



Effect of feeding extruded flaxseed with different grains on the performance of dairy cows and milk fatty acid profile

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ABSTRACT

Sixteen Holsteins cows were used in a Latin square design experiment to determine the effects of extruded flaxseed (EF) supplementation and grain source (i.e., corn vs. barley) on performance of dairy cows. Extruded flaxseed diets contained 10% [dry matter (DM) basis] of an EF product that consisted of 75% flaxseed and 25% ground alfalfa meal. Four lactating Holsteins cows fitted with rumen fistulas were used to determine the effects of dietary treatments on ruminal fermentation. Intakes of DM (23.2 vs. 22.2 kg/d), crude protein (4.2 vs. 4.0 kg/d), and neutral detergent fiber (8.3 vs. 7.9 kg/d) were greater for cows fed EF diets than for cows fed diets without EF. Milk yield and composition were not affected by dietary treatments. However, 4% fat-corrected milk (30.5% vs. 29.6 kg/d) and solids-corrected milk (30.7 vs. 29.9 kg/d) were increased by EF supplementation. Ruminal pH and total volatile fatty acid concentration were not influenced by EF supplementation. However, feeding barley relative to corn increased molar proportions of acetate and butyrate and decreased that of propionate. Ruminal NH₃-N was lower for cows fed barley than for cows fed corn. Milk fatty acid composition was altered by both grain source and EF supplementation. Cows fed EF produced milk with higher polyunsaturated and lower saturated fatty acid concentrations than cows fed diets without EF. Feeding EF or corn increased the milk concentration of C18:0, whereas that of C16:0 was decreased by EF supplementation only. Extruded flaxseed supplementation increased milk fat α -linolenic acid content by 60% and conjugated linoleic acid content by 29%. Feeding corn relative to barley increased milk conjugated linoleic acid by 29% but had no effect on milk α -linolenic concentration. Differences in animal performance and milk fatty acid composition were mainly due to EF

supplementation, whereas differences in ruminal fermentation were mostly due to grain source.

Key words: flaxseed, milk composition, fatty acids, corn and barley

INTRODUCTION

Flaxseed is a rich source of both protein and fat. On average it contains 40% oil, 20% CP, and 30% NDF (Petit, 2010). Flaxseed has been fed to lactating dairy cows in various forms such as whole or ground (Caroprese et al., 2010), extruded (Gonthier et al., 2005), and micronized (Mustafa et al., 2003). Feeding different forms of flaxseed to dairy cows increases the concentration of unsaturated fatty acids and decreases the concentration of SFA, particularly C16:0, in milk (Neveu et al., 2013). However, minimal effects on the concentrations of C18:1 and C18:2 have been reported as a result of flaxseed supplementation (Mustafa et al., 2003; Gonthier et al., 2005; Loor et al., 2005a). This is likely due to the ruminal biohydrogenation of flaxseed PUFA. Altering the physical structure (e.g., heat treatment) of flaxseed may help to protect dietary oilseed FA from ruminal biohydrogenation. Application of heat treatments, such as extrusion, to oilseeds can denature the protein matrix surrounding the fat droplet and therefore protect FA from ruminal biohydrogenation (Kennelly, 1996; Gonthier et al., 2005). A major disadvantage of feeding vegetable oils and oilseeds to dairy cows is the significant reduction in milk fat concentration and yield mainly due to the formation of several *trans* and conjugated FA isomers during ruminal biohydrogenation, which negatively affects *de novo* milk FA synthesis (Loor et al., 2005b; Chilliard et al., 2007; Glasser et al., 2008). Several dietary factors may influence ruminal pH and the microbial population and therefore alter the extent and rate of ruminal biohydrogenation of dietary FA. These include type and concentration of supplementary fat, forage:concentrate ratio, forage type, and composition of basal diet. Modification of ruminal FA biohydrogenation may alter duodenal flow of FA and consequently milk FA composition (Gonthier et al., 2004, 2005; Loor et al., 2004, 2005a).

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Type and concentration of fermentable carbohydrates can have a major effect on ruminal pH and therefore the rate and extent of ruminal biohydrogenation of dietary PUFA. Sources of fermentable carbohydrates such as grains may vary in their rate of starch fermentation and therefore on their effects on ruminal fermentation parameters such as pH and VFA (Kiran and Mutsvangwa, 2007). Mutsvangwa et al. (2012) showed that changing the availability of ruminally fermentable carbohydrate (through barley processing) and the source of supplemental fat altered duodenal FA flows and milk FA composition. However, no studies have evaluated the interaction between grain source (corn vs. barley) and extruded flaxseed (**EF**) on the performance of lactating dairy cows and milk FA composition.

