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Diet-induced milk fat depression: Association with changes in milk fatty acid composition and fluidity of milk fat

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Abstract

This experiment was designed to examine changes in milk fatty acids during fish oil-induced milk fat depression (MFD) and to test the theory that these changes are related to milk fat fluidity. The experiment was divided into three periods: 1) Baseline: all cows ($n=12$) received a high fiber diet without fish oil (FO) for 12 days; 2) Treatment: 4 cows/group received the following treatments for 21 days: a) Low fiber diet without FO (LF), b) High fiber diet+FO (HF+FO) and c) Low fiber diet+FO (LF+FO); 3) Post-treatment: cows returned to the baseline diet and were monitored for 12 days. FO was included at 1.6% DM and HF and LF diets had 40 and 26% NDF, respectively. Milk fat content and yield were unchanged by the LF diet, but were reduced by FO diets at both dietary fiber levels and recovered in the post-treatment period. FO diets caused a pronounced reduction in stearic and oleic acid concentrations in milk fat and an equally pronounced increase in trans-18:1 fatty acid concentrations. Milk fat mean melting point (MMP) was correlated with MFD ($r=0.73$) and with milk oleic acid concentration ($r=-0.92$). The ratio of oleic:stearic in milk fat increased gradually and consistently in response to FO. Trans-C18:1 isomers with double bounds at carbon ≤ 10 increased with greater MFD and those with double bonds at carbon ≥ 11 decreased with greater MFD. Trans-9 cis-11 CLA explained more than 80% of MFD and was strongly correlated with trans-10 C18:1. Maintenance of MMP below 39–40 °C suggests that the mammary gland was able to secrete only milk fat with adequate fluidity and that MFD could be an adaptation mechanism to prevent secretion of milk with higher MMP.

Keywords: Fish oil; Milk fat depression; Trans-fatty acids; Conjugated linoleic acids; Milk fat fluidity

1. Introduction

When milk-fat depression (MFD) is induced by feeding low-fiber diets to dairy cows, concentration of trans-10 C18:1 increases in milk fat, indicating a shift in rumen biohydrogenation intermediates (Griinari et al., 1998). In such conditions, increased concentration of trans-10

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Table 1
Ingredients (% DM) and chemical composition of high fiber (HF) and low fiber (LF) diets with or without fish oil (FO)

	Diet			
	HF	LF	HF+FO	LF+FO
Ingredients				
Corn silage	70.1	35.2	70.1	35.1
Ground corn	6.4	41.6	3.8	40.2
Soybean meal	21.0	20.5	21.7	20.4
Dicalcium Phosphate	0.1	–	0.1	–
Limestone	1.0	1.3	1.0	1.3
Salt	0.5	0.5	0.5	0.5
Vitamin–mineral premix [a]	1.0	1.0	1.0	1.0
Fish oil [b]	–	–	1.6	1.6
Chemical composition				
NDF (%)	39.1	26.4	40.2	25.3
ADF (%)	21.4	14.0	22.5	14.3
Lignin (%)	3.43	3.27	3.86	2.95
CP (%)	16.1	17.0	16.5	16.6
Lipid (%)	2.58	2.77	3.81	3.97
Ca (%)	0.89	0.75	0.83	0.89
P (%)	0.35	0.41	0.36	0.38
NE_L (Mcal/kg DM) [c]	1.62	1.84	1.65	1.90

[a] Composition per kg (Ca: 230 g, P: 90 g, S: 15 g, Mg: 20 g, Na: 48 g, Co: 100 mg, Cu: 700 mg, Fe: 2000 mg, I: 80 mg, Mn: 1250 mg, Se: 20 mg, Zn: 2700 mg, Vit.A: 200000 IU, Vit.D3: 60000 IU, Vit.E: 60 IU).
[b] Salmon oil.
[c] Estimated by CNCPS (4.1).

C18:1 is associated with increased concentration of trans-10 cis-12 conjugated linoleic acid (CLA) in milk fat (Piperova et al., 2000). This CLA isomer was identified as a potent inhibitor of milk fat synthesis (Baumgard et al., 2000) that decreases activity of several lipogenic enzymes in the mammary gland (Baumgard et al., 2002). When MFD is induced by fish oil (FO), however, although increased concentration of trans-10 C18:1 is a common observation (Offer et al., 1999; Offer et al., 2001; Loor et al., 2005a), only a small or even no increase in milk fat trans-10 cis-12 CLA has been observed in some studies (Whitlock et al., 2002; Loor et al., 2005a). Low concentrations of trans-10 cis-12 CLA in milk fat are consistent with lower amounts of this CLA isomer leaving the rumen of cows fed FO (Shingfield et al., 2003).

FO contains the long-chain polyunsaturated fatty acids eicosapentaenoic acid (EPA; C20:5 *n*-3) and docosahexaenoic (DHA; C22:6 *n*-3), which are beneficial to human health (Williams, 2000). However, transfer efficiency of these compounds to milk is generally low due to extensive rumen biohydrogenation (Chilliard et al., 2001). Although MFD has been observed in studies involving post-ruminal infusion of FO, more pronounced MFD occurs when FO is included in the diet (Loor et al., 2005a), which suggests that EPA and DHA alter rumen biohydrogenation, resulting in compounds other than trans-10 cis-12 CLA that inhibit milk fat secretion.

Increased percentages of several C18:1 isomers (including most trans-C18:1 isomers and certain cis-C18:1 isomers) have been observed in milk fat from cows with FO-induced MFD (Loor et al., 2005a), some of which have also been associated with MFD induced by high-concentrate diets supplemented with plant oil (Loor et al., 2005b). Shingfield et al. (2005) provided the first

Table 2
Fatty acid profiles of fish oil (FO) and experimental diets

		Diet			
	FO [a]	HF	LF	HF+FO	LF+FO
Fatty acid	(g/100 g total fatty acids)				
C14:0	6.80	0.68	nd	2.66	2.05
C14:1 c9	nd	nd	nd	nd	nd
C16:0	22.4	20.9	19.8	21.40	20.58
C16:1 c9	9.20	0.31	0.27	3.18	2.97
C18:0	5.62	3.09	2.56	3.91	3.48
C18:1 c9	31.0	25.8	28.9	27.49	29.53
C18:2 c9c12	2.49	42.8	45.2	29.80	32.28
C18:3 c9c12c15	nd	5.64	2.74	3.82	1.91
C20:0	1.00	0.34	0.30	0.55	0.51
C20:1 c11	1.35	0.35	0.26	0.67	0.59
C20:4	0.57	nd	nd	0.18	0.17
C20:5	7.48	nd	nd	2.42	2.26
C22:0	nd	nd	nd	nd	1.80
C22:6	12.1	nd	nd	3.91	3.66

nd = not detectable (<0.05 g/100 g).
[a] Salmon oil.

Table 3
Dry matter intake (DMI), milk production and milk composition of cows in response to LF(1), HF+FO(2) and LF+FO(3) dietary treatments

Variable	Treatments			SE	Contrast[a]		
	LF	HF+FO	LF+FO		1×2	1×3	2×3
DMI (kg/day)	19.8	13.3	11.4	1.41	**	**	NS
Milk production (kg/day)	19.7	15.4	17.8	1.19	NS	NS	NS
Milk fat (%)	3.86	2.85	2.62	0.24	*	**	NS
Milk fat yield (kg/day)	0.73	0.45	0.44	0.06	**	**	NS
Milk protein (%)	3.32	3.13	3.12	0.12	NS	NS	NS
Milk protein yield (kg/day)	0.66	0.49	0.53	0.05	*	NS	NS
Milk lactose (%)	4.76	4.85	4.93	0.07	NS	NS	NS
Milk lactose yield (kg/day)	0.95	0.77	0.84	0.06	NS	NS	NS
Milk N–urea (mg/dL)	16.2	17.9	15.0	0.76	NS	NS	NS

HF = high fiber; LF = low fiber; FO = fish oil.
NS = not significant ($P>0.05$) *$P<0.05$ **$P<0.01$.
[a] Significance of differences between treatment means.

positive identification of trans-9, cis-11 CLA in milk fat of MFD cows and subsequently reported an inverse association with milk fat content (Shingfield et al., 2006). Changes in milk fatty acids associated with FO-induced MFD have been addressed in studies where cows were fed diets with varying concentrate:forage ratios. Therefore, the influence of an altered rumen environment on FO-induced MFD is not as well-established as for MFD induced by more traditional diets (Griinari et al., 1998).

