J. Dairy Sci. 96:3886–3894
http://dx.doi.org/10.3168/jds.2012-6189


# Effect of feeding extruded flaxseed with different forage:concentrate ratios on the performance of dairy cows

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## ABSTRACT

Twenty Holstein cows were used in a Latin square design experiment with a 2 × 2 factorial arrangement to determine the effects of extruded flaxseed (EF) supplementation with 2 different forage to concentrate ratios on the performance of dairy cows. Extruded flaxseed diets contained 9% (dry matter basis) EF product which consisted of 75% EF and 25% ground alfalfa meal. Four lactating Holsteins cows fitted with rumen fistulae were used to determine the effects of dietary treatments on ruminal fermentation. Intakes of dry matter and crude protein were not influenced by dietary treatments. However, neutral detergent fiber intake was greater for the high-forage (8.4 kg/d) than the low-forage (7.8 kg/d) diet. Milk yield (average 40.2 kg/d) was similar for all dietary treatments. However, cows fed the high-forage diets produced milk with higher fat (3.76 vs. 2.97%) and total solids (12.58 vs. 11.95%) concentrations, but lower protein (3.19 vs. 3.33%) and lactose (4.66 vs. 4.72%) contents. Ruminal pH and total volatile fatty acid concentration were not affected by dietary treatments. However, feeding high forage relative to low forage diets increased molar proportion of acetate but decreased that of propionate. Ruminal $NH_3$-N was reduced by feeding high forage relative to low forage diets. Milk fatty acid composition was altered by both forage level and EF supplementation. Feeding diets containing EF or low forage reduced the concentrations of saturated fatty acids and increased those of mono-unsaturated fatty acids. Concentrations of poly-unsaturated fatty acids were increased by feeding EF or low-forage diets. Extruded flaxseed supplementation increased milk fat α-linolenic acid content by 100% and conjugated linoleic acid by 54%. It was concluded that differences in animal performance and ruminal fermentation observed in this study were mostly due to differences in forage to concentrate ratio. However, EF supplementation caused most of the differences observed in milk fatty acid composition.
**Key words:** dairy cow, extrusion, fatty acid, flaxseed

## INTRODUCTION

Flaxseed is a rich source of linolenic acid, averaging 18% of the total seed weight and constituting 53% of the total FA (Gonthier et al., 2004). Feeding whole, rolled, or extruded flaxseed (**EF**) to dairy cows increased concentrations of milk unsaturated FA and decreased the concentrations of SFA, particularly C16:0 (Mustafa et al., 2003; Akraim et al., 2007). However, feeding flaxseed produced relatively small changes in the concentrations of C18:2 and C18:3 in milk due to the extensive biohydrogenation of these FA in the rumen. Altering the physical structure of flaxseed (e.g., through heat treatment) may help to protect dietary FA of oilseeds from ruminal biohydrogenation (Chouinard et al., 1997; Sterk et al., 2011). Application of heat treatments, such as extrusion, to oilseeds can denature the protein matrix surrounding the fat droplet and therefore protects fat from ruminal biohydrogenation (Kennelly, 1996). During the extrusion process of oilseeds, the rapid release of intracellular oil may lead to considerable oil losses. Therefore, addition of a binder may help to reduce oil losses during extrusion of oilseeds (Akraim et al., 2007).

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 that have adverse effects on de novo FA synthesis (Loor et al., 2005; Chilliard et al., 2007; Glasser et al., 2008). Several dietary factors may affect the extent of FA metabolism in the rumen by altering rumen pH and the microbial population; these include type and concentration of supplementary fat, forage to concentrate ratio, forage type, and composition of basal diet. Previous studies have shown that the effect of supplementary flaxseed oil on milk FA composition is influenced by the forage to concentrate ratio and forage type (Shingfield et al., 2010; Sterk et al., 2011). To our knowledge, the

Received September 23, 2012.
Accepted February 19, 2013.
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effects of EF addition to diets formulated with different forage to concentrate ratios on the performance and milk FA profile of dairy cows has not yet been reported. The objectives of this study were to determine the effects of supplementation of an EF product (OmegaPlus, Belisle Solution Nutrition Inc., Saint Mathias, Canada) and the manipulation of the forage to concentrate ratio on the performance and milk FA composition of lactating dairy cows.

