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Effects of Linseed Oil or Whole Linseed Supplementation on Performance and Milk Fatty Acid Composition of Lactating Dairy Cows

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ABSTRACT: The objective of this study was to determine the effects of linseed oil or whole linseed supplementation on performance and milk fatty acid composition of lactating dairy cows. Thirty six Holstein Friesian crossbred lactating dairy cows were blocked by milking days first and then stratified random balanced for milk yields and body weight into three groups of 12 cows each. The treatments consisted of basal ration (53:47; forage:concentrate ratio, on a dry matter [DM] basis, respectively) supplemented with 300 g/d of palm oil as a positive control diet (PO), or supplemented with 300 g/d of linseed oil (LSO), or supplemented with 688 g/d of top-dressed whole linseed (WLS). All cows were received *ad libitum* grass silage and individually fed according to the treatments. The experiment lasted for 10 weeks including the first 2 weeks as the adjustment period, followed by 8 weeks of measurement period. The results showed that LSO and WLS supplementation had no effects on total dry matter intake, milk yield, milk composition, and live weight change; however, the animals fed WLS had higher crude protein (CP) intake than those fed PO and LSO ($p<0.05$). To compare with the control diet, dairy cow's diets supplemented with LSO and WLS significantly increased milk concentrations of *cis*-9,*trans*-11-conjugated linoleic acid (CLA) ($p<0.05$) and n-3 fatty acids (FA) ($p<0.01$), particularly, *cis*-9,12,15-C18:3, C20:5n-3 and C22:6n-3. Supplementing LSO and WLS induced a reduction of medium chain FA, especially, C12:0-C16:0 FA ($p<0.05$) while increasing the concentration of milk unsaturated fatty acids (UFA) ($p<0.05$). Milk FA proportions of n-3 FA remarkably increased whereas the ratio of n-6 to n-3 decreased in the cows supplemented with WLS as compared with those fed the control diet and LSO ($p<0.01$). In conclusion, supplementing dairy cows' diet based on grass silage with WLS had no effect on milk yield and milk composition; however, *trans*-9-C18:1, *cis*-9,*trans*-11-CLA, n-3 FA and UFA were increased while saturated FA were decreased by WLS supplementation. Therefore, it is recommended that the addition 300 g/d of oil from whole linseed should be used to lactating dairy cows' diets. (**Key Words:** Linseed Oil, Whole Linseed, Milk Production, Milk Composition, Milk Fatty Acids, Dairy Cows)

INTRODUCTION

The inclusion of saturated fats of animal origin in human diets may increase the risk for cardiovascular diseases (Joyce et al., 2009). It has been estimated that dairy products contribute up to 60% of saturated fatty acids (SFA) to human diets in some European countries (Chilliard et al., 2007). Furthermore, dietary oilseed supplements generally increase the concentration of *trans* fatty acids (FA) in bovine milk (Glasser et al., 2008), which, depending on the position and number of double bonds, may have detrimental effects on human health (Shingfield et al., 2008). Limited evidence exists to confirm putative adverse or neutral effects of *trans* 18:1 isomers from ruminant products is relative to partially hydrogenated vegetable oils (Brouwer et al., 2010). In contrast, dietary consumption of n-3 FA is beneficial for human health (Gebauer et al., 2006), and conjugated linoleic acid (CLA) from ruminant fat has been shown to exhibit potent anti-inflammatory and anti-carcinogenic activities, and is reported to improve biomarkers of cardiovascular health in animal models or studies with human cell lines (Huth et al., 2006; Shingfield et al., 2008; Gebauer et al., 2011). For above reasons, public

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health guidelines in most developed countries have recommended population-wide reduction in saturated and *trans* FA and an increase in 18:3n-3, 20:5n-3, and 22:6n-3 in the human food chain to decrease the incidence of chronic disease (WHO, 2003). Although increasing the proportion of polyunsaturated fatty acids (PUFA) in milk is limited due to extensive biohydrogenation of these FA in the rumen, supplementing linseed to dairy cattle increased the proportion of *cis*-9,*trans*-11-18:2 (*c*9,*t*11 CLA) and n-3 FA, particularly linolenic acid, EPA and DHA in milk (Glasser et al., 2008, Lerch et al., 2012; Puppel et al., 2013). The objective of this study was to characterize milk FA composition responses to different forms of dietary linseed supplements in lactating dairy cows fed a diet based on grass silage. The hypothesis of this study was that feeding different physical forms of linseed in dairy cattle diets leads to an improvement in milk n-3 FA and CLA.