Besides changes in putative inhibitors of milk fat synthesis, MFD diets have been associated with a lack of endogenously synthesized oleic acid for triglyceride formation (Loor and Herbein, 2003). The role of endogenously formed oleic acid as a possible regulatory step in triglyceride synthesis and maintenance of milk fat fluidity is well-established (Kinsella, 1972; Parodi, 1979). Substitution of trans-18:1 isomers for oleic acid could increase melting point of milk fat and further inhibit milk fat secretion (Chilliard et al., 2000). Loor et al. (2005a) suggested that a combined high trans-C18:1, along with reduced availability of stearic acid, could inhibit milk fat secretion in cows fed FO due to inability of the mammary gland to maintain a suitable milk fat fluidity. However, this theory has not been evaluated directly.

The objectives of the current work were: 1) To examine the role of putative milk fat inhibitors in FO-induced MFD; 2) To test the theory of reduced milk fat fluidity as a factor contributing to FO-induced MFD. These objectives were achieved by evaluating temporal variations in milk fatty acids in relation to MFD and milk fat fluidity using mean melting point of milk fatty acids as a proxy.

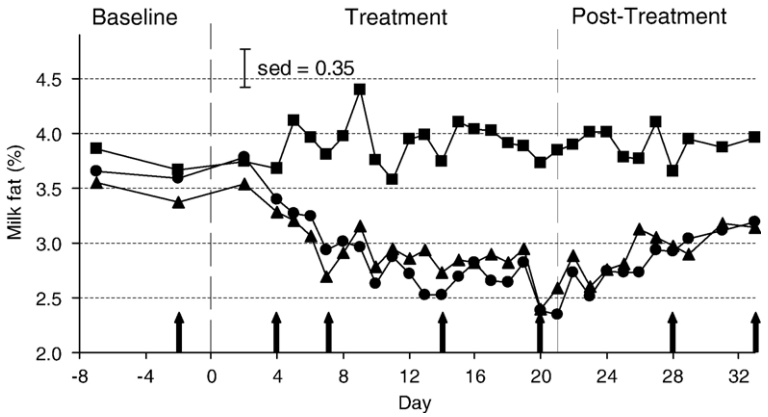

Fig. 1. Milk fat content for cows fed a high fiber diet during baseline (days −7 to −1) and post-treatment (days 22 to 33) periods, and low fiber (■), low fiber plus fish oil (●), or high fiber plus fish oil (▲) diets, during a treatment period (days 1 to 21). Arrows indicate days when milk samples were collected for fatty acid analysis. Error bar is SED for comparing diet means for any day within treatment period (diet $P<0.05$; day $P<0.001$; diet × day $P<0.05$).

2. Materials and methods

2.1. Animals, treatments and experimental periods

The experiment was conducted in a research center of the University of São Paulo (USP), located at Pirassununga City, São Paulo State, Brazil. Twelve multiparous Holstein cows in mid lactation (150±30 days) were housed in a pen of 250 m^2.

The experiment was divided into three periods: 1) Baseline: all cows (n=12) received a high fiber diet without FO (Baseline diet) for 12 days; 2) Treatment: 4 cows/group received the following diets for 21 days: a low fiber diet without FO (LF), a low fiber diet with FO (LF+FO), a high fiber diet with FO (HF+FO), and 3) Post-treatment: cows returned to the baseline diet and were evaluated for 12 days. A continuous treatment design was chosen instead of a crossover design because fish oil alters the rumen environment and biohydrogenation to an extent that there can be a significant carry over (Shingfield et al., 2003). The transition from baseline diet to low fiber diets (LF and LF+FO) was done gradually by replacing 25, 50, 75 and 100% of the baseline diet during the first 4 days of the treatment period.

FO (Salmon oil provided by Nutron Alimentos Ltda) was included at 1.6% of diet DM to allow an intake of approximately 200 g/day. Inclusions of FO between 1 and 2% of diet DM had been shown to induce MFD previously (Donovan et al., 2000; Whitlock et al., 2002). Corn silage represented approximately 70% of diet DM in HF and 35% in LF diets (Table 1). Diets were fed twice daily as total mixed rations (TMR) after morning and afternoon milkings.

2.2. Sample collection

Milk samples were collected to determine fat, protein, lactose, urea–N and fatty acid profile. In the baseline period, samples were collected 7 and 2 days before the beginning of the treatment period to provide baseline values for all variables. In the treatment and post-treatment periods, milk samples were collected daily for components and on days −2, 4, 7, 14, 20, 28 and 33 for fatty acid profile.

Diet samples were collected weekly to determine chemical composition (Table 1) and fatty acid profile (Table 2). A sample of FO was analyzed for acidity (3.23 mg NaOH/g), peroxide index (38 mEq/kg fat) and fatty acid profile (Table 2).

2.3. Dry matter intake (DMI) and milk production

Individual DMI was recorded daily using electronically controlled gates (Calan Gates). The baseline period was initiated after adaptation to this system. Milk production was

Table 4
Milk fatty acid profiles (g/100 g of total fatty acids) of cows in response to LF(1), HF+FO(2) and LF+FO(3) dietary treatments

Fatty acid	Treatment diet			SE	Contrast[a]		
	LF	HF+FO	LF+FO		1×2	1×3	2×3
C4:0 to C10:0	9.60	9.15	8.60	0.34	NS	NS	NS
C12:0 to C16:1	48.8	56.0	48.8	1.30	**	NS	**
C18:0	7.88	2.03	2.49	0.83	**	**	NS
C18:1 c9	22.3	8.87	10.8	1.82	**	**	NS
C18:1 c11	0.63	1.45	1.75	0.16	**	**	NS
C18:1 c12	0.41	0.15	0.15	0.05	*	*	NS
C18:1 c13	0.13	0.23	0.18	0.02	*	NS	NS
C18:1 t4	0.01	0.04	0.04	0.01	NS	NS	NS
C18:1 t5	0.02	0.05	0.06	0.01	NS	*	NS
C18:1 t6/8	0.37	0.43	0.77	0.12	NS	NS	NS
C18:1 t6/9	0.37	0.90	1.02	0.09	**	**	NS
C18:1 t10	0.65	2.57	3.76	0.62	NS	NS	NS
C18:1 t11	1.92	8.60	11.4	1.50	**	**	NS
C18:1 t12	0.54	1.05	0.96	0.09	**	*	NS
C18:1 t13/14	0.20	0.44	0.52	0.05	*	**	NS
C18:1 t15	0.28	0.38	0.33	0.03	NS	NS	NS
C18:1 c14/t16	0.38	0.22	0.19	0.03	**	**	NS
C18:2 c9c12	3.08	1.62	2.24	0.20	**	**	*
C18:2 t9c12	0.21	0.27	0.24	0.03	NS	NS	NS
C18:3 c9c12c15	0.19	0.17	0.20	0.01	NS	NS	NS
CLA c9t11	0.67	2.74	3.21	0.38	**	**	NS
CLA t9c11	0.014	0.034	0.050	0.008	NS	NS	NS
CLA t10c12	0.002	0.014	0.016	0.002	*	**	NS
C20:5 n-3 (EPA)	0.05	0.30	0.27	0.04	**	**	NS
C22:6 n-3 (DHA)	0.11	0.56	0.54	0.07	*	**	NS

HF = high fiber; LF = low fiber; FO = fish oil.
NS = not significant (P>0.05) *P<0.05 **P<0.01.
[a] Significance of differences between treatment means.

recorded daily and means in the baseline period were used to block cows before allocation to treatment diets.