## 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. Twenty lactating Holsteins cows (664 ± 60.8 kg of BW; 98 ± 29.2 DIM) with different parities were used in a replicated (n = 5) 4 × 4 Latin square experiment with 21-d periods (14 d of adaptation and 7 d of data collection). Cows were housed in tiestalls and had free access to water. Cows were milked twice daily at 0530 and 1700 h.

### Dietary Treatments and Sample Collection

Four diets were formulated to meet nutrient requirements of lactating dairy cows in early lactation (NRC, 2001; Table 1). Dietary treatments were a high-forage (60% DM) diet with no EF, a high-forage diet with EF, a low-forage (40% DM) diet with no EF, and a low-forage diet with EF. The EF product (OmegaPlus, Belisle Solution Nutrition Inc.) consisted of 75% flaxseed and 25% ground alfalfa meal (Table 2). Extrusion was carried out using an Insta-Pro 2000RC extruder (model 2000RC, Insta-Pro International, Des Moines, IA) outfitted with an 8100RC volumetric feeder. Extrusion temperature was maintained around 122°C.

Diets were fed as a TMR once daily for ad libitum intake. Feed offered and weigh backs of each cow were measured daily during data collection periods (d 15–21 of each period) to determine daily feed intake. Diets were sampled daily during the data collection periods (d 15–21 of each period) and were 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 samples (200 g) were collected from each cow 4 times daily over a 12-h period on d 15 to 17 of each period and dried at 60°C in a forced-air oven. Samples were composited by cow, 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 Corporation, Macedon, NY) and incubated in the rumen of a fistulated cow for 12 d. Following incubation, the nylon bags were removed and washed under cold tap water until the wash water was clear from residues. The bags were then oven-dried at 60°C for 48 h. The residues were analyzed for ADF and total fecal output was calculated by determining the intake of IADF and dividing IADF intake by IADF concentration in the feces (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 volume, and analyzed for fat, protein, lactose, and MUN using an infrared analyzer (Valacta, Sainte-Anne-de-Bellevue, Canada) according to AOAC (1990, method no. 972.16). Milk samples were also analyzed for TS using standard procedures (AOAC, 1990). Portions of composited samples were frozen for later analysis of FA.

### Ruminal Fermentation

Four multiparous lactating cows (119 ± 76.7 DIM) fitted with rumen fistula were used 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 with 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 and 21 of each period. On d 18, rumen fluid was collected before feeding (0 h) and at 2, 4, 6, 8, 10, 12 h post feeding. On d 21, rumen fluid was collected at 1, 3, 5, 7, 9, 11 h postfeeding. Ruminal pH was determined immediately using an Accumet pH meter (Fisher Scientific, Montreal, 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 also preserved by adding 5 mL of 0.1 N HCl for $NH_3$-N analysis. Samples were immediately frozen (−20°C) for later analysis.

### Chemical Analysis

Dry matter and ash contents of diets and fecal samples were determined using standard procedures (AOAC, 1990). Neutral (Van Soest et al., 1991) and acid (AOAC, 1990) detergent fiber for feed and fecal

