
Barley (ground)	0·0	9·5
Protein supplement†	17·7	15·4
Chemical composition		
DM, %	36·3	42·6
	%, DM	
CP (N × 6·25)	18·2	17·1
Soluble protein	6·0	3·9
Ruminal undegradable protein‡	45·1	39·3
ADF	27·0	21·0
NDF	40·0	32·7
Lignin	4·5	3·2
Ether extract	2·4	2·0
Ash	7·8	6·4
NFC§	31·6	41·8
Starch	12·6	24·1
NE_L ¶, Mcal/kg	1·50	1·66
Est. Starch fermentation rate††, %/h	25·6	34·5

†Contained (% of DM): 48%-soybean meal, 24·8; high-protein corn gluten meal, 19·8; canola meal, 10·1; roasted soybean (whole), 10·4; fish meal (herring), 5·1; beet pulp, 1·3; calcium carbonate (limestone), 3·9; dicalcium phosphate, 4·9; soybean hulls (ground), 5·9; sodium bicarbonate, 4·4; salt, 2·8; molasses (in pelleter), 1·8; urea, 2·8; magnesium oxide, 1·0; Organic Ruminant Micro Premix (Floradale Feed Mill Limited, Floradale, ON, Canada), 0·7; sulphur flour (99·5%), 0·4; Rovimix Biotin (H-2, DSM Nutritional Products, Inc., Parsippany, NJ), 0·02

‡,¶Estimated using CPM-Dairy v 3.0.8 (Miner Institute, Chazy, NY) using the chemical analysis of feed ingredients

§Nonfibre carbohydrates = 100 − (NDF + CP + ether extract + ash)

††Estimated fermentation rate of dietary starch calculated from the CPM-Dairy default values of starch content of dietary ingredients and the ingredients' fermentation rates

Table 2. Fatty acid profile (g/100 g fatty acids) of high forage:concentrate (HFC) and moderate forage:concentrate (MFC) total mixed rations (TMR)

Item/TMR	HFC	MFC
10:0	0·025	0·023
12:0	0·235	0·253
14:0	0·380	0·446
15:0	0·112	0·110
16:0	16·6	18·6
16:1 *cis*-7	0·265	0·306
16:1 *cis*-9	0·492	0·473
17:0	0·147	0·162
18:0	4·55	4·69
18:1 *cis*-9	19·6	19·5
18:1 *cis*-11	2·14	1·97
18:1 *cis*-12	0·181	0·364
18:1 *cis*-13	0·090	0·102
18:1 *cis*-14	0·511	0·499
18:2 n-6	39·1	38·5
18:3 n-3	9·05	7·53
19:0	0·126	0·175
20:0	0·625	0·525
20:1 *cis*-11	0·770	0·833
20:2 n-6	0·045	0·045
20:4 n-6	0·020	0·015
20:5 n-3	0·102	0·120
22:0	0·516	0·488
22:6 n-3	0·123	0·088
23:0	0·147	0·138
24:0	0·376	0·401
PUFA (total)	48·4	48·9
Unidentified	3·6	3·7

(Shingfield et al. 2009) were identified as antilipogenic, nonetheless, more studies are needed to confirm their role in MFD.

Previously, AlZahal et al. (2009) demonstrated that diets with moderate forage level, rich in rapidly fermentable starch, and low in PUFA content induced ruminal pH depression, did not cause MFD. Further, the concentrations of BH intermediates known to inhibit lipogenesis in dairy cows (*trans*-10, *cis*-12; *trans*-9, *cis*-11 CLA) were not different among treatments and were lower than those levels known to cause MFD. In the current study, we hypothesized that PUFA-induced MFD is greater when cows are fed moderate- compared with high-forage diets. The objective of this study was to investigate the effect of ruminal infusion of soybean oil (SBO) with either a moderate- or high-forage-to-concentrate diet on fat concentration, yield and composition in milk from dairy cows.

Materials and Methods

Animals, experimental design and feeding

As described in the previous study (AlZahal et al. 2009), six rumen-fistulated multiparous lactating Holstein cows housed in a tie-stall facility at Elora Dairy Research Centre, University of Guelph, Guelph, Ontario and cared for and handled in accordance with the Canadian Council on Animal Care regulations were used in the study.

