J. Dairy Sci. 95:5276–5284
http://dx.doi.org/10.3168/jds.2012-5415
© American Dairy Science Association®, 2012.

Transfer rate of α-linolenic acid from abomasally infused flaxseed oil into milk fat and the effects on milk fatty acid composition in dairy cows

U. Moallem,*[1] D. Vyas,† B. B. Teter,† P. Delmonte,‡ M. Zachut,* and R. A. Erdman†
*Department of Dairy Cattle, Institute of Animal Sciences, Volcani Center, PO Box 6, Bet-Dagan, 50250 Israel
†Department of Animal and Avian Sciences, University of Maryland, College Park 20742
‡Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD 20742

ABSTRACT

The objectives of the present study were to evaluate the transfer efficiency of α-linolenic acid (ALA) from the abomasum into milk fat, its interaction with milk fat content and yield, and the relationship between ALA and C16:0 in milk fat. Three rumen-fistulated multiparous Holstein cows at midlactation were used in a 3 × 3 Latin square design. Treatments consisted of abomasal infusion of (1) 110 mL of water/d (control), (2) 110 mL of flaxseed oil/d (low flaxseed oil, LFO), and (3) 220 mL of flaxseed oil/d (high flaxseed oil, HFO). Experimental periods were continued for 2 wk and fat supplements were infused abomasally during the last 7 d of each period. Average dry matter intake and milk yield were not affected by oil infusion. Milk fat and lactose content tended to be greater with flaxseed infusion compared with the control. Plasma ALA was 2.9- and 4.0-fold greater with LFO and HFO, respectively. The apparent transfer efficiency of ALA to milk was 44.8 and 45.7% with LFO and HFO, respectively. The C16:0 content in milk fat was decreased by 3.59 and 5.25 percentage units, whereas the ALA content was increased by 1.68 and 3.09 percentage units with LFO and HFO, respectively. Similarly, C18:2n-6 was increased by 0.95 and 1.31 percentage units with LFA and HFO, respectively, without changes in other fatty acids (FA). Total polyunsaturated FA was 4.4 and 2.7% lower in the HFO and LFO, respectively, than in the control. Furthermore, C16:0 content in the milk fat was reduced to a greater extent than the increase in ALA content, as a 1.68 and 3.09 percentage unit increase occurred in ALA compared with a 3.6 and 5.25 percentage unit decrease in C16:0 for LFO and HFO, respectively, such that a negative correlation existed between ALA and C16:0 ($r = -0.72$). In conclusion, abomasal infusion of flaxseed oil dramatically increased the ALA content in plasma and milk fat. Because the replacement of C16:0 with ALA and C18:2n-6 occurred without changes in other FA presumed to be synthesized de novo in the mammary gland, this suggests that the preformed C16:0 was replaced, rather than being caused, by an overall suppression of de novo FA synthesis in the mammary gland.
Key words: α-linolenic acid, flaxseed oil

INTRODUCTION

Omega-3 FA plays significant roles in several biological pathways (Deckelbaum et al., 2006). The chain length and the position of the double bonds provide distinct characteristics to these FA that confer them unique biological capabilities that are beneficial to human health; for example, they have been shown to decrease the incidence of cardiovascular diseases, hypertension, and arthritis (Simopoulos, 2002). Therefore, a growing interest exists in enhancing the proportion of these FA in milk fat.

Flaxseed is a rich source of n-3 FA, containing approximately 50% α-linolenic acid (**ALA**). Studies on feeding flaxseed to dairy cows resulted in an increased n-3 FA content and a decreased n-6/n-3 ratio in milk fat. Several studies investigated the effect of feeding a variety of flaxseed forms to test the magnitude of transfer of ALA into milk fat and the results were inconsistent. The rate of increase of ALA in milk fat ranged from 1.8 to 3 fold (Petit et al., 2004; Ambrose et al., 2006). Differences in the apparent transfer rate between studies may be ascribed to the difference in the proportion of ALA in the control groups and to the form of flaxseed fed.

In a recent study (Zachut et al., 2010), feeding high rates of extruded flaxseed (9.2% of diet, providing 402.5 g of ALA/d per cow) to dairy cows increased the proportion of ALA in milk by up to 2 percentage points. However, enrichment of ALA in milk fat was also negatively correlated with milk fat percentage. Although no change in de novo synthesized FA of less than 16 carbons was found, C16:0 yields were markedly decreased. Furthermore, the yield of C16:0 was nega-

Received February 7, 2012.
Accepted June 3, 2012.
[1]Corresponding author: uzim@volcani.agri.gov.il

tively correlated with ALA content in milk fat (Zachut et al., 2010).

We hypothesized that ALA per se suppresses de novo synthesis of C16:0 in the mammary gland without impairing the synthesis of short-chain FA. Direct feeding of fat sources containing high concentrations of ALA such as flaxseed would likely alter production of rumen biohydrogenation end products such *trans*-10,*cis*-12 conjugated linoleic acid (**CLA**) and other C18:1 and C18:2 *trans* isomers, which are responsible for depressing milk fat synthesis and altering milk FA composition; indeed, several studies reported higher concentrations of CLA and C18:1 *trans* isomers in milk fat of cows supplemented with extruded flaxseed (Mustafa et al., 2003; Gonthier et al., 2005; Chilliard et al., 2009). Therefore, abomasal infusion of flaxseed oil allows for study of the effects of ALA on milk fat synthesis and composition without the confounding effects of changes in rumen biohydrogenation. The objectives of the present experiment were to evaluate the transfer efficiency of postruminal ALA from flaxseed oil to milk fat, the interaction of ALA with milk fat content and yield, and the relationship between ALA and C16:0 in milk fat.