**Table 1.** Ingredients and chemical composition of dietary treatments

| | Treatment | | | |
|---|---|---|---|---|
| | 60:40 forage:concentrate | | 40:60 forage:concentrate | |
| Item | No flaxseed | Flaxseed | No flaxseed | Flaxseed |
| Ingredients, % | | | | |
| Corn silage | 13.3 | 13.3 | 13.4 | 13.3 |
| Alfalfa haylage | 43.4 | 41.3 | 20.2 | 18.1 |
| Grass hay | 3.3 | 3.3 | 6.5 | 6.5 |
| High moisture corn | 28.8 | 26.0 | 39.0 | 34.7 |
| Soyhulls | | | 5.1 | 5.0 |
| OmegaPlus[1] | | 9.0 | | 8.7 |
| Soybean meal | 1.5 | | 9.0 | 6.8 |
| RTM Amino | 6.6 | 5.6 | 5.0 | 4.9 |
| Mineral mix[2] | 1.5 | 1.5 | | |
| Mineral mix[3] | | | 2.0 | 2.0 |
| Megalac[4] | 1.6 | | | |
| DM, % | 48.7 ± 1.09 | 49.0 ± 0.91 | 47.3 ± 1.10 | 47.9 ± 1.34 |
| Chemical composition, % of DM | | | | |
| Ash | 7.1 ± 0.53 | 7.3 ± 0.40 | 6.4 ± 0.4 | 6.5 ± 0.18 |
| CP | 17.0 ± 0.35 | 16.6 ± 0.66 | 16.9 ± 1.2 | 16.8 ± 0.63 |
| Neutral detergent-insoluble CP, % of DM | 2.9 ± 0.13 | 3.0 ± 0.17 | 2.9 ± 0.2 | 3.0 ± 0.11 |
| Acid detergent-insoluble CP, % of DM | 1.0 ± 0.02 | 1.1 ± 0.08 | 1.0 ± 0.1 | 0.9 ± 0.13 |
| Ether extract | 3.5 ± 0.32 | 3.6 ± 0.18 | 2.6 ± 0.3 | 3.0 ± 0.43 |
| NDF | 31.2 ± 0.87 | 31.3 ± 1.89 | 28.8 ± 2.8 | 29.9 ± 1.45 |
| ADF | 21.1 ± 0.53 | 22.6 ± 0.29 | 20.6 ± 0.7 | 20.1 ± 2.00 |
| ADL | 4.7 ± 0.29 | 4.9 ± 0.31 | 3.8 ± 0.4 | 3.6 ± 0.48 |
| Starch | 23.0 ± 2.73 | 20.6 ± 0.99 | 27.2 ± 1.9 | 27.6 ± 2.07 |
| $NE_L$,[5] Mcal/kg | 1.64 | 1.63 | 1.67 | 1.68 |
| FA, % of total FA | | | | |
| C16 :0 | 23.4 ± 0.50 | 14.9 ± 0.31 | 17.8 ± 0.85 | 15.2 ± 1.29 |
| C18 :0 | 2.8 ± 0.09 | 2.6 ± 0.11 | 2.5 ± 0.12 | 2.9 ± 0.27 |
| C18 :1 | 18.4 ± 0.66 | 15.4 ± 0.26 | 17.9 ± 0.58 | 18.1 ± 1.75 |
| C18 :2 | 29.0 ± 1.33 | 29.2 ± 1.35 | 40.5 ± 1.85 | 35.5 ± 1.75 |
| C18 :3 | 15.6 ± 0.65 | 27.7 ± 1.17 | 10.7 ± 0.50 | 19.2 ± 0.84 |

[1]Manufactured by Belisle Solution Nutrition Inc., Saint Mathias, Canada. [2]Contained 40.65% dicalcium phosphate, 18.2% sodium chloride, 13.63% calcium carbonate, 7.67% Mg, 4.9% K, 4.52% Ca, 3.4% Na, 0.85% Zn, 0.72% Mn, 0.45% Cu, 0.02% Co, 0.01% I, 0.01% selenium yeast, 1.52% mineral oil, 3.11% canola meal, 1, 350 kIU of vitamin E/kg, 400,000 IU of vitamin A/kg, and 150, 000 IU of vitamin D/kg.[3]Contained 28.01% dicalcium phosphate, 16.86% sodium chloride, 33.98% calcium carbonate, 5.97% Mg, 5.10% K, 1.74% Ca, 2.28% Na, 0.71% Zn, 0.60% Mn, 0.38% Cu, 0.02% Co, 0.01% I, 0.01% selenium yeast, 1.52% mineral oil, 2.53% canola meal, 1, 150 kIU of vitamin E/kg, 400, 000 IU of vitamin A/kg, and 100, 000 IU of vitamin D/kg.[4]Church and Dwight Inc., Princeton, NJ.

[5]Calculated using the equation of Weiss et al. (1992).

samples were analyzed using an Ankom fiber Analyzer (Ankom Technology). The analysis for NDF was performed without the inclusion of sodium sulfite and with the inclusion of heat-stable α-amylase. Acid detergent lignin analysis of feed samples was conducted following AOAC (1990) procedures. Crude protein (N × 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 of the diets were determined by analyzing NDF and ADF residues, respectively, for total N.

Frozen milk samples were thawed and fat was extracted by centrifugation at 15,000 × *g* for 25 min and 0.5 g of fat was used for FA methyl ester synthesis (O'Fallon et al., 2007). OmegaPlus was 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 methyl esters was

