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Milk Yield, Composition, and Fatty Acid Profile in Dairy Cows Fed a High-concentrate Diet Blended with Oil Mixtures Rich in Polyunsaturated Fatty Acids

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ABSTRACT: To evaluate the effects of feeding linseed oil or/and sunflower oil mixed with fish oil on milk yield, milk composition and fatty acid (FA) profiles of dairy cows fed a high-concentrate diet, 24 crossbred primiparous lactating dairy cows in early lactation were assigned to a completely randomized design experiment. All cows were fed a high-concentrate basal diet and 0.38 kg dry matter (DM) molasses per day. Treatments were composed of a basal diet without oil supplement (Control), or diets of (DM basis) 3% linseed and fish oils (1:1, w/w, LSO-FO), or 3% sunflower and fish oils (1:1, w/w, SFO-FO), or 3% mixture (1:1:1, w/w) of linseed, sunflower, and fish oils (MIX-O). The animals fed SFO-FO had a 13.12% decrease in total dry matter intake compared with the control diet ($p<0.05$). No significant change was detected for milk yield; however, the animals fed the diet supplemented with SFO-FO showed a depressed milk fat yield and concentration by 35.42% and 27.20%, respectively, compared to those fed the control diet ($p<0.05$). Milk *c*9, *t*11-conjugated linoleic acid (CLA) proportion increased by 198.11% in the LSO-FO group relative to the control group ($p<0.01$). Milk C18:3*n*-3 (ALA) proportion was enhanced by 227.27% supplementing with LSO-FO relative to the control group ($p<0.01$). The proportions of milk docosahexaenoic acid (DHA) were significantly increased ($p<0.01$) in the cows fed LSO-FO (0.38%) and MIX-O (0.23%) compared to the control group (0.01%). Dietary inclusion of LSO-FO mainly increased milk *c*9, *t*11-CLA, ALA, DHA, and *n*-3 polyunsaturated fatty acids (PUFA), whereas feeding MIX-O improved preformed FA and unsaturated fatty acids (UFA). While the lowest *n*-6/*n*-3 ratio was found in the LSO-FO, the decreased atherogenecity index (AI) and thrombogenicity index (TI) seemed to be more extent in the MIX-O. Therefore, to maximize milk *c*9, *t*11-CLA, ALA, DHA, and *n*-3 PUFA and to minimize milk *n*-6/*n*-3 ratio, AI and TI, an ideal supplement would appear to be either LSO-FO or MIX-O. (**Key Words:** Linseed Oil, Sunflower Oil, Fish Oil, Milk Yield, Milk Fatty Acids, Dairy Cows)

INTRODUCTION

The inclusion of saturated fats in human diets may bring the risk of cardiovascular diseases (Joyce et al., 2009). However, for milk processors, milk with high saturated fatty acids (SFA) may improve the keeping quality but may be detrimental to human health. The negative effects of SFA seem to outweigh their positive functions in milk, so more research is needed to reduce the amount of SFA in milk (Nantapo et al., 2014). It has been showed that consumption of dietary *n*-3 fatty acid (FA), mainly alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is useful for human health, especially cardio- and cerebro-vascular diseases (Siegel and Ermilov, 2012), and conjugated linoleic acid (CLA) from ruminant fat has been reported to exert anti-carcinogenic benefits in various experimental animal models and human cancer cells (Gebauer et al., 2011; Grądzka, 2013). Dairy cows' diets supplemented with linseed oil rich in ALA has been shown to increase milk polyunsaturated fatty acids (PUFA), especially ALA and *c*9,*t*11-CLA proportions (Mach et al., 2012). AbuGhazaleh (2008) reported that sunflower oil and fish oil inclusion in dairy cattle diet led to increase milk *c*9,*t*11-CLA and vaccenic acid proportions.

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Nevertheless, ruminal biohydrogenation (BH), which transforms unsaturated fatty acids (UFA) into SFA, is a major concern due to SFA is detrimental for human health. The BH process has long been known to occur in the rumen as the result of microbial metabolic activity (Kellens et al., 1986). Thus, if ruminal BH of UFA to further form stearic acid is controlled to become an incomplete process, it may be possible to improve the healthiness of ruminant meats and milk by increasing their UFA composition, particularly CLA and *n*-3 FA (Côrtes et al., 2010). Inclusion of fish oil containing EPA and DHA has been shown to inhibit the complete BH of C18 UFA, resulting in an increase of *trans*-18:1 isomers (Chow et al., 2004; Wąsowska et al., 2006) which are vailable for further synthesis of CLA isomers, particularly *c*9,*t*11-CLA, in animal adipose tissues (Silva et al., 2014). Consequently, the EPA and DHA supplement mixed with linoleic acid (LA, precursor of CLA isomers) and ALA sources may improve EPA, DHA, CLA isomers, and ALA in animals' products.

Hence, feeding fish oil in combination with linseed oil or/and sunflower oil to dairy cattle diet may increase milk beneficial FA as concerned above. However, inclusion of oil high in PUFA in the ruminant diets could induce adverse effects on feed intake resulting in decrease milk yield and milk fat yield (Chilliard et al., 2009). For this reason, feeding proper oil mixture rich in PUFA in dairy cows should be studied so that it enhances milk beneficial PUFA for human health without any effect on animal performance. The objectives of this study were to measure the effects of feeding different oil mixtures rich in PUFA on milk yield, milk composition, and FA profiles of dairy cows fed a high-concentrate diet. The hypothesis of this experiment was that dairy cows' diet supplemented with oil compounds comprising high *n*-3 PUFA could improve *n*-3 PUFA and CLA isomers in cows' milk.