MATERIALS AND METHODS

Animals, experimental design, and treatments

Thirty six Holstein Friesian crossbred lactating dairy cows, averaging 45±8 days in milk, 17.3±0.9 kg of milk and 452±18 kg body weight, were blocked by milking days first and then stratified randomly based on milk yield and body weight into three groups of 12 cows each. All cows were fed with 8 kg/d of 21% crude protein (CP) concentrate. The experimental design was a randomized complete block design with twelve replicates per each treatment. The treatments consisted of basal ration based on grass silage supplemented with 300 g/d of palm oil as a positive control diet (PO), or supplemented with 300 g/d linseed oil (LSO), or supplemented with 688 g/d of top-dressed whole linseed (4.46% dry matter (DM) intake, WLS). The WLS contained 43.7% fat, thus the supplementation of 688 g/d of WLS provided 300 g/d of LSO, respectively. The level of oil supplement calculated as percent to DM intake was 1.9% in LSO and WLS treatments. The PO was added to balance energy concentration in the diets. The basal diet was formulated to meet NRC (2001) requirements (Table 1). All cows received *ad libitum* grass silage (*Brachiaria ruziziensis*; 50 d cutting age), had free access to clean water, were individually housed in a free-stall unit, and individually fed according to treatments. The experiment lasted for 10 weeks with the first 2 weeks for adjustment, followed by 8 weeks of measurement period.

Measurements, sample collection, and chemical analysis

Residual feeds were weighed for two consecutive days weekly. Feed samples were taken and dried at 60℃ for 48 hours. At the end of the experimental period, feed samples were composited and subsamples were taken for further chemical analysis. Samples were ground through a 1 mm screen and subjected to proximate analysis. Crude protein content was determined by Kjeldahl method (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 were determined using the method described by Van Soest et al. (1991), adapted for Fiber Analyzer. Chemical analysis was expressed on the basis of final DM.

Cows were milked twice daily at 05.00 and 15.00 h, and milk yields were recorded for each cow. Milk samples (evening and morning) were collected at each milking for

Table 1. Chemical composition of feed used in the experiment

% Dry matter (DM)	Concentrate[1]	Grass silage	Palm oil	Linseed oil	Whole linseed
DM	91.02	28.34			91.70
CP	21.35	7.61			21.34
Lipid	3.87	2.23	100.00	100.00	43.70
Ash	9.01	8.37			3.30
CF	12.28	30.25			6.21
NFC[2]	33.81	27.91			3.15
NDF	38.27	55.19			36.20
ADF	16.04	34.28			22.30
ADL	3.28	4.16			4.27
NE_{LP}[3] (Mcal/kg)	1.63	1.33	4.63	4.63	3.12
*dg*DM[4]	63.87	48.33			-
*dg*CP[4]	66.83	56.27			-

CP, crude protein; CF, crude fiber; NFC, non-fiber carbohydrate; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; NE_{LP}, net energy for lactation at production level.

[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 5,000 IU of vitamin A, 2,200 IU of vitamin D_3, 15 IU of vitamin E, 8.5 g of Ca, 6 g of P, 9.5 g of K, 2.4 g of Mg, 2.1 g of Na, 3.4 g of Cl, 3.2 g of S, 0.16 mg of Co, 100 mg of Cu, 1.3 mg of I, 64 mg of Mn, 64 mg of Zn, 64 mg of Fe, and 0.45 mg of Se.

[2] Calculated as 100– (CP+NDF+lipid+ash). [3] Calculated using published values of NRC (2001).

[4] *dg*DM = effective degradability of DM; *dg*CP = effective degradability of CP. Obtained from nylon bag technique (Ø rskov and McDonald, 1979).

two consecutive days weekly and stored at 4℃ with a preservative (bronopol tablet; D&F Control System, San Ramon, CA, USA) until analyzed for fat, protein, lactose and solid-not-fat contents using a Milko-Scan S50 analyzer (Foss Tecator, Hillerød, Denmark). All cows were weighed at the start and end of the experiment.