MATERIALS AND METHODS

The experimental protocol and procedures were approved by the University of Maryland Institutional Animal Care and Use Committee (College Park). Three rumen-fistulated multiparous Holstein cows in midlactation (49 ± 20 DIM; mean ± SD) were used in a 3 × 3 Latin square design, with 14-d experimental periods. Treatments consisted of twice-daily (0630 and 1900 h) abomasal infusion of the following: (1) 110 mL of water/d (control), (2) 110 mL of flaxseed oil/d (low flaxseed oil, **LFO**), and (3) 220 mL of flaxseed oil/d (high flaxseed oil, **HFO**). Cows were not infused during d 1 to 7 of each experimental period to reduce carryover effects of the previous treatment. Abomasal infusion of different treatments was from d 8 to 14 of each period. Oil was filled in syringes and was infused via Tygon tubing (0.48-cm i.d., 0.64- cm o.d.; VWR Scientific Inc., Bridgeport, NJ) as described by Kadegowda et al. (2008). The daily amounts of infused flaxseed oil or water were split into 2 equal portions and manually infused twice daily, at 0630 and 1900 h. Patency and location of the infusion line inside the cow were checked on alternate days.

Cows were housed in individual tie-stalls and were fed a basal diet containing 55% forage and 45% concentrate (DM basis) to meet NRC (2001) nutrient specifications for a 650-kg cow producing 40 kg of milk containing 3.7% milk fat and 3.1% milk protein. Ingredients and chemical composition of the basal diet are shown in Table 1. Diets were fed as TMR once daily at 0800 h. Corn silage DM was determined weekly, and the TMR was adjusted accordingly to maintain a constant forage-to-concentrate ratio on a DM basis. Amounts of feed offered and refused were recorded once daily. Cows were milked twice per day at 0600 and 1600 h, and milk production was recorded electronically at each milking.

Samples for milk composition and FA analysis were collected from 2 consecutive milkings on d 7 of each period, before infusion, and from 6 consecutive milkings on d 12 to 14 of each experimental period. Milk fat, CP, and SCC were determined by infrared analysis (MilkoScan; Foss Food Technology Corp., Eden Prairie, MN) on fresh samples from individual milkings. A subset of samples from each milking was composited and frozen at −20°C for subsequent analysis of FA profile in milk fat.

Table 1. Ingredient and chemical composition of the basal diet

Composition	Value
Ingredient, % of DM	
Corn silage	55.04
Corn grain, ground	22.01
Soybean meal	19.19
Corn gluten meal	0.45
Limestone	0.62
BioPhos[1]	0.43
Magnesium oxide	0.16
Sodium bicarbonate	0.57
Dynamate[2]	0.13
Salt	0.38
Trace minerals and vitamins[3]	0.46
Megalac[4]	0.56
Chemical composition (DM basis; % of DM unless noted)	
DM, %	58.16
CP	16.39
RUP	42.63[5]
ADF	15.19
NDF	26.22
NE_L, Mcal/kg	1.56[5]
Ca	0.75
P	0.43
Mg	0.26
K	1.36
S	0.23
Na	0.29
Cl	0.39
DCAD, mEq/100 g of DM	21.78

[1]IMC-Agrico, Bannockburn, IL.

[2]The Mosaic Co. (Plymouth, MN).

[3]Trace mineral and vitamin mix combined (per kilogram of mix): 1,732 mg of Co, 2,207 mg of Cu, 1,196 mg of Fe, 141 mg of I, 980 mg of Mn, 8,153 mg of Zn, 75 mg of Se, 819,000 IU of vitamin A, 273,000 of vitamin D, and 4,880 IU of vitamin E.

[4]Church and Dwight Co. Inc., Princeton, NJ.

[5]Calculated value.

Blood samples were collected twice per week before the evening milking on Monday and Thursday. They were collected from the tail vein into vacuum tubes containing lithium heparin (Becton Dickinson Systems, Cowley, UK), and plasma was immediately collected after centrifugation at 1,500 × *g* for 20 min, and stored at −18°C pending analysis.

Plasma FA Composition

Total FA in plasma (100 μL) were extracted according to Moallem et al. (1999). Briefly, the samples were saponified in a mixture of 60% KOH and ethanol, extracted with petroleum ether, and methylated with 5% sulfuric acid in methanol. The FA methyl esters (**FAME**) were analyzed with a model 7890N gas chromatograph (Agilent Technologies Inc., Santa Clara, CA) equipped with a DB-23 capillary column (60 m × 0.25 mm, 0.25-μm film width; Agilent Technologies Inc.) equipped with a flame ionization detector. The initial temperature of the column was set at 130°C, increased at 6.5°C/min to 170°C, and then increased at 2.75°C/min to 215°C, at which it was held for 18 min. Then, the temperature was increased to 230°C at 40°C/min for the remainder of the analysis. The carrier gas was hydrogen, flowing at a linear velocity of 1.6 m/min; the injection volume was 1 μL.