The cows were randomly assigned to one of two dietary treatments, a high forage:concentrate (HFC; 74:26; % of DM) or a moderate forage:concentrate (MFC; 56:44; % of DM) total mixed ration. Ingredients and chemical analyses and FA profiles of the experimental TMR are presented in Tables 1 and 2, respectively. The HFC diet was designed to provide a large amount of fibre to maintain high ruminal pH. On the other hand, the MFC diet provided an adequate amount of fibre to maintain DMI and milk production and yet included a large amount of starch (24% of DM) derived from wheat and barley, which are rapidly and extensively fermented in the rumen. The estimated

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total dietary starch fermentation rate was 34·6%/h for the MFC diet compared with 25·6%/h for the HFC diet (Table 1). The chemical analyses of MFC and HFC TMR agreed closely with formulation targets. The study consisted of 4 weeks of adaptation during which each cow received one of the two TMR without ruminal SBO infusion (AlZahal et al. 2009), followed by 3 weeks (current experiment) during which, cows continued receiving the same diet but with ruminal infusion of SBO [Morrison Bros Ltd., Wingham, Ontario, Canada (g/100 g FA; 17, 16:0; 12, 18:0; 34, 18:1; 28,18:2 n-6; 3,18:3 n-3; and 7, others)].

The TMR (HFC and MFC) were fed twice daily at 07·00 and 13·00 h. The amount of feed was adjusted based on average DMI of the previous week to allow a maximum of 5 kg/d of refusals (as-fed basis). Soybean oil was dosed into the rumen during the experimental period through the cows' fistulae at 13·00 h using a plastic funnel connected to a 1-metre tube. The SBO was pulse-dosed over 10 minutes and distributed evenly into the different compartments of the rumen. The rumen contents were mixed within the rumen through the cannula for 2 min. The amount of added SBO equalled 2% of the individual cow's average DMI of the previous week.

Experimental measures and samples analyses

Ruminal pH was measured and recorded continuously every min for 3 d per week using a pH recording system as described by AlZahal et al. (2007). pH electrodes were calibrated weekly using standard buffer solutions of pH 4·00 and 7·00 (Fisher Scientific, Fairlawn, NJ). Feed intake and milk yield were monitored daily throughout the experimental period. Total mixed ration samples from each dietary treatment and ort samples from each individual animal were collected 3 times per week and frozen at −20 °C until analysis. The orts samples were pooled per cow per week proportionally to the amount of the orts. The TMR samples were pooled per week per treatment. Pooled TMR and orts samples were dried for 48 h in a forced-air oven to determine the DM content for that week. At the end of the experiment, dried TMR samples were ground through a 1-mm screen (Wiley Mill, Arthur A. Thomas Co., Philadelphia, PA) and pooled by treatment across all weeks. Samples were analyzed at Agri-Food Laboratory, Guelph, Ontario, Canada as described previously by AlZahal et al. (2007).

Cows were milked twice daily at 05·00 and 15·00 h and milk samples were collected in duplicate 3 times per week during morning and afternoon milking throughout the experiment. Milk samples for FA analysis were frozen immediately at −20 °C until analysis. Milk samples for component analysis were preserved with 2-bromo-2-nitropropane-1-2-diol and stored at 4 °C. Every week, milk samples for components analysis were pooled by cow by day based on a constant proportion of 60:40 (am:pm, respectively) and then were pooled by week using equal proportions and submitted to Laboratory Services Division (Guelph, Ontario, Canada) for analysis using a near-infrared analyzer (Foss System 4000, Foss Electric, HillerØd, Denmark).

Lipids for FA analysis were extracted from pooled milk and feed samples as explained by Or-Rashid et al. (2009).

Particle size distribution

The particle size of the experimental TMR was assessed weekly (on the second day of pH recording of each week) in duplicate using the Penn State Forage Particle Size Separator with three sieves and a solid bottom pan (model C24682N, Nasco, Fort Atkinson, WI), as described by Kononoff et al. (2003). The materials remaining on each sieve and pan was then removed, weighed and oven-dried at 100 °C to determine the distribution of feed DM retained on each sieve and in the pan. The peNDF (of particles >1·18 mm) was determined by multiplying NDF content by the proportion of DM of particles retained on the top, middle, and bottom screens of the separator (Mertens, 1997).

Statistical analysis

Statistical analysis was conducted on weekly averages of DMI, milk yield, milk components and ruminal pH characteristics and FA data. Proc Mixed of SAS (SAS Institute, 2004) was used using the following model: $Y_{ijk}=\mu+D_i+W_j+(D\times W)_{ij}+e_{ij}$ where Y_{ij}=the dependent variable, μ=overall mean, D_i=effect of diet ($_i$=1, 2), W_j=effect of week ($_j$=1, 2, 3), $(D\times W)_{ij}$=effect of diet x week ($_{ij}$=1,.., 6), and e_{ij}=random residual error.