Milk samples were collected from individual cow on day 56 of the experiment. Milk samples were centrifuged at 2,000×g to fat cake and extraction. Lipid extraction was that of the procedures described by Hara and Radin (1978), using a volume of 18 mL of hexane and isopropanol (3:2, v/v)/g of fat cake. After vortexing, a sodium sulfate solution (6.7% $NaSO_4$ in distilled H_2O) was added at a volume of 12 mL/g of fat cake. The hexane layer was transferred to a tube containing 1 g of $NaSO_4$, and after 30 min, the hexane layer was removed and stored at –20℃ until methylation.

Fatty acid methyl esters (FAME) were prepared by procedure described by Ostrowska et al. (2000). Briefly, the procedure required that approximately 30 mg of the extracted oil was placed into a 15-mL reaction tube fitted with a teflon-lined screw cap and then 1.5 mL of 0.5 M sodium hydroxide in methanol was added. The tubes were flushed with nitrogen, capped, heated at 100℃ for 5 min with occasional shaking and then cooled to room temperature. One mL of C17:0 internal standard (2 mg/mL in hexane) and 2 mL of 14% boron trifluoride in methanol were added and heated at 100℃ for 5 min with occasional shaking. After methylation was completed, 10 mL of deionized water was added. The solution was transferred to a 40-mL centrifuged tube, and 5 mL of hexane was added for FAME extraction. The solution was centrifuged at 2,000 ×g, at 10℃ for 20 min and the hexane layer was then dried over sodium sulfate and was taken into vial for analyzed by gas chromatography (GC) (Hewlett Packard GC system HP6890 A; Hewlett Packard, Avondale, PA, USA) equipped with a 100 m×0.25 mm fused silica capillary column (SP2560, Supelco Inc, Bellefonte, PA, USA). Injector and detector temperatures were 240℃. The column temperature was kept at 70℃ for 4 min, then increased at 13℃/min to 175℃ and held at 175℃ for 27 min, then increased at 4℃/min to 215℃ and held at 215℃ for 31 min.

Statistical analysis

Measurements of intake, milk production, milk composition, milk FA and body weight change were analyzed by ANOVA for a randomized complete block design using the Statistical Analysis System (SAS, 1996). Differences between treatment means were statistically compared using Least Significant Differences (Steel and Torrie, 1980).

RESULTS

The chemical compositions of the feeds are presented in Table 1. Palm oil and linseed oil were used only as a source of lipid, while WLS was rich in concentrations of both lipid (43.70%) and crude protein (21.34%). The palm oil was high in SFA (38.24%) and low in n-3 FA (0.26%); in contrast, the supplementation of LSO and WLS was chosen to reduce SFA (10.23% and 10.80%, respectively) and increase n-3 FA (52.46% and 49.62%, respectively) (Table 2). No significant differences were found for DM and net energy for lactation at production level (NE_{LP}) intakes among groups (Table 3); however, the animals supplemented with WLS had greater total CP intake than those fed the other diets ($p<0.05$). In particular, CP intake of the animals fed WLS was 88 and 81 g/d higher than those fed palm oil and linseed oil, respectively. In contrast to CP intake, no changes were found for milk yield and compositions among the treatments ($p>0.05$) (Table 4). The amount of dietary fat did not affect live weight of the cows over the course of the trial; however, live weight was lost at 54 and 107 g/d in the animals fed PO and LSO, respectively, the animals fed diet added WLS increased live weight at 71

Table 2. Fatty acid composition of feeds (% of total fatty acids) used in the experiment

Fatty acid	Concentrate	Grass silage	Palm oil	Linseed oil	Whole linseed
C12:0	30.89	3.97	0.23	0.02	nd
C14:0	13.67	3.01	0.85	0.36	0.04
C16:0	11.98	28.76	36.77	5.07	3.53
C18:0	2.76	15.74	0.39	4.78	7.23
cis-9-C18:1	19.29	15.08	49.48	20.3	20.59
cis-9,12-C18:2	12.05	16.68	11.74	13.98	18.47
cis-9,12,15-C18:3	nd	6.07	0.26	52.46	49.62
Unknown	9.36	10.69	0.28	0.03	0.52
SFA[1]	59.3	51.48	38.24	10.23	10.80
UFA[2]	31.34	37.83	61.48	86.74	88.68
UFA/SFA	0.53	0.73	1.61	8.48	8.21
n-6[3]/n-3[4]	-	2.75	45.15	0.27	0.37

nd, not detected; SFA, saturated fatty acids; UFA, unsaturated fatty acids.
[1] SFA = C12:0+C14:0+C16:0+C18:0. [2] UFA = *cis*-9-C18:1+*cis*-9,12-C18:2+*cis*-9,12,15-C18:3. [3] *cis*-9,12-C18:2. [4] *cis*-9,12,15-C18:3.