MATERIALS AND METHODS

Animals, experimental design, and diets

All experimental procedures were conducted following the Ethical Principles and Guidelines for the Use of Animals issued by National Research Council of Thailand. Twenty four primiparous Holstein Friesian lactating dairy cows in early lactation averaging 26.67±9.20 days in milk, 12.25±0.57 kg of milk and 347.21±30.80 kg body weight, housed in individual tie stalls and offered daily rations as equal meals at 06:00 and 17:00 h. The animals had free access to water and mineral block, and they had enough space to walk. The animals were assigned to a completely randomized design with six replicates per each treatment. The experiment lasted for 6 weeks consisting a former 2-week for adjustment, followed by a latter 4-week for sample collection. The cows were fed a high-concentrate basal diet and 0.38 kg dry matter (DM) molasses per day. Treatments were composed of the basal diet without oil supplement (Control), or diets of (DM basis) 3% linseed and fish oils (1:1, w/w, LSO-FO), or 3% sunflower and fish oils (1:1, w/w, SFO-FO), or 3% mixture (1:1:1, w/w) of linseed, sunflower, and fish oils (MIX-O). Three oil compounds were daily blended as above ratios and then mixed with a 21% crude protein (CP) concentrate before feeding. The concentrate was formulated to meet the nutrient requirements of dairy cows (NRC, 2001). Corn silage was offered *ad libitum* as a main roughage source. Molasses was added as a top-dressing on corn silage.

The dietary ingredients and chemical compositions of the individual feeds and experimental diets used in the current study are presented in Tables 1 and 2. Concentrate was used as the main source of protein (21.13% CP) whereas corn silage was fed as the major source of fiber due to its high neutral detergent fiber (NDF) content (65.47%). Linseed, sunflower, and fish oils were selected as sources of supplemented oil in the diets. Table 1 shows that linseed oil was particularly great in ALA (55.82%), while sunflower oil was rich in LA (59.10%), and only fish oil was greater source of EPA and DHA (8.12% and 36.30%, respectively). Therefore, the LSO-FO mixture led to increase three main *n*-3 FA, specially ALA, EPA, and DHA contents in the diet; SFO-FO induced to improve LA, EPA, and DHA; and combination of linseed, sunflower and fish oils resulted in a perfectly potential compound rich in *n*-3 PUFA (ALA, EPA, and DHA) and *c*9,*t*11-CLA precursor, namely LA (Table 2).

Sampling, measurements, and chemical analysis

Feeds offered and the residuals were recorded daily during the collection period, and feed samples were collected for two consecutive days weekly to calculate daily feed intake. Feed samples were taken and dried at 60℃ for 48 h. At the end of the experimental period, feed and oil samples were pooled and representative samples were taken for further chemical analysis. Samples were ground through a 1-mm screen and subjected to proximate analysis. Crude protein was determined by Kjeldahl mothod (procedure 928.08, AOAC, 1998). Ether extract was determined by using petroleum ether in a Soxtec System (procedure 948.15, AOAC, 1998). Neutral detergent fiber and acid detergent fiber (ADF) were determined using the method described by Van Soest et al. (1991), adapted for Fiber Analyzer. The NDF analysis used sodium sulfite in the neutral detergent solution. Both NDF and ADF are expressed inclusive of residual ash. The net energy for lactation (NE_L; Mcal/kg DM) of feeds and oils was calculated according to the equations of NRC (2001). All chemical components were expressed on DM basis. The animals were weighed at the start and end of the experiment.

The dairy cows were milked daily at 5:00 and 16:00 h,

Table 1. Chemical composition and major fatty acid composition of individual feeds

Item	Experimental feed					
	Concentrate[1]	Corn silage	LSO	SFO	FO	Molasses
Chemical composition (% DM)						
DM	91.18	23.45	-	-	-	75.63
OM	90.84	90.97	-	-	-	88.44
CP	21.13	9.75	-	-	-	1.23
EE	3.79	1.84	100	100	100	
Ash	9.16	9.03	-	-	-	11.56
NFC[2]	24.54	13.91	-	-	-	-
NDF	41.38	65.47	-	-	-	-
ADF	28.43	41.69	-	-	-	-
Lignin (sa)	3.60	4.40	-	-	-	-
NE_L (Mcal/kg)[3]	1.79	1.35	4.39	4.39	4.39	1.88
Fatty acid composition (g/100 g FA)						
C12:0	20.16	2.98	0.01	0.03	0.07	-
C14:0	6.62	4.78	0.07	0.11	3.80	-
C16:0	14.38	33.04	5.56	6.52	22.84	-
C18:0	2.92	17.38	3.20	3.38	6.21	-
*c*9-C18:1	26.56	12.46	17.92	27.19	12.51	-
*c*9,*c*12-C18:2	23.66	20.15	16.40	59.10	1.67	-
C18:3*n*-3	2.03	0.00	55.82	1.60	0.10	-
C20:5*n*-3	0.00	0.00	0.00	0.00	8.12	-
C22:6*n*-3	0.00	0.00	0.00	0.00	36.30	-
SFA	47.51	67.38	9.06	10.28	37.52	-
UFA	52.49	32.62	90.94	89.72	62.48	-
MUFA	26.80	12.47	18.05	27.30	13.66	-
PUFA	25.69	20.15	72.89	62.42	48.82	-
n-3	2.03	0.00	56.09	1.80	44.69	-
n-6	23.66	20.15	16.80	60.62	4.13	-

LSO, linseed oil; SFO, sunflower oil; FO, fish oil; DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NFC, non fiber carbohydrate; NDF, neutral detergent fiber; ADF, acid detergent fiber; NE_L, net energy for lactation; FA, fatty acids; SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

[1] Contained (as DM basis): 32% cassava distillers dried meal, 20% soybean meal, 17.5% corn distillers dried grains with solubles, 10% rice bran, 10% wheat bran, 8% molasses, and 2.5% mineral and vitamin mix. Mineral and vitamin mix: provided per kg of concentrate including vitamin A, 5,000 IU; vitamin D_3, 2,200 IU; vitamin E, 15 IU; Ca, 8.5 g; P, 6 g; K, 9.5 g; Mg, 2.4 g; Na, 2.1 g; Cl, 3.4 g; S, 3.2 g; Co, 0.16 mg; Cu, 100 mg; I, 1.3 mg; Mn, 64 mg; Zn, 64 mg; Fe, 64 mg; Se, 0.45 mg.

[2] Calculated as NFC = 100–(CP+NDF+EE+ash).

