Effect of Linseed Oil Supplementation on Production, Composition and n-6/n-3 Fatty Acid Ratio in Cow's Milk

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ABSTRACT

The effects of linseed oil supplementation on milk production, milk composition, and n-6/n-3 ratio of dairy cow's milk were studied. Twenty-four Holstein Friesian crossbred lactating dairy cows were assigned into a 2x2 Factorial arrangement. All cows were fed approximately 6 kg/d of 21% CP concentrate. Treatments were: 1) concentrate plus 300 g/d of palm oil (PO) together with ad libitum corn silage (CS); 2) concentrate plus 300 g/d of linseed oil (LSO) together with ad libitum CS; 3) concentrate plus 300 g/d of PO together with ad libitum fresh grass (FG); and 4) concentrate plus 300 g/d of LSO together with ad libitum FG. Supplementation with LSO had no effect on DMI, milk production and milk composition. Milk fat content was also not affected by LSO supplementation. However, the milk C18:3n3 percentage was increased while n-6/n-3 FA ratio was decreased by LSO supplementation. It was concluded that the milk FA composition can be altered by 300 g/d LSO supplementation with increasing concentrations of potentially health beneficial FA and decreasing concentrations of SFA. Finally, LSO supplemented with ad libitum FG lowed n-6/n-3 FA ratio in dairy cow's milk.

Keywords: Linseed oil, Milk fatty acid, n-6/n-3 Fatty acid ratio, Dairy Cow's milk

INTRODUCTION

The nutritional contribution of milk and the potential health effects of its main components (fat, protein, antioxidants, vitamins, and minerals) have been reviewed extensively (Steijns, 2008). However, milk contains a high proportion of saturated fatty acids (SFA) because of extensive biohydrogenation of dietary unsaturated fatty acids (UFA) in the rumen and de novo synthesis of short- and medium chain saturates in the mammary gland (Shingfield et al., 2008). SFA and trans fatty acids in milk fat are relative proportions of high and low density lipoprotein cholesterol resulting in coronary heart disease (CHD). Thus, the milk industry aims to improve the nutritional quality of milk fat by reducing SFA and increasing the content of n-3 series FA, including alpha linolenic acid (ALA), which is recognized as minimizing the risk of cardiovascular disease and is equally essential for the functional development of the central nervous system (Ambroise, 2001). The fatty acid distribution in milk fat is dependent on dietary composition (Dewhurst et al., 2003). It is now well established that supplementation of cow's diet with UFA affects milk FA profiles (Harvatine et al., 2009). The main sources of unsaturated lipids are oilseed lipids, among which linseed, rapeseed, soybean, and sunflower seeds (Glasser et al., 2008). Linseed oil contains the essential alpha–linolenic acid (ALA) which is oil from linseed (*Linumusitatissimum*) produced predominantly in the Great Plains and Canada. LSO supplementation caused a quadratic increase in milk fat and protein contents and supplementing grazing dairy cow diets with LSO at up to 510 g/d can improve the nutritional value of milk without compromising milk composition or cow performance (Flowers et al., 2008). Previous studies compared the effects of TMR containing mixtures of fish oil and different sources of UFA or concentrate: forage ratio and LSO. The results suggested that the nature of supplemental PUFA added to high-concentrate diets likely altered the profile and amount of hydrogenation intermediates available for secretion in milk (Loor et al., 2005). Thus, the objective of this study was to determine the effects of LSO supplementation on milk production, milk composition, and n-6/n-3 ratio of dairy cow's milk.

MATERIALS AND METHODS

Experimental design and treatments

Twenty four Holstein Friesian crossbred lactating dairy cows, averaging 106±43 days in milk, 12.1±3.0 kg of milk and 387±41 kg body weight, were blocked by parity first and then stratified random balanced for milk yield, milking days and body weight into four groups of 6 cows each. They were then assigned into a 2x2 Factorial arrangement. All cows were fed approximately 6 kg/d of 21% CP concentrate. The concentrate fed [(g/kg) 100 cassava chip, 250 cassava meal, 100 rice bran, 120 palm kernel meal, 100 soybean meal, 70 bush bean, 75 corn gluten feed, 80 cassava ethanol, 60 liquid molasses, 20 urea, 20 dicalcium