2.4. Analytical procedures

Milk components (fat, protein and lactose) were determined by near-infrared reflectance spectroscopy (Bentley2000, Bentley Instruments). Milk urea–N (MUN) was analyzed by colorimetry using a commercial kit (Sigma Diagnostics, St. Louis, MO). Milk fat was extracted using the method of Hara and Radin (1978) and fatty acid methyl esters were prepared by base-catalyzed transmethylation according to Christie (1982). Fatty acid methyl esters were quantified using a gas chromatograph (model 6890 Agilent Technologies, Stockport, UK) fitted with a flame-ionization detector and equipped with a CP7421-Varian fused silica capillary column (200 m × 0.25 mm, coating select FAME, Varian Inc, Yarnton, UK). Initial oven temperature was 80 °C. Three minutes after sample injection, oven temperature was increased at 10 °C/min to 165 °C and held for 5 min. After that, oven temperature was increased at 0.3 °C/min to 195 °C (held for 2 min) and then at 2 °C/min to 240 °C (held for 10 min). Finally, oven temperature was increased at 4 °C/min to 280 °C and held for 10 min. The hydrogen carrier gas flow rate was 1.2 ml/min at a constant pressure of 33.47 psi; injector temperature was 240 °C and detector temperature was 250 °C. A split ratio of 50:1 was used. Detector hydrogen flow was 40 ml/min; airflow was 450 ml/min and the flow of nitrogen make-up gas was 45 ml/min. A butter oil reference standard (CRM 164; Commission of the European Community Bureau of References, Brussels, Belgium) was used to determine recoveries and correction factors for individual fatty acids. Individual FA peaks were identified by comparing their retention time to those observed in CRM164 and pure CLA isomers, which had previously been characterized in our lab by GC-MS and comparison with published elution orders. Diet lipids were extracted with hexane for 2 h at 85 °C using Soxhlet apparatus. Transmethylation was acid-catalyzed (sulfuric acid 10%) according to AOCS (1991) and fatty acid methyl esters were identified using a gas chromatograph (Varian 3400) fitted with a flame-ionization detector and equipped with a LM-100 capillary column (60 m × 0.25 mm × 1 μm). Hydrogen was used as carrier

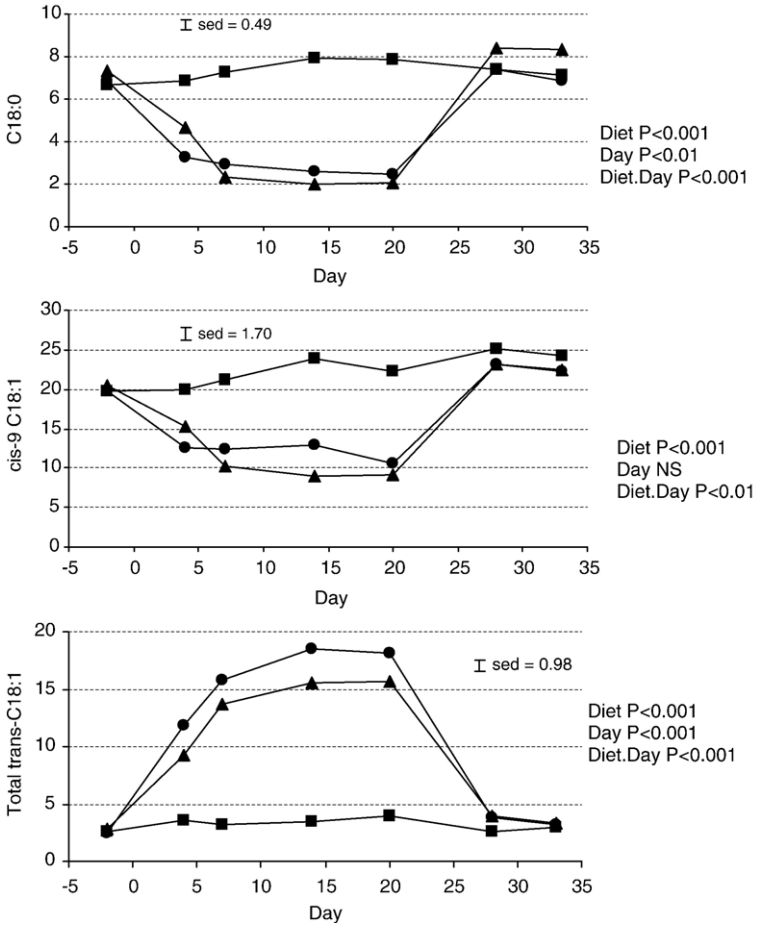

Fig. 2. Major C18 fatty acids in milk from cows fed a high fiber diet during baseline (Day −2) and post-treatment (Days 28 and 33) periods, and low fiber (■), low fiber plus fish oil (●), or high fiber plus fish oil (▲) diets, during a treatment period (Days 4 to 20). Error bars show SED for comparing diet means for any day within treatment period.

Table 5
Indices of desaturase activity and MMP for cows in LF(1), HF+FO(2), and LF+FO(3) dietary treatment groups

	Treatment diet			SE	Contrast [a]		
	LF	HF+FO	LF+FO		1×2	1×3	2×3
Desaturase index [b]							
C12:1 cis-9/C12:0	0.007	0.016	0.011	0.002	NS	NS	NS
C14:1 cis-9/C14:0	0.059	0.063	0.079	0.005	NS	NS	NS
C16:1 cis-9/C16:0	0.059	0.091	0.069	0.008	NS	NS	NS
C18:1 cis-9/C18:0	2.88	4.54	4.47	0.306	*	*	NS
cis-9, trans-11 CLA/trans-11 C18:1	0.39	0.31	0.30	0.023	NS	NS	NS
MMP (°C)	36.1	38.4	37.2	0.59	*	NS	NS

HF = high fiber; LF = low fiber; FO = fish oil.
NS = not significant ($P>0.05$) *$P<0.05$ **$P<0.01$.
[a] Significance of differences between treatment means.
[b] Product/precursor ratios.

gas at a flow rate of 1 ml/min; injector temperature was 220 °C and detector temperature was 240 °C. A split ratio of 20:1 was used, and oven temperature was increased from 70 °C to 230 °C at 4 °C/min. Individual fatty acids were identified by comparison of retention times to those of pure standards (Sigma Diagnostics, PA).

2.5. Calculation of milk fat depression (MFD) and melting point (MMP) of milk fat

MFD was calculated for each cow by subtracting values of milk fat content observed during the third week of the treatment period (days 14 to 21) from those observed during the baseline period. MMP was calculated by summing the products of molar fraction and melting point (Small, 1986) for the main fatty acids (molar fraction >0.1%) in milk fat, assuming additivity of melting points (Holman et al., 1991).

2.6. Statistical analysis

Treatments imposed in the treatment period (LF, LF+FO and HF+FO) were compared by the GLM procedure of SAS (SAS Institute Inc., 2001). Average values of milk components and milk production for the third week of the treatment period (days 14 to 21), when MFD had stabilized, were used as responses and data from the baseline period were used for covariate adjustment. For milk fatty acids and MMP data, values at day 20 of the treatment period were compared using values at day −2 for covariance adjustment.