MATERIALS AND METHODS

Animals and Experimental Design

Experimental procedures were approved by the Animal Care Committee of the Faculty of Agricultural and Environmental Sciences of McGill University. Sixteen lactating Holstein cows (93.1 ± 49.9 DIM) of different parities (10 multiparous and 6 primiparous) were used

in a 4×4 Latin square experiment with 21-d periods (14 d of adaptation and 7 d of data collection). Dietary treatments were allotted randomly to cows within each block (i.e., DIM). Cows were housed in tiestalls, had free access to water, and were milked twice daily at 0530 and 1700 h.

Dietary Treatments and Sample Collection

Four diets were formulated to meet the nutrient requirements of lactating dairy cows in early lactation (NRC, 2001; Table 1). Dietary treatments were a corn-based diet with no EF, a corn-based diet with EF, a barley-based diet with no EF, and a barley-based diet with EF. The EF product (OmegaPlus, Belisle Solution Nutrition Inc., Saint Mathias, QC, Canada) consisted of 75% flaxseed and 25% ground alfalfa meal (Table 2). The extrusion was carried out using an Insta-Pro extruder (model 2000RC; Insta-Pro International, Des Moines, IA) fitted with an 8100RC volumetric feeder. Extrusion temperature was maintained at 112°C.

Diets were fed as TMR once daily at 0800 h for ad libitum intake. Feed offered and weigh-backs (10% orts as fed) for each cow were measured daily during data collection periods to determine daily feed intake. Diets

Table 1. Ingredients and chemical composition of dietary treatments (DM basis)

Item	Corn		Barley	
	No flaxseed	Flaxseed	No flaxseed	Flaxseed
Ingredients, %				
Corn silage	25.7	25.9	27.3	27.1
Alfalfa haylage	25.8	25.9	27.3	27.1
Grass hay	7.8	7.9	8.3	8.2
High moisture corn	25.6	19.1		
Rolled barley			24.0	17.44
Extruded flaxseed		10.0		10.0
Soybean meal	11.8	7.9	9.6	6.2
Mineral mix ¹	3.3	3.4	3.4	3.4
DM, %	46.8 ± 1.9	48.1 ± 2.5	48.5 ± 1.5	48.7 ± 2.2
Chemical composition, % of DM				
Ash	7.0 ± 0.5	7.3 ± 0.4	7.3 ± 0.4	6.7 ± 0.9
Ether extract	2.3 ± 0.2	2.7 ± 0.3	1.7 ± 0.2	2.3 ± 0.2
NDF	34.4 ± 2.0	35.1 ± 3.9	36.5 ± 1.2	36.2 ± 2.2
ADF	24.3 ± 2.3	25.8 ± 2.7	23.9 ± 0.7	24.9 ± 1.3
ADL	3.4 ± 0.5	3.9 ± 0.6	3.8 ± 0.4	4.1 ± 0.8
CP	17.6 ± 1.7	17.8 ± 1.0	18.0 ± 1.5	18.6 ± 0.9
Neutral detergent insoluble protein	2.5 ± 0.4	2.6 ± 0.4	2.9 ± 0.6	3.0 ± 0.6
Acid detergent insoluble protein	0.9 ± 0.1	0.9 ± 0.2	0.9 ± 0.1	1.0 ± 0.2
Starch	19.3 ± 1.2	16.5 ± 1.5	18.1 ± 0.6	17.4 ± 1.4
NE _L , ² Mcal/kg	1.62	1.60	1.56	1.59
FA, % of total FA				
C16:0	17.5 ± 1.19	16.1 ± 1.32	19.5 ± 0.47	16.6 ± 1.08
C18:0	2.1 ± 0.12	2.4 ± 0.25	2.0 ± 0.03	2.2 ± 0.08
C18:1	13.0 ± 0.40	13.4 ± 0.31	9.6 ± 0.93	11.6 ± 0.92
C18:2	37.6 ± 2.67	31.8 ± 2.48	36.2 ± 1.14	32.3 ± 0.50
C18:3	18.0 ± 1.06	25.8 ± 1.18	19.9 ± 0.67	27.0 ± 1.82

¹Contained 16.14% Ca, 2.85% P, 5.12% Mg, 0.48% K, 12.31% Na, 7.57 mg/kg Se.

²Calculated using the equation of Weiss et al. (1992).