[3] Calculated using published formulas of NRC (2001).

and milk yields were recorded at each milking. Milk from both morning and afternoon milking were sampled weekly in 2 consecutive milking days. The morning milk samples were pooled to one composite sample; afternoon samples were also pooled. The composite milk samples of both milking times were analyzed for milk composition including fat, protein, casein, lactose, solid not fat, total solid, urea, free FA, citric acid, and acidity using a MilkoScan FT2 infrared automatic analyser (Foss, Hillerød, Denmark).

To measure milk FA composition, milk samples were taken from individual cow on two consecutive days (d 41 and 42) of the experiment and stored at –20℃ until further analysis. Briefly, lipids in milk samples were extracted in a mixture of dichloromethane and methanol (2:1, v/v) following a method of Romeu-Nadal et al. (2004) with small adjustments. Lipids in feed samples were extracted in a mixture of chloroform and methanol (2:1, v/v) according to a modified method described by Folch et al. (1957). After lipid extraction, 10 mL of the extracts were transferred to new culture tubes fitted with a teflon-lined screw cap, evaporated to exact dryness under a N_2 stream, and then methylated. Approximately 30 mg of the extracted lipid were added with 1 mL of internal standard (2 mg C17:0/mL hexane) and 2 mL of boron trifluoride (BF_3) in methanol. The samples were mixed for 30 s by a vortexer, and then methylated with 1.5 mL of NaOH in methanol (0.5 M) at 90℃ for 30 min. The samples were then cooled down at room temperature, and 10 mL of deionized water were added. The top-layer solution was transferred to a 40 mL

Table 2. Calculated chemical composition and major fatty acid composition of dietary treatments

Item	Experimental treatment[1]			
	Control	LSO-FO	SFO-FO	MIX-O
Ingredient composition (% DM)				
Concentrate	55.07	54.00	53.92	54.25
Corn silage	41.51	39.30	39.17	39.08
Linseed oil	-	1.47	-	0.98
Sunflower oil	-	-	1.48	0.98
Fish oil	-	1.47	1.48	0.98
Molasses	3.42	3.76	3.94	3.72
Chemical composition (% DM)				
DM	62.53	61.30	61.33	61.44
OM	90.81	88.13	88.10	88.12
CP	15.73	15.29	15.26	15.32
EE	2.85	5.71	5.73	5.72
Ash	9.19	8.93	8.93	8.93
NFC	19.29	18.72	18.68	18.75
NDF	49.96	48.08	47.96	48.03
ADF	32.96	31.74	31.66	31.72
Lignin (sa)	3.81	3.67	3.66	3.67
NE_L (Mcal/kg)	1.61	1.70	1.70	1.70
Fatty acid composition (g/100 g FA)				
C12:0	15.56	7.62	7.59	7.63
C14:0	6.13	3.97	3.97	3.66
C16:0	19.38	16.65	16.88	15.31
C18:0	6.79	5.67	5.71	5.43
*c*9-C18:1	22.78	18.93	21.31	21.00
*c*9,*c*12-C18:2	22.72	15.69	26.70	24.28
C18:3*n*-3	1.49	15.12	1.16	10.61
C20:5*n*-3	0.00	2.09	2.10	1.49
C22:6*n*-3	0.00	9.35	9.40	6.23
SFA	52.83	37.56	37.78	35.29
UFA	47.17	62.44	62.22	64.71
MUFA	22.96	19.35	21.73	21.33
PUFA	24.21	43.09	40.49	43.38
n-3	1.49	26.67	12.76	18.35
n-6	22.72	16.42	27.73	25.03

DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NFC, non fiber carbohydrate; NDF, neutral detergent fiber; ADF, acid detergent fiber; NE_L, net energy for lactation; FA, fatty acid; SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

[1] Control, basal diet without oil supplement; LSO-FO, 3% linseed and fish oils at 1:1; SFO-FO, 3% sunflower and fish oils at 1:1; MIX-O, 3% mixture of linseed, sunflower, and fish oils at 1:1:1.

centrifuged tube, and 5 mL of hexane were added for extraction of FA methyl esters. The mixture was centrifuged at 2,000 g, at 10℃, for 20 min, and the hexane layer was then dried over Na_2SO_4. Finally, 1 mL of sample at top-layer was transferred into vial for analyzing FA by gas chromatography (Hewlett-Packard 7890A series, Agilent Technology, Palo Alto, CA, USA) equipped with a 100 m× 0.25 mm×0.2 μm film fused silica capillary column (SP1233, Supelco Inc, Bellefonte, PA, USA) and a flame ionization detector. Injector and detector temperatures were 250℃. The column temperature was kept at 70℃ for 4 min, then increased at 13℃/min to 175℃ and held for 27 min, then increased at 4℃/min to 215℃ and held for 17 min, then increased at 4℃/min to 240℃ and held for 10 min. Fatty acids were identified by comparison of retention times with external FAME standards (Food Industry 37 FAME mix, 35077 Restek Co., Bellefonte, PA, USA). The CLA mixture (Sigma-Aldrich, Louis, MO, USA) contained *c*9,*t*11-18:2, *t*10,*c*12-18:2, *c*9,*c*11-18:2, and *t*9,*t*11-18:2.

Atherogenecity index (AI) and thrombogenicity index (TI) were calculated using equations proposed by Ulbricht and Southgate (1991):

AI = [C12:0+4(C14:0)+C16:0]
/[monounsaturated fatty acids (MUFA)+PUFA]

TI = (C14:0+C16:0+C18:0)
/[(MUFA+*n*-6 PUFA)/2+3(*n*-3 PUFA)
+(*n*-3 PUFA/*n*-6 PUFA)]

Dietary and intake of FA were calculated from individual experimental feeds as follow:

Dietary FA
= sum of {% DM of individual feed in the diet
×[individual feed lipid content (g/100 g DM)/100]
×[% of FA (g/100 g total FA in feed)/100]}
/dietary lipid content

FA intake
= sum of {individual feed intake (g DM)
×[individual feed lipid content (g/100 g DM)/100]
×[% of FA (g/100 g total FA in feed)/100]}
(Stergiadis et al., 2014)

Statistical analysis

Data on feed intakes, milk yield and milk composition were analyzed according to a completely randomized design with the repeated measures (weeks) using PROC MIXED procedure of SAS (SAS, 2002). The statistical model used was as follows:

$$Y_{ijk} = \mu + A_i + T_j + W_k + (T \times W)_{jk} + \varepsilon_{ijk},$$

where Y_{ijk} = the dependent variable, μ = the overall mean, A_i = the random effect of animal, T_j = the fixed effect of treatment, W_k = the fixed effect of week, $(T \times W)_{jk}$ = the fixed effect of interaction between treatment and week, and ε_{ijk} = the random residual error.