phosphate, and 5 premix] was fed 3 times daily. The oil supplementation was offered twice daily on top of concentrate fed. Treatments were: 1) concentrate plus 300 g/d of palm oil (PO) together with ad libitum corn silage (CS); 2) concentrate plus 300 g/d of LSO together with ad libitum CS; 3) concentrate plus 300 g/d of PO together with ad libitum fresh grass (FG) (Napier; (*Pennisetum purpureum x Pennisetum americanum*) older not more than 50 days). and 4) concentrate plus 300 g/d of LSO together with ad libitum FG. All cows also had free access to clean water and were individually housed in a free-stall unit and individually fed according to treatments. The experiment lasted for 40 days of measurement period.

Analytical procedures

Feed offered and left after eating of individual cow were collected on 2 consecutive days of each 5 d period and dried at 60 °C for 48 h. At the end of the experiment, feed samples were pooled to make representative samples for proximate and detergent analyses. Samples were ground through 1 mm screen and analyzed for dry matter (DM, hot air oven at 60°C for 48 h), crude protein (CP, Kjeldahl analysis) (AOAC, 1990), ether extract (EE, petroleum ether in a Soxtec System). Fiber fraction, neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the method described by Van Soest et al. (1991), adapted for Fiber Analyzer. Ash content was determined by ashing in a muffle furnace at 600°C for 3 h. The chemical analysis was expressed on the basis of the final DM. Fatty acids composition of concentrates, fresh grass and corn silage were extracted using a modified of the method used by Folch et al. (1957) and Metcalfe et al. (1966) for analyzing by gas chromatography (GC) (7890A GC System, Agilent Technology, USA). Cows were weighed at the start and at the end of the experiment. Cows were milked twice daily at 05.00 and 15.00 h and milk yields were recorded for each cow. Milk samples from both the morning and evening milking were collected on 2 consecutive days of each 5-d period and stored at 4 °C with a preservative until analyzed for fat, protein, lactose and solid not fat content using Milkoscan FT 6000® (Foss Electric; Hillerod, Denmark). In addition, milk samples were extracted for fatty acid using a modified method used by Romeu-Nadal et al. (2004). From a well-mixed aliquot of milk, 3 ml was placed in 50 ml centrifuge tubes. Then added 27 ml of a dichloromethane-methanol solution (2:1, v/v) to each tube. The mixture was shaken mechanically for 15 min and centrifuged at 2,500×g for 8 min at 4 °C. Approximately 8 ml of distilled water was pipette into each tube and, after shaking for a further 15 min, the sample was, again centrifuged at 2,500 × g for 8 min at 4 °C. As much of the upper aqueous fraction as possible was carefully removed with a pipette. The organic layer was washed with 8 ml of a saturated solution of the sodium chloride, and finally mixed mechanically for 15 min and centrifuged for 8 min at 2,500 ×g at 4 °C. The upper aqueous fraction was carefully removed with a pipette. The organic fraction was carefully transferred to a separating funnel and filtered through 1PS paper (Whatman, Maidstone, UK) containing anhydrous sodium

sulfate, and 3-5 ml of dichloromethane was passed through the filter. The fat solution was taken in pre-weighed conical flask. Finally, the extract was concentrated by removing dichloromethane in a rotatory evaporator and dried under a gentle stream of nitrogen. The weight difference of the conical flask before/after was assumed to be fat. The fat was stored at -20 °C and redissolved in dichloromethane (3%, w/v) intermediately analyzing by gas chromatography (GC) (7890A GC System, Agilent Technology, USA). Fatty acid methyl esters (FAME) were prepared by the procedure described by Ostrowska et al. (2000). The procedure involved placing approximately 30 mg of the extracted oil into a 15 ml reaction tube fitted with a teflon-lined screw cap. One and a half ml of 0.5 M sodium hydroxide in methanol was added. The tubes were flushed with nitrogen, capped, heated at 100 ºC for 5 min with occasional shaking and then cooled to room temperature. One ml of C17:0 internal standard (2.00 mg/mL in hexane) and 2 ml of boron trifluoride in methanol were added and heated at 100 °C for 5 min with occasional shaking and 10 ml of deionized water were added. The solution was transferred to a 40 ml centrifuged tube and 5 ml of hexane were added for FAME extraction. The solution was centrifuged at 2,000 g, at 10 °C for 20 min and then the hexane layer was dried over sodium sulfate and transferred into vial for analyzing by gas chromatography (GC) (7890A GC System, Agilent Technology, USA) equipped with a 100 m x 0.25 mm x 0.2 μm film fused silica capillary column (SP1233, SupelcoInc, Bellefonte, PA, USA). Injector and detector temperatures were 250 °C. The column temperature was kept at 70 °C for 4 min, then increased at 13 °C/min to 175 °C and held at 175 °C for 27 min, then increased at 4 °C/min to 215 °C and held at 215 °C for 17 min, then increased at 4 °C/min to 240 °C and held at 240 °C for 10 min.