Flaxseed and Milk FA Composition

Milk fat was extracted according to reference procedure (IDF 1C:1987, IDF 16C: 1987; International Dairy Federation, Brussels, Belgium) as adopted by Shingfield et al. (2003) and Shingfield et al. (2006). Briefly, the lipids were extracted from 1 mL (~30–35 mg of lipids) using a mixture of diethyl ether and hexane (5:4, vol/vol). The procedure was repeated 3 times and the combined organic extracts were evaporated to dryness in a water bath at 37°C under nitrogen. Samples were further dissolved in hexane and the FAME were prepared by mild transesterification with 1.4 mol/L of H_2SO_4 in methanol (Christie, 1982). Similarly, the amount of flaxseed oil used to prepare FAME provided approximately 20 to 30 mg of total lipids.

Separations of FAME were achieved using an Agilent 6890N gas chromatograph (Agilent Technologies Inc., Wilmington, DE) equipped with a SLB-IL111 capillary column and a flame ionization detector. Hydrogen was used as carrier gas at 1 mL/min constant flow with the linear velocity of 30 cm/s. Air flow was maintained at 400 mL/min. Nitrogen was used as the makeup gas with flow rate of 33 mL/min. The oven was maintained at 169°C isothermal temperature, the injection port at 250°C, and the detector at 250°C. The split ratio was set to 1:100 and the typical injection volume was 1μL.

Short- and medium- chain FA (C4–C14) in milk were analyzed as FA butyl esters (**FABE**), which were mathematically converted to FAME and normalized to the FAME chromatogram (Gander et al., 1962) with C14:0 used as cutoff point. The original FABE procedure was modified as follows: milk samples were heated in screw-capped test tubes at 80°C for 1 h. in the presence of 1.4 *N* H_2SO_4 in butanol and then extracted with hexane in the presence of saturated KCl and distilled water. Samples were then centrifuged at 500 × *g* for 5 min. An aliquot of the upper hexane layer was injected directly into a Hewlett-Packard 5880 gas-liquid chromatograph equipped with a split injector, a flame ionization detector, and a 25-m × 0.2-mm fused silica capillary column coated with HP1 (Hewlett-Packard, Avondale, PA). Helium was used as the carrier gas at a flow rate of 2 mL/min with a split ratio of 45:1. The injector temperature and detector temperature were set at 250°C, whereas the column temperature started at 130°C. The ramp was set at 6°C/min to 290°C, followed by 4°C/min to 260°C, and finally holding at 260°C for 20 min. Standard mixtures, including GLC-60 (Nu-Chek Prep Inc., Elysian, MN), were converted to FABE to aid in the identification and quantification of FA.

Statistical Analysis

Dry matter intake, milk production, milk components, plasma, and milk FA composition data were analyzed as 3 × 3 Latin square design using the MIXED procedure of SAS (SAS Institute, 2002). Treatment was analyzed as a fixed effect, whereas cow and period were analyzed random effects in the statistical model

$$Y_{ijkl} = \mu + T_i + P_j + C_k + e_{ijkl},$$

where Y_{ijkl} is the observation, μ is the overall mean, T_i is the effect of the ith treatment (i = 1, 2, or 3), P_j is the effect of the jth period (j = 1, 2, or 3), C_k is the effect of the kth cow (k = 1, 2, or 3), and e_{ijkl} is the residual error. The PROC REG procedure of SAS (SAS Institute, 2002) was used for correlation analysis. Data are presented as least squares means, with $P < 0.05$ used as the accepted level of significance unless otherwise stated.

RESULTS AND DISCUSSION

The FA profile of the basal diet and the flaxseed oil are in Table 2. α-Linolenic acid was a minor constituent of the basal diet FA, whereas flaxseed oil contained

Table 2. The FA composition (g/100 g) of the basal diet and abomasally infused flaxseed oil

FA,[1] g/100 g of FAME	Basal diet	Flaxseed oil
C12:0	0.34	
C12:1	0.28	
C14:0	0.57	
C14:1 *c*9	0.34	
C16:0	21.27	5.33
C16:1 *c*9	1.73	
C18:0	3.57	4.27
C18:1 *c*9	21.54	21.78
C18:1 *c*11	0.82	0.51
C18:2 *c*9,*c*12	34.88	16.09
C18:3 *c*9,*c*12,*c*15	6.33	50.80
C20:0	0.95	0.17
C20:2n-6	0.03	
C20:3n-6	0.17	
C22:0	1.18	
C22:1 *c*11	0.34	
C24:0	1.35	
C24:1	0.20	
Others[2]	4.00	1.05
Total FA, %	3.06	87.35

[1]FAME = FA methyl esters; *c* = *cis*.
[2]Other minor FA.

50.8% ALA. The flaxseed oil in the LFO and HFO treatments provided 52 and 104 g of ALA/d, respectively.

Effects on DMI

Postruminal infusion of ALA-rich flaxseed oil did not affect DMI (Table 3). Several studies have examined the effects of various FA on DMI in dairy cows. Abomasal infusion of 400 g of ALA-rich flaxseed oil/d did not affect DMI; however, intraruminal delivery of various flaxseed products (400 g of flaxseed oil and 1.8 kg of flax hulls) decreased DMI (Kazama et al., 2010). Similarly, duodenal infusion of ALA-rich FFA mixture (40 to 160 g/d) had no effects on DMI (Khas-Erdene et al., 2010). Abomasal infusion of increasing amounts of oleic acid (18:1 *cis*-9; 250–1,000 g/d) dramatically decreased DMI and milk yields (Drackley et al., 2007). Abomasal infusion of canola oil (62.5% C18:1 and 24.1% C18:2) or high oleic sunflower oil (86% C18:1) up to 400 g/d decreased DMI (LaCount et al., 1994). Kadegowda et al. (2008) infused either 400 g/d of butterfat as a source of short- and long-chain FA, or 250 g of long-chain FA/d without effects on DMI. Drackley et al. (2007) suggested that unsaturated FA, but not SFA, were potent inhibitors of DMI. The above findings demonstrate different effects of postruminal infusion of various FA on DMI, suggesting FA-specific regulation of feed intake. For example, in several studies, postruminal infusion of ALA did not reduce DMI (Kazama et al., 2010; Khas-Erdene et al., 2010), which agrees with our findings, whereas infusion of various amounts of C18:1 (LaCount et al., 1994; Jenkins, 1999; Drackley et al., 2007) showed a negative effect on DMI. It appears that the effect of unsaturated FA on DMI, as suggested by Drackley et al. (1992), is FA specific and not necessarily related to the energy provided by postruminal fat supplementation.