Table 2. Effects of extruded flaxseed supplementation and grain type on performance of lactating cows

Item	Corn		Barley		SEM	<i>P</i> -value ¹		
	No flaxseed	Flaxseed	No flaxseed	Flaxseed		F	G	F × G
Intake								
DM, kg/d	22.31	23.16	22.05	23.16	1.402	0.01	0.68	0.78
CP, kg/d	3.94	4.14	3.99	4.33	0.350	<0.01	0.11	0.34
NDF, kg/d	7.68	8.14	8.06	8.35	0.543	0.01	0.05	0.57
OM, kg/d	20.73	21.51	20.42	21.61	1.303	<0.01	0.77	0.50
Yield, kg/d								
Milk	30.92	31.57	31.53	32.33	1.460	0.12	0.15	0.87
ECM	31.57	32.20	31.87	32.90	1.380	0.06	0.25	0.64
4% FCM	29.50	30.21	29.79	30.81	1.291	0.03	0.26	0.69
SCM	29.70	30.29	30.00	31.11	1.333	0.04	0.16	0.53
Fat	1.13	1.17	1.14	1.20	0.052	0.02	0.39	0.47
Protein	0.98	0.97	1.01	0.98	0.051	0.40	0.23	0.54
Lactose	1.44	1.47	1.49	1.50	0.066	0.21	0.15	0.81
TS	3.94	3.95	3.89	4.06	0.176	0.08	0.13	0.60
SNF	2.74	2.80	2.78	2.87	0.131	0.20	0.12	0.71
Composition, %								
Fat	3.69	3.74	3.65	3.73	0.117	0.19	0.63	0.67
Protein	3.15	3.11	3.12	3.13	0.118	0.25	0.65	0.11
Lactose	4.69	4.69	4.71	4.70	0.036	0.78	0.09	0.56
TS	12.57	12.56	12.51	12.65	0.186	0.28	0.72	0.21
SNF	8.74	8.82	8.87	8.92	0.111	0.94	0.19	0.12
MUN, mg/dL	1,286	13.12	13.12	14.05	0.924	0.06	0.06	0.28
Feed efficiency	1.38	1.41	1.42	1.44	0.094	0.16	0.07	0.98

¹F = flaxseed, G = grain type, F × G = flaxseed × grain type interaction.

were sampled daily during the data collection periods and pooled by period. The pooled samples were oven-dried at 60°C for 48 h and ground through a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA). Dried and ground samples were then stored at room temperature for later analysis. Fecal grab samples (200 g) were collected 4 times daily from each cow on d 15 to 17 of each period and dried at 60°C in a forced-air oven. Samples were composited by cow within period, ground and stored for later analysis. Indigestible ADF (**IADF**) was used as an internal marker to estimate total fecal output (Huhtanen et al., 1994). Approximately 5 g of 1-mm-ground fecal samples and feed samples were weighed (in duplicate) into nylon bags (20 × 10 cm, 50 µm pore size, Ankom Technology Corp., Macedon, NY) and incubated in the rumen of a fistulated cow for 12 d. Following incubation, the bags were removed and washed under cold tap water until the wash water was clear of residues. The bags were then oven-dried at 60°C for 48 h. Residues were analyzed for ADF (AOAC, 1990), and total fecal output was calculated by determining the intake of IADF and dividing IADF intake by fecal IADF concentration (Kelzer et al., 2009).

Milk samples were collected on d 16 and 18 of each data collection period from morning and evening milkings, combined according to production, preserved with bronopol-based tablets, and analyzed for fat, protein, lactose, and MUN using an infrared analyzer

(MilkoScan, Foss 4000, Foss Food Technology, Hillerød, Denmark). Milk samples were also analyzed for TS according to AOAC (1990). Portions of composited samples were frozen for later analysis of FA.

Rumen Fermentation

Three multiparous lactating cows (average 176.67 ± 24.19 DIM) fitted with rumen fistula were used in an incomplete Latin square design to determine the effects of dietary treatments on ruminal fermentation. Experimental periods consisted of 14 d of diet adaptation and 7 d of data collection. Cows were housed in tiestalls and had continuous access to water. Dietary treatments were the same as in the production study. Samples of rumen fluid were collected from different parts of the rumen on d 18 and 21 of each period using a syringe screwed to a stainless-steel tube with a fine metal mesh (RT rumen Fluid Collection Tube, Bar Diamond Inc., Parma, ID). On d 18, rumen fluid was collected before feeding (0 h) and at 2, 4, 6, 8, 10, and 12 h postfeeding; on d 21, rumen fluid was collected at 1, 3, 5, 7, 9, and 11 h postfeeding. Ruminal pH was determined immediately using an Accumet pH meter (Fisher Scientific, Montreal, QC, Canada). Immediately after pH determination, 50 mL of ruminal fluid was preserved by adding 5 mL of 25% of metaphosphoric acid for VFA analysis, and 50 mL of ruminal fluid was preserved by adding 5 mL of 0.1 N HCl for NH₃-N

analysis. Samples were immediately frozen (-20°C) for later analysis.