Statistical analysis

Measured data of intake, milk production, milk composition, and body weight change were analyzed by ANOVA for 2x2 Factorial in randomized complete block design using the Statistical Analysis System (SAS, 2001). Significant differences among treatment were assessed by Duncan's new multiple range test. A significant level of $P<0.05$ was used (Steel and Torries, 1980).

RESULTS

Feed chemical and fatty acid composition

The average EE content and energy values of the oil were higher than the concentrate diet, CS and FG, respectively (Table 1) C18:3n3 was the major fatty acid in the FG and LSO accounting for approximately 48.89% and 56.20% of total fatty acid, respectively. The second major fatty acid in the FG and LSO was

C18:2n6 accounting for 19.03% and 17.04% of total fatty acid, respectively (Table 2). The LSO had similar proportion of n-3 FA to FG, but n-3 FA was almost absent from the PO.

Table 1. Chemical compositions of the experimental diets (% of DM).

Item	21% CP Concentrate	Palm oil/ Linseed oil	Corn silage	Fresh grass
Dry matter	94.40	-	24.09	12.50
Ash	7.66	-	7.43	12.40
Crude protein	20.50	-	6.69	10.07
Ether extract	2.80	100	0.90	1.78
Crude fiber	12.67	-	29.28	36.04
Neutral detergent fiber	45.88	-	61.64	64.42
Neutral detergent insoluble N	1.51	-	0.46	0.32
Acid detergent fiber	22.79	-	26.51	34.43
Acid detergent insoluble N	0.81	-	0.54	0.35
Acid detergent lignin	7.17	-	3.41	2.62
TDN_{1x}(%)[1]	62.01	184.15	57.67	55.05
DE_{1x} (Mcal/kg)[2]	2.96	7.71	2.55	2.51
DE_p (Mcal/kg)[3]	2.87	5.79	2.54	2.51
ME_p (Mcal/kg)[4]	2.45	5.79	2.12	2.08
NE_{lp} (Mcal/kg)[5]	1.53	4.63	1.30	1.27

Note: [1]Total digestible nutrients, TDN_{1X} (%) = tdNFC + tdCP + (tdFA x 2.25) + tdNDF – 7 (NRC, 2001); [2]Digestible energy, DE_{1X} (Mcal/kg) = [(tdNFC/100)x4.2]+[(tdNDF/100) x 4.2]+[(tdCP/100) x 5.6]+[(FA/100) x 9.4] –0.3; [3]DE_P (Mcal/kgDM) = DE1X x Discount (NRC, 2001); [4]Metabolisable energy, MEp = [1.01 x (DEp) – 0.45] + [0.0046 x (EE – 3)] (NRC, 2001); [5]Net energy for lactation, NElp = ([0.703 x MEp (Mcal/kg)] – 0.19) + ([(0.097 x MEp + 0.19)/97] x [EE– 3]) (NRC, 2001).

Table 2. Fatty acid compositions of control concentrate (21% CP), fresh grass, corn silage, palm oil and linseed oil.