The effects of week and diet were considered as fixed effects. Week of experiment was used as a repeated measurement with cow within dietary treatment as the subject. Orthogonal polynomial contrast was used to describe the linear and quadratic terms of week effect and week by diet interaction. For each analyzed variable, cow was subjected to five covariance structures: compound symmetry, heterogeneous compound symmetry, autoregressive order 1, heterogeneous autoregressive order 1 and unconstructured covariance structure. The covariance structure that gave the smallest Bayesian information criterion was used (Littell et al. 1996). The fold-change in a given variable by week or treatment, in case of lack of an interaction, was calculated from the main effects (not shown). In case of a significant interaction, the proportions were calculated for each factor within the levels of the other factor.

Results and Discussion

Ruminal pH

Diet had a significant effect on mean ruminal pH and duration of ruminal pH below 6·0 during adaptation

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Table 3. Effect of diet (D), week (W), and their interaction (D × W) on ruminal pH characteristics, DMI, and milk yield and components, HFC = high forage : concentrate, MFC = moderate forage : concentrate

	HFC TMR			MFC TMR				*P* value†				
Item	W 1	W 2	W 3	W 1	W 2	W 3	SE	D	W^L	W^Q	D × W^L	D × W^Q
Mean pH	6·26	6·32	6·24	5·95	6·06	6·08	0·068	*	NS	NS	NS	NS
<6·0‡, min/d	293	223	270	736	611	574	109	*	NS	NS	NS	NS
DMI§, kg/d	21·8	21·9	22·3	23·9	23·7	23·6	1·38	NS	NS	NS	NS	NS
Milk, kg/d	30·3	32·2	30·4	31·4	32·3	30·8	6·26	NS	NS	NS	NS	NS
Fat, %	4·19	3·89	3·25	4·41	3·61	2·54	0·360	NS	***	NS	**	NS
Fat, kg/d	1·22	1·21	0·96	1·36	1·12	0·74	0·141	NS	***	NS	*	NS
Protein, %	3·25	3·40	3·39	3·82	3·77	3·74	0·311	NS	NS	NS	**	NS
Protein, kg/d	0·96	1·05	0·99	1·15	1·18	1·11	0·142	NS	NS	*	NS	NS
Lactose, %	4·71	4·59	4·61	4·68	4·72	4·70	0·124	NS	NS	NS	*	*
Lactose, kg/d	1·44	1·49	1·42	1·48	1·54	1·47	0·325	NS	NS	NS	NS	NS

†L, Q = linear and quadratic effects, respectively
‡Duration ruminal fluid pH was below 6·0
§Including ruminally infused soybean oil (2% of DMI)
*,**, ***Significant at $P<0·05$, $P<0·01$ and $P<0·001$, respectively; NS, non significant $P \geqslant 0·05$

(AlZahal et al. 2009) and during SBO infusion (current experiment, $P<0·05$, Table 3). Ruminal pH below 6·0 is considered suboptimal for cellulolytic bacteria growth (Russell & Wilson, 1996) and the pH remained below 6·0 for greater than 9·5 h/d in the MFC diet.

Although the MFC diet contained sufficient amount of peNDF (29·3% of DM), the provision of highly fermentable carbohydrate (starch from ground wheat and barley) led to a significant depression in ruminal pH. This was in agreement with Zebeli et al. (2008) who showed that increasing the concentration of ruminally degradable starch can depress ruminal pH despite the provision of a diet containing 31% peNDF (DM basis). This emphasizes the need to take into account total diet starch fermentation rate during formulating lactating cow rations.

Dry matter intake, milk yield and components

The main effect of diet, week, and their interaction had no effect on DMI and milk yield ($P \geqslant 0·05$, Table 3). Additionally, diet had no effect on protein percentage and yield (Table 3). However, there was a significant ($P<0·05$) quadratic week effect on milk protein yield (kg/d). The increase in milk protein yield during week two likely reflected the tendency ($P=0·06$) for milk yield to increase during that week.