Table 3. Effects of treatment diets on DM, CP, and NE_{LP} intakes

Intake	Treatment[1]			SEM	p-value
	PO	LSO	WLS		
DM (kg/d)					
Concentrate	7.28	7.28	7.28		
Grass silage	8.13[ab]	8.22[a]	7.53[b]	0.21	0.04
PO/WLS	0.30[b]	0.30[b]	0.63[a]	0.08	0.02
Total	15.71	15.80	15.44	0.23	0.54
CP (g/d)					
Concentrate	1536	1536	1536		
Grass silage	619[ab]	626[a]	573[b]	16.13	0.05
PO/WLS	0	0	134		
Total	2155[b]	2162[b]	2243[a]	16.15	0.03
NE_{LP} (Mcal/d)					
Concentrate	11.87	11.87	11.87		
Grass silage	10.81	10.94	10.01	0.31	0.26
PO/WLS	1.39[b]	1.39[b]	1.97[a]	0.13	0.03
Total	24.07	24.20	23.85	0.31	0.45

DM, dry matter; CP, crude protein; NE_{LP}, net energy for lactation at production level; SEM, standard error of the mean.

[1] PO = supplemented with 300 g/d palm oil as positive control diet; LSO = supplemented with 300 g/d linseed oil; WLS = supplemented with 688 g/d whole linseed.

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

g/d (Table 4).

In the current study, linseed-containing diets resulted in marked alternations in milk FA composition and yield relative to the diet added palm oil (Tables 5 and 6). The LSO and WLS diets led to a decrease of C12:0-C16:0 FA ($p<0.05$) and an increase of *trans*-9-C18:1, *trans*-9,12-C18:2 and *cis*-9,12,15-C18:3 ($p<0.01$) compared to the PO diet. However, *cis*-9-C18:1, *cis*-9,12-C18:2, and *cis*-6,9,12-18:3 remained unchanged among the treatments ($p>0.05$). The diets containing linseed resulted in greater milk fat *cis*-9,12,15-C18:3, representing respectively 0.91% and 1.03% of the total milk FA from animals fed LSO and WLS diets compared to 0.54% in the animals fed the control diet ($p<0.01$). As the result of biohydrogenation of linoleic acid in the rumen and synthesis of CLA from *trans*-9-C18:1 in the mammary gland, milk *cis*-9,*trans*-11-CLA significantly increased in the LSO and WLS diets (1.62% and 1.72%, respectively) compared to 0.70% in the control diet ($p<0.05$). The concentrations of milk EPA and DHA, n-3 FA, were also improved fromanimals fed diets supplemented with LSO and WLS ($p<0.01$). As a result, the animals fed LSO and WLS diets decreased milk SFA (49.91% and 49.14%) and increased milk unsaturated fatty acids (UFA) (37.83% and 38.64%) compared to the animals fed PO diet (60.59% and 31.56%, respectively), accompanied by increasing UFA to SFA ratio in the linseed-containing groups relative to the control group ($p<0.01$). The proportion of total n-3 FA significantly increased ($p<0.01$) in the animals fed diets containing linseed that led to a decrease ($p<0.01$) in the ratio of n-6 to n-3 FA in their milk fat. The animals fed diets supplemented with LSO and WLS

Table 4. Effects of treatment diets on milk yield, milk composition, and live weight change

Yield	Treatment[1]			SEM	p-value
	PO	LSO	WLS		
Yield					
Milk (kg/d)	18.1	18.3	18.6	0.97	0.69
3.5% FCM[2] (kg/d)	18.5	19.0	19.4	0.96	0.67
Fat (g/d)	659	681	703	62	0.35
Protein (g/d)	529	551	564	51	0.82
Lactose (g/d)	896	899	917	76	0.70
Solid-not-fat (g/d)	1,627	1,636	1,642	103	0.68
Total solid (g/d)	2,286	2,317	2,345	142	0.61
Composition (g/100 g of raw milk)					
Fat	3.64	3.72	3.78	0.22	0.66
Protein	2.92	3.01	3.03	0.14	0.60
Lactose	4.95	4.91	4.93	0.34	0.87
Solid-not-fat	8.99	8.94	8.83	0.41	0.74
Total solid	12.63	12.66	12.61	0.45	0.81
Live weight change					
Initial live weight (kg)	454	451	453	11.7	0.58
Final live weight (kg)	451	445	457	12.4	0.67
Live weight gain (g/d)	–54	–107	+71	103	0.56

SEM, standard error of the mean; FCM, fat-corrected milk.