% of total FA	21%CP Concentrate	Fresh grass	Corn silage	Palm oil	Linseed oil
C8:0	1.03	ND	ND	0.05	0.05
C10:0	1.09	ND	ND	0.02	ND
C12:0	15.89	1.42	1.14	0.19	ND
C14:0	5.75	0.74	2.09	0.96	0.06
C16:0	16.26	19.66	19.03	38.29	4.91
C18:0	2.92	3.18	5.56	4.42	3.46
C18:1n9c	30.11	6.55	2.52	40.61	17.88
C18:2n6c	25.29	19.03	14.21	13.77	16.97
C20:0	ND	0.54	3.00	0.04	ND
C18:3n3	0.34	48.89	8.00	0.26	55.87
SFA[1]	42.93	25.53	30.81	44.05	8.70
MUFA[2]	30.11	6.55	2.52	41.07	17.96
PUFA[3]	26.97	67.92	66.67	14.89	73.34
Total n6[4]	26.63	19.03	58.66	14.46	17.04
Total n3[5]	0.34	48.89	8.00	0.43	56.20
PUFA:SFA	0.63	2.66	2.16	0.34	8.43
n-6/n-3	79.49	0.39	7.33	33.69	0.30

Note: [1]SFA = Sum of saturated fatty acid from C4: 0 – C20: 0; [2]MUFA = Sum of monounsaturated fatty acid from C14:1 – C22:1; [3]PUFA = Sum of polyunsaturated fatty acids from C18: 2 – C22: 6; [4]Sum of n6 fatty acids C18: 2n-6 – C22: 4n-6;[5] Sum of n3 fatty acids C18:3n-3 – C22:6n-3.

Intake and live weight

The effects of linseed lipid supplement on feed intake and live weight of dairy ruminants are slightly inconsistent; the results of intake of nutrients including of dry matter intake (DMI) (Table 3), crude protein intake (CPI), ether extract intake (EEI) and net energy for lactation from a specified feed ($NE_{LPintake}$) (Mcal/day) were no interaction of main treatment effects, final live weight (FLW, kg) and live weight change (LWC, g/d) were reduced by dietary fresh grass (P>0.05).

Table 3. Effect of Linseed Oil Supplementation on DM, CP, fat and fatty acid intakes of experimental cattle.

Item	CS		FG		SEM	Pr>F		
	300g/d PO	300 g/d LSO	300g/d PO	300 g/d LSO		Roughage	Oil	Roughage×Oil
ILW (kg)	402	397	374	378	2.84	0.195	0.992	0.790
FLW (kg)	404	401	369	374	8.20	0.077	0.964	0.822
LWC (g/d)	+67	+128	-150	-150	116	0.300	0.897	0.897
DMI, kg/d								
Concentrate	6.1	6.1	6.1	6.1	-	-	-	-
Roughage	9.6	9.8	5.8	6.1	0.23	<0.01	0.560	0.990
Oil	0.3	0.3	0.3	0.3	-	-	-	-
Total	16.0	16.3	12.3	12.3	0.30	<0.01	0.658	0.990
DMI, g/BW0.75	174.9	180.4	141.4	143.3	3.31	<0.01	0.589	0.789
CPI, g/d								
Concentrate	1,280	1,280	1,280	1,280	-	-	-	-
Roughage	639	657	588	616	19.90	0.259	0.575	0.899
Total	1,920	1,937	1,869	1,896	51.05	0.656	0.826	0.960
EEI, g/d								
Concentrate	171	171	171	171	-	-	-	-
Roughage	86	88	103	109	3.31	<0.01	0.561	0.981
Oil	300	300	300	300	-	-	-	-
Total	558	560	518	520	7.14	0.195	0.801	0.930
NDFI, kg/d								
Concentrate	281.4	281.4	281.4	281.4	-	-	-	-
Roughage	588.9	609.5	376.0	393.8	14.43	<0.01	0.513	0.961
Total	870.3	890.9	657.4	675.2	16.57	<0.01	0.568	0.967
$NE_{LPintake}$, Mcal/d								
Concentrate	9.4	9.4	9.4	9.4	-	-	-	-
Roughage	10.4	12.8	7.4	7.8	0.53	<0.01	0.223	0.364
Oil	1.39	1.39	1.39	1.39	-	-	-	-
Total	21.2	23.5	18.1	18.5	0.64	<0.01	0.310	0.451

Milk production and milk composition

The supplementing the diets of dairy cows by treatments had no effect on milk production and composition (Table 4). A higher 5% LSO supplementation in a grass hay based diet did not affect milk production either.