Effects on Milk and Milk Component Responses

Flaxseed oil infusion had no effect on milk and milk solid yields despite a calculated additional 0.58 and 1.16 Mcal of NE_L/d available with LFO and HFO treatments, respectively. Milk yield was maintained in studies with postruminal oil infusion when DMI was not decreased (Kadegowda et al., 2008; Khas-Erdene et al., 2010). However, when high rates of postruminal oil infusion decreased DMI, milk yield was depressed (Gagliostro and Chilliard, 1991; Drackley et al., 2007). Similar negative effects on milk yield have been ob-

Table 3. Least squares means for DMI, milk production, and milk composition from cows fed a basal diet and abomasally infused with 110 mL of water/d (control), 110 mL of flaxseed oil/d (LFO), or 220 mL of flaxseed oil/d (HFO)

	Treatment				
Item	Control	LFO	HFO	SEM	*P*-value[1]
DMI, kg/d	26.0	25.4	26.6	1.60	0.49
DMI total,[2] kg/d	26.0	25.5	26.8	1.61	0.49
Milk, kg/d	44.8	45.0	46.0	2.30	0.55
Fat, %	3.34	3.60	3.57	0.12	0.10
Protein, %	3.00	3.08	3.03	0.04	0.44
Lactose, %	5.72	5.65	5.67	0.13	0.07
Fat, kg/d	1.54	1.61	1.68	0.11	0.55
Protein, kg/d	1.36	1.37	1.43	0.08	0.65
Lactose, kg/d	2.60	2.52	2.68	0.19	0.52

[1]Probability that treatment means are not different.
[2]Including infused oil.

served when infusion or feeding flaxseed oil resulted in decreased DMI (Kazama et al., 2010). However, Côrtes et al. (2011) observed decreased DMI with abomasal infusion of flaxseed oil without effects on milk production.

Milk fat percentage tended ($P = 0.10$) to be higher in the LFO and HFO than in the control. Similar responses were observed earlier with abomasal infusion of flax oil (Côrtes et al., 2011), with ALA in FFA form (Khas-Erdene et al., 2010) or with C18:1 *cis*-9 infusion (Drackley et al., 2007). In studies where flaxseed was fed rather than infused postruminally, milk fat percentage was decreased (Mustafa et al., 2003; Petit et al., 2007; Chilliard et al., 2009; Zachut et al., 2010, 2011), but not in the others (Gonthier et al., 2005; Akraim et al., 2007). Milk fat responses to feeding flaxseed are most likely due to generation of unique FA isomers during rumen biohydrogenation of unsaturated FA (Bauman and Griinari, 2003). Indeed, several studies have reported greater concentrations of CLA and C18:1 *trans* isomers, associated with milk fat depression, in milk fat of cows supplemented with extruded flaxseed (Mustafa et al., 2003; Gonthier et al., 2005; Chilliard et al., 2009). However, postruminal infusion of flaxseed oil prevented generation of these isomers (Tables 5 and 6), which would explain the trend for increase in milk fat content with LFO and HFO, rather than a decrease observed in previous feeding studies. No differences were observed in milk protein percentage between treatments, which was also found in other reports (Khas-Erdene et al., 2010; Côrtes et al., 2011); however, in the present study, lactose percentage tended to be lower the LFO than in the control (Table 3), which was not reported elsewhere.

Effects on Plasma and Milk FA Composition

Abomasal infusion of flaxseed oil increased the ALA concentrations in plasma (Table 4) and milk (Table 5). The proportion of ALA in total plasma lipid was 2.9- and 4.0-fold greater in the LFO and HFO treatment groups than in the control group, respectively. In a previous study, the plasma proportion of ALA in cows that were fed extruded flaxseed, providing 376.2 g of ALA/d, was 5.43%, which was 5.3-fold higher than in the control group (1.03%; Zachut et al., 2010). However, in the present study, the proportion of ALA in the plasma of the control group was 2.8-fold higher than in the control group of the previous study (2.9 vs. 1.03%, respectively), probably due to greater ALA content in

Table 4. Least squares means for FA composition in total plasma lipids of cows fed a basal diet and abomasally infused with 110 mL of water/d (control), 110 mL of flaxseed oil/d (LFO), or 220 mL of flaxseed oil/d (HFO)