Temporal changes in milk fat content and fatty acid concentrations during the treatment period were examined with repeated measurements analysis using the residual maximum likelihood (REML) method of Genstat 10 (Lawes Agricultural Trust, Rothamsted, UK). A mixed model was specified, with Diet, Time and their interaction as fixed effects, and with Cow and data from the baseline period as random effects.

Regression analyses were performed using the Guided Data Analysis (GDA) procedure of SAS. Pearson correlation coefficients were generated for associations among fatty acids, MMP and MFD. Only data from the third week of treatment (average of days 14 and 20) were used in the data analysis to eliminate confounding effects between treatment and time.

3. Results

Supplementation with FO significantly ($P<0.05$) reduced milk fat content (Table 3). The reduction occurred over the first two weeks of treatment ($P<0.05$ from day 5

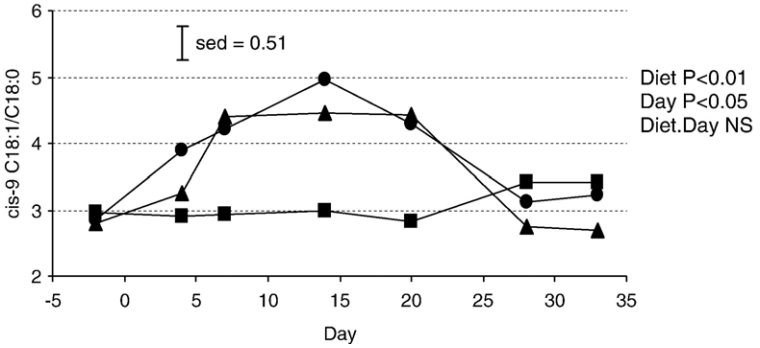

Fig. 3. Temporal variation in milk fat oleic acid:stearic acid ratio from cows fed a high fiber diet during baseline (day −2) and post-supplementation (days 28 and 33) periods, and low fiber (■), low fiber plus fish oil (●), or high fiber plus fish oil (▲) diets, during a supplementation period (days 4 to 20). Error bar is SED for comparing diet means for any day within treatment period.

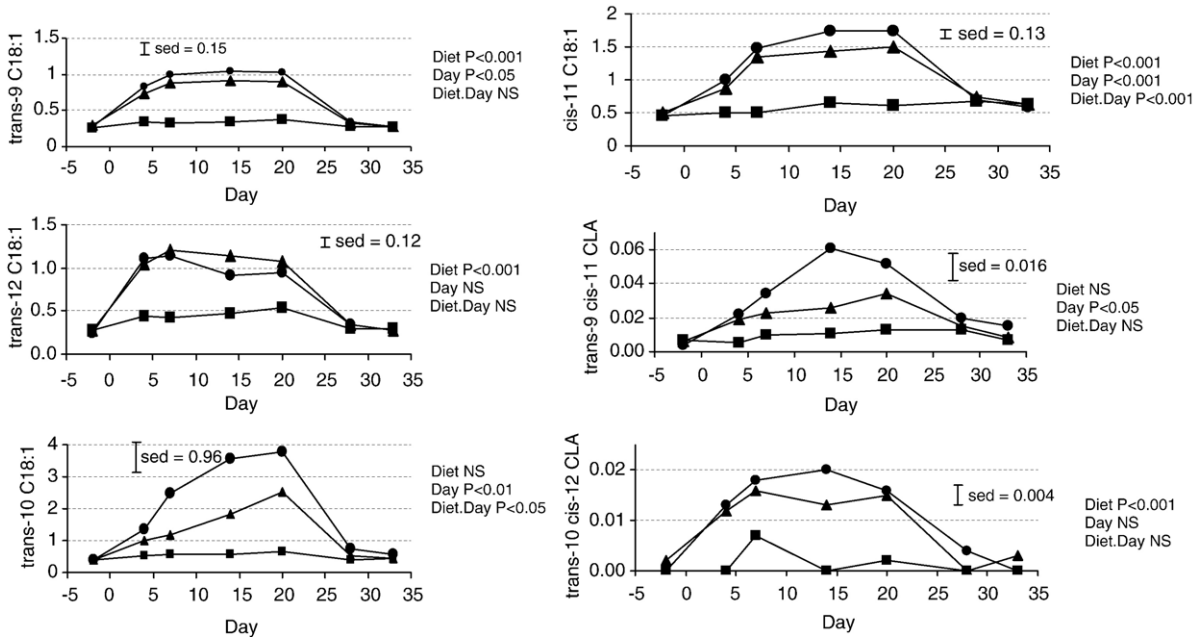

Fig. 4. Trans-9 C18:1, trans-12 C18:1, trans-10 C18:1, cis-11 C18:1, trans-9 cis-11 CLA and trans-10 cis-12 CLA fatty acids (g/100 g) in milk from cows fed a high fiber diet during baseline (day −2) and post-treatment (days 28 and 33) periods, and low fiber (■), low fiber plus fish oil (●), or high fiber plus fish oil (▲) diets, during a treatment period (days 4 to 20). Error bars show SED for comparing diet means for any day within treatment period.

onwards), after which milk fat content remained constant (Fig. 1). Reductions in milk fat content induced by FO were 20% for HF+FO (Baseline 3.5% fat, Treatment 2.8% fat; SED 0.16%) and 28% for LF+FO (Baseline 3.6% fat, Treatment 2.6% fat; SED 0.31%). Diet LF did not alter milk fat content (Baseline 3.8% fat, Treatment 3.9% fat; SED 0.30%).

In week three of the treatment period, cows on diets HF+FO and LF+FO had lower DMI, milk fat content and milk fat yield, and cows on diet HF+FO had lower milk protein yield, than cows on diet LF, but there was no difference between HF+FO and LF+FO treatments for any production variable (Table 3). There was no difference between diets in concentration of short-chain fatty acids (C4:0 to C10:0), but diet HF+FO resulted in higher concentrations of medium-chain fatty acids (C12:0 to C16:1) than LF and LF+FO diets (Table 4).

Compared with diet LF, FO supplementation reduced concentrations of stearic acid by ≈70% and oleic acid by ≈60% (Table 4) and also reduced concentrations of cis-12 C18:1, cis-14/trans-16 C18:1 and cis-9 cis-12 C18:2 (Table 4). In contrast, FO supplementation increased concentrations of most trans-C18:1 fatty acids and CLA isomers, with increases ranging from ≈80% for trans-12 C18:1 to ≈420% for trans-11 C18:1, and also increased concentrations of cis-11 C18:1 by ≈150%, cis-9 trans-11 CLA by ≈350% and trans-10 cis-12 CLA by ≈650% (Table 4). Temporal variations in stearic acid and oleic acid were inversely associated with changes in total trans-C18:1 and differences between FO supplemented diets and LF were significant throughout the treatment period (Fig. 2). The ratio of oleic acid to stearic acid increased about 50% over the first two weeks of treatment for both FO diets (Table 5

Table 6
Milk fatty acids secretion (g/day) in response to LF(1), HF+FO(2), and LF+FO(3) dietary treatments

Fatty acids	Treatment diet			SE	Contrast[a]		
	LF	HF+FO	LF+FO		1x2	1x3	2x3
Short chain (C4–C10)	71	42	37	6.6	**	**	NS
Medium chain (C12–C16)	357	256	213	32.9	**	**	NS
Long chain (>18C)	294	145	180	23.6	**	**	NS

HF = high fiber; LF = low fiber; FO = fish oil.
NS = not significant ($P>0.05$) *$P<0.05$ **$P<0.01$.
[a] Significance of differences between treatment means.

and Fig. 3). Compared with LF, the oleic acid to stearic acid ratio was significantly ($P<0.05$) elevated from day 4 for LF+FO and from day 7 for HF+FO (Fig. 3).