Data of live weight and milk FA were analyzed by ANOVA procedure of SAS (SAS, 2002) for a completely

randomized design with the following statistical model:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij},$$

where Y_{ij} = the dependent variable, μ = the overall mean, T_i = the treatment effect, and ε_{ij} = the random residual error.

Significant differences among treatment means were assessed by Tukey's multiple comparison tests after a significant F-test. Overall differences between treatment means were considered to be significant at $p<0.05$. Data are expressed as mean±standard error of the mean (SEM), which represents the pooled SEM for the model.

RESULTS

Intakes of main components and major fatty acids

Supplementation of SFO-FO did result in a 13.12% decrease of total dry matter intake (DMI) compared with the control diet ($p<0.05$) (Table 3). As the result of DMI reduction, CP intake was decreased by supplementing oil mixtures ($p<0.01$), the lowest value was in the animals fed the SFO-FO diet (1.48 kg/d) versus the greatest one in those fed the control diet (1.76 kg/d). The SFO-FO-supplemented group led to increased intake of *c*9,*c*12-C18:2 ($p<0.01$) while intake of C18:3*n*-3 was remarkably improved in the animals fed LSO-FO ($p<0.01$) compared to the control

Table 3. Intakes of main components and major fatty acids

Item	Treatment[1]				SEM	p-value
	Control	LSO-FO	SFO-FO	MIX-O		
Live weight (LW, kg)	350.67	337.17	338.25	341.25	34.64	0.90
Intake of main components						
DM (kg/d)						
Total	11.05^{a}	10.06^{ab}	9.60^{b}	10.15^{ab}	1.10	0.02
Concentrate	6.09^{a}	5.43^{b}	5.18^{b}	5.51^{b}	0.38	0.01
Corn silage	4.59	3.96	3.76	3.97	1.00	0.11
Molasses	0.38	0.38	0.38	0.38	-	-
Added oil	0.00^{b}	0.30^{a}	0.28^{a}	0.30^{a}	0.02	0.01
Added oil/DMI (%)	0.00^{b}	2.95^{a}	3.00^{a}	2.97^{a}	0.27	0.01
C[2] (%)	57.74	58.12	58.59	58.63	6.20	0.97
R[2] (%)	42.26	41.88	41.41	41.37	6.20	0.97
DMI/LW (g/kg)	31.84	30.09	28.79	29.87	4.50	0.58
CP (kg/d)	1.76^{a}	1.56^{b}	1.48^{b}	1.57^{b}	0.13	0.01
NE_L (Mcal/d)	17.80	17.08	16.30	17.24	1.84	0.23
Intake of FA (g/d)						
C12:0	49.03^{a}	43.81^{b}	41.75^{b}	44.38^{b}	3.00	0.01
C14:0	19.31^{b}	22.84^{a}	21.86^{a}	21.28^{a}	1.52	0.01
C16:0	61.07^{b}	95.66^{a}	92.89^{a}	89.00^{a}	7.78	0.01
C18:0	21.41^{b}	32.58^{a}	31.41^{a}	31.55^{a}	3.56	0.01
*c*9-C18:1	71.79^{c}	108.77^{b}	117.27^{ab}	122.05^{a}	7.44	0.01
*c*9,*c*12-C18:2	71.59^{c}	90.12^{b}	146.92^{a}	141.13^{a}	8.69	0.01
C18:3*n*-3	4.68^{c}	86.87^{a}	6.40^{c}	61.64^{b}	4.50	0.01
C20:5*n*-3	0.00^{c}	12.01^{a}	11.57^{a}	8.10^{b}	0.68	0.01
C22:6*n*-3	0.00^{c}	53.68^{a}	51.71^{a}	36.28^{b}	3.05	0.01
SFA	166.49^{b}	215.77^{a}	207.90^{a}	205.14^{a}	16.70	0.01
UFA	148.63^{c}	358.72^{ab}	342.34^{b}	376.06^{a}	23.39	0.01
MUFA	72.36^{c}	111.17^{b}	119.54^{ab}	123.95^{a}	7.56	0.01
PUFA	76.28^{c}	247.55^{a}	222.79^{b}	252.11^{a}	16.03	0.01
n-3	4.68^{d}	153.21^{a}	70.21^{c}	106.61^{b}	8.10	0.01
n-6	71.59^{c}	94.35^{b}	152.59^{a}	145.50^{a}	8.95	0.01
Total FA	315.12^{b}	574.49^{a}	550.23^{a}	581.20^{a}	38.33	0.01

SEM, standard error of the mean; DM, dry matter; DMI, dry matter intake; CP, crude protein; NE_L, net energy for lactation; FA, fatty acids; SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

[1] Control, basal diet without oil supplement; LSO-FO, 3% linseed and fish oils at 1:1; SFO-FO, 3% sunflower and fish oils at 1:1; MIX-O, 3% mixture of linseed, sunflower, and fish oils at 1:1:1.

[2] C, concentrate and R, roughage were calculated as percent to total concentrate and corn silage intakes.

[a-d] Means within a row with different superscripts are significantly different ($p<0.05$).

group. Oil inclusion in the basal diet led to increase intake of total FA as compared with the control diet ($p<0.01$).