Chemical Analysis

Dry matter and ash contents of dried diets and fecal samples were determined using standard procedures (AOAC, 1990). Neutral detergent fiber (Van Soest et al., 1991) and ADF (AOAC, 1990; nonsequential) of diets and fecal samples were determined using an Ankom fiber analyzer (Ankom Technology Corp.). The NDF analysis was performed using heat-stable α -amylase without the inclusion of sodium sulfite (Van Soest et al., 1991). Neutral detergent fiber and ADF were expressed inclusive of residual ash. Acid detergent lignin and ether extract of feed samples were analyzed following the AOAC (1990) procedures. Crude protein ($\text{N} \times 6.25$) was analyzed for both feed and fecal samples using a Leco Nitrogen Analyzer (FP-428 Nitrogen Determinator, Leco Corp., St. Joseph, MI). Neutral and acid detergent insoluble protein for feed samples were determined by analyzing NDF and ADF residues, respectively, for total N. Starch analysis of feed samples was conducted as described by McCleary et al. (1997).

Frozen milk samples were thawed and fat was extracted by centrifugation at $15,000 \times g$ for 25 min and 0.5 g of fat was used for FA methyl ester synthesis (O'Fallon et al., 2007). Feed samples were analyzed using the same procedure. The internal standard used was tridecanoic acid (C13:0; Nu-Chek Prep Inc., Elysian, MN). Fatty acid composition of the FA methyl esters was determined by capillary gas chromatography (Varian model 3900 equipped with flame-ionization detector at 260°C and model 1177 auto injector; Varian, Palo Alto, CA) fitted with a fused silica capillary column (CP7489, $100 \text{ m} \times 0.25 \text{ mm}$; Varian). The carrier gas was H_2 and the flow rate was 0.8 mL/min. Injector and detector temperatures were 260°C , and the split ratio was 50:1. Column temperature was set at 70°C for 4 min, and then increased to 130°C at a rate of $12.0^{\circ}\text{C}/\text{min}$ and was maintained for 3 min. It was then increased to 175°C at a rate of $4^{\circ}\text{C}/\text{min}$ and was maintained for 27 min. Finally, the temperature was increased to 214°C at a rate of $4^{\circ}\text{C}/\text{min}$ and maintained for 11 min and increased to 225°C at a rate of $4^{\circ}\text{C}/\text{min}$ and held for 5.5 min; therefore, total run time was 79.25 min. Fatty acids were identified by comparing their retention times with FA methyl standards (Nu-Chek Prep Inc.).

Samples of ruminal fluid preserved for VFA analysis were centrifuged for 10 min at $10,000 \times g$ and analyzed for acetic, propionic, and butyric acids using HPLC (Andersson and Hedlund, 1983). The HPLC system included a Milton Roy 711 pump (Milton Roy, Sun-

derland, UK), a Valco CV-6-UHP injection valve, and an R401 differential refractometer (both from Milton Roy). Separation of VFA was carried out using an Aminex HPX-87H column ($300 \times 7.8 \text{ mm}$; Bio-Rad, Hercules, CA) with a mobile phase of $0.013 \text{ M H}_2\text{SO}_4$, and a flow rate of 0.6 mL/min. Detection was made at 210 nm. Ruminal $\text{NH}_3\text{-N}$ was determined by colorimetry with a multichannel Lachat Autoanalyzer (Lachat Instruments, Milwaukee, WI).

Statistical Analysis

Data of the production study and total-tract nutrient digestibility were analyzed using the MIXED procedure (SAS Institute, 1989) for a 4×4 Latin square design with a 2×2 factorial arrangement of treatments. Variables used in the statistical analyses were Y = the dependent variable, μ = overall mean, C_i = cow ($i = 1, \dots, 16$), P_j = period ($j = 1, 2, 3, 4$), E_k = EF supplementation (1, 2), G_l = grain source treatment (1, 2), and ε = residual error. In the statistical models, interaction terms were represented by combinations of the letters used to represent variables. In all analyses, cows were treated as random variables.