Temporal variations in specific milk fatty acids (Fig. 4) followed patterns similar to those observed for milk fat content (Fig. 1). For HF+FO and LF+FO, concentrations of trans-10 C18:1 and trans-9 cis-11 CLA increased over the first two weeks of the treatment period and differences from LF were significant only for LF+FO from day 14 onwards. In contrast, increases in trans-9 C18:1, trans-12 C18:1, cis-11 C18:1 and trans-10 cis-12 CLA were more immediate, with significant differences from LF and 60 to 90% of maximum levels being reached after 4 days of FO supplementation. Total daily secretion of short, medium and long-chain fatty acids was lower for HF+FO and LF+FO than for LF (Table 6).

Correlations of selected C18 fatty acids, measured in the third week of FO treatment, with MFD and MMP are presented in Table 7. All trans-C18:1 fatty acids except trans-13 C18:1 were significantly correlated with MFD. Negative associations (increased fatty acid concentration with greater MFD) were observed for fatty acids

Table 7
Pearson correlation coefficients for relationships between concentrations of C18 milk fatty acids (g/100 g of fatty acids), milk fat depression (MFD) and mean melting point (MMP) of milk fat from cows fed FO (mean data from samples taken in the third week of treatment)

	MFD	MMP
MMP	0.73*	
C18:0	−0.70*	−0.83**
C18:1 c9	−0.82**	−0.92***
C18:1 c11	−0.60	−0.85**
C18:1 c12	0.75*	0.36
C18:1 c13	0.64	0.51
C18:1 t6–8	−0.90**	−0.71*
C18:1 t9	−0.82**	−0.76*
C18:1 t10	−0.91***	−0.74*
C18:1 t11	0.68*	0.19
C18:1 t12	0.87**	0.57
C18:1 t13/14	−0.06	−0.15
C18:1 t15	0.83*	0.59
C18:1 t16	0.74*	0.62
C18:2 c9c12	−0.78*	−0.92***
CLA c9t11	0.61	0.32
CLA t9c11	−0.91***	−0.75*
CLA t7c9	−0.87**	−0.64*
CLA t10c12	−0.66	−0.59
C18:3 c9c12c15	−0.90**	−0.83**
Totals		
C18:1 t≤10	−0.90***	−0.74*
C18:1 t≤11	0.73*	0.25
C18:1 t6–16	0.08	−0.54
CLA	0.49	0.17

*$P<0.05$, **$P<0.01$, ***$P<0.001$.

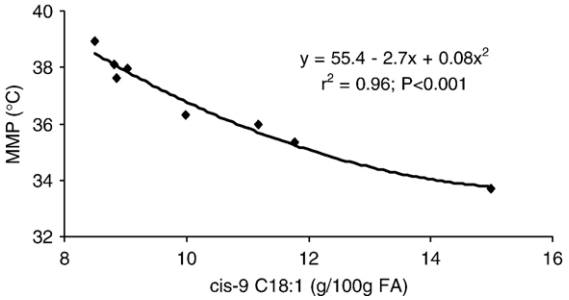

Fig. 5. Relationship between concentration of oleic acid and mean melting point (MMP) of milk fat from cows fed FO (mean data from third week of FO treatment).

with trans-double bonds at carbon 10 or less and positive associations for fatty acids with trans-double bonds at carbon 11 or more. Trans-9 cis-11 CLA was closely and inversely correlated with MFD but trans-10 cis-12 had a weaker and not significant association with MFD. Cis-11 C18:1 and oleic acid were negatively related to MFD; cis-12 C18:1 and cis-13 C18:1 were positively related to MFD. Stearic acid was inversely related to MFD and closely associated with oleic acid ($r=0.76$, $P<0.001$). MMP was higher for HF+FO than for LF (Table 5) and inversely related to milk oleic acid concentration in cows fed FO (Fig. 5). MFD was positively related to MMP (Table 7).

A positive association between trans-10 C18:1 and trans-10 cis-12 was observed ($r=0.56$, $P<0.05$), but the strongest correlation was between trans-10 C18:1 and trans-9 cis-11 CLA ($r=0.97$, $P<0.001$). This CLA isomer was strongly associated also with trans-6/8 C18:1 ($r=0.67$, $P<0.01$) and trans-9 C18:1 ($r=0.64$, $P<0.01$), but not with cis-11 C18:1 ($r=0.47$, $P>0.05$).

4. Discussion

4.1. Diet effects on milk production and composition

Reductions in milk fat by FO are within the range observed in other studies involving FO supplementation up to 450 g/day (Chilliard et al., 2001; Donovan et al., 2000; Whitlock et al., 2002). The LF diet did not result in MFD, although others have observed MFD when high concentrate diets comparable to the LF diet have been fed (Griinari et al., 1998; Loor et al., 2005b). In the present study, corn silage was the only forage source in the LF diet (35% of diet DM) and NDF content was 26%, which is close to the minimum level of fiber considered to support proper rumen function (NRC, 2001). Although ruminal pH was not measured in the present study, feces showed a loose consistency in response to LF diets, which

is a recognized signal of fiber deficiency. Therefore, we assume that the LF diet in the current study had the potential to alter rumen biohydrogenation to result in formation of milk fat inhibitors. The LF diet contained no added plant oil, however, so precursors of milk fat inhibitors (PUFA) might have been too low for MFD to be expressed, as demonstrated in the study by Griinari et al. (1998).

Addition of FO to the LF diet resulted in MFD comparable to the HF+FO diet. Thus the PUFA x fiber-level interaction effect on milk fat yield observed in studies with plant oil as a PUFA source (Griinari et al., 1998; Loor et al., 2005b) does not seem to hold true with FO. Pronounced MFD in cows fed HF+FO diet is consistent with results from studies in which FO has induced MFD in diets with adequate fiber levels (Chilliard et al., 1999; Offer et al., 1999).

Reductions in DM intake by FO treatments could have been due to changes in palatability, fiber digestibility in the rumen, or metabolic signals of satiety with decreased milk energy output (due to MFD). In studies where forage and concentrates have been fed separately and fish oil has been included in the concentrate portion of the diet, reduction in intake was due primarily to reductions in forage intake (Shingfield et al., 2003).

4.2. Diet effects on milk fatty acids

Total daily secretion of fatty acids in short, medium and long chain fatty acid classes was reduced by both FO diets, indicating that inhibitory effects of FO on milk fat secretion involved all milk fatty acid classes.

The higher concentration of medium chain fatty acids for HF+FO probably reflects differences in fatty acid supply because estimated palmitic acid intake for HF+FO was 22% greater than for LF and 34% greater than for LF+FO (data not shown). This also explains the slight differences between HF+FO and LF+FO in yields of medium and long chain fatty acids.

Within the long-chain fatty acid class, FO diets resulted in a switch from unsaturated and cis-fatty acids to trans-fatty acids so that trans-C18:1 to cis-C18:1 ratio was about 7-fold higher in milk from cows fed FO diets. As a percentage of total C18 fatty acids, trans-C18 fatty acids contributed 14% for LF and 55% for FO treatments. The lower concentration of total cis-C18:1 in milk from cows fed FO was largely attributable to an extensive drop in milk oleic acid concentration. Concentration of cis-11 C18:1 increased in cows fed FO, however, as observed in other studies (Offer et al., 1999; Donovan et al., 2000; Whitlock et al., 2002).