Milk yield and composition

In contrast to DM and CP intakes, no significant changes ($p>0.05$) were detected for milk yield (Table 4). However, the animals fed diet supplemented with SFO-FO induced to depress milk fat yield and concentration by 35.42% and 27.20%, respectively, compared to those fed the control diet ($p<0.05$). Further analysis across week of experiment showed that added oils had effect on milk fat percentage after 2-week feeding ($p<0.01$). The lowest milk fat concentration was observed in the SFO-FO group at 4-week supplementation ($p<0.05$, Figure 1). While milk contents of protein, casein, lactose and solid not fat remained unchanged among the treatments ($p>0.05$), reduced milk fat content reflected to decrease milk total solid content in the animals supplemented with SFO-FO, compared to the control group ($p<0.05$). Similar trend to milk fat depression (MFD), milk total FA was decreased in the SFO-FO group (2.62%) compared to 3.60% in the control group ($p<0.05$). The supplementing of oils in the cattle diets had no effect on milk urea concentration; however, added oils significantly increased milk citric acid concentration ($p<0.05$), accompanied by decreasing milk acidity in the SFO-FO group related to the control group ($p<0.05$). Feed efficiency was not affected by feeding oil mixtures in the current study ($p>0.05$).

Milk fatty acid composition

Milk FA profiles were strongly modified by oil supplementation (Table 5). The proportion of total C18:1*n*-9 in the milk fat were remarkably enhanced ($p<0.01$) to 29.85%, 31.00%, and 33.67% corresponding to the feeding

Table 4. Milk yield and composition

Variable	Treatment[1]				SEM	p-value
	Control	LSO-FO	SFO-FO	MIX-O		
Yield						
Milk (kg/d)	12.41	11.29	11.02	12.02	1.21	0.13
3.5% FCM[2] (kg/d)	13.14^{a}	11.26^{ab}	9.77^{b}	11.30^{ab}	2.27	0.02
ECM[3] (Mcal/d)	12.89^{a}	11.11^{ab}	9.92^{b}	11.29^{ab}	1.90	0.01
Fat (kg/d)	0.48^{a}	0.39^{ab}	0.31^{b}	0.38^{ab}	0.13	0.01
Protein (kg/d)	0.35^{a}	0.31^{ab}	0.31^{b}	0.33^{ab}	0.03	0.03
Casein (kg/d)	0.26^{a}	0.22^{b}	0.23^{b}	0.24^{ab}	0.03	0.02
Lactose (kg/d)	0.53	0.49	0.48	0.51	0.06	0.34
Solid-not-fat (kg/d)	1.02	0.91	0.91	0.98	0.10	0.07
Total solid (kg/d)	1.48^{a}	1.27^{b}	1.21^{b}	1.36^{ab}	0.16	0.01
Composition						
Fat (%)	3.86^{a}	3.52^{ab}	2.81^{b}	3.12^{ab}	1.03	0.04
Protein (%)	2.82	2.73	2.79	2.75	0.14	0.45
Casein (%)	2.12	1.96	2.07	1.99	0.26	0.39
Lactose (%)	4.29	4.31	4.33	4.24	0.21	0.72
Solid-not-fat (%)	8.22	8.06	8.27	8.15	0.34	0.32
Total solid (%)	11.95^{a}	11.34^{ab}	10.98^{b}	11.33^{ab}	0.99	0.04
Urea N (mg/dL)	18.56	16.41	15.98	16.29	3.39	0.34
FFA (mekv/L)	0.71	0.78	0.75	0.86	0.39	0.78
Citric acid (%)	0.17^{b}	0.19^{ab}	0.21^{ab}	0.22^{a}	0.04	0.04
Acidity (°TH)	17.79^{a}	15.67^{ab}	14.91^{b}	14.97^{ab}	2.46	0.04
Fatty acid (%)	3.60^{a}	3.28^{ab}	2.62^{b}	2.91^{ab}	0.57	0.04
Feed efficiency						
Milk/DMI (kg/kg)	1.14	1.13	1.16	1.20	0.17	0.81
3.5% FCM/DMI (kg/kg)	1.21	1.23	1.03	1.12	0.24	0.37
ECM/DMI (kg/kg)	1.18	1.11	1.04	1.12	0.21	0.48
ECM/NE_Lintake (kg/Mcal)	0.74	0.65	0.61	0.66	0.12	0.18

SEM, standard error of the mean; FCM, fat-corrected milk; ECM, energy corrected milk; N, nitrogen; FFA, free fatty acids; mekv, milliequivalents; °TH, Thorner degrees; DMI, dry matter intake; NE_L, net energy for lactation.

[1] Control, basal diet without oil supplement; LSO-FO, 3% linseed and fish oils at 1:1; SFO-FO, 3% sunflower and fish oils at 1:1; MIX-O, 3% mixture of linseed, sunflower, and fish oils at 1:1:1.

[2] 3.5% FCM = [0.432×milk (kg)]+[16.216×fat (kg)] (Dairy Records Management Systems, 2014).

[3] ECM = [0.327×milk (kg/d)]+[12.86×fat (kg/d)]+[7.65×protein (kg/d)] (Peterson et al., 2012).

[a-b] Means within a row with different superscripts are significantly different ($p<0.05$).

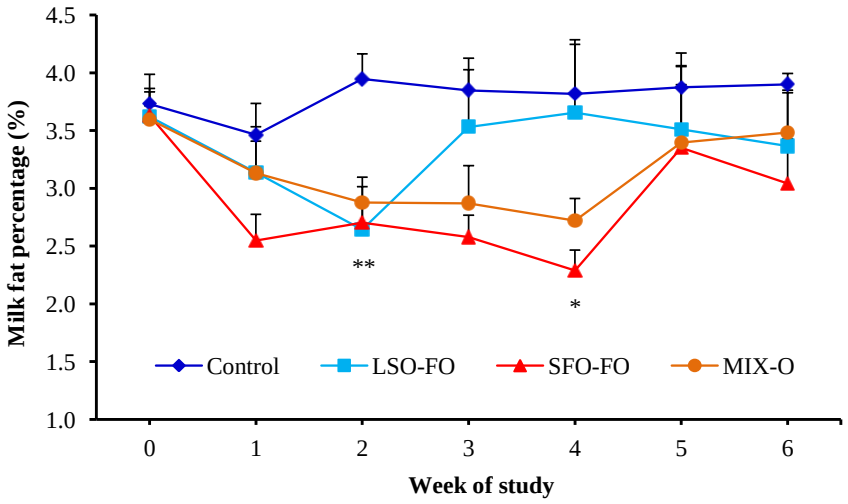

Figure 1. Average milk fat percentage changes of treatment groups during entire experiment. * p<0.05; ** p<0.01. Diets were statistical significance (p = 0.03), but no significant differences were detected for week and treatment×week interaction (p>0.05).