**Table 2.** Chemical composition of extruded flaxseed product (OmegaPlus, Belisle Solution Nutrition Inc., Saint Mathias, Canada)

| Item | % DM |
|---|---|
| Ash | 6.0 |
| CP | 28.9 |
| NDF | 33.3 |
| ADF | 24.8 |
| Total FA | 15.5 |
| FA | |
| C14:0 | 0.01 |
| C16:0 | 1.06 |
| C16:1 | 0.01 |
| C18:0 | 0.48 |
| C18:1n-9c | 2.62 |
| C18:2n-6c | 2.62 |
| C18:3n-3 | 8.60 |
| C20:0 | 0.02 |
| C20:5n-3 | 0.02 |
| C22:0 | 0.02 |

determined by capillary gas chromatography (Varian model 3900 equipped with flame ionization detector at 260°C and model 1177 auto injector) fitted with a fused silica capillary column (CP7489, 100 m × 0.25 mm; Varian, CA). 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°C/min and maintained for 3 min. It was then increased to 175°C at a rate of 4°C/min and was maintained for 27 min. Finally the temperature was increased to 214°C at a rate of 4°C/min and maintained for 11 min, then increased to 225°C at a rate of 4°C/min and held for 5.5 min; therefore, total run time was 79.25 min. Fatty acids were identified by comparing their retentions times with FA methyl standards (NuChek Prep Inc.).

Samples of ruminal fluid preserved for VFA analysis were centrifuged for 10 min at 10,000 × $g$ and analyzed for acetic, propionic, and butyric acid using HPLC (Andersson and Hedlund, 1983). The HPLC system included a Milton Roy 711 pump (Milton Roy, Sunderland, UK), a Valco CV-6-UHP injection valve, and an R 401 differential refractometer (Waters, Milford, MA). Separation of VFA was carried out using an Aminex HPX-87H column (300 × 7.8 mm; Bio-Rad, Hercules, CA) with a mobile phase of 0.013 $M$ of $H_2SO_4$, and a flow rate of 0.6 mL/min. Detection was made at 210 nm. Ruminal $NH_3$-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 utilization were analyzed using the MIXED procedure of SAS (SAS Institute, 1989) as a 4 × 4 Latin square design with a 2 × 2 factorial arrangement with the following model:

$$Y_{ijk} = \mu + E_i + F_l + E_i \times F_l + P_j + C_k + e_{ijk},$$

where $Y_{ijk}$ = observation; $\mu$ = population mean; $E_i$ = EF effect ($i$ = 1,2); $F_l$ = forage to concentrate ratio effect ($l$ = 1, 2); $P_j$ = period ($j$ = 1, 2, 3, or 4); $C_k$ = random effect of cow ($k$ = 1, 2, …, or 20); $C_k$ = ~ $N\left(0, \sigma^2_{\text{cow}}\right)$; and $e_{ijk}$ = residual error, $e_{ijk}$ ~ $N\left(0, \sigma^2_e\right)$. Data of ruminal fermentation were analyzed as repeated measurements across time using the MIXED procedure of SAS (SAS Institute, 1989) with the following model:

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

where $Y_{ijkl}$ = observation; $\mu$ = population mean; $T_i$ = treatment ($i$ = 1, 2, 3, or 4); $P_j$ = period ($j$ = 1, 2, 3, or 4); $C_k$ = random effect of cow ($k$ = 1, 2, 3, or 4), $C_k$ = ~ $N\left(0, \sigma^2_{\text{cow}}\right)$; $S_l$ = sampling time ($l$ = 0, 1,…, or 12h); $T_i \times S_l$ = treatment × time interaction; and $e_{ijk}$ = residual error, $e_{\text{ijk}}$ ~ $N\left(0, \sigma^2_{\text{cow}}\right)$. Significance was declared at $P < 0.05$.

## RESULTS AND DISCUSSION

### Feed Intake, Milk Yield, and Milk Composition

The chemical composition of the EF product (OmegaPlus) used in this study is shown in Table 2. The product contained less FA and C18:3 and more CP than traditional EF (Gonthier et al., 2004). This can be attributed to the partial loss of oil and the addition of ground alfalfa meal. Intakes of DM and CP were not affected by dietary treatment and averaged 26.7 and 4.5 kg/d, respectively (Table 2). However, cows fed the high-forage diets consumed more ($P < 0.05$) NDF than cows fed the low-forage diets, likely due to their higher NDF contents. Dry matter intake for high-producing cows in early lactation can be limited by high dietary NDF due to rumen fill effect (Allen, 2000). For example, diets containing more than 32% NDF are expected to limit DMI of high-producing dairy cows (Zebeli et al., 2008). However, all diets in the present study contained less than 32% NDF. The lack of effect of EF supplementation on DMI agrees with previous studies which showed that inclusion of processed flaxseed in diets of dairy cows in early lactation up to 10% of the diet DM had no negative effect on DMI (Gonthier et al., 2005; Hurtaud et al., 2010; Petit, 2010).