	Treatment				
FA, g/100 g of FAME[1]	Control	LFO	HFO	SEM	*P*-value[2]
C14:0	3.5	3.0	3.6	0.73	0.53
C16:0	24.4	28.9	27.1	2.26	0.23
C16:1	1.6	1.5	2.1	0.75	0.75
C16:2	1.3	1.2	0.9	0.32	0.08
C16:3	0.6	0.7	0.4	0.21	0.47
C18:0	12.0	11.1	10.4	0.60	0.36
C18:1n-9	5.4	3.9	4.1	1.24	0.71
C18:1n-7	1.3	1.5	1.5	0.57	0.91
C18:2n-6	34.3	29.6	28.0	1.74	0.16
C18:3n-6	1.2	0.6	0.5	0.17	0.13
C18:3n-3	2.9[a]	8.4[b]	11.7[c]	1.18	0.01
C18:4	0.2	0.3	0.8	0.26	0.38
C20:1n-9	0.6	0.5	0.4	0.03	0.07
C20:3	1.3	1.0	0.6	0.16	0.12
C20:4n-6	1.4	1.2	1.0	0.16	0.22
C20:4n-3	0.1	0.4	0.3	0.06	0.11
C20:5n-3	6.6	5.2	5.3	0.80	0.38
C22:1n-9	0.6[a]	0.5[ab]	0.3[b]	0.07	0.05
C22:5n-3	0.2	0.3	0.2	0.10	0.84
C22:6n-3	0.4	0.3	0.5	0.24	0.84
Sum n-3 FA	10.2[a]	14.5[b]	18.1[c]	1.79	0.01
Total plasma lipids, mg/dL	506.7[c]	629.3[a]	580.4[b]	49.8	0.001

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]FAME = FA methyl esters.

[2]Probability that treatment means are not different.

Table 5. Least squares means for milk FA concentrations (g/100 g of FAME[1]) from cows fed a basal diet and abomasally infused with 110 mL of water/d (control), 110 mL of flaxseed oil/d (LFO), or 220 mL of flaxseed oil/d (HFO)

	Treatment				
FA,[2] g/100 g of FAME	Control	LFO	HFO	SEM	*P*-value[3]
C4:0	3.84	3.98	4.13	0.294	0.65
C6:0	2.13	2.27	2.38	0.139	0.44
C8:0	1.33	1.40	1.45	0.067	0.09
C10:0	3.12	3.25	3.31	0.237	0.22
C12:0	3.96	3.90	3.76	0.298	0.59
C13:0	0.30[a]	0.29[a]	0.23[b]	0.025	0.05
C14:0	12.36	11.65	11.37	0.443	0.15
C14:1 *c*9	1.17[a]	0.89[ab]	0.81[a]	0.065	0.04
C15:0	1.86	1.84	1.46	0.150	0.02
C15:1 *c*9	0.02	0.01	0.01	0.006	0.63
C16:0	36.25[a]	32.66[b]	31.00[b]	1.272	0.02
C16:1[4]	2.18	1.67	1.55	0.167	0.13
C17:0	1.08	1.09	1.04	0.108	0.93
C18:0	6.74	7.86	8.26	0.572	0.19
C18:1[5]	19.12	19.99	20.13	0.446	0.14
C18:2 *c*9,*c*12	2.58[b]	3.53[ab]	3.89[a]	0.258	0.04
C18:3 *c*9,*c*12,*c*15	0.21[c]	1.89[b]	3.30[a]	0.426	0.02
C20:0	0.12	0.13	0.14	0.007	0.21
C20:1 *c*9	0.13	0.12	0.14	0.005	0.18
C20:2n-6	0.04[b]	0.04[b]	0.06[a]	0.003	0.05
C20:3n-6	0.09	0.11	0.11	0.014	0.14
C20:4n-3	0.00	0.00	0.01	0.005	0.37
C20:5n-3	0.01	0.02	0.02	0.003	0.19
C22:0	0.01	0.02	0.03	0.005	0.22
C22:1 *c*11	0.03	0.03	0.03	0.003	0.61
C22:4n-6	0.03[b]	0.03[ab]	0.04[a]	0.003	0.08
C22:5n-3	0.05[b]	0.08[a]	0.08[a]	0.007	0.03
*t*10,*c*12 CLA	0.03	0.03	0.03	0.003	0.87
*c*9,*t*11 CLA	0.23	0.21	0.22	0.017	0.62
n-3	0.28[c]	1.98[b]	3.40[a]	0.433	0.05
SFA	73.1[a]	70.4[b]	68.6[b]	0.641	0.03
MUFA	22.63	22.71	22.67	0.526	0.99
PUFA	3.01[b]	5.70[a]	7.51[a]	0.642	0.03
Total FA	3.12	3.36	3.33	0.110	0.15

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]FAME = FA methyl esters.

[2]*c* = *cis*; *t* = *trans*; CLA = conjugated linoleic acid.

[3]Probability that treatment means are not different.

[4]16:1 *c*7 + *c*9 + *c*11 + *c*13.

[5]18:1 *t*5 + *t*7 + *t*8 + *t*9 + *t*10 + *t*11 + *t*13/14 + *t*16 + *c*9 + *c*11 + *c*12 + *c*13 + *c*14 + *c*15.

the control diet of the present experiment. Differences between studies might also be related to stage of lactation and diet composition.

As expected, the FA composition of milk fat was altered by ALA-rich flaxseed oil infusion (Table 5). The percentage of C16:0 in cows infused with LFO and HFO was 3.6 ($P < 0.02$) and 5.25 percentage points ($P < 0.01$) lower than in the control, respectively. The proportion of C18:2n-6 was greater in cows that were infused with HFO than in the control, but not different from LFO. The percentage of ALA in cows infused with LFO and HFO was 9- and 15.7-fold higher than in the control, and that of C22:5n-3 was similar between LFO and HFO, but both were higher than in the control. Total SFA were 2.7 and 4.5 percentage points higher in the control than in the LFO ($P < 0.05$) and HFO ($P < 0.02$), respectively, with no differences in MUFA. Total PUFA were 4.5 and 1.8 percentage units higher in the HFO and LFO, respectively, than in the control ($P < 0.03$).