Table 4. Effect of Linseed Oil Supplementation on Milk yield (MY) and milk composition of dairy cows.

Item	CS		FG		SEM	Pr>F[2]		
	300g/d PO	300 g/d LSO	300g/d PO	300 g/d LSO		Roughage	Oil	Roughage×Oil
MY, kg/d	13.5	12.8	12.1	12.4	0.58	0.447	0.831	0.686
3.5%FCM (kg/d)	14.59	13.41	12.86	13.28	0.49	0.361	0.706	0.430
Fat								
%	4.07	3.88	3.93	4.01	0.13	0.987	0.829	0.604
g/d	549	497	476	497	18.05	0.367	0.649	0.322
Protein								
%	3.05	2.98	3.02	3.01	0.05	0.988	0.707	0.775
g/d	412	381	365	373	13.54	0.323	0.713	0.586
Lactose								
%	4.8	4.78	4.7	4.63	0.04	0.209	0.645	0.747
g/d	648	612	569	574	27.98	0.319	0.737	0.767
SNF								
%	8.57	8.46	8.44	8.36	0.08	0.484	0.578	0.927
g/d	1,157	1,083	1,021	1,037	45.21	0.333	0.724	0.683
TS								
%	12.64	12.39	12.38	12.35	0.18	0.688	0.702	0.754
g/d	1,706	1,586	1,498	1,531	59.24	0.303	0.707	0.564

Milk fatty acid composition

Supplementing 300 g/d LSO with *ad libitum* FG significantly increased MUFA, PUFA, n6 FA and n3 FA concentrations, but decreased milk fat SFA concentration and n-6/n-3 ratio (P<0.01, Table 5), whereas LSO supplementation with roughage sources significantly increased concentration of C4:0, C8:0 and decreased C12:0 to C16:0 (P<0.01). Therefore, there was an interaction effect of oil supplement and roughage source for milk fat (C18:3n-3 FA). Among these intermediates, C18:2n6t and C18:1n9t showed that the largest increase observed at 3% of dietary supplementation of LSO (Table 5).

Table 5. Effect of Linseed Oil Supplementation on Milk fatty acid composition of dairy cows (% of total FA).

% of total FA	CS		FG		SEM	Pr>F		
	300g/d PO	300 g/d LSO	300g/d PO	300 g/d LSO		Roughage	Oil	Roughage×Oil
C4:0	1.58	2.58	1.74	2.57	0.053	0.506	<0.01	0.450
C6:0	1.50	1.53	1.60	1.58	0.042	0.372	0.961	0.760
C8:0	0.68	0.99	0.94	1.17	0.014	<0.01	<0.01	0.164
C10:0	1.55	1.71	1.36	1.36	0.012	<0.01	<0.01	0.003
C12:0	1.88	1.58	1.76	1.36	0.009	<0.01	<0.01	0.010
C14:0	8.08	7.53	7.62	6.80	0.010	<0.01	<0.01	<0.01
C14:1	0.75	0.68	0.67	0.68	0.005	<0.01	<0.01	<0.01
C16:0	28.23	25.30	27.17	23.97	0.068	<0.01	<0.01	0.336
C16:1	1.49	1.40	1.55	1.41	0.010	0.069	<0.01	0.214
C18:0	12.27	12.55	12.55	12.45	0.020	0.036	0.028	<0.01
C18:1n9t	6.22	8.35	6.94	9.55	0.018	<0.01	<0.01	<0.01
C18:1n9c	30.26	29.13	30.22	30.17	0.073	<0.01	<0.01	<0.01
C18:2n6t	0.53	0.85	0.60	1.08	0.017	<0.01	<0.01	0.027
C18:2n6c	2.29	2.10	2.22	2.04	0.028	0.406	<0.01	0.720
C20:0	0.22	0.17	0.20	0.14	0.003	<0.01	<0.01	0.463
C18:3n6	0.01	0.01	0.01	0.01	0.006	0.646	0.640	0.646
C20:1	0.06	0.02	0.04	0.02	0.002	0.013	<0.01	0.013
C18:3n3	0.56	1.06	0.77	1.17	0.013	<0.01	<0.01	0.150
CLA c9,t11	1.15	1.39	1.25	1.71	0.011	<0.01	<0.01	<0.01
C20:2	0.10	0.07	0.11	0.05	0.003	0.451	<0.01	0.011
C22:0	0.11	0.45	0.11	0.05	0.056	0.091	0.218	0.096
C20:3n6	0.02	0.02	0.02	0.02	0.002	0.255	0.817	0.490
C22:1n9	0.02	0.02	0.01	0.02	0.002	0.168	0.639	0.168
C20:3n3	0.01	0.02	0.01	0.02	0.001	0.063	<0.01	0.781
C20:4n6	0.17	0.15	0.16	0.12	0.004	0.014	<0.01	0.420
C22:2	0.02	0.02	0.01	0.01	0.002	0.239	1.000	1.000
C24:0	0.03	0.04	0.04	0.03	0.005	0.931	0.931	0.794
C22:6n3	0.04	0.09	0.12	0.13	0.008	<0.01	0.056	0.246
C20:5n3	0.04	0.04	0.05	0.04	0.003	0.233	0.628	0.469
SFA[1]	56.14	54.42	55.08	51.48	0.081	<0.01	<0.01	<0.01
MUFA[2]	38.80	39.59	39.44	41.85	0.078	<0.01	<0.01	<0.01
PUFA[3]	5.06	5.99	5.48	6.67	0.038	<0.01	<0.01	0.098
n-6[4]	4.31	4.70	4.42	5.23	0.038	<0.01	<0.01	0.011
n-3[5]	0.66	1.22	0.97	1.41	0.017	<0.01	<0.01	0.085
n-6/n-3	6.66	3.87	4.59	3.79	0.114	<0.01	<0.01	<0.01