Milk yield was not influenced by dietary treatment; however, ECM, 4% FCM, and SCM were all higher ($P < 0.05$) for cows fed the high-forage diets than for those fed the low-forage diets (Table 2). In agreement with our findings, Loor et al. (2005) found no difference in milk yield of cows fed a low- (35:65) or high- (65:35) concentrate to forage diet. The lack of effect of dietary treatments on milk yield is likely due to similar intakes of DM and $NE_L$. Milk yield is generally correlated to DMI (NRC, 2001), and in studies where DMI of cows responded positively to low-forage diets, an increase in milk yield has also been observed (Voelker et al., 2002). The lack of response in milk yield as a result of flaxseed supplementation agrees with previous studies (Mustafa et al., 2003; Gonthier et al., 2005). In a review of the literature, Petit (2010) reported no effect of flaxseed supplementation (up to 11% of diet DM) on milk yield of dairy cows in early lactation.

Milk composition was influenced by the forage to concentrate ratio, but not by EF inclusion (Table 3). Cows fed high-forage diets produced milk with higher ($P < 0.05$) fat and TS concentrations but lower ($P <$

0.05) protein and lactose concentrations than cows fed the low-forage diets (Table 3). Our results agree with previous studies, which showed no effect of flaxseed inclusion up to 12% of the diet DM on milk composition (Martin et al., 2008; Petit et al., 2009). However, few studies showed a reduction in milk fat concentration when unheated (Mustafa et al., 2003) or EF (Moallem, 2009) were fed to dairy cows.

Reducing the forage to concentrate ratio is generally associated with lower milk fat and higher milk protein concentration (Yang and Beauchemin, 2007; Aguerre et al., 2011). A significant decrease in milk fat concentration is usually observed when diets contain 60% concentrates or higher (Chilliard et al., 2007). The higher NDF to starch ratio in the high-forage than the low-forage diets (1.43 vs. 1.05) may explain the difference in milk fat concentration between dietary treatments. Previous studies reported a positive relationship between NDF to starch ratio and milk fat concentration (Beckman and Weiss, 2005). Slater et al. (2000) indicated that increasing ruminally degradable starch decreases milk fat concentration without affecting milk yield. Feeding low-forage diets is a major cause of diet-induced milk fat depression (Palmquist and Beaulieu, 1993; Bauman and Griinari, 2003). This is mainly due to the fact that low-forage diets modify ruminal biohydrogenation of dietary FA resulting in the accumulation of *trans* FA, which inhibits FA synthesis in the mammary gland. The reduction in milk protein and milk lactose concentrations associated with high-forage diets is likely due to a reduction in the supply of gluconeogenic precursors such as propionate and metabolizable protein (Broderick, 2003; Jenkins and McGuire, 2006; Sterk et al., 2011).

### *Ruminal Fermentation and Total-Tract Digestibility*

No treatment × time interactions were observed; therefore, only mean effects of dietary treatment were presented (Table 4). Ruminal pH and total VFA concentration were not influenced by dietary treatments (Table 4). In agreement with our findings, Yang et al. (2001) showed no effects of forage to concentrate ratio (35:65 vs. 55:45) on ruminal pH or total VFA. Molar proportion of acetate was higher ($P < 0.05$); whereas that of propionate was lower ($P < 0.05$) for cows fed the high-forage diets relative to cows fed the low-forage diets. Consequently, acetate to propionate ratio was reduced by feeding cows with high-concentrate diets. Other researchers reported similar effects of forage to concentrate ratio on molar proportions of ruminal acetate and propionate (Yang et al., 2001; Voelker et al., 2002). A recent study by Maxin et al. (2011) suggests that propionate can contribute to milk fat depression when feeding cows with low-forage diets by reducing the concentrations of the main precursor of de novo