Average daily yields of C16:0 tended to be lower in the LFO ($P < 0.08$) and HFO ($P < 0.1$) than in the control (Table 6). Daily yields of ALA were 16- and 8.5-fold higher in the HFO ($P < 0.006$) and LFO ($P < 0.02$), respectively, than in the control. Yields of C22:5n-3 were greater in the HFO ($P < 0.02$) and LFO ($P < 0.04$) than in the control. Polyunsaturated FA yields were 2.7- ($P < 0.007$) and 1.8- ($P < 0.03$) fold higher in the HFO and LFO, respectively, than in the control.

Table 6. Least squares means for yield of FA (g/d) in milk from cows fed a basal diet and abomasally infused with 110 mL of water/d (control), 110 mL of flaxseed oil/d (LFO), or 220 mL of flaxseed oil/d (HFO)

	Treatment				
FA,[1] g/100 g of FAME	Control	LFO	HFO	SEM	*P*-value[2]
C4:0	56.4	57.7	65.5	7.54	0.15
C6:0	31.1[b]	32.9[b]	37.7[a]	3.80	0.04
C8:0	19.4[b]	20.2[b]	23.1[a]	2.05	0.02
C10:0	45.3[b]	46.5[ab]	52.4[a]	4.03	0.05
C12:0	57.4	55.6	59.4	4.17	0.63
C13:0	4.4[a]	4.1[ab]	3.7[b]	0.43	0.04
C14:0	180.2	166.6	179.3	9.46	0.39
C14:1 *c*9	17.2	12.7	12.7	1.25	0.15
C15:0	27.2	26.4	23.1	3.01	0.11
C15:1 *c*9	0.2	0.2	0.2	0.09	0.56
C16:0	532.8	470.3	487.7	41.85	0.15
C16:1[3]	32.2	24.0	24.4	3.35	0.20
C17:0	15.8	15.5	16.4	1.99	0.93
C18:0	97.7[b]	113.8[ab]	130.3[a]	11.30	0.04
C18:1[4]	280.1	288.3	318.0	25.50	0.18
C18:2 *c*9,*c*12	37.6[b]	50.4[ab]	60.9[a]	3.40	0.04
C18:3 *c*9,*c*12,*c*15	3.1[c]	26.4[b]	50.6[a]	4.76	0.01
C20:0	1.7[c]	1.9[ab]	2.1[a]	0.14	0.05
C20:1 *c*9	1.9[ab]	1.8[b]	2.1[a]	0.15	0.05
C20:2n-6	0.6[b]	0.6[b]	0.9[a]	0.09	0.01
C20:3n-6	1.3[b]	1.5[ab]	1.7[a]	0.27	0.03
C20:4n-3	0.0	0.1	0.1	0.06	0.40
C20:5n-3	0.1	0.3	0.3	0.05	0.19
C22:0	0.2	0.3	0.4	0.08	0.19
C22:1 *c*11	0.4	0.4	0.5	0.07	0.59
C22:4n-6	0.4	0.5	0.6	0.07	0.12
C22:5n-3	0.8[b]	1.1[a]	1.3[a]	0.07	0.04
*t*10,*c*12 CLA	0.4	0.4	0.4	0.05	0.93
*c*9,*t*11 CLA	3.4	3.1	3.5	0.42	0.11
Sum n-3	4.0[c]	27.8[b]	52.2[a]	4.82	0.01
Sum C4–C15	438.7	423.3	457.1	30.57	0.26
SFA	1,069.5	1,012.9	1,081.1	73.09	0.29
MUFA	332.1	327.7	357.8	28.80	0.38
PUFA	43.9[c]	80.9[b]	116.4[a]	6.22	0.01
Total FA	1,440	1,500	1,490	35.03	0.60

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]FAME = FA methyl esters; *c* = *cis*; *t* = *trans*; CLA = conjugated linoleic acid.

[2]Probability that treatment means are not different.

[3]16:1 *c*7 + *c*9 + *c*11 + *c*13.

[4]18:1 *t*5 + *t*7 + *t*8 + *t*9 + *t*10 + *t*11 + *t*13/14 + *t*16 + *c*9 + *c*11 + *c*12 + *c*13 + *c*14 + *c*15.

The ALA percentage in milk fat was 1.89 and 3.3% of total FA for the LFO and HFO, respectively (Table 5). Abomasal infusion of 250 and 500 g of flaxseed oil/d resulted in 7.5 and 12.4% of ALA in milk fat (Côrtes et al., 2011), whereas duodenal infusion of 80, 120, and 160 g of ALA-rich fat/d resulted in 12.4, 18.8, and 25.4% of ALA in milk fat, respectively (Khas-Erdene et al., 2010). These differences between studies can be attributed to the amounts infused and total milk yield, which averaged 34 and 16 kg/d in Côrtes et al. (2011) and Khas-Erdene et al. (2010), respectively, compared with a milk yield of 45 kg/d in the present study. To the best of our knowledge, the HFO group achieved the highest milk FA yields (g/d) of ALA (50.6 g/d) ever reported.