Data on ruminal fermentation measurements were analyzed as repeated measurements across time using the MIXED procedure (SAS Institute, 2008) with a model that included the effects of cow, periods, treatments, treatment interactions, sampling time, and time \times treatment interactions. Significance was declared at $P < 0.05$ and tendencies at $0.05 < P < 0.10$.

RESULTS AND DISCUSSION

Feed Intake, Milk Yield, and Milk Composition

The chemical composition of the extruded flaxseed (OmegaPlus) used in this study was reported by Neveu et al. (2013). Intakes of DM, CP, and NDF were greater ($P < 0.05$) for cows fed diets with EF than for cows fed diets without EF (Table 2). The increase in DMI of dairy cows as a result of EF supplementation is consistent with the findings of Zachut et al. (2010). It has been shown that low to moderate inclusion levels of ground or extruded flaxseed in the diets of dairy cows (up to 10% of the diet DM) have no effect on DMI (Gonthier et al., 2005; Hurtaud et al., 2010; Petit, 2010), whereas high inclusion levels (21% of the diet DM) reduce DMI (Chilliard et al., 2009). Source of grain had no effect on DM or CP intakes. Previous studies also showed no effects of feeding barley or corn on DM or OM intakes of lactating cows (Yang et al., 1997b; Khorasani et al., 2001). Intake of NDF tended to be higher ($P = 0.05$) for cows fed barley than for cows fed corn, likely due to

the higher NDF content of the barley diets relative to the corn diets (Table 1).

Milk yield was not influenced by dietary treatments (Table 2). However, 4% FCM and SCM were higher ($P < 0.05$) for cows fed EF than for cows fed no EF. Energy-corrected milk tended ($P = 0.06$) to be greater for cows fed EF than for cows fed diets with no EF. In a previous study, Neveu et al. (2013) observed no effects of feeding EF (9% of diet DM) at 2 different forage:concentrate ratios on yields of milk, 4% FCM, and SCM of lactating dairy cows. In contrast, Moallem (2009) reported a 2.7% increase in milk yield for early lactating cows fed extruded flaxseed at 4% of the diet DM. Discrepancies between studies can be attributed to several factors, such as inclusion level of flaxseed, type of flaxseed fed, type of forage, forage:concentrate ratio, and stage of lactation (Gonthier et al., 2005; Petit, 2010; Neveu et al., 2013). In a literature review, Petit (2010) reported no effect of flaxseed supplementation (up to 11% of diet DM) on milk yield of dairy cows in early lactation, which is consistent with our findings. Yields of milk components were similar for all dietary treatments, with the exception of fat yield, which was greater ($P < 0.05$) for cows fed EF than for cows fed no EF.

Milk composition was not influenced by dietary treatments (Table 2). These results were in agreement with other studies that showed no effects of flaxseed supplementation on milk protein, lactose, and TS concentrations (da Silva et al., 2007; Moallem, 2009; Caroprese et al., 2010). However, the effect on milk fat concentration is inconsistent. Although many studies have shown no effect of flaxseed supplementation on milk fat concentration (Gonthier et al., 2005; Martin et al., 2008; Petit and Côrtes, 2009), a few studies have reported a negative effect of flaxseed supplementation on milk fat concentration (Mustafa et al., 2003; Moallem, 2009). The lack of effects of grain type on milk composition was in agreement with findings of others (Yang et al., 1997a,b; Khorasani et al., 2001).

Ruminal Fermentation and Total-Tract Digestibility

No dietary treatment \times time interactions were observed for ruminal fermentation; therefore, only main effects are reported (Table 3). Ruminal pH and $\text{NH}_3\text{-N}$ tended ($P = 0.05$) to be greater for cows fed barley than for cows fed corn, whereas total VFA concentration was not influenced by grain source. Replacing corn with barley in diets of lactating cows has been shown to decrease (Overton et al., 1995), have no effect (Khorasani et al., 2001), or increase (Fellner et al., 2008) ruminal pH. The inconsistent response of ruminal pH to grain source can be due to various factors such as grain variety, extent of grain processing, and forage level and source. Differences in dietary starch and NDF concentrations may also help to explain this inconsistency. The response of ruminal $\text{NH}_3\text{-N}$ in the present study is in agreement with the findings of Casper et al. (1990, 1999), Feng et al. (1995), and Fellner et al. (2008). In contrast, Surber and Bowman (1998) reported higher ruminal $\text{NH}_3\text{-N}$ concentration for steers fed barley than for steers fed corn, and Khorasani et al. (2001) reported no treatment differences. The higher ruminal $\text{NH}_3\text{-N}$ concentration for cows fed barley than for cows fed the corn diets might indicate less synchronization between energy and protein in the rumen for the barley diets. However, for all diets, ruminal $\text{NH}_3\text{-N}$ concentration was >5 mg/dL, which is thought to maximize growth of ruminal bacteria (Satter and Slyter, 1974).