Note: [1] SFA = Sum of saturated fatty acid from C4:0 – C20:0; [2] MUFA = Sum of monounsaturated fatty acid from C14:1 – C22:1; [3] PUFA = Sum of polyunsaturated fatty acids from C18:2 – C22:6; [4] Sum of n6 fatty acids C18:2n-6 – C22:4n-6; [5] Sum of n3 fatty acids C18:3n-3 – C22:6n-3; SEM is standard error of mean.

DISCUSSION

No interaction of main treatment effects occurred for intake of nutrients (Table 3). However, the ANOVA of main effects showed that cows fed diets based on corn silage consumed more total dry matter (DM) than fresh grass ($P<0.01$) which had greater CF, NDF and ADF compared to corn silage. In the case of fresh forage, high water content is often mentioned as a factor regulating DM intake (Forbes, 1995). Phillips et al. (1991) showed that the high water content of forages could limit intake in cattle and sheep. Tafaj et al. (2007) reported that DMI linearly decreased with increasing dietary NDF. Forage NDF is a major factor affecting feed intake and rumen fill in high-producing cows. However, DMI in the current experiment (4.92 g/100 g of concentrate, DM) were not reduced by oil supplementation. There was no interaction between oil and roughage sources on ether extract intake (EEI, g/d) and net energy for lactation intake (NELP intake). However, cows fed diets based on corn silage consumed more EE and NELP than those cows on fresh grass ($P<0.01$).

Milk production and composition were unaffected by treatments (Table 4). Loor et al. (2005) did not observe any effect of less than 5% LSO on milk production when added either to forage based or concentrate based diets of dairy cows. A higher 5% LSO supplementation in a grass hay based diet did not affect milk production either. In the present experiment, adding oil to dairy cow diets did also not affect milk composition. Similarly, inclusion of plant lipids in the concentrate had no effect on milk yield or milk composition in cow fed red clover silage, possibly because the intake of DM and ME was similar across treatments (Filleau et al., 2011). Milk fat content was unaffected by treatments (Table 4). Chilliard et al. (2009) and Shingfield et al. (2010) reported no effect of dietary oil supplementations on milk fat content. In the present experiment, LSO did not affect milk protein (Table 4). The effect of oils on milk protein content has also been variable. Bu et al. (2007) reported no effect of LSO addition on milk protein. In contrast, Loor et al. (2005) and Flowers et al. (2008) observed an increase in milk protein content of grazing cows supplemented with LSO. Variability between studies in milk composition response to oil supplementation could be explained not only by the amount of oil added to the diet but also by the composition of the basal diet. No significant relationships were found between the amount of fat added (ranged from 200 to 1,000 g/day) and the response in milk or FCM production, or between the milk production response and the level of milk production of the cows (ranged from 11 to 31 kg/day) (Schroeder et al., 2004)