In a previous study, AlZahal et al. (2009) utilized dietary treatments that were low in lipid [2·0 to 2·4% of DM, linoleic acid (LA) intake was approximately 170 g/d/cow for a cow consuming approximately 20 kg/d] to investigate the effect of dietary forage level (74 to 56% of DM, corn-silage-haylage based forage) on MF and demonstrated that forage level had no effect on MF, when low PUFA diets were fed. In the current experiment, which is a continuation of the previous experiment, the same base diets were utilized and SBO was pulse-dosed intra-ruminally daily for 3 continuous weeks. Results showed that both MF concentration and MF yield (kg/d) dropped linearly overtime, with cows receiving the MFC diet having a greater drop in MF concentration and MF yield than cows receiving the HFC diet ($P<0·05$, significant linear week × diet interaction; Table 3). By the third week of SBO infusion, milk fat concentration dropped by 22 and 42% for the HFC and MFC diet, respectively (Table 3). Similarly, milk fat yield (kg/d) dropped by 21 and 45% for HFC and MFC diet, respectively (Table 3).

The effect of dietary plant oil supplementation on milk fat secretion is well established (Bauman & Griinari, 2003). However, there are many factors that mediate the effect of plant oil supplementation on MF content, yield, and FA profile and thus contribute to differences among studies. These factors are the composition of the basal diet used in the study, plant oil type, and duration of plant oil supplementation (Loor et al. 2005; Roy et al. 2006; Shingfield et al., 2008).

Roy et al. (2006) observed a reduction in MF content by 39 and 52% with 48 : 52 and 27 : 73 forage to concentrate corn-silage-based diets, respectively, following 18 d of sunflower oil supplementation (5% of DM) as the source of LA. Additionally, Roy et al. (2006) showed no change in milk fat content when a similar amount of linseed oil was supplemented to a 64 : 36 grass-hay-based diet. Furthermore, Shingfield et al. (2008) demonstrated that an incremental increase in supplemental linseed oil (from 0 to 750 g/d) to a grass-silage-based diet (60 : 40, forage : concentrate) had no effect on MF.

Milk fatty acids

Feeding high amounts of PUFA to ruminants inhibit ruminal BH and generate a wide range of BH intermediates that are transferred to the milk, including *trans*-18 : 1 FA

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and CLA isomers, some of which inhibit milk fat synthesis in the mammary gland (Bauman & Griinari, 2001, 2003). Griinari & Bauman (1999) suggested a minor BH pathway in which LA is isomerised to form *trans*-10, *cis*-12 CLA. This isomer in turn is reduced to *trans*-10 18:1 and subsequently to C18:0. Recent studies confirmed that *trans*-10, *cis*-12 CLA can be synthesized from LA when incubated with *Probionibacterium acnes* and mixed ruminal bacteria (Wallace et al. 2007) and in-vivo (Shingfield et al. 2008). Other CLA such as *cis*-10, *trans*-12 CLA (Sæbø et al. 2005) and *trans*-9, *cis*-11 CLA (Perfield et al. 2007), both intermediates of LA (Wallace et al., 2007), were suggested as antilipogenic. Furthermore, *trans*-10, *trans*-12 CLA (Sæbø et al. 2005) and *trans*-9, *trans*-11 CLA (Perfield et al. 2007) showed no effect on lipogenesis but caused a decrease in Δ^9-desaturation indices. The *trans* 18:1 intermediates investigated include *trans*-9 (Rindsig & Schultz, 1974), *trans*-11, and *trans*-12 (Griinari et al. 2000). These studies showed no direct effect on milk fat synthesis. Studies showed that *trans*-10 18:1 had a significant association with MFD in dairy cows (Bauman & Griinari, 2001). A study by Lock et al. (2007) demonstrated that *trans*-10 18:1 had no direct role in MF synthesis in the mammary gland. Lock et al. (2007) infused post-ruminally 42·6 g/d of a pure preparation (95%) of *trans*-10 18:1, which increased the concentration of milk *trans*-10 18:1 from 0·47 to 1·11 (g/100 g FA). Provided that during MFD the levels of milk *trans*-10 18:1 can exceed in some cases 10 g/100 g FA, the study by Lock et al. (2007) has been criticized by not infusing a sufficient amount of *trans*-10 18:1 to induce MFD (Kadegowda et al. 2008). Most recently, Shingfield et al. (2009) post-ruminally infused a mixture of 18:1 FAME that supplied 92 g/d *trans*-10 18:1 and provided convincing evidence that *trans*-10 18:1 may contribute to MFD. However, more studies are needed to confirm the role of *trans*-10 18:1 on milk fat synthesis using pure isomer at different doses.

In the current study, MFD was associated with a significant shift in milk FA profile during the SBO infusion weeks (week effect). This shift included a proportional decrease in MF concentration (g/100 g FA) of *de novo* synthesized FA (FAC 6 to <C16, $P<0·05$) and a proportional increase in MF concentration (g/100 g FA) of long chain FA (FA >C16, $P<0·05$) and most BH intermediates (Tables 4, 5 & 6). These changes were more pronounced for cows receiving the MFC diet (week × diet interaction).