**Table 3.** Effects of extruded flaxseed supplementation and forage to concentrate ratio on performance of dairy cows

| | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 60:40 forage:concentrate | | 40:60 forage:concentrate | | | *P*-value[1] | | |
| Item | No flaxseed | Flaxseed | No flaxseed | Flaxseed | SEM | 1 | 2 | 3 |
| Intake | | | | | | | | |
| DM, kg/d | 26.6 | 27.2 | 26.2 | 26.7 | 0.75 | 0.13 | 0.23 | 0.84 |
| DM, % BW | 4.1 | 4.2 | 4.1 | 4.1 | 0.16 | 0.15 | 0.29 | 0.75 |
| CP, kg/d | 4.4 | 4.7 | 4.5 | 4.6 | 0.15 | 0.07 | 0.44 | 0.29 |
| NDF, kg/d | 8.1 | 8.6 | 7.7 | 7.8 | 0.29 | 0.01 | <0.01 | 0.12 |
| NDF, % BW | 1.2 | 1.3 | 1.2 | 1.2 | 0.06 | 0.01 | <0.01 | 0.10 |
| Yield, kg/d | | | | | | | | |
| Milk | 39.7 | 39.7 | 40.0 | 41.4 | 2.21 | 0.54 | 0.38 | 0.54 |
| ECM | 41.2 | 40.7 | 37.9 | 38.3 | 2.13 | 0.92 | 0.01 | 0.64 |
| 4% FCM | 38.7 | 37.9 | 34.2 | 34.4 | 2.10 | 0.75 | <0.01 | 0.63 |
| SCM | 38.4 | 38.2 | 35.6 | 35.6 | 1.94 | 0.92 | 0.01 | 0.88 |
| Fat | 1.52 | 1.547 | 1.21 | 1.19 | 0.089 | 0.39 | <0.01 | 0.74 |
| Protein | 1.24 | 1.26 | 1.33 | 1.36 | 0.059 | 0.32 | <0.01 | 0.76 |
| Lactose | 1.84 | 1.85 | 1.89 | 1.95 | 0.103 | 0.46 | 0.18 | 0.55 |
| TS | 4.98 | 4.99 | 4.82 | 4.87 | 0.249 | 0.83 | 0.26 | 0.89 |
| SNF | 3.47 | 3.53 | 3.61 | 3.68 | 0.179 | 0.48 | 0.12 | 0.95 |
| Composition, % | | | | | | | | |
| Fat | 3.82 | 3.70 | 3.04 | 2.89 | 0.135 | 0.09 | <0.01 | 0.86 |
| Protein | 3.16 | 3.21 | 3.34 | 3.32 | 0.054 | 0.81 | <0.01 | 0.30 |
| Lactose | 4.65 | 4.66 | 4.71 | 4.73 | 0.035 | 0.39 | <0.01 | 0.91 |
| TS | 12.57 | 12.60 | 12.10 | 11.81 | 0.175 | 0.27 | <0.01 | 0.16 |
| SNF | 8.75 | 8.91 | 9.06 | 8.92 | 0.135 | 0.92 | 0.07 | 0.08 |
| MUN, mg/dL | 13.3 | 13.6 | 14.0 | 13.0 | 0.60 | 0.08 | 0.83 | 0.37 |
| Feed efficiency (milk/ DMI) | 1.5 | 1.4 | 1.3 | 1.3 | 0.07 | 0.27 | <0.01 | 0.57 |

[1]1 = extruded flaxseed; 2 = forage to concentrate ratio; and 3 = extruded flaxseed × forage to concentrate ratio.

**Table 4.** Effects of flaxseed supplementation and forage to concentrate ratio on ruminal fermentation

| | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 60:40 forage:concentrate | | 40:60 forage:concentrate | | | *P*-value[1] | | |
| Item | No flaxseed | Flaxseed | No flaxseed | Flaxseed | SEM | 1 | 2 | 3 |
| pH | 5.82 | 5.87 | 5.73 | 5.80 | 0.06 | 0.36 | 0.27 | 0.92 |
| $NH_3$-N, mg/dL | 14.67 | 16.67 | 10.35 | 11.11 | 1.13 | 0.31 | 0.02 | 0.61 |
| Total VFA, m*M* | 147.6 | 162.6 | 159.0 | 157.2 | 6.45 | 0.36 | 0.67 | 0.28 |
| Molar proportion, % | | | | | | | | |
| Acetic acid | 50.4 | 51.8 | 46.9 | 45.8 | 1.05 | 0.90 | 0.02 | 0.32 |
| Propionic acid | 30.1 | 30.4 | 35.2 | 35.3 | 1.36 | 0.90 | 0.03 | 0.93 |
| Butyric acid | 19.3 | 17.8 | 18.5 | 18.6 | 0.81 | 0.47 | 0.98 | 0.39 |
| Acetate:propionate | 1.74 | 1.74 | 1.41 | 1.34 | 0.10 | 0.63 | 0.03 | 0.71 |

[1]1 = extruded flaxseed; 2 = forage to concentrate ratio; and 3 = extruded flaxseed × forage to concentrate ratio.

milk FA synthesis (i.e., acetate and BHBA). Ruminal $NH_3$-N was lower ($P < 0.05$) for cows fed the low-forage diets than for cows fed the high-forage diets (Table 4). High-concentrate diets provide more fermentable carbohydrates for ruminal bacteria, which increases the capture of $NH_3$-N for microbial growth and therefore reduces the amount of free $NH_3$-N in the rumen.