[1] PO = supplemented with 300 g/d palm oil as positive control diet; LSO = supplemented with 300 g/d linseed oil; WLS = supplemented with 688 g/d whole linseed.

[2] 3.5% FCM = (0.432×kg of milk)+(16.216×kg of milk fat).

Table 5. Milk fatty acid composition (g/100 g FA) of cows fed linseed oil or whole linseed

Fatty acid	Treatment[1]			SEM	p-value
	PO	LSO	WLS		
C4:0	1.36	1.25	1.22	0.07	0.56
C6:0	1.21	1.06	1.08	0.07	0.58
C8:0	0.68	0.57	0.55	0.06	0.47
C10:0	1.49	1.25	1.20	0.12	0.28
C12:0	1.76^{a}	1.51^{b}	1.45^{b}	0.08	0.04
C14:0	7.84^{a}	6.92^{b}	6.87^{b}	0.16	0.03
C16:0	28.97^{a}	23.46^{b}	22.97^{b}	0.62	0.01
C17:0	0.91	0.83	0.82	0.03	0.09
C18:0	16.37^{a}	13.06^{b}	12.98^{b}	0.97	0.02
trans-9- C18:1	3.72^{b}	8.55^{a}	9.02^{a}	0.83	0.01
cis-9-C18:1	24.13	23.27	23.23	1.21	0.28
trans-9,12-C18:2	0.26^{b}	1.15^{a}	1.22^{a}	0.13	0.01
cis-9,12-C18:2	2.02	1.87	1.85	0.12	0.12
cis-6,9,12-18:3	0.29	0.31	0.30	0.04	0.87
cis-9,12,15-C18:3	0.54^{b}	0.91^{a}	1.03^{a}	0.07	0.01
cis-9,*trans*-11-CLA	0.70^{b}	1.62^{a}	1.72^{a}	0.30	0.04
trans,*trans* -CLA	0.12^{b}	0.28^{ab}	0.33^{a}	0.06	0.04
C20:5n-3, EPA	0.01^{b}	0.04^{a}	0.06^{a}	<0.01	0.01
C22:4n-6	0.02^{b}	0.04^{a}	0.05^{a}	<0.001	0.01
C22:5n-3	0.03^{b}	0.06^{a}	0.07^{a}	<0.001	0.01
C22:6n-3, DHA	0.01^{b}	0.04^{a}	0.06^{a}	<0.001	0.01
Unknown	7.85	12.26	12.22	1.68	0.12
SFA	60.59^{a}	49.91^{b}	49.14^{b}	0.97	0.02
UFA	31.56^{b}	37.83^{a}	38.64^{a}	0.97	0.02
n-6 FA[2]	2.60^{b}	3.41^{a}	3.48^{a}	0.05	0.01
n-3 FA[3]	0.59^{c}	1.05^{b}	1.22^{a}	0.03	0.01
n-6/n-3	4.41^{a}	3.25^{a}	2.85^{b}	0.09	0.01
Indices[4]					
M/S	0.46^{b}	0.64^{a}	0.66^{a}	0.02	0.01
P/S	0.05^{b}	0.09^{a}	0.09^{a}	0.01	0.01
U/S	0.52^{b}	0.76^{a}	0.79^{a}	0.01	0.01

SEM, standard error of the mean; CLA, conjugated linoleic acid; SFA, saturated fatty acids; UFA, unsaturated fatty acids.

[1] PO = supplemented with 300 g/d palm oil as positive control diet; LSO = supplemented with 300 g/d linseed oil; WLS = supplemented with 688 g/d whole linseed.

[2] Calculated as *trans*-9,12-C18:2+*cis*-9,12-C18:2+*cis*-6,9,12-18:3+C22:4n-6.

[3] Calculated as *cis*-9,12,15-C18:3+C20:5n-3+C22:5n-3+C22:6n-3.