By the third week, MF concentration (g/100 g FA) of FA<C16 was reduced by 7·9 and 13·7% for the HFC and MFC treatments, respectively. The concentrations of C6:0, 7:0, 10:0, 11:0, 12:0, 12:1, *iso*-13:0, *ai*-13:0 and 15:0 FA were, however, reduced to a greater extent with the MFC treatment (week and week × diet, $P<0·05$). On the other hand, the concentrations (g/100 g of FA) of total *trans*-18:1 FA, total CLA and long chain FA were increased to a greater extent with the MFC diet (week and week × diet effect, $P<0·05$), namely *iso*-17:0; *trans*-5 18:1; *trans*-10 18:1; *trans*-12 18:1; *cis*-11 18:1; *trans*-9, *cis*-11 CLA; *trans*-9, *trans*-11+*trans*-10, *trans*-12 CLA (unresolved peak); *cis*-11 20:1 and 20:2 n-6. Milk concentration of *cis*-9, *trans*-11 CLA was increased over week but this increase was more pronounced for the HFC treatment than the MFC diet (42 vs. 28%). Additionally, milk concentration of *trans*-10, *cis*-12 CLA was increased over time ($P<0·05$) but without interaction with diet.

Milk concentration of *trans*-10, *cis*-12 CLA was increased when a high-concentrate diet was supplemented with an oil rich in LA (Loor et al. 2005; Roy et al. 2006). Whilst, milk fat concentration of *trans*-9, *cis*-11 CLA was increased when a high-concentrate diet was supplemented with fish oil (Shingfield et al. 2005) and sunflower oil (Roy et al. 2006).

Plant oil supplementation has been shown to increase the amount of *trans*-11 18:1 leaving the rumen and thus milk concentration of endogenously-synthesized *cis*-9, *trans*-11 CLA (Bauman et al. 2003). However, the persistency of the response of MF *trans*-18:1; *cis*-9, *trans*-11 CLA; and total CLA to oil supplementation was influenced by the composition of basal diet, oil supplement source, and duration of oil supplementation (Roy et al. 2006). The authors demonstrated that the concentration of *cis*-9, *trans*-11 CLA in MF was consistent over time with high-forage diets supplemented with linseed oil, whereas, the increase in MF concentration of *cis*-9, *trans*-11 CLA was rapid and transient and declined over-time with lucerne and maize silage based diets supplemented with sunflower oil. This decline in *cis*-9, *trans*-11 CLA was associated with an increase in MF concentration (g/100 g FA) of *trans*-10 18:1 likely mediated by a time-dependent shift in ruminal biohydrogenation.

In the current study, there was a quadratic increase ($P<0·05$) in milk concentration (g/100 g FA) of *trans*-11 18:1 and the concentration (g/100 g FA) of *cis*-9, *trans*-11 CLA. During week two of SBO infusion, the concentration of *trans*-11 18:1 was 3·7 and 4·18 (g/100 g of FA) for the HFC and MFC treatments, respectively. The decline in *trans*-11 18:1 from week two to week three was associated with an increase in milk concentration (g/100 g FA) of *trans*-10 18:1 for the MFC treatment (from 1·5 to 2·8 g/100 g FA), whereas there was no change in *trans*-10 18:1 concentration from week two to week three for the HFC treatment (1·06 and 1·13 g/100 g FA; week two and week three 3; respectively). These results, suggest that there was a shift in the pathway of ruminal BH towards a higher *trans*-10 to *trans*-11 18:1 ratio when dietary fibre was replaced with grain in agreement with Bauman & Griinari (2003), and Roy et al. (2006). The shift in the BH pathway can be explained by the fact that LA to *cis*-9, *trans*-11 CLA formation in the rumen is mediated by *Butyrivibrio fibrisolvens* (Kepler & Tove, 1967), which are cellulolytic bacteria that can be inhibited directly by low ruminal pH (Russell & Dombrowski 1980). Whereas, the formation of LA to form *trans*-10, *cis*-12 CLA and other CLA is mainly mediated by lactic acid bacteria (i.e., *Propionibacterium* and *Lactobacillus*), which despite their

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Table 4. Milk fatty acid composition (g/100 g total FA), HFC = high forage : concentrate, MFC = moderate forage : concentrate