Increased total trans-C18:1 and reduced C18:0 in milk fat from cows fed FO in the present study suggests inhibition of the last biohydrogenation step in the rumen, which is consistent with several studies where FO was fed (Wonsil et al., 1994; Donovan et al., 2000; Whitlock et al., 2002; Shingfield et al., 2002). Concentrations of stearic acid in milk fat from cows fed FO in the present study were among the lowest found in the literature. This might have resulted from low C18 PUFA content of diets. In cows fed diets with 2% FO content and a similarly low C18 PUFA content, Whitlock et al. (2002) found only 3% stearic acid in milk fat. In contrast, they found approximately 6% stearic acid in milk fat when FO was added to a diet containing extruded whole soybean (a source of linoleic acid).

Temporal data in the present study reveal that concentrations of both stearic acid and oleic acid declined over the first seven days of FO treatment and remained low until the end of the treatment period. The initial responses are of similar magnitude and timescale to temporal changes reported by Shingfield et al. (2006) for cows supplemented with FO. In marked contrast to the present study, however, Shingfield et al. (2006) found that low concentrations of stearic and oleic acids were transitory; both started to increase after approximately 10-days FO supplementation and approached control concentrations after 18–24 days, even though MFD persisted until the end of treatment at 28 days. The major difference between studies is that the treatment diet of Shingfield et al. (2006) included 3% sunflower oil in addition to 1.5% FO. This further supports the suggestion that stearic and oleic acid concentrations were limited by low C18 PUFA content of FO diets in the present study.

Of all the milk trans-C18:1 isomers, the greatest increase in response to FO supplementation occurred for trans-11 C18:1 (vaccenic acid) which represented more than 60% of total trans-C18:1. Significant increases were observed also for trans-9 C18:1, trans-10 C18:1, trans-12 C18:1 and trans-13/14 C18:1. In general, temporal variation in trans-fatty acids mirrored changes in stearic acid and oleic acid. Again, changes were maintained throughout the treatment period, in contrast to the transitory changes observed by Shingfield et al. (2006). There were, however, distinct differences in the temporal increases among the trans-C18:1 isomers: trans-9 C18:1 and trans-12 C18:1 concentrations increased immediately following introduction of FO diets whereas concentrations of trans-10 C18:1, cis-11 C18:1, trans-10 cis-12 CLA, and trans-9 cis-11 CLA took longer to plateau, suggesting changes in rumen microbial populations.

4.3. MFD and associated changes in putative milk fat inhibitors

Concentration of milk trans-10 cis-12 CLA increased gradually in response to FO, but MFD was greater than would be predicted based on trans-10 cis-12 CLA concentration in milk fat from cows fed classical MFD-inducing diets (Piperova et al., 2000; Peterson et al., 2002). Moreover, some studies have observed FO-induced MFD without any increase in milk trans-10 cis-12 content (Chilliard et al., 1999; Loor et al., 2005a). This suggests that FO-induced MFD involves other inhibitors or mechanisms and could be related to the more global change in biohydrogenation observed in the current study. An inhibitory effect of trans-C18:1 isomers on milk fat synthesis has been proposed (Wonsil et al., 1994). Elevated concentration of milk trans-10 C18:1 is a common observation in MFD induced by either FO (Offer et al., 1999; Chilliard et al., 1999, 2001; Loor et al., 2005a) or low fiber diets (Griinari et al., 1998; Piperova et al., 2000). In the present study, concentration of trans-10 C18:1 in milk fat was closely correlated with MFD in cows fed FO. Although a raised concentration of milk trans-10 C18:1 is a consistent observation in all types of diet-induced MFD, a recent study found no MFD with a short-term (4 days) abomasal infusion of trans-10 C18:1 (Lock et al., 2007). Similarly, short-term (3 or 4-days) abomasal infusion of trans-9 C18:1 (Rindsig and Schultz, 1974) or a mixture of trans-11 C18:1 plus trans-12 C18:1 (Griinari et al., 2000) found no effect of these trans-C18:1 isomers on milk fat synthesis. This suggests that either close associations between certain milk fatty acids and MFD observed in several studies might not indicate cause–effect relationships, or that longer-term exposure of the mammary gland to trans-fatty acids, at higher concentrations than achieved in these infusion studies, is necessary to establish significant inhibition of milk fat synthesis.

The patterns of gradual increase in trans-10 C18:1, trans-10 cis-12 CLA and trans-9 cis-11 CLA were similar to those observed by Shingfield et al. (2006). However, the reduction in milk fat yield was more pronounced and occurred during four weeks of FO supplementation in Shingfield et al. (2006), whereas it remained constant, at a reduced level, after the second week of treatment in the present study. Compared with the present study, concentrations of trans-10 C18:1, trans-9 cis-11 CLA and trans-10 cis-12 at day 21 were considerably higher in Shingfield et al. (2006), with trans-10 cis-12 CLA concentrations reaching values seen during abomasal infusion of pure trans-10 cis-12 CLA (Baumgard et al., 2000). This suggests that formation of these and other putative milk fat inhibitors was gradually induced, resulting in reduced milk fat secretion that continued to decline beyond the second week of supplementation.

In contrast to trans-10 cis-12 CLA, trans-9 cis-11 CLA was strongly correlated with FO-induced MFD in the present study. The close association observed between trans-9 cis-11 CLA and trans-9 C18:1 suggests that trans-9 C18:1 in milk fat might be formed in the rumen from biohydrogenation of trans-9 cis-11 CLA. In addition, trans-9 C18:1 could be formed from oleic acid isomerization (Mosley et al., 2002) which is consistent with the correlation between these fatty acids ($r=0.72$, $P<0.05$). However, the lower correlation between trans-10 cis-12 CLA and trans-10 C18:1 ($r=0.56$, $P<0.05$) suggests that formation of trans-10 C18:1 in the rumen when FO is fed may not involve trans-10 cis-12 CLA as an intermediate. Interestingly, we observed a close association between trans-9 cis-11 CLA and trans-10 C18:1 ($r=0.97$, $P<0.001$). The reason for such a strong relationship is not clear: the possibility that these two biohydrogenation intermediates have a common precursor warrants further investigation.

Overall, the data suggest that milk fatty acids other than trans-10 cis-12 CLA might contribute to MFD induced by FO diets. As found in several studies, some trans-C18:1 and CLA isomers appear more closely related to MFD than others. However, clear distinction regarding inhibitory effects cannot be drawn between individual trans-18:1 isomers. The strong correlation with MFD in this study suggests that trans-9 cis-11 CLA is the most likely candidate for a milk fat inhibitor when FO is fed, which is supported by a recent infusion study (Perfield et al., 2007).

4.4. Diet effects on melting point of milk fat

The melting point of milk fat is important because milk fat must be in liquid form when secreted. Timmen and Patton (1989) pointed out that the necessity for liquidity of the milk fat globule requires that most fatty acids be esterified to triglycerides in combinations that have a melting point at or below 39 °C, the body temperature of the cow. This selectivity of esterification indicates that esterification is directed to produce the required triglycerides regardless of changes in dietary fatty acids. The quantities of triglycerides will change, but not their structure (Jensen, 2002). Because they have relatively low melting points, C4:0 to C10:0 are thought to be used alternately with oleic acid at position sn-3 as the main regulators of milk fluidity during the final step of triglyceride synthesis (Timmen and Patton, 1989).