Transfer Efficiency of ALA to Milk Fat

Apparent transfer efficiency of ALA was estimated by dividing the amount of ALA in milk fat (minus the control cows' yields) divided by the amounts of ALA infused. The apparent transfer efficiency for ALA was 44.8 and 45.7% for the LFO and HFO, respectively. Similarly, the apparent transfer efficiency of ALA in a recent study (Côrtes et al., 2011) was 44.7 and 41.0% for cows infused with 256 and 521 g/d of flaxseed oil, respectively. It should be indicated that the similarity in transfer rate of ALA between both studies was not influenced by milk yields, which were greatly different. In contrast, the transfer rate of ALA from diet to milk fat averaged 5.9% in cows fed extruded flaxseed,

providing 376.2 g of ALA/d per cow (Zachut et al., 2010), and only 2% for cows fed a variety of flaxseed sources (Gonthier et al., 2005). The extremely low apparent transfer efficiency for PUFA in the diet is almost certainly attributable to high rates of rumen biohydrogenation of ALA, reducing the supply reaching the site of absorption in the small intestine, limiting the supply available to the mammary gland for incorporation into milk fat, as suggested by Gonthier et al. (2005).

Relationship Between PUFA and C16:0 in Milk Fat

Flaxseed supplies not only ALA but also C18:2n-6. The increase in ALA proportion and, to a lesser extent, C18:2n-6 content in milk fat was accompanied by a marked reduction of C16:0 but not of other de novo synthesized FA. These results are comparable to previous studies with other ALA sources (Khas-Erdene et al., 2010; Côrtes et al., 2011).

Amounts of ALA infused were negatively correlated with proportions of C16:0 in milk fat ($r = -0.75$, $P < 0.02$), whereas the correlation between infused ALA and proportion of ALA in milk fat was highly positive ($r = 0.90$; $P < 0.001$). Furthermore, the milk fat proportions of ALA and C16:0 were negatively correlated ($r = -0.72$; $P < 0.03$) in our experiment. Similar responses were observed in cows fed extruded flaxseed (Zachut et al., 2010). The C16:0 content in milk fat was decreased by 3.59 and 5.25 percentage points, whereas ALA content was increased by 1.68 and 3.09 percentage points with LFO and HFO, respectively. Similarly, C18:2n-6 was increased by 0.95 and 1.31 percentage points with LFA and HFO, respectively. Because the reduction in other FA was minor, it seems that an inverse relationship exists between ALA and C18:2n-6 and C16:0 in milk fat. Using the data from both studies, we hypothesize that PUFA availability may either depress C16:0 synthesis in the mammary gland or replace the portion derived from circulating (dietary and mobilized) sources. As other short-chain (de novo) FA were not affected, our data suggest the latter. Regardless, the change in C16:0 decreased the SFA:PUFA ratio in milk fat from 24.4 for the control to 13.0 and 9.4 for LFO and HFO, respectively ($P < 0.005$). The decrease in the SFA:PUFA ratio in milk products may be desirable and may contribute to production of more healthy milk products, independent of the direct affect of n-3 FA.

CONCLUSIONS

Abomasal infusion of ALA-rich flaxseed oil tended to increase milk fat content in contrast with previous feeding studies where milk fat was depressed. Most likely, this is simply due to limiting the effect on rumen biohydrogenation by abomasal infusion. Concentrations and yields of ALA in milk fat were increased with flaxseed oil infusion and corresponded with a relatively high apparent transfer efficiency of 45 to 46%. This suggested that if ALA is presented to the small intestine, it will be absorbed and incorporated into milk fat. Increased contents of ALA and C18:2n-6 were accompanied by reduced C16:0, suggesting an inverse relationship between both of these FA. The replacement of C16:0 with ALA and C18:2n-6 occurred without changes in other FA presumed to be synthesized de novo in the mammary gland; this suggests that the preformed C16:0 was replaced, rather than being caused, by an overall suppression de novo FA synthesis in the mammary gland.

ACKNOWLEDGMENTS

The authors thank Michael Dwyer and Brian Spielman at the Central Maryland Research and Education Center (CMREC, Ellicott City, MD) for assistance with feeding, infusions, and animal care during this study.

REFERENCES

Akraim, F., M. C. Nicot, P. Juaneda, and F. Enjalbert. 2007. Conjugated linolenic acid (CLnA), conjugated linoleic acid (CLA) and other biohydrogenation intermediates in plasma and milk fat of cows fed raw or extruded linseed. Animal 1:835–843.

Ambrose, D. J., J. P. Kastelic, R. Corbett, P. A. Pitney, H. V. Petit, J. A. Small, and P. Zalkovic. 2006. Lower pregnancy losses in lactating dairy cows fed a diet enriched in α-linolenic acid. J. Dairy Sci. 89:3066–3074.

Bauman, D. E., and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annu. Rev. Nutr. 23:203–227.

Chilliard, Y., C. Martin, J. Rouel, and M. Doreau. 2009. Milk fatty acids in dairy cows fed whole crude linseed, extruded linseed, or linseed oil, and their relationship with methane output. J. Dairy Sci. 92:5199–5211.

Christie, W. W. 1982. Lipid Analysis. 2nd ed. Page 22. Pergamon Press Ltd., Oxford, UK.

Côrtes, C., R. Kazama, D. da Silva-Kazama, C. Benchaar, L. M. Zeoula, G. T. Santos, and H. V. Petit. 2011. Digestion, milk production and milk fatty acid profile of dairy cows fed flax hulls and infused with flax oil in the abomasum. J. Dairy Res. 78:293–300.