	HFC TMR			MFC TMR				P value†				
Item	W 1	W 2	W 3	W 1	W 2	W 3	SE	D	W^L	W^Q	D × W^L	D × W^Q
6:0	1·76	1·68	1·64	1·77	1·62	1·34	0·126	NS	***	NS	***	NS
7:0	0·015	0·012	0·009	0·036	0·025	0·017	0·0027	**	***	NS	*	NS
8:0	0·878	0·759	0·713	1·010	0·822	0·643	0·0773	NS	**	NS	NS	NS
9:0	0·014	0·012	0·009	0·031	0·023	0·017	0·0029	*	**	NS	NS	NS
10:0	2·283	2·091	1·957	2·771	2·460	2·027	0·0888	*	***	NS	**	NS
11:0	0·251	0·243	0·223	0·373	0·305	0·221	0·0186	NS	***	NS	***	NS
12:0	2·72	2·49	2·33	3·45	3·09	2·79	0·106	*	***	NS	*	NS
12:1	0·075	0·067	0·069	0·128	0·110	0·090	0·0069	*	***	NS	**	NS
13:0 *iso*	0·023	0·025	0·024	0·022	0·022	0·021	0·0015	NS	NS	NS	NS	NS
13:0 *anteiso*	0·065	0·065	0·062	0·090	0·078	0·074	0·0076	NS	**	NS	*	NS
13:0	0·066	0·065	0·059	0·105	0·089	0·079	0·0098	NS	***	NS	**	NS
14:0 *iso*	0·131	0·119	0·132	0·087	0·068	0·058	0·0092	**	NS	NS	NS	NS
14:0	10·72	10·15	10·10	11·58	10·98	10·94	0·211	*	**	NS	NS	NS
14:1 *cis*-9	0·790	0·840	0·845	1·018	0·948	1·111	0·1545	NS	NS	NS	NS	NS
15:0 iso	0·196	0·187	0·177	0·159	0·145	0·128	0·0046	***	***	NS	NS	NS
15:0 *anteiso*	0·396	0·377	0·386	0·360	0·349	0·328	0·0089	**	*	NS	NS	NS
15:0	1·08	1·03	1·01	1·38	1·23	1·14	0·063	NS	**	NS	*	NS
16:0 *iso*	0·311	0·294	0·290	0·248	0·215	0·189	0·0313	NS	*	NS	NS	NS
16:0	30·03	27·94	27·40	30·26	27·12	26·26	0·865	NS	***	*	NS	NS
16:1 *cis*-9	1·07	1·02	0·94	1·36	1·09	1·31	0·257	NS	NS	NS	NS	NS
16:1 *trans*-9	0·127	0·169	0·159	0·115	0·170	0·167	0·0161	NS	***	***	NS	NS
17:0 *iso*	0·275	0·279	0·277	0·296	0·310	0·382	0·0153	NS	**	NS	**	NS
17:0 *anteiso*	0·515	0·482	0·456	0·463	0·453	0·494	0·0125	NS	NS	NS	**	NS
17:0	0·474	0·431	0·451	0·462	0·455	0·446	0·0198	NS	NS	NS	NS	NS
18:0	12·23	11·91	12·19	9·82	10·13	9·80	0·567	*	NS	NS	NS	NS
18:1 *cis*	21·07	22·63	23·62	19·80	21·18	22·36	0·748	NS	**	**	NS	NS
18:1 *trans*	7·55	9·01	8·83	7·16	9·86	10·73	0·556	NS	***	***	**	NS
18:1 total	28·62	31·64	32·45	26·96	31·04	33·09	0·839	NS	***	***	NS	NS
18:2‡	2·20	2·40	2·49	2·96	3·15	3·71	0·140	***	*	*	NS	NS
CLA§	1·20	1·75	1·68	1·24	2·08	1·67	0·108	NS	***	***	NS	*
18:3 n-3	0·384	0·417	0·407	0·438	0·448	0·469	0·0195	*	NS	NS	NS	NS
18:3 n-6	0·020	0·020	0·020	0·023	0·013	0·020	0·0028	NS	NS	NS	NS	NS
19:0	0·046	0·040	0·042	0·040	0·040	0·043	0·0051	NS	NS	NS	NS	NS
20:0	0·172	0·157	0·161	0·134	0·137	0·123	0·0086	NS	**	NS	NS	**
20:1 *cis*-9	0·144	0·153	0·153	0·127	0·124	0·120	0·0072	*	NS	NS	NS	NS
20:1 *cis*-11	0·085	0·091	0·091	0·091	0·094	0·104	0·0035	NS	***	NS	NS	*
20:2 n-6	0·024	0·025	0·021	0·023	0·024	0·034	0·0016	NS	*	NS	**	*
20:3 n-3	0·007	0·007	0·009	0·008	0·008	0·009	0·0011	NS	NS	NS	NS	NS
20:3 n-6	0·099	0·085	0·080	0·093	0·073	0·076	0·0109	NS	***	**	NS	NS
20:4 n-3	0·041	0·035	0·032	0·028	0·027	0·024	0·0028	**	NS	NS	NS	NS
20:4 n-6	0·090	0·079	0·077	0·101	0·089	0·082	0·0110	NS	*	NS	NS	NS
20:5 n-3	0·040	0·032	0·038	0·038	0·035	0·030	0·0022	NS	*	NS	NS	*
22:0	0·068	0·060	0·064	0·051	0·049	0·047	0·0058	NS	NS	NS	NS	NS
22:1 *cis*-13	0·005	0·006	0·006	0·006	0·009	0·008	0·0011	NS	*	NS	NS	NS
22:2 n-6	0·018	0·015	0·016	0·014	0·016	0·015	0·0020	NS	NS	NS	NS	NS
22:4 n-3	0·007	0·006	0·006	0·009	0·006	0·008	0·0016	NS	NS	NS	NS	NS
22:4 n-6	0·017	0·016	0·016	0·019	0·018	0·017	0·0024	NS	NS	NS	NS	NS
22:5 n-3	0·055	0·043	0·046	0·058	0·052	0·052	0·0050	NS	NS	NS	NS	NS
22:6 n-3	0·044	0·040	0·037	0·051	0·039	0·036	0·0016	NS	***	NS	*	NS
23:0	0·028	0·023	0·021	0·021	0·018	0·020	0·0041	NS	NS	NS	NS	NS
24:0	0·032	0·028	0·030	0·024	0·024	0·018	0·0038	NS	*	NS	NS	*
24:1 *cis*-15	0·006	0·004	0·006	0·005	0·006	0·006	0·0014	NS	NS	NS	NS	NS
26:0	0·019	0·017	0·015	0·015	0·014	0·015	0·0028	NS	NS	NS	NS	NS
Summation by source¶												
<16 C	21·45	20·21	19·76	24·37	22·36	21·02	0·420	*	***	NS	*	NS
16:0 and 16:1	31·53	29·42	28·79	31·98	28·60	27·92	1·014	NS	***	*	NS	NS
>16 C	47·02	50·37	51·45	43·65	49·04	51·06	1·293	NS	***	*	*	NS