Molar proportions of acetate and butyrate were higher ($P < 0.05$) and that of propionate was lower ($P < 0.05$) for cows fed barley than for cows fed corn (Table 3). Consequently, the acetate:propionate ratio was decreased ($P < 0.05$) by feeding corn. The effects of grain source on molar proportions of VFA are inconsistent. Compared with corn, barley increases the molar proportion of acetate without affecting that of propionate (Fellner et al., 2008). Although molar proportions of acetate and propionate were unaffected by grain source, the molar percentage of butyrate was

Table 3. Effects of extruded flaxseed supplementation and grain type on ruminal fermentation

Item	Corn		Barley		SEM	<i>P</i> -value ¹		
	No flaxseed	Flaxseed	No flaxseed	Flaxseed		F	G	F \times G
pH	5.82	5.81	5.89	5.88	0.14	0.83	0.05	0.89
$\text{NH}_3\text{-N}$, mg/L	14.6	13.9	15.3	16.0	1.31	0.99	0.05	0.29
Total VFA, mM	124.1	119.7	117.7	124.3	3.95	0.70	0.76	0.05
Molar proportions								
Acetic acid	55.6	56.1	58.0	56.6	0.89	0.46	0.01	0.10
Propionic acid	29.9	27.9	26.1	25.5	1.89	0.02	<0.01	0.19
Butyric acid	14.6	16.0	15.9	17.9	1.66	<0.01	<0.01	0.62
Acetate:propionate	1.99	2.01	2.26	2.28	0.162	0.34	<0.01	0.19

¹F = flaxseed, G = grain type, F \times G = flaxseed \times grain type interaction.

Table 4. Effects of extruded flaxseed supplementation and grain type on digestion

Total-tract digestibility, %	Corn		Barley		SEM	<i>P</i> -value ¹		
	No flaxseed	Flaxseed	No flaxseed	Flaxseed		F	G	F × G
DM	65.9	67.1	64.6	64.7	2.80	0.33	0.01	0.45
OM	65.9	67.0	64.5	65.1	2.81	0.23	0.02	0.68
CP	64.7	67.6	64.7	65.0	2.35	0.09	0.16	0.15
NDF	40.9	44.5	41.1	40.8	3.26	0.13	0.11	0.08

¹F = flaxseed, G = grain type, F × G = flaxseed × grain type interaction.

higher for cows fed barley than for cows fed corn (Yang et al., 1997a). However, Khorasani et al. (2001) found that replacing barley with corn in the diets of lactating cows decreased the molar proportion of acetate and increased that of butyrate.

Extruded flaxseed supplementation had no influence on ruminal pH, NH₃-N, or total VFA concentration (Table 3). This is consistent with the results of Gonthier et al. (2004), Doreau et al. (2009), and Côrtes et al. (2010), which showed no effects of different forms of flaxseed supplementation on ruminal pH, NH₃-N, or VFA concentration. Molar proportion of propionate was lower ($P < 0.05$) and that of butyrate was higher ($P < 0.05$) for cows fed EF than for cows fed no EF. However, the molar proportion of acetate was not influenced by EF supplementation. Diets containing EF had less starch, which may explain the greater molar proportion of propionate for cows fed diets without EF than for cows fed EF diets. Feeding flaxseed at 4.1% of diet DM had no effect on total and molar proportions of individual VFA (Côrtes et al., 2010). However, feeding flaxseed at a higher level (12.5% of the diet DM) reduced the molar proportion of acetate and increased that of propionate, resulting in a lower acetate:propionate ratio (Gonthier et al., 2004).

We observed no interaction between EF supplementation and grain source on total-tract digestibility (Table 4). Dry matter and OM digestibility were greater ($P < 0.05$) for cows fed corn than for cows fed barley, whereas digestibility of CP and NDF were not influenced by grain source (Table 4). Total-tract digestibilities of CP and NDF for cows fed corn and barley diets were in agreement with other researchers (Yang et al., 1997b; Khorasani et al., 2001). However, Surber and Bowman (1998) reported higher total-tract DM and CP digestibilities when steers were fed barley-based diets than when they were fed corn-based diets. Differences between studies might be due to differences in quantities of grains included in the diets. Extruded flaxseed supplementation had no effect on total-tract digestibility except for CP digestibility, which tended ($P = 0.09$) to be higher for cows fed EF than for cows

fed diets without EF. These results are consistent with the findings of da Silva et al. (2007).