of LSO-FO, SFO-FO, and MIX-O while in the control group it was only 21.60%. Milk *c*9,*t*11-CLA increased by 198.11% in the LSO-FO group relative to the control group (p<0.01). In the current study, milk proportion of *t*10,*c*12-CLA was increased (p<0.01) because of oil supplementation rich in PUFA, regardless of different oil mixtures. Milk C18:3*n*-3 proportion was enhanced 227.27% by supplementing with LSO-FO relative to the control group (p<0.01). It was found in the current study that the proportion of DHA was significantly increased (p<0.01) in the cows fed the LSO-FO (0.38%) and MIX-O (0.23%) compared with the control group (0.01%), but the SFO-FO supplementation didn't have any significant additive effect. Oil supplementation led to significantly reduced proportions of short- and medium chain FA (<16 carbons) in the milk fat compared to the control diet (p<0.01), the extent of the decrease was greatest for the animals fed the MIX-O diet. In contrast, preformed FA (>16 carbons) proportion in the milk fat was increased in the animals fed MIX-O compared with the control animals and even LSO-FO group (p<0.01). Feeding oil mixtures improved C18 UFA proportion in the milk fat (43.84% to 57.00%) relative to the control (p<0.01), the greatest increase was observed for MIX-O group. The oil inclusion in the diet led to significantly reduce SFA, AI, and TI while increased UFA including MUFA and PUFA compared to the control diet (p<0.01). The greater increase of the total *n*-3 FA (p<0.01) led to decrease the ratio of *n*-6 to *n*-3 FA (p<0.01) in the milk fat of the animals fed diets added oils (2.28% to 3.46%) compared to the control animals (11.63%). Further calculation of yield of some selected FA in milk fat showed that milk yields of *c*9,*t*11-CLA, C18:3*n*-3, EPA+DHA, *n*-3 PUFA were greater in the LSO-FO group than those in the control group (p<0.05). In contrast, the transfer of C18:2*n*-6, C18:3*n*-3 and PUFA from feed to milk fat was higher in the control group compared to the other groups (p<0.01).

DISCUSSION

Intakes

The decreased total DMI in the current study was in agreement with some previous studies (Martin et al., 2008; Chilliard et al., 2009). However, the lesser effect as total lipid concentration in the diet higher than 6% DM was also documented (Huang et al., 2008; Angulo et al., 2012; Benchaar et al., 2012). Diets supplemented with oil sources rich in unprotected PUFA often causes a decrease in DM intake, and the mechanisms of this effect are attributed to effects on ruminal fermentation, gut motility, palatability of added fat diets, release of gut hormones, and oxidation of fat in the liver (Allen, 2000). In this study, to compare with LSO-FO and MIX-O, SFO-FO supplementation might highly relate to some causes concerning above which led to be lower DMI.

Milk yield and composition

Oil supplementation at 3% had no effect on milk yield. This result was supported by Angulo et al. (2012) and Vafa et al. (2012). A similar result was also recently published by Neveu et al. (2014). The decreased milk fat percentage and yield in the animals fed SFO-FO was consistent with some previous studies in bovines (Murphy et al., 2008; Angulo et al., 2012). This effect is known as MFD and often occurs in dairy cows fed oil/fats rich PUFA (Shingfield et al., 2006; Huang et al., 2008). The MFD is related to an alteration of ruminal BH resulting from the production of different ruminal intermediates that have a negative effect on the gene expression of lipogenic enzymes (Bauman and Griinari, 2001). In general, oils with a high degree of