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Table 4 (*Cont.*)

	HFC TMR			MFC TMR				P value†				
Item	W 1	W 2	W 3	W 1	W 2	W 3	SE	D	W^L	W^Q	D × W^L	D × W^Q
Total Saturates	64·84	61·03	60·30	65·09	60·32	57·75	0·695	NS	***	*	*	NS
Total MUFA††	30·92	33·99	34·73	29·81	33·60	36·00	0·756	NS	***	NS	NS	NS
Total PUFA‡‡	4·25	4·98	4·97	5·10	6·08	6·25	0·193	**	**	*	NS	NS

† D, W, L, Q = diet, week, linear and quadratic effects, respectively
‡ Sum of 18 : 2 FA excluding isomers of conjugated linoleic acid
§ Total conjugated linoleic acid
¶ FA <16 C originated from *de novo* synthesis, FA >16C were preformed FA taken up by the mammary gland, and 16 : 0 and 16 : 1 FA came from both *de novo* and preformed sources
†† Monounsaturated FA
‡‡ Polyunsaturated FA
*,**, *** Significant at $P<0·05$, $P<0·01$ and $P<0·001$, respectively; NS, non significant $P\geqslant 0·05$

Table 5. Milk concentration (g/100 g total FA) of 18 : 1 FA, HFC = high forage : concentrate, MFC = moderate forage : concentrate