The MUFA, PUFA, n6 FA and n3 FA concentrations increased throughout from 300 g/d LSO supplementation with *ad libitum* FG, whereas a significantly decrease in SFA concentration and n-6/n-3 ratio were detected in milk fat ($P<0.01$). The FA composition of milk fat depends on various dietary characteristics including roughage to concentrate ratio (Sterk et al., 2011), FA

intake, FA metabolism in the mammary gland (Chilliard et al., 2007). LSO supplementation with roughage sources significantly increased concentration of C4:0, C8:0 and decreased C12:0 to C16:0 (P<0.01). Furthermore, 300 g/d LSO with ad libitum FG decreased C14:0 to C16:0 in milk fat (P<0.01; Table 5). These effects are consistent with the reduction in de novo FA synthesis due to feeding unsaturated oils, which occurs because of greater uptake and secretion of dietary or ruminal derived FA (Benchaar et al., 2012).

The increased concentration of C18:0 in milk fat can be expected due to extensive metabolism of long chain PUFA in the rumen which leads to an increase in the amount of C18:0 for absorption (Jensen, 2002). The decreased in milk SFA content with LSO supplementation was similar between the CS and FG diets (P<0.01; Table 5). The faster rate of oil release into the rumen from LSO could result in the higher production of trans FA in the rumen and, thus an inhibitory effect on de novo mammary lipogenesis (Chilliard et al., 2007). Furthermore, a meta-analysis citing a range of studies in which linseed and other oil supplements decreased concentrations of long chain UFA (Glasser et al., 2008).

Feeding LSO increased C18:1n9t, C18:1n9c and CLA (C9, T11) (P<0.01; Table 5) in milk fat. This can be explained by the result from the ruminal BH of C18:2 and C18:3 (Shingfield et al., 2010). In particular, C18:1 could be derived from the reduction of C18:2 and C18:3n3 and during ruminal metabolism, which both increased when the diet was supplemented with LSO (Benchaar et al., 2012). In the present study, the roughage sources, FG and CS, which led to an increase in C18:1 in milk fat could, therefore, be explained by the higher supply of dietary C18:2 (Table 5) or by the overall ruminal UFA load, as suggested by Lock (2010). Duodenal flow and secretion of C18:1 in milk fatwas shown to be highly dependent on the composition of the basal diet. Feeding LSO in a diet rich in starch and based on CS as the sole forage increased milk fat content of C18:1 (Chilliard et al., 2007). In the contrast, supplemental LSO decreased C18:1when the diet was based on grass hay rich in fiber (Roy et al., 2006).

Milk fat content of CLA was increased with LSO supplementation (Table 5). It has been established that a great proportion of this CLA isomer found in the milk is produced endogenously in the mammary gland using C18:1 produced in the rumen as a substrate (Griinari et al., 2000). Nevertheless, for each type of forage, the specific FAs were correlated with milk fat content. Loor et al. (2005) showed that C18:2 was enhanced in the duodenal digesta in response to natural grassland hay based diets containing LSO as a source of C18:3n3. There was a significant effect of oil supplement and roughage source (P<0.01) interaction on C18:3n-3 FA in milk fat. The decrease in transfer efficiency observed with increasing supply of LSO could be explained by 1) an increased efficiency in biohydrogenation with higher supply of free LSO in the diet, 2) a lower intestinal digestibility with an increasing amount of C18:3n-3 escaping ruminal

fermentation as dietary LSO supply increased, or 3) a lower efficiency of mammary uptake as the arterial concentration of C18:3n-3 increased (Benchaar et al., 2012). In this regard, Loor et al. (2005) observed an increase in intestinal digestibility of C18:3n-3 with dietary supplement of LSO, which does not support a limitation in absorption capacity. At the level of the mammary gland, Enjalbert et al. (1998) reported that extraction of arterial FA, either nonesterified or in the form of triglycerides, increased with arterial concentration following duodenal infusion of long chain FA. Finally, Khas-Erdene et al. (2010) observed a quite stable efficiency of transfer to milk of duodenal infused C18:3n-3 over a wide range of FA supply. The decrease in apparent recovery of dietary C18:3n3 can be explained by the efficiency BH process, which has been shown by Loor et al. (2005) to be greater when free LSO was added in the diet.