[4] M/S = sum of monounsaturated fatty acids/sum of saturated fatty acids; P/S = sum of polyunsaturated fatty acids/sum of saturated fatty acids; U/S = sum of unsaturated fatty acids/sum of saturated fatty acids.

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

had respectively 68.5% and 90.7% more *cis*-9,12,15-C18:3 and 131% and 146% higher *cis*-9,*trans*-11-CLA, and 130% and 142% higher milk *trans*-C18:1 than those fed PO diet. The LSO and WLS supplementation significantly increased the yields of *trans*-C18:1, *cis*-9,12,15-C18:3 ($p<0.01$), and *cis*-9,*trans*-11-CLA ($p<0.01$) while it did not affect the main of n-6 FA (*cis*-9,12-C18:2; $p>0.10$). Decreased SFA and increased UFA yields were also found as the animals fed diets comprising LSO and WLS ($p<0.05$). The average daily yields of total n-3 FA in the LSO and WLS diets were improved respectively up to 7.15 and 8.58 g/d, 83.8% and 121% higher than PO diet ($p<0.01$). Consequently, the ratio of n-6 to n-3 FA was reduced in the animals fed WSO-containing diet (2.85) compared to 4.41 in the PO diet-fed animals ($p<0.01$).

DISCUSSION

The foremost consideration to supplement linseed in dairy cow feeding is potential adverse effects of FA from linseed on feed intake and lactation performance. The intakes of DM and NE_{LP} in the current study were in agreement with most literature reports that showed lesser effects of concentration and type of fat supplement when

Table 6. Milk fatty acid yield (g/d) of cows fed linseed oil or whole linseed

Fatty acid	Treatment[1]			SEM	p-value
	PO	LSO	WLS		
C4:0	8.96	8.51	8.58	0.477	0.56
C6:0	7.97	7.22	7.59	0.477	0.58
C8:0	4.48	3.88	3.87	0.408	0.47
C10:0	9.82	8.51	8.44	0.817	0.28
C12:0	11.60	10.28	10.19	0.545	0.61
C14:0	51.65	47.11	48.30	1.089	0.22
C16:0	190.87^{a}	159.71^{b}	161.50^{b}	4.221	0.01
C17:0	6.00	5.65	5.77	0.204	0.39
C18:0	107.85	88.91	91.26	6.604	0.42
trans-9- C18:1	24.51^{b}	58.20^{a}	63.42^{a}	5.650	0.01
cis-9-C18:1	158.98	158.41	163.33	8.237	0.28
trans-9,12-C18:2	1.71^{b}	7.83^{a}	8.58^{a}	0.885	0.01
cis-9,12-C18:2	13.31	12.73	13.01	0.817	0.12
cis-6,9,12-18:3	1.91	2.11	2.11	0.272	0.87
cis-9,12,15-C18:3	3.56^{b}	6.19^{a}	7.24^{a}	0.477	0.01
cis-9,*trans*-11-CLA	4.61^{b}	11.03^{a}	12.09^{a}	2.042	0.04
trans,*trans* -CLA	0.79	1.91	2.32	0.408	0.23
C20:5n-3, EPA	0.07^{c}	0.27^{b}	0.42^{a}	<0.01	0.01
C22:4n-6	0.13^{c}	0.27^{b}	0.35^{a}	<0.001	0.01
C22:5n-3	0.20^{c}	0.41^{b}	0.49^{a}	<0.001	0.01
C22:6n-3, DHA	0.07^{c}	0.27^{b}	0.42^{a}	<0.001	0.01
Unknown	51.72	83.46	85.92	1.68	0.12
SFA	399.19^{a}	339.77^{c}	345.49^{b}	0.97	0.02
UFA	207.93^{c}	257.53^{b}	271.67^{a}	0.97	0.02
n-6 FA[2]	17.13^{c}	23.21^{b}	24.47^{a}	0.340	0.01
n-3 FA[3]	3.89^{c}	7.15^{b}	8.58^{a}	0.204	0.01
n-6/n-3	4.41^{a}	3.25^{a}	2.85^{b}	0.09	0.01
Indices[4]					
M/S	0.46^{b}	0.64^{a}	0.66^{a}	0.02	0.01
P/S	0.05^{b}	0.09^{a}	0.09^{a}	0.01	0.01
U/S	0.52^{b}	0.76^{a}	0.79^{a}	0.01	0.01

SEM, standard error of the mean; CLA, conjugated linoleic acid; SFA, saturated fatty acids; UFA, unsaturated fatty acids.