Oleic acid is converted from stearic acid by stearoyl-CoA desaturase (SCD) to maintain milk fluidity (Jensen,

2002). Thus, inhibition of SCD might affect milk fat secretion by reducing supply of oleic acid for triglyceride synthesis. For example, Bickerstaffe and Johnson (1972) observed a 29% decrease in milk fat concentration when SCD was inhibited by a 14-day intravenous sterculic acid infusion in a lactating goat, and Corl et al. (2001) observed a 9% decrease in milk fat concentration with a 4-day abomasal infusion of sterculic oil in lactating dairy cows. In contrast, a 4-day abomasal infusion of sterculic oil inhibited SCD activity but milk fat secretion was unchanged in cows fed fresh pasture (Kay et al., 2004), suggesting that the effect is diet-dependant.

In cows fed FO diets, increased concentrations of trans-C18:1 combined with reduced availability of stearic acid for oleic acid synthesis through SCD might reduce the ability of the mammary gland to maintain a desirable milk fat fluidity. This theory was proposed by Loor et al. (2005a,b) as a possible explanation for high-concentrate diet plus plant oil and FO-induced MFD. Because trans-isomers of fatty acids always have a higher melting point than equivalent cis-isomers, the shift from oleic acid to trans-C18:1 in response to FO would be expected to decrease milk fat fluidity. This effect can be observed in Fig. 5 ($r^2 = 0.96$, $P < 0.001$) by using MMP as a proxy for fluidity.

The oleic:stearic acid ratio increased by 56% in response to FO treatments, which is consistent with other studies, where increases in the oleic:stearic acid ratio with FO supplementation have ranged from 23% (Loor et al., 2005b) to 65% (Ahnadi et al., 2002). A smaller increase (17%) in response to FO treatments was observed in the ratio of myristoleic:myristic, which is usually considered the best indicator of mammary SCD activity because both fatty acids are synthesized only within the mammary gland. Other studies have observed a higher increase (+27%; Whitlock et al., 2002) or a reduction (−13%; Loor et al., 2005a) in the myristoleic:myristic ratio. Furthermore, Ahnadi et al. (2002) observed that dietary addition of unprotected FO had no effect on SCD mRNA abundance in mammary tissue of dairy cows. In contrast to oleic:stearic, the ratio of cis-9 trans-11 CLA:trans-11 was reduced by 23% in the present study, and similar responses (−7%; Loor et al., 2005a and −18%; Whitlock et al., 2002) were observed in other studies with FO. These results indicate either an increase in specific activity of SCD for stearic acid when cows are fed FO or increased uptake of oleic acid from other tissues. Both responses may result from a physiological adjustment to the increased supply of trans-C18:1 fatty acids and decreased supply of stearic acid for mammary oleic acid synthesis.

For combined FO treatments, there was a close association between milk oleic acid concentration and MMP (Fig. 5). This association suggests that the mammary gland is able to work within a wide range of oleic acid concentrations above 8% in order to maintain MMP below 39–40 °C, which is close to the physiological limit proposed by Timmen and Patton (1989). It seems reasonable to expect that milk fat globules with MMP higher than 39–40 °C could not be secreted. The shortage of stearic acid for oleic acid synthesis could, therefore, impair the ability of the mammary gland to maintain adequate milk fat fluidity in milk fat globules, resulting in MFD.

Reduced milk fat synthesis maybe represents a physiological condition where milk fat secretion can continue, but at a lower rate. In the study of Shingfield et al. (2006), the initial (days 5 to 7) decrease in concentrations of stearic and oleic acids in milk fat (2.2 and 8%, respectively, comparable to reductions in the current study) was followed by almost complete recovery of stearic and oleic acid concentrations by day 28. During this time, milk fat yield remained constant at an approximately 50% reduced level. Increases in stearic and oleic acid concentrations occurred in response to a pronounced decrease in proportions of C4 to C16 fatty acids. These shifts in milk fat fatty acid composition suggest further mechanisms by which the mammary gland can adapt to altered supply of milk fat precursors, albeit at reduced rate.

Although the fluidity theory is discussed here in relation to secretion of milk fat, change in MMP of milk fat precursor fatty acids could be acting at other points in the transfer of fatty acids into milk fat. Increase in MMP of fatty acids leaving the rumen, or a switch from cis- to trans-configuration, could affect digestion, absorption, transport and metabolism of fatty acids in the body, as well as mammary uptake, triglyceride synthesis and fat globule formation. It should be noted also that studies of milk fat depression/composition measure only fatty acids that have been successfully secreted; it is possible that some fatty acids that are not secreted at the same rate as the majority of fatty acids accumulate in mammary cells to inhibit synthetic processes. Thus, a clear interpretation of the relationship between MMP of secreted milk fat and MFD can be difficult.

5. Conclusions

The mechanism by which FO induces MFD seems to differ from other types of diet-induced MFD. In particular, FO supplementation was shown to induce similar MFD when associated with both high and low fiber diets and concentration of trans-10 cis-12 CLA in milk fat was poorly associated with MFD. In contrast, most trans-C18:1 isomers with a double bond at carbon 10 or less and

trans-9 cis-11 CLA demonstrate a strong inverse correlation with milk fat content. Correlation of some of these milk fatty acids with FO-induced MFD was stronger than has been predicted from trans-10 cis-12 CLA in classical diet-induced MFD (low fiber plus plant oils). Trans-9 cis-11 CLA appears to be a common inhibitor of milk fat synthesis in different types of diet-induced MFD.

Supplementation with FO induced a sustained shift from oleic and stearic acid to trans-C18:1 in milk fat, which was associated with a slight increase in MMP of secreted milk fat in spite a higher oleic:stearic ratio. In general, our results suggest that possible adaptation mechanisms used to regulate milk fat fluidity were insufficient to cope with increased trans-C18:1 supply to the mammary gland at the expense of oleic acid availability in cows fed FO, so MFD occurred. Maintenance of MMP below 39–40 °C suggests that the mammary gland was able to secrete only milk fat with adequate fluidity and that MFD could be an adaptation mechanism to prevent secretion of milk with higher MMP.

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References

Ahnadi, C.E., Beswick, N., Delbecchi, L., Kennelly, J.J., Lacasse, P., 2002. Addition of fish oil to diets for dairy cows. II. Effects on milk fat and gene expression of mammary lipogenic enzymes. J. Dairy Res. 69, 521–531.

AOCS, 1991. 5th ed. Official Methods and Recommended Practices of the American Oil Chemists Society, vol. I. AOCS, Champaign.

Baumgard, L.H., Corl, B.A., Dwyer, D.A., Saebo, A., Bauman, D.E., 2000. Identification of the conjugated linoleic acid isomer that inhibits fat synthesis. Am. J. Physiol., Regul. Integr. Comp. Physiol. 278, R179–R184.

Baumgard, L.H., Matitashvili, E., Corl, B.A., Dwyer, D.A., Bauman, D.E., 2002. Trans-10, cis-12 conjugated linoleic acid decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. J. Dairy Sci. 85, 2155–2163.

Bickerstaffe, R., Johnson, A.R., 1972. The effect of an intravenous infusion of sterculic acid on milk fat synthesis. Brit. J. Nutr. 27, 561–570.

Chilliard, Y., Chardigny, J.M., Chabrot, J., Ollier, A., Sébédio, J.L., Dureau, M., 1999. Effects of ruminal or postruminal fish oil supply on conjugated linoleic acid (CLA) content of cow milk fat. Proc. Nutr. Soc. 58, 70.

Chilliard, Y., Ferlay, A., Mansbridge, R.M., Doreau, M., 2000. Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids. Ann. Zootech. 49, 181–205.

Chilliard, Y., Ferlay, A., Doreau, M., 2001. Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. Livest. Prod. Sci. 70, 31–48.

Christie, W.W., 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesterol esters. J. Lipid Res. 23, 1072–1075.