Deckelbaum, R. J., T. S. Worgall, and T. Seo. 2006. n-3 Fatty acids and gene expression. Am. J. Clin. Nutr. 83(Suppl.):1520S–1525S.

Drackley, J. K., T. H. Klusmeyer, A. M. Trusk, and J. H. Clark. 1992. Infusion of long-chain fatty acids varying in saturation and chain length into the abomasum of lactating dairy cows. J. Dairy Sci. 75:1517–1526.

Drackley, J. K., T. R. Overton, G. Ortiz-Gonzalez, A. D. Beaulieu, D. M. Barbano, J. M. Lynch, and E. G. Perkins. 2007. Responses to increasing amounts of high-oleic sunflower fatty acids infused into the abomasum of lactating dairy cows. J. Dairy Sci. 90:5165–5175.

Gagliostro, G., and Y. Chilliard. 1991. Duodenal rapeseed oil infusion in early and midlactation cows. 2. Voluntary intake, milk production, and composition. J. Dairy Sci. 74:499–509.

Gander, G. W., R. G. Jensen, and J. Sampugna. 1962. Analysis of milk fatty acids by gas-liquid chromatography. J. Dairy Sci. 45:323–328.

Gonthier, C., A. F. Mustafa, D. R. Ouellet, P. Y. Chouinard, R. Berthiaume, and H. V. Petit. 2005. Feeding micronized and extruded

flaxseed to dairy cows: Effects on blood parameters and milk fatty acid composition. J. Dairy Sci. 88:748–756.
Jenkins, T. C. 1999. Lactation performance and fatty acid composition of milk from Holstein cows fed 0 to 5% oleamide. J. Dairy Sci. 82:1525–1531.
Kadegowda, A. K. G., L. S. Piperova, P. Delmonte, and R. A. Erdman. 2008. Abomasal infusion of butterfat increases milk fat in lactating dairy cows. J. Dairy Sci. 91:2370–2379.
Kazama, R., C. Côrtes, D. da Silva-Kazama, N. Gagnon, C. Benchaar, L. M. Zeoula, G. T. D. Santos, and H. V. Petit. 2010. Abomasal or ruminal administration of flax oil and hulls on milk production, digestibility, and milk fatty acid profile of dairy cows. J. Dairy Sci. 93:4781–4790.
Khas-Erdene, J. Q. Wang, D. P. Bu, L. Wang, J. K. Drackley, Q. S. Liu, G. Yang, H. Y. Wei, and L. Y. Zhou. 2010. Short communication: Responses to increasing amounts of free α-linolenic acid infused into the duodenum of lactating dairy cows. J. Dairy Sci. 93:1677–1684.
LaCount, D. W., J. K. Drackley, S. O. Laesch, and J. H. Clark. 1994. Secretion of oleic acid in milk fat in response to abomasal infusions of canola or high oleic sunflower fatty acids. J. Dairy Sci. 77:1372–1385.
Moallem, U., Y. Folman, A. Bor, A. Arav, and D. Sklan. 1999. Effect of calcium soaps of fatty acids and administration of somatotropin on milk production, preovulatory follicular development, and plasma and follicular fluid lipid composition in high yielding dairy cows. J. Dairy Sci. 82:2358–2368.
Mustafa, A. F., P. Y. Chouinard, and D. A. Christensen. 2003. Effects of feeding micronized flaxseed on yield and composition of milk from Holstein cows. J. Sci. Food Agric. 83:920–926.
NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academy Press, Washington, DC.
Petit, H. V., C. Germiquet, and D. Lebel. 2004. Effect of feeding whole, unprocessed sunflower seeds and flaxseed on milk production, milk composition, and prostaglandin secretion in dairy cows. J. Dairy Sci. 87:3889–3898.
Petit, H. V., M. F. Palin, and L. Doepel. 2007. Hepatic lipid metabolism in transition dairy cows fed flaxseed. J. Dairy Sci. 90:4780–4792.
SAS Institute. 2002. SAS User's Guide. Statistics. Version 9.2 ed. SAS Inst. Inc., Cary, NC.
Shingfield, K. J., S. Ahvenjärvi, V. Toivonen, A. Ärölä, K. V. V. Nurmela, P. Huhtanen, and J. M. Griinari. 2003. Effect of dietary fish oil on biohydrogenation of fatty acids and milk fatty acid content in cows. Anim. Sci. 77:165–179.
Shingfield, K. J., C. K. Reynolds, G. Hervás, J. M. Griinari, A. S. Grandison, and D. E. Beever. 2006. Examination of the persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. J. Dairy Sci. 89:714–732.
Simopoulos, A. P. 2002. Omega-3 fatty acids in inflammation and autoimmune diseases. J. Am. Coll. Nutr. 21:495–505.
Zachut, M., A. Arieli, H. Lehrer, L. Livshitz, S. Yakoby, and U. Moallem. 2010. Effects of increased supplementation of n-3 fatty acids to transition dairy cows on performance and fatty acid profile in plasma, adipose tissue, and milk fat. J. Dairy Sci. 93:5877–5889.
Zachut, M., A. Arieli, and U. Moallem. 2011. Incorporation of dietary n-3 fatty acids into ovarian compartments in dairy cows and the effects on hormonal and behavioral patterns around estrus. Reproduction 141:833–840.

Copyright of Journal of Dairy Science is the property of Elsevier Science and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.