Table 5. Milk fatty acid composition

Fatty acid (g/100 g FA)	Treatment[1]				SEM	p-value
	Control	LSO-FO	SFO-FO	MIX-O		
Individual FA						
C4:0	2.41^{a}	2.10^{ab}	1.69^{b}	1.74^{b}	0.33	0.01
C6:0	1.78^{a}	1.05^{b}	0.93^{bc}	0.79^{c}	0.15	0.01
C8:0	1.12^{a}	0.55^{b}	0.49^{b}	0.40^{b}	0.12	0.01
C10:0	2.44^{a}	1.25^{b}	1.11^{b}	0.88^{b}	0.33	0.01
C11:0	0.32^{a}	0.13^{b}	0.10^{bc}	0.07^{c}	0.02	0.01
C12:0	7.06^{a}	5.48^{b}	5.13^{b}	4.47^{b}	0.64	0.01
C13:0	0.25^{a}	0.17^{b}	0.15^{b}	0.12^{c}	0.02	0.01
C14:0	12.51^{a}	11.00^{b}	10.11^{bc}	9.23^{c}	0.75	0.01
C14:1	1.08	0.96	0.87	0.69	0.27	0.13
C15:0	1.02	1.02	1.05	0.97	0.07	0.27
C16:0	35.83	34.01	34.18	32.55	3.03	0.34
C16:1	2.24	2.29	2.37	2.00	0.48	0.59
C17:1	0.19	0.23	0.22	0.23	0.04	0.34
C18:0	7.73^{a}	5.06^{b}	7.07^{a}	8.02^{a}	1.10	0.01
C18:1*n*-9	21.60^{b}	29.85^{a}	31.00^{a}	33.67^{a}	2.54	0.01
*c*9-C18:1	17.91^{ab}	15.06^{b}	15.31^{b}	19.18^{a}	2.19	0.01
*t*9,*t*12-C18:2	0.19^{ab}	0.27^{a}	0.15^{b}	0.22^{ab}	0.07	0.04
*c*9,*c*12-C18:2	0.99^{b}	0.18^{ab}	1.06^{b}	1.38^{a}	0.17	0.01
*c*9,*t*11-CLA	0.53^{b}	1.58^{a}	0.74^{b}	0.94^{ab}	0.44	0.01
*t*10,*c*12-CLA	0.02^{b}	0.16^{a}	0.21^{a}	0.18^{a}	0.04	0.01
*c*9,*c*11-CLA	0.01^{b}	0.02^{b}	0.07^{a}	0.05^{a}	0.01	0.01
*t*9,*t*11-CLA	0.08^{b}	0.21^{a}	0.16^{a}	0.16^{a}	0.05	0.01
C18:3*n*-3	0.11^{c}	0.36^{a}	0.20^{b}	0.25^{b}	0.06	0.01
C20:0	0.15	0.14	0.18	0.16	0.03	0.09
C20:1	0.08^{b}	0.22^{a}	0.19^{a}	0.18^{a}	0.03	0.01
C20:2	0.03	0.07	0.04	0.06	0.04	0.21
C20:3*n*-6	0.07	0.05	0.06	0.05	0.03	0.65
C20:4*n*-6	0.09	0.13	0.12	0.13	0.04	0.33
C24:0	0.03	0.06	0.14	0.07	0.11	0.34
C20:5*n*-3	0.00	0.00	0.08	0.08	0.08	0.13
C22:6*n*-3	0.01^{c}	0.38^{a}	0.19^{bc}	0.23^{ab}	0.11	0.01
FA groups						
De novo[2]	30.00^{a}	23.72^{b}	21.51^{bc}	19.38^{c}	1.86	0.01
Mixed[3]	38.08	36.30	36.55	34.56	3.30	0.36
Preformed[4]	31.93^{c}	39.98^{b}	41.95^{ab}	46.07^{a}	3.21	0.01
C18 UFA	23.63^{b}	33.99^{a}	33.79^{a}	37.10^{a}	2.70	0.01
SFA	72.68^{a}	62.04^{b}	62.26^{b}	59.49^{b}	2.50	0.01
UFA	27.32^{b}	37.96^{a}	37.74^{a}	40.51^{a}	2.50	0.01
MUFA	25.19^{b}	33.54^{a}	34.65^{a}	36.78^{a}	2.33	0.01
PUFA	2.13^{c}	4.42^{a}	3.09^{b}	3.72^{ab}	0.58	0.01
n-6	1.34^{b}	1.63^{ab}	1.38^{b}	1.78^{a}	0.19	0.01
n-3	0.12^{b}	0.74^{a}	0.48^{a}	0.57^{a}	0.17	0.01
EPA+DHA	0.01^{b}	0.38^{a}	0.27^{a}	0.31^{a}	0.14	0.01
Indices						
UFA/SFA	0.38^{b}	0.62^{a}	0.61^{a}	0.68^{a}	0.06	0.01
MUFA/SFA	0.35^{b}	0.55^{a}	0.56^{a}	0.62^{a}	0.05	0.01
PUFA/SFA	0.03^{c}	0.07^{a}	0.05^{b}	0.06^{ab}	0.01	0.01
n-6/*n*-3	11.63^{a}	2.28^{b}	3.21^{b}	3.46^{b}	1.12	0.01
Atherogenecity index	3.42^{a}	2.24^{b}	2.10^{b}	1.83^{b}	0.31	0.01
Thrombogenicity index	4.11^{a}	2.54^{b}	2.59^{b}	2.35^{b}	0.39	0.01

FA, fatty acid; SEM, standard error of the mean; CLA, conjugated fatty acid; UFA, unsaturated fatty acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

[1] Control, basal diet without oil supplement; LSO-FO, 3% linseed and fish oils at 1:1; SFO-FO, 3% sunflower and fish oils at 1:1; MIX-O, 3% mixture of linseed, sunflower, and fish oils at 1:1:1.

[2] *De novo* FA originate from mammary *de novo* synthesis (<16 carbons).

[3] Mixed FA originate from both sources (C16:0 and C16:1).

[4] Preformed FA originate from extraction from plasma (>16 carbons).

[a-c] Means within a row with different superscripts are significantly different ($p<0.05$).

unsaturation are able to disturb ruminal fermentation and fiber digestibility, leading to lower acetate production and therefore milk fat synthesis (Coppock and Wilks, 1991). Moreover, some studies where cows were fed milk fat-depressing diets including low forage and high-oil diets show down regulation of genes involved in mammary lipid synthesis, which was associated with increased milk *t*10-18:1 and *t*10,*c*12-CLA (Ahnadi et al., 2002; Harvatine and Bauman, 2006; Angulo et al., 2012), but *c*9,*t*11-CLA does not (Baumgard et al., 2000). In the current study, therefore increase of *t*10,*c*12-CLA proportion in milk fat was found in the SFO-FO group which resulted in the strong depression of milk fat percentage and yield. The molecular mechanism mediating the inhibitory effect of CLA isomers, particularly *t*10,*c*12-CLA, on MFD is not well understood up to now. Nevertheless, Harvatine and Bauman (2006) showed that the sterol response element binding protein transcription factor system, by binding to response elements located in lipogenic enzyme genes, may be a central signaling pathway by which CLA regulates fatty acid synthesis in the mammary gland. Thyroid hormone responsive spot 14, which is down regulated during diet-induced MFD, may also be involved in the molecular mechanism of MFD, possibly as a secondary cellular signal for sterol response element binding protein 1.

Milk fatty acid composition

In the current study, increases in milk fat C18:1*n*-9, isomers of C18:1, when dairy cows were fed oil mixtures rich in C18 UFA was in agreement with Bu et al. (2007) and Huang et al. (2008). The lower proportion of C18:0 in milk fat from cows fed LSO-FO could be ascribed to an incomplete BH process in the rumen of either C18:3*n*-3 or C18:1 to C18:0, resulting in increased milk C18:3*n*-3, C18:1 isomers and CLA isomers. The combination of LSO and FO had additive effects on *c*9,*t*11-CLA and C22:6*n*-3 proportions in milk fat, but the combination of SFO and FO did not have any clear additive effect, indicating that a quantity of *t*11-C18:1, *c*9,*t*11-CLA, and C22:6*n*-3 was able to bypass ruminal BH in the LSO-FO group while this was not in the SFO-FO group. Milk C18:3*n*-3 proportion was increased to the greatest extent by feeding LSO-FO, indicates that ruminal BH of C18:3*n*-3 was diminished.