Corl, B.A., Baumgard, L.H., Dwyer, D.A., Griinari, J.M., Phillips, B.S., Bauman, D.E., 2001. The role of delta(9)-desaturase in the production of cis-9, trans-11 CLA. J. Nutr. Biochem 12, 622–630.

Donovan, D.C., Schingoethe, D.J., Baer, R.J., Ryali, J., Hippen, A.R., Franklin, S.T., 2000. Influence of dietary fish oil on conjugated linoleic acid and other fatty acids in milk fat from lactating dairy cows. J. Dairy Sci. 83, 2620–2628.

Griinari, J.M., Dwyer, D.A., McGuire, M.A., Bauman, D.E., Palmquist, D.L., Nurmela, K.V.V., 1998. Trans-octadecenoic acids and milk fat depression in lactating dairy cows. J. Dairy Sci. 81, 1251–1261.

Griinari, J.M., Corl, B., Lacy, S., Chouinard, P., Nurmela, K., Bauman, D.E., 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by delta-9 desaturase. J. Nutr. 130, 2285–2291.

Hara, A., Radin, N.S., 1978. Lipid extraction of tissues with low-toxicity solvent. Anal. Biochem. 90, 420–426.

Holman, R.T., Johnson, S.B., Ogburn, P.L., 1991. Deficiency of essential fatty acids and membrane fluidity during pregnancy and lactation. Proc. Natl. Acad. Sci. 88, 4835–4839.

Jensen, R.G., 2002. The composition of bovine milk lipids: January 1995 to December 2000. J. Dairy Sci. 85, 295–350.

Kay, J.K., Mackle, T.R., Auldist, M.J., Thomson, N.A., Bauman, D.E., 2004. Endogenous synthesis of cis-9, trans-11 conjugated linoleic acid in dairy cows fed fresh pasture. J. Dairy Sci. 87, 369–378.

Kinsella, J.E., 1972. Stearyl CoA as a precursor of oleic acid and glycerolipids in mammary microsomes from lactating bovine: possible regulatory step in milk triglyceride synthesis. Lipids 7, 349–355.

Lock, A.L., Tyburczy, C., Dwyer, D.A., Harvatine, K.J., Destaillats, F., Mouloungui, Z., Candy, L., Bauman, D.E., 2007. Trans-10 octadecenoic acid does not reduce milk fat synthesis in dairy cows. J. Nutr. 137, 71–76.

Loor, J., Herbein, J., 2003. Reduced de novo synthesis and desaturation due to exogenous trans-10, cis-12 CLA in cows fed oleic or linoleic oil. J. Dairy Sci. 86, 1354–1369.

Loor, J.J., Doreau, M., Chardigny, J.M., Ollier, A., Sebedio, J.L., Chilliard, Y., 2005a. Effects of ruminal or duodenal supply of fish oil on milk fat secretion and profiles of trans-fatty acids and conjugated linoleic acid isomers in dairy cows fed maize silage. Anim. Feed Sci. Technol. 199, 227–246.

Loor, J.J., Fearlay, A., Ollier, A., Doreau, M., Chilliard, Y., 2005b. Relationship among trans and conjugated fatty acids and bovine milk fat yield due to dietary concentrate and linseed oil. J. Dairy Sci. 88, 726–740.

Mosley, E.E., Powell, G.L., Riley, M.B., Jenkins, T.C., 2002. Microbial biohydrogenation of oleic acid to trans isomers in vitro. J. Lipid Res. 43, 290–296.

Offer, N.W., Marsden, M., Dixon, J., Speake, B.K., Thacker, F.E., 1999. Effect of dietary fat supplements on levels of *n*-3 polyunsaturated fatty acids, trans acids and conjugated linoleic acid in bovine milk. Anim. Sci. 69, 613–625.

Offer, N.W., Marsden, M., Phipps, R.H., 2001. Effect of oil supplementation of a diet containing a high concentration of starch on levels of *trans* fatty acids and conjugated linoleic acids in bovine milk. Anim. Sci. 73, 533–540.

Parodi, P.W., 1979. Stereospecific distribution of fatty acids in bovine milk fat triglycerides. J. Dairy Res. 46, 75–81.

Perfield, J.W., Lock, A.L., Griinari, J.M., Saebo, A., Delmonte, P., Dwyer, D.A., Bauman, D.E., 2007. Trans-9, cis-11 conjugated linoleic acid reduces milk fat synthesis in lactating dairy cows. J. Dairy Sci. 90, 2211–2218.

Peterson, D.G., Baumgard, L.H., Bauman, D.E., 2002. Short communication: milk fat response to low doses of trans-10, cis-12 conjugated linoleic acid (CLA). J. Dairy Sci. 85, 1764–1766.

Piperova, L.S., Teter, B.B., Bruckental, I., Sampugna, J., Mills, S.T., Yurawecz, M.P., Fritsche, J., Ku, K., Erdman, R.A., 2000. Mammary lipogenic enzyme activity, trans fatty acids and conjugated linoleic acids are altered in lactating dairy cows fed a milk fat-depressing diet. J. Nutr. 130, 2568–2574.

Rindsig, R.B., Schultz, L.H., 1974. Effects of abomasal infusions of safflower oil or elaidic acid on blood lipids and milk fat in dairy cows. J. Dairy Sci. 57, 1459–1466.

SAS Institute, 2001. SAS/STAT Users Guide, release 8.0. SAS Inst., Inc., Cary, NC.

Shingfield, K.J., Ahvenjarvi, S., Toivonen, V., Arola, A., Huhtanen, P., Griinari, J.M., 2002. Fish oil inhibits the biohydroganation of fatty acids in the rumen causing an increase in milk trans-octadecenoic and conjugated linoleic acid content. J. Anim. Sci. 80 (Suppl.1). J. Dairy Sci. 85 (Suppl.1), 143. (Abstr.).

Shingfield, K.J., Ahvenjarvi, S., Toivonen, V., Arola, A., Nurmela, K.V.V., Huhtanen, P., Griinari, J.M., 2003. Effect of dietary fish oil on biohydrogenation of fatty acids and milk fatty acid content in cows. Anim. Sci. 77, 165–179.

Shingfield, K.J., Reynolds, C.K., Lupoli, B., Toivonen, V., Yurawecz, M.P., Delmonte, P., Griinari, J.M., Grandison, A.S., Beever, D.E., 2005. Effect of forage type and proportion of concentrate in the diet on milk fatty acid composition in cows given sunflower oil and fish oil. Anim. Sci. 80, 225–238.

Shingfield, K.J., Reynolds, C.K., Hervás, G., Griinari, J.M., Grandison, A.S., Beever, D.E., 2006. Examination of the persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. J. Dairy Sci. 89, 714–732.

Small, D.M., 1986. Thermal data for fatty acids. The physical chemistry of lipids from alkanes to phospholipids. In: Small, D.M. (Ed.), Handbook of Lipid Research, vol. 4. Plenum Press, New York.

Timmen, H., Patton, S., 1989. Milk fat globules: fatty acid composition, size, and in vivo regulation of fat liquidity. Lipids 26, 685–689.

Whitlock, L.A., Schingoethe, D.J., Hippen, A.R., Kalscheur, R.J., Baer, R.J., Ramaswamy, N., Kasperson, K.M., 2002. Fish oil and extruded soybeans fed in combination increase conjugated linoleic acids in milk of dairy cows more than when fed separately. J. Dairy Sci. 85, 234–243.

Williams, C.M., 2000. Dietary fatty acids and human health. Ann. Zootech. 49, 165–180.

Wonsil, B.J., Herbein, J.H., Watkins, B.A., 1994. Dietary and ruminally derived trans-18:1 fatty acids alter bovine milk lipids. J. Nutr. 124, 556–565.