	HFC TMR			MFC TMR				P value†				
Item	W 1	W 2	W 3	W 1	W 2	W 3	SE	D	W^L	W^Q	D × W^L	D × W^Q
cis-9	18·66	20·29	21·19	17·37	18·59	19·46	0·730	NS	**	NS	NS	NS
cis-11	1·48	1·47	1·45	1·40	1·55	1·97	0·079	*	*	NS	*	NS
cis-12	0·875	0·823	0·925	0·973	0·996	0·879	0·0850	NS	NS	NS	NS	NS
cis-13	0·051	0·052	0·061	0·047	0·053	0·056	0·0046	NS	*	NS	NS	NS
trans-4	0·032	0·033	0·042	0·027	0·035	0·036	0·0028	NS	**	NS	NS	NS
trans-5	0·026	0·025	0·032	0·019	0·027	0·024	0·0034	NS	***	NS	NS	***
trans-6+7+8	0·565	0·604	0·565	0·476	0·494	0·616	0·0562	NS	NS	NS	NS	NS
trans-9	0·683	0·797	0·753	0·592	0·696	0·774	0·0312	NS	**	NS	NS	NS
trans-10	0·93	1·06	1·13	1·08	1·49	2·79	0·216	*	***	NS	***	NS
trans-11	2·77	3·70	3·47	2·65	4·18	3·51	0·349	NS	**	***	NS	NS
trans-12	1·005	1·142	1·149	0·971	1·286	1·315	0·0489	NS	***	*	*	NS
trans-13+14	1·26	1·34	1·35	1·12	1·37	1·36	0·074	NS	*	NS	NS	NS
trans-16+*cis*-14	0·292	0·315	0·341	0·237	0·280	0·296	0·0195	NS	***	NS	NS	NS
Ratio												
trans-10/*trans*-18 : 1	0·12	0·12	0·13	0·15	0·15	0·26	0·014	*	***	***	***	**
trans-10/*trans*-11	0·34	0·29	0·33	0·41	0·36	0·84	0·074	NS	**	**	**	*

† D, W, L, Q = diet, week, linear and quadratic effects, respectively
*,**, ***Significant at $P<0·05$, $P<0·01$ and $P<0·001$, respectively; NS, non significant $P\geqslant 0·05$

Table 6. Milk concentration (g/100 g total FA) of 18 : 2 FA, HFC = high forage : concentrate, MFC = moderate forage : concentrate

	HFC TMR			MFC TMR				P value†				
Item	W 1	W 2	W 3	W 1	W 2	W 3	SE	D	W^L	W^Q	D x W^L	D x W^Q
Non conjugated												
cis-9, *cis*-12	2·01	2·17	2·28	2·80	2·92	3·50	0·133	***	*	NS	NS	NS
trans-9, *cis*-12	0·141	0·181	0·162	0·118	0·186	0·162	0·0127	NS	*	**	NS	NS
trans-11, *cis*-15	0·050	0·053	0·045	0·042	0·052	0·045	0·0067	NS	NS	*	NS	NS
Conjugated												
cis-9, *trans*-11	1·06	1·60	1·50	1·08	1·85	1·39	0·105	NS	***	***	NS	*
trans-9, *cis*-11	0·047	0·059	0·072	0·058	0·097	0·128	0·0080	**	***	NS	*	NS
trans-10, *cis*-12	0·019	0·024	0·025	0·016	0·019	0·029	0·0023	NS	**	NS	NS	NS
trans-11, *trans*-13	0·027	0·021	0·024	0·028	0·034	0·034	0·0046	NS	NS	NS	*	*
trans, *trans*‡	0·046	0·054	0·061	0·054	0·078	0·089	0·0039	*	***	NS	***	NS

† D, W, L, Q = Diet, week, linear and quadratic effects, respectively
‡ Unresolved peak of *trans*-9, *trans*-11 and *trans*-10, *trans*-12 conjugated linoleic acid
*, **, ***Significant at $P<0·05$, $P<0·01$ and $P<0·001$, respectively; NS, non significant $P\geqslant 0·05$

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lower occurrence in the rumen environment, are more abundant with concentrate feeding, hence, the increase in *trans*-10, *cis*-12 CLA with concentrate diets (Jenkins et al. 2008). Additionally, mechanisms responsible for CLA synthesis in the rumen differ based on bacteria involved in CLA formation and CLA formed (Wallace et al. 2007).

Cows receiving the MFC treatment had, on average, 43% greater MF LA concentration than the HFC treatment (main effect of diet, $P<0{\cdot}05$). Additionally, MF concentration of LA was increased (20%) over time (week effect, $P<0{\cdot}05$). The difference in LA concentration among treatments might be in response to an inhibition in ruminal lipid lipolysis by low ruminal pH (Van Nevel & Demeyer, 1996).

Results showed that cows receiving SBO infusion and the MFC diet had a greater degree of milk fat depression than cows receiving SBO infusion and the HFC diet and greater milk concentration of *trans*-FA and CLA, namely, *trans*-10 18:1 and *trans*-9, *cis*-11 CLA. The results of this study emphasized the two conditions necessary for milk fat depression, namely, the presence of PUFA and low fibre/high concentrate level in the diet.

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