FA Profile

We observed no EF × grain type interactions between major FA; therefore, only main effects are reported (Table 5). Milk FA composition was affected by both grain source and EF supplementation (Table 5). Dietary treatments had little or no effect on concentrations of short-chain fatty acids (C4 to C10), suggesting little effect of diets on de novo fatty acid synthesis. Our findings are in agreement with those of Moallem (2009), Zachut et al. (2010), and Côrtes et al. (2010), who found no effect of flaxseed supplementation on the synthesis of milk short-chain fatty acids. In a meta-analysis of the response of cow milk FA to lipid supplements from various oilseeds, Glasser et al. (2008) reported that oilseeds rich in C18:3, such as flaxseed, have less inhibitory effects on de novo synthesis of milk short-chain fatty acid than oilseeds rich in C18:2. Glasser et al. (2008) also reported a greater inhibitory effect of oils than oilseeds.

Milk concentration of C16:0 was decreased ($P < 0.05$) by EF supplementation and unaffected by grain source. The negative effect of EF feeding on milk C16:0 concentration is likely due to the inhibitory effects of long-chain fatty acids on de novo synthesized C16:0 (Glasser et al., 2008). Similar effects of EF supplementation on C16:0 concentration have been reported by Gonthier et al. (2005) and Moallem (2009).

Extruded flaxseed supplementation and corn diets increased ($P < 0.05$) the concentration of milk C18:0 (Table 5). However, the increase was more pronounced for EF supplementation than for grain source, as indicated by EF × grain source interaction. We expected that dietary PUFA would be extensively biohydrogenated in the rumen, which would increase the flow of C18:0 into the small intestine (Loor et al., 2005a). The increase in the concentration of C18:0 with EF is likely due to an increase in mammary uptake of C18 fatty acids in the small intestine (Gonthier et al., 2004).

Table 5. Effects of extruded flaxseed supplementation and grain type on milk fatty acid profile of lactating cows