Short- and medium chain FA proportions (<16 carbons) were decreased while the long-chain FA (particularly C18 UFA) were increased in diets blended with linseed, sunflower, and fish oils compared to the control diet (Table 5). This suggested that the supplementation of oil mixtures rich in PUFA had strongly inhibited the *de novo* synthesis of FA in mammary fat tissues, because almost milk C4:0-C14:0 and about half of C16:0 are synthesized *de novo* by the mammary epithelial cells. Dietary supply of long-chain UFA has been shown to improve their secretion in milk fat and decrease the *de novo* synthesis of short- and medium-chain FA in the mammary gland (Grummer, 1991), and it is probable that the decreased part in endogenous mammary FA synthesis when UFA are fed is related to increased formation of specific BH intermediates in the rumen. The

Table 6. Milk fatty acids secreted relative to corresponding dietary fatty acids

Item	Treatment[1]				SEM	p-value
	Control	LSO-FO	SFO-FO	MIX-O		
Yield of milk fatty acids (g/d)						
*c*9,*c*12-C18:2	5.11	4.84	3.56	5.48	1.28	0.09
*c*9,*t*11-CLA	2.39^{b}	5.95^{a}	2.41^{b}	3.38^{ab}	1.97	0.01
C18:3*n*-3	0.48^{b}	1.30^{a}	0.59^{b}	0.91^{ab}	0.32	0.01
C20:5*n*-3+C22:6*n*-3	0.05^{b}	1.37^{a}	0.79^{ab}	1.09^{a}	0.48	0.01
PUFA	9.61^{b}	16.29^{a}	8.96^{b}	13.30^{ab}	3.79	0.01
n-3 PUFA	0.53^{c}	2.67^{a}	1.40^{bc}	2.00^{ab}	0.69	0.01
n-6 PUFA	6.06	5.96	4.02	6.33	1.53	0.06
Transfer into milk (g/100 g intake)						
C18:2*n*-6[2]	7.20^{a}	5.40^{ab}	2.43^{c}	3.88^{bc}	1.33	0.01
*c*9,*t*11-CLA[3]	3.16^{ab}	3.37^{a}	1.40^{b}	1.68^{ab}	1.16	0.01
C18:3*n*-3	10.27^{a}	1.52^{b}	9.15^{a}	1.47^{b}	2.05	0.01
C20:5*n*-3+C22:6*n*-3	-	2.11	1.24	2.46	-	-
PUFA	12.72^{a}	6.61^{b}	4.02^{b}	5.27^{b}	2.09	0.01
n-3 PUFA	11.41^{a}	1.77^{b}	1.98^{b}	1.87^{b}	1.64	0.01
n-6 PUFA	8.54^{a}	6.34^{ab}	2.63^{c}	4.34^{bc}	1.51	0.01

SEM, standard error of the mean; CLA, conjugated linoleic acid; PUFA, polyunsaturated fatty acids.

[1] Control, basal diet without oil supplement; LSO-FO, 3% linseed and fish oils at 1:1; SFO-FO, 3% sunflower and fish oils at 1:1; MIX-O, 3% mixture of linseed, sunflower, and fish oils at 1:1:1.

[2] Calculated as 100×(Sum of milk *c*9,*c*12-C18:2 and *t*9,*t*12-C18:2) / *c*9,*c*12-C18:2 intake.

[3] Calculated as 100×(milk *c*9,*t*11-CLA (g) / (sum of *c*9,*c*12-C18:2 and C18:3*n*-3 intakes).

[a-c] Means within a row with different superscripts are significantly different ($p<0.05$).

improved proportion of milk C18 UFA in this study was in agreement with previous studies (Caroprese et al., 2010; Lerch et al., 2012). This was also supported by Chow et al. (2004) that fish oil inclusion in the *in vitro* study containing sunflower oil and linseed oil led to increase the accumulation of C18:1 UFA. Milk from cows receiving oil inclusion rich in PUFA showed an improvement in UFA proportion and a decrease in SFA proportion. These were in agreement with previous studies (Caroprese et al., 2010; Lerch et al., 2012; Neveu et al., 2014).

Some FA can help to prevent or promote coronary thrombosis and atherosclerosis based upon their effects on low-density lipoprotein-cholesterol concentrations and serum cholesterol (Ulbricht and Southgate, 1991). The equations proposed by Ulbricht and Southgate (1991) for the atherogenic and thrombogenic indices showed that C12:0, C14:0, and C16:0 FA are atherogenic while C14:0, C16:0, and C18:0 are thrombogenic. The *n*-3 PUFA, *n*-6 PUFA, and MUFA are anti-atherogenic and anti-thrombogenic. The ratio between the saturated and unsaturated FA is used to calculate the atherogenic and thrombogenic (Table 5). The supplementation of oil mixtures rich in PUFA in the current study resulted in reduced the AI, TI, and *n*-6 to *n*-3 ratio that can counteract the detrimental effect of high SFA and *n*-6 FA in the milk. Decreased milk atherogenic and thrombogenic indices in the present study were supported by Huang et al. (2008). That the transfer efficiency of selected FA including C18:2*n*-6, C18:3*n*-3, PUFA, *n*-3 PUFA, and *n*-6 PUFA in milk was higher for cows fed the control diet is in agreement with Dewhurst et al. (2003) and Côrtes et al. (2011) who reported that the lower apparent transfer was observed in the diets containing higher UFA concentration.

CONCLUSIONS

A supplementation of dairy cow diet with LSO-FO or MIX-O had no effects on total DMI, milk yield, and milk composition, whereas the diet supplemented with FSO-FO caused negative effects on total DMI, milk fat percentage, and yields of milk fat, protein, and total solid. Dietary supplementation with LSO-FO mainly increased milk *c*9,*t*11-CLA, ALA, DHA, and *n*-3 PUFA whereas feeding MIX-O improved preformed FA and UFA. The lowest *n*-6/*n*-3 ratio was found in the LSO-FO, and the decreased AI and TI seemed to be remarkable in the MIX-O. To maximize milk *c*9,*t*11-CLA, ALA, DHA, and *n*-3 PUFA and to minimize milk *n*-6/*n*-3 ratio, AI and TI, an ideal supplement would look to be either LSO-FO or MIX-O.

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