In this case, the extent of ruminal BH has been assessed by calculating the proportion of dietary PUFA disappearing during their passage in the rumen (not found in the small intestine). However, this calculation does not provide any information about the efficiency of the overall series of reactions (e.g., the final production of C18:0). According to Harfoot (1981), the BH pathway of C18:3n3 involves the production of C18:3n3, C18:2n6t and C18:1n9t, which can all be absorbed and incorporated in to milk fat. Among these intermediates, C18:2n6t and C18:1n9t showed a response to the level of LSO supplementation, with the largest increase observed at 3% of dietary supplementation (Table 5). Therefore, it speculates that dietary C18:3n3 rapidly undergoes BH by being reduction of double bonds at carbons 6 and 9 to yield C18:2n6t and C18:1n9t, which accumulate in the rumen as the efficiency of the first step in the pathway increases. The average concentrations of C18:3n3 and C18:2n6 in milk fat of 300 g/d LSO with *ad libitum* FG cows were 1.17 and 2.04 % of total FA (Table 5). The n-6/n-3 ratio of 3.79 ($P<0.01$) falls within the recommended range, from 1:1 to 4:1, that is considered to be important for human homeostasis and normal development (Simopoulos, 2008).

For cows, main sources of C18:3n3 and C18:2n6 were from LSO, grass and corn silage, respectively (Chilliard et al., 2001). C18:3n3 and C18:2n6 that escape rumen BH will probably, as in other mammals, be beta-oxidized (Cunnane et al., 2003), stored in body tissues, or incorporated in milk fat. Desaturation of C20:4n3 causes the formation of C20:5n3, which is subsequently elongated to form C22:5n3. In humans, the main products formed out of C18:3n3 are C20:5n3 and C22:5n3; the latter may have beneficial effects (Kaur et al., 2011) and may serve as a substrate for metabolic retro conversion to C20:5n3 (Russo, 2009). However, the absorption levels of C22:5n3 and the conversion rate to C20:5n3 have never been reported. In the generally accepted n-3 FA partway, C22:5n3 is further converted into C24:5n-3, C24:6n-3, and finally C22:6n3. The conversion of C22:5n3 is the rate limiting step for the conversion of C18:3n-3 to C22:6n-3 (Arterburn et al., 2006). Unfortunately, the intermediates C24:5n-3 and C22:6n-3 could not be determined in the present study, which makes it impossible

to determine the actual rate-limiting step. Concentrations of C22:6n-3 were lower than 0.20% of total FA of fat in all treatments, which is in agreement with the low conversion rates from C18:3n-3 (<0.1%) that are frequently reported in the literature (Williams and Burdge, 2006). In the present analyses, we used the average FA content of milk. Milk FA composition is not constant and feeding trials have shown that concentrations of very long chain PUFA may increase significantly due to variation in the diets of dairy cows. For example, the C22:6n-3 concentration may be increased from 0.04 to 0.13 % of total FA upon supplementation of the dairy cow diet with linseed oil (Zachut et al., 2010).

CONCLUSION

Supplementing 300 g/d oil (PO and LSO) to roughage sources (CS and FG) did not negatively affect DMI, milk yield, milk production and milk composition. Milk fatty acid proportions of n-3FA increased, whereas total SFA decreased with the addition of LSO in the diet. As a result of changes in these FAs, the ratio of n-6 to n-3 was lowered in dairy cow supplemented with LSOFG as compared with other treatments. It can be concluded that 300 g/d LSO can be safely supplemented to forage-based diets of dairy cows to enrich milk with potential health-beneficial FA without causing any detrimental effect on animal performance.

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