Fatty acid, g/100 g of FA	Corn		Barley		SEM	<i>P</i> -value ¹		
	No flaxseed	Flaxseed	No flaxseed	Flaxseed		F	G	F × G
C4:0	0.82	0.89	0.84	0.83	0.026	0.01	0.15	0.10
C6:0	1.04	1.00	1.03	1.00	0.035	0.06	0.91	0.90
C8:0	0.91	0.89	0.86	0.89	0.034	0.08	0.23	0.99
C10:0	2.46	2.49	2.59	2.60	0.124	0.91	<0.01	0.72
C11:0	0.22	0.22	0.22	0.24	0.017	0.20	0.16	0.76
C12:0	3.27	3.17	3.49	3.36	0.184	0.06	<0.01	0.06
C13:0	0.37	0.42	0.36	0.42	0.018	<0.01	<0.01	0.18
C14:0	11.62	11.65	12.02	11.76	0.412	0.45	0.09	0.35
C14:1 <i>iso</i>	0.21	0.20	0.21	0.21	0.009	0.76	0.22	0.21
C14:1 <i>cis</i> -9	1.06	0.97	1.09	0.99	0.086	<0.01	0.21	0.76
C15:0	1.42	1.13	1.52	1.32	0.077	<0.01	<0.01	0.47
C16:0	34.5	32.02	35.46	32.11	0.839	<0.01	0.25	0.41
C16:1 <i>trans</i> -9	0.29	0.26	0.21	0.24	0.074	0.13	0.03	0.86
C16:1	2.11	1.64	2.7	1.95	0.149	<0.01	<0.01	0.18
C17:0	0.65	0.66	0.77	0.74	0.0266	<0.01	<0.01	0.75
C17:1 <i>trans</i> -10	0.27	0.21	0.27	0.23	0.017	<0.01	0.32	0.28
C18:0	8.38	9.80	7.98	9.34	0.569	<0.01	<0.01	0.05
C18:1 <i>trans</i> -9	0.24	0.21	0.24	0.20	0.011	0.01	0.02	0.03
C18:1 <i>trans</i> -11	0.54	0.78	0.54	0.80	0.086	<0.01	0.51	0.42
C18:1 <i>cis</i> -9	20.94	21.05	19.5	20.95	1.036	0.13	0.25	0.30
C18:1 <i>cis</i> -11	0.79	0.63	0.68	0.38	0.0656	<0.01	0.03	0.04
C18:1 <i>cis</i> -12	0.33	0.44	0.28	0.36	0.023	<0.01	<0.01	0.29
C18:1 <i>cis</i> -13	0.08	0.08	0.08	0.08	0.011	0.45	0.61	0.98
C18:1 <i>cis</i> -14	0.33	0.45	0.28	0.46	0.020	<0.01	<0.01	0.14
C18:1 <i>cis</i> -16	0.14	0.21	0.13	0.21	0.010	<0.01	0.41	0.67
C18:2 <i>cis</i> -15, <i>trans</i> -11	0.22	0.33	0.22	0.31	0.024	<0.01	0.06	0.33
18:2 <i>trans</i> -9, <i>trans</i> -12	0.13	0.13	0.13	0.13	0.010	0.82	0.51	0.91
C18:2 <i>trans</i> -9, <i>trans</i> -12	0.14	0.09	0.13	0.07	0.001	<0.01	0.02	0.83
C18:2 <i>cis</i> -9, <i>trans</i> -12	0.07	0.16	0.04	0.15	0.010	<0.01	0.03	0.16
C18:2 <i>cis</i> -9, <i>cis</i> -12	1.48	1.63	1.56	1.63	0.073	0.17	<0.01	0.31
C20:0	0.08	0.09	0.08	0.10	0.010	0.65	0.52	0.06
C18:3 <i>cis</i> -6, <i>cis</i> -9, <i>cis</i> -12	0.08	0.08	0.08	0.08	0.08	0.10	0.05	0.23
C18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	0.54	0.81	0.54	0.75	0.044	<0.01	0.87	0.48
C18:2 <i>cis</i> -9, <i>trans</i> -11 CLA	0.44	0.49	0.3	0.44	0.024	<0.01	<0.01	0.82
C22:0	0.05	0.05	0.06	0.06	0.003	0.22	0.08	0.07
C20:3n-6	0.08	0.07	0.08	0.07	0.007	0.15	0.15	0.52
C20:4n-6	0.098	0.09	0.13	0.14	0.004	<0.01	<0.01	0.72
C20:5n-3	0.16	0.09	0.09	0.14	0.048	0.01	0.42	0.60
C24:0	0.08	0.07	0.08	0.07	0.008	0.14	0.20	0.89
C22:5n-3	0.12	0.38	0.18	0.41	0.099	0.86	0.45	<0.01
Unknown	3.26	3.26	2.91	3.47	0.138	0.01	0.48	0.01
SFA	66.02	65.11	67.35	64.77	0.954	<0.01	0.34	0.17
MUFA	27.27	27.26	27.59	27.62	0.936	0.16	0.37	0.17
PUFA	3.48	4.44	3.82	4.18	0.146	<0.01	0.69	<0.01

¹F = flaxseed, G = grain type, F × G = flaxseed × grain type interaction.

The concentration of *cis*-9 C18:1 was not influenced by dietary treatments (Table 5). In agreement with our findings, da Silva et al. (2007) found that feeding ground flaxseed to dairy cows up to 12% of the diet DM had no effect of milk C18:1. However, others reported an increase in the concentration of milk C18:1 as a result of feeding different forms of flaxseed (Gonthier et al., 2005; Côrtes et al., 2010; Zachut et al., 2010). Concentrations of C18:3 and CLA were increased ($P < 0.05$) by EF supplementation, whereas feeding corn increased the concentration of CLA but not C18:3 (Table 5). Despite the significant increase in the concentrations of health-promoting milk FA such as C18:3

and CLA as a result of EF supplementation, the levels of these FA remained <1.0% of total milk FA. These findings suggest extensive ruminal biohydrogenation of dietary PUFA. The low transfer efficiency of dietary PUFA from flaxseed supplementation to milk is well documented (Chilliard et al., 2001; Gonthier et al., 2005; Côrtes et al., 2010). It has been reported that extrusion increases ruminal biohydrogenation of C18:3 and therefore reduces the amount of C18:3 reaching the duodenum (Gonthier et al., 2004). Gonthier et al. (2004) also suggested that the proportion of C18:3 reaching the duodenum of cows fed EF is less available for intestinal digestion or absorption.

CONCLUSIONS

Most of the effects observed on dairy cow performance in this study were due to EF supplementation. However, differences observed in ruminal fermentation measurements were mainly due to the type of grain. The inclusion level of EF used in this study improved milk yield without adversely affecting milk fat composition or yield. We observed no major interaction between grain source and EF supplementation.

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