[1] PO = supplemented with 300 g/d palm oil as positive control diet; LSO = supplemented with 300 g/d linseed oil; WLS = supplemented with 688 g/d whole linseed.

[2] Calculated as *trans*-9,12-C18:2+*cis*-9,12-C18:2+*cis*-9,12,15-C18:3+C22:4n-6.

[3] Calculated as *cis*-9,12,15-C18:3+C20:5n-3+C22:5n-3+C22:6n-3.

[4] M/S = sum of monounsaturated fatty acids/sum of saturated fatty acids; P/S = sum of polyunsaturated fatty acids/sum of saturated fatty acids; U/S = sum of unsaturated fatty acids/sum of saturated fatty acids.

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

total fat concentration was less than 6% of the DM (Lunsin et al., 2012; Dirandeh et al., 2013; Mach et al., 2013). The animals fed WLS had greater CP intake due to the higher CP concentration in the whole linseed compared to palm oil and linseed oil. The effect of unsaturated FA from LSO and WLS on feed intake has been variable among previous studies. Chilliard et al. (2009) reported that dairy cows' diet based on corn silage and grass hay (65.1:34.9 forage:concentrate [F:C] ratio, DM basis) supplemented with oil from whole crude linseed at 4.2% of dietary DM had no effect on feed intake. In addition, Shingfield et al. (2011) found no effect of 3% LSO supplementation to a corn silage-based diet (60:40 F:C, DM basis) of growing steers on DMI. This result was supported by Benchaar et al. (2012) that LSO supplementation at 2%, 3%, or 4% DM to lactating dairy cows fed a total mixed ration with a R/C ratio of 50:50 had no effect on DM intake and digestibility of nutrients. In contrast, Martin et al. (2008) concluded that lactating dairy cows fed a diet supplemented with LSO at 5.7% DM had significantly lower DMI compared to the control diet, while no negative effects were found when dairy cows were supplemented with crude linseed or extruded linseed. A similar result was also found in the recent study of Lerch et al. (2012) where dairy cows were

supplied with 3.0% oil (DM basis) from extruded linseed in the basal diet containing 60:40 F:C ratio. It is possible that FA intake has a direct inhibitory effect on voluntary intake via inhibition of rumino-recticular motility (Chilliard, 1993). In general, these results suggest that the effect of unsaturated fat from linseed on DMI varies with the amount of added fat, fat type, and F:C ratio of the diet.

Supplementation of LSO and oil from WLS at 300 g/d had no effect on milk yield. Unchanged milk yield was also reported in the studies (Caroprese et al., 2010; Lerch et al., 2012; Weisbjerg et al., 2013). Moreover, dairy cows were supplemented with 13% extruded linseed increased milk yield (Mach et al., 2013). In contrast, supplementation of linseed oil decreased milk yield of dairy cows (Brown et al., 2008; Martin et al., 2008) Indeed, the decrease in milk production reported in some previous studies was associated with a depression in DMI and diet digestibility due to disturbances in rumen function caused by a high level of LSO intake (i.e., >5% of DMI). Discrepancies among studies on effect of linseed supplementation on milk yield of dairy cows might be due to the form of oil, added level, and different duration of the experiment. Allen (2000) concluded that supplementing FA from hydrogenated fat and oilseeds at approximately 23 and 30 g/kg DM added fat, respectively, has no effect on DMI, which may contribute to the explainatioon of why milk yield of dairy cows fed high PUFA-containing diet was similar to those fed high mono- and saturated FA-comprising diet in the current study.

In the current study, supplementing LSO or oil from WLS had no effect on milk concentrations and yields of fat, protein, and lactose which agreed with previous studies (Côrtes et al., 2010; Petit and Côrtes, 2010; Lerch et al., 2012). Similarly, inclusion of 56 g/kg DM ground rapeseed plus 19 g/kg DM ground linseed in the diet of Danish Holstein cows had no effect on milk fat concentration and yield (Weisbjerg et al., 2013). In contrast, dairy cows fed a diet supplemented with 3.1% LSO plus DHA Gold algae (Angulo et al., 2012) had lower milk fat yield compared to the control group fed a basal diet supplemented with 3.1% protected saturated fat. A latter research also reported reduction of milk fat concentration and yield as dairy cows were supplemented with 4.03% DM extruded linseed (Dirandeh et al., 2013); however, no effect was found in concentration of milk protein and lactose. The decrease in milk fat concentration in some previous studies was attributed to lower DMI and nutrient digestibility, particularly fiber, due to the high level of oil ingested (Martin et al., 2008) and lower mammary lipogenesis resulting from supplementing polyunsaturated oil when cows were fed a starch-rich diet (Chilliard et al., 2007).

Dairy milk fat contains over 400 individual FA and their isomers. Cow milk contains large amounts of SFA, particularly C14:0 and C16:0, which lead to physiological dysfunctions including higher plasma cholesterol, and small amounts of mono-unsaturated fatty acids (MUFA), PUFA, and n-3 FA which have beneficial effects on human health. The important concern is how to alter the milk FA profile to make it more suitable for human health. One suggestion is to use an organic system (Tudisco et al., 2010), or feed whole oleaginous plants or oils from them to the cows. Oil from linseed may influence milk FA levels as follows: i) decrease SFA, ii) increase UFA, iii) increase the proportion of the CLA and of the α-linolenic acid, and iv) decrease the n-6 FA to n-3 FA ratio. These possible changes rely on the very high level of C18:3n-3 in flax seeds (40% to 45%). In this study, the lower concentration of milk medium chain FA, particularly C12:0-C16:0 FA, in the LSO and WLS diets indicated less *de novo* FA synthesis occurred with the animals fed these treatments. For this reason, feeding high PUFA oils are typically associated with a reduction in the *de novo* synthesis of short- and medium- chain FA (Brown et al., 2008; Lerch et al., 2012; Weisbjerg et al., 2013). The decrease in milk C12:0-C16:0 concentrations from linseed-supplemented cows may be a positive goal from a human health perspective because high proportions of C14:0 and C16:0 have been shown to combine with human cardiovascular problems (Noakes et al., 1996). The improvement of milk concentrations and yields of n-3 FA, particularly linolenic acid, and CLA in the cows fed diets containing LSO or WLS in the current study was in agreement with some previous studies (Lerch et al., 2012; Mach et al., 2013; Weisbjerg et al., 2013). The higher milk *cis*-9,*trans*-11-CLA originates from ruminal biohydrogenation of linoleic acid as an intermediate product or from endogenous synthesis in the mammary gland from vaccenic acid (VA) (Griinari and Bauman, 1999). The endogenous synthesis of *cis*-9,*trans*-11-CLA from VA has been showed as the major pathway of *cis*-9,*trans*-11-CLA synthesis in lactating cows, accounting for an estimated 80% of the *cis*-9,*trans*-11-CLA in milk fat (Mosley et al., 2006). Milk from LSO and WLS-supplied cows together with the high CLA concentration was characterized by low atherogenic and thrombogenic indices, suggesting that its utilization has less detrimental effects concerning the atherosclerosis and coronary thrombosis risk associated with the consumption of milk and dairy products, consequently being potentially healthier for humans. The average concentration of milk linolenic acid in the study was in agreement with Moate et al. (2007) who reported on 28 publications, that the average level of *cis*-9,12,15-C18:3 in the milk was 5.9% of the total FA, with a range from 0.2 to 19. A decrease of n-6 FA alongside an increase of n-3 FA led to a decrease of n-6 FA to n-3 FA ratio in milk of cows fed LSO and WLS. Lowering the ratio of n-6 to n-3 FA in food products has been recommended to prevent or modulate certain diseases in humans (Connor, 2000). Milk

from cows supplemented with LSO and WLS represented an improvement in the FA profile, with an increase in the MUFA, PUFA, and total UFA concentrations and a decrease in SFA concentration, resultant in increased indices of MUFA, PUFA and UFA to SFA. These results were in agreement with recent studies (Caroprese et al., 2010; Petit and Côrtes, 2010; Lerch et al., 2012), which reported similar changes in milk FA when flaxseed supplements were used.

CONCLUSION

The present study clearly indicated that supplementation of whole linseed to lactating dairy cows had no effect on milk yield and milk composition; however, *trans*-9-C18:1, *cis*-9,*trans*-11-CLA, n-3 FA and unsaturated FA were increased and SFA were decreased by WLS supplementation. Therefore, 300 g/d of oil from whole linseed should be supplemented to lactating dairy cows' diets.

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