Total-tract digestibility of DM ($P = 0.05$) and NDF ($P < 0.01$) were higher for high-forage than low-forage diets, but not influenced by EF inclusion (Table 5). However, dietary treatment had no effect on total-tract digestibility of OM and CP. Neutral detergent fiber from low-forage diets is expected to have faster passage rate, a shorter ruminal retention time, and therefore lower total-tract digestibility than NDF from high-forage diets (Voelker et al., 2002).

### *Milk FA Profile*

Milk FA composition was affected by both forage to concentrate ratio and EF supplementation (Table 6). Cows fed high-forage diets had higher ($P < 0.05$) concentrations of C4 to C8 FA but lower ($P < 0.05$) concentrations of C12:0 and C14:0 than cows fed low-forage diets. Concentrations of milk FA up to C14 were not influenced by EF supplementation, which is in agreement with Moallem (2009), who reported no effect of feeding EF (4% of diet DM) on milk short-chain FA concentrations. However, our results are in contrast with other studies that showed significant reduction in de novo FA synthesis as a result of flaxseed supplementation (Mustafa et al., 2003; Gonthier et al., 2005); in the present study, the level of supplemental flaxseed might not have been large enough to inhibit de novo FA synthesis. It is well documented that the change in the concentration of milk FA is proportional to the inclusion level of flaxseed (Petit and Gagnon, 2009; Petit, 2010). 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 milk FA synthesis than oilseed rich in C18:2. The authors also reported a greater inhibitory effect of oils than oilseeds.

Milk concentration of C16:0 was higher ($P < 0.05$) for cows fed the high-forage diets relative to cows fed the low-forage diets, and was decreased ($P < 0.05$) by EF supplementation (Table 6). Our results on the effects of forage to concentrate ratio on milk C16:0 concentration are in agreement with Loor et al. (2005). The negative effect of EF supplementation on milk C16:0 concentration is likely due to the inhibitory effects of long-chain FA on de novo synthesized C16:0 (Glasser et al., 2008). A similar effect of EF supplementation on C16:0 concentration has been reported by Gonthier et al. (2005) and Moallem (2009).

**Table 5.** Effects of extruded flaxseed supplementation and forage to concentrate ratio on total-tract digestibility of lactating cows

| | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 60:40 forage:concentrate | | 40:60 forage:concentrate | | | *P*-value[1] | | |
| Item | No flaxseed | Flaxseed | No flaxseed | Flaxseed | SEM | 1 | 2 | 3 |
| Total-tract digestibility, % | | | | | | | | |
| DM | 62.8 | 63.2 | 62.0 | 61.1 | 1.56 | 0.72 | 0.05 | 0.39 |
| OM | 63.8 | 64.7 | 63.6 | 62.6 | 1.52 | 0.95 | 0.16 | 0.25 |
| CP | 61.3 | 62.2 | 61.1 | 60.7 | 2.09 | 0.75 | 0.32 | 0.48 |
| NDF | 35.6 | 34.3 | 29.0 | 29.1 | 2.39 | 0.66 | <0.01 | 0.62 |

[1]1 = extruded flaxseed; 2 = forage to concentrate ratio; and 3 = extruded flaxseed × forage to concentrate ratio.

**Table 6.** Effects of extruded flaxseed supplementation and forage to concentrate ratio on milk FA profile of lactating cows

| | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 60:40 forage:concentrate | | 40:60 forage:concentrate | | | *P*-value[1] | | |
| FA | No flaxseed | Flaxseed | No flaxseed | Flaxseed | SEM | 1 | 2 | 3 |
| 4:0 | 1.0 | 1.0 | 0.7 | 0.7 | 0.04 | 0.97 | <0.01 | 0.85 |
| 6:0 | 1.2 | 1.2 | 0.9 | 0.9 | 0.04 | 0.87 | <0.01 | 0.51 |
| 8:0 | 1.0 | 1.0 | 0.9 | 0.8 | 0.04 | 0.75 | <0.01 | 0.39 |
| 10:0 | 2.6 | 2.6 | 2.7 | 2.6 | 0.11 | 0.93 | 0.51 | 0.26 |
| 11:0 | 0.2 | 0.2 | 0.2 | 0.2 | 0.01 | 0.26 | 0.46 | 0.02 |
| 12:0 | 3.2 | 3.2 | 3.8 | 3.6 | 0.11 | 0.61 | <0.01 | 0.14 |
| 13:0 | 0.3 | 0.3 | 0.5 | 0.5 | 0.03 | 0.36 | <0.01 | 0.27 |
| 14:0 | 11.5 | 11.8 | 12.6 | 12.4 | 0.21 | 0.72 | <0.01 | 0.04 |
| 14:0 isot | 0.9 | 1.0 | 1.4 | 1.4 | 0.11 | 0.49 | <0.01 | 0.54 |
| 14:1 *cis*-9 | 1.1 | 1.1 | 1.7 | 1.5 | 0.06 | 0.16 | <0.01 | 0.04 |
| 16:0 | 34.7 | 30.2 | 32.8 | 28.8 | 0.83 | <0.01 | <0.01 | 0.47 |
| 17:0 iso | 0.3 | 0.3 | 0.3 | 0.4 | 0.02 | <0.01 | 0.26 | 0.96 |
| 16:1 *trans*-9 | 0.2 | 0.2 | 0.2 | 0.2 | 0.01 | 0.07 | 0.97 | 0.63 |
| 16:1 | 1.6 | 1.6 | 2.3 | 2.1 | 0.13 | 0.23 | <0.01 | 0.48 |
| 17:0 | 0.6 | 0.6 | 0.7 | 0.7 | 0.03 | 0.74 | <0.01 | 0.15 |
| 17:1 | 0.2 | 0.2 | 0.3 | 0.3 | 0.03 | 0.45 | <0.01 | 0.61 |
| 18:0 | 9.1 | 10.3 | 7.1 | 7.4 | 0.38 | <0.01 | <0.01 | <0.01 |
| 18:1 *trans*-9 | 0.5 | 0.6 | 0.8 | 1.2 | 0.13 | 0.07 | <0.01 | 0.10 |
| C18:1 *trans*-11 | 0.7 | 1.0 | 1.5 | 2.0 | 0.24 | 0.03 | <0.01 | 0.54 |
| 18:1 *cis*-9 | 20.0 | 20.2 | 18.4 | 19.2 | 0.64 | 0.11 | <0.01 | 0.28 |
| 18:1 *cis*-11 | 0.6 | 0.6 | 0.9 | 0.8 | 0.05 | 0.14 | <0.01 | 0.38 |
| 18:1 *cis*-12 | 0.6 | 0.7 | 0.6 | 0.6 | 0.06 | 0.04 | 0.18 | 0.24 |
| 18:1 *cis*-13 | 0.1 | 0.1 | 0.1 | 0.1 | 0.01 | <0.01 | <0.01 | 0.44 |
| 18:1 *cis*-14 | 0.5 | 0.7 | 0.3 | 0.4 | 0.03 | <0.01 | <0.01 | 0.03 |
| 18:1 *cis*-15 | 0.2 | 0.4 | 0.2 | 0.4 | 0.02 | <0.01 | 0.09 | 0.40 |
| 18:1 *cis*-16 | 0.0 | 0.0 | 0.0 | 0.1 | 0.02 | 0.02 | <0.01 | 0.04 |
| 18:2 *cis*-15,*trans*-11 | 0.4 | 0.5 | 0.4 | 0.6 | 0.04 | <0.01 | <0.01 | 0.26 |
| 18:2 *trans*-9, *trans*-12 | 0.2 | 0.3 | 0.2 | 0.3 | 0.02 | <0.01 | 0.16 | 0.02 |
| 18:2 *cis*-9, *trans*-12 | 0.1 | 0.1 | 0.0 | 0.1 | 0.01 | <0.01 | <0.01 | 0.82 |
| 18:2 *trans*-9,*cis*-12 | 0.5 | 0.4 | 0.1 | 0.4 | 0.12 | 0.17 | 0.01 | <0.01 |
| 18:2 *cis*-9,*cis*-12 | 1.3 | 1.6 | 2.2 | 2.4 | 0.15 | 0.02 | <0.01 | 0.72 |
| 18:2 *cis*-9,*cis*-15 | 0.1 | 0.3 | 0.1 | 0.1 | 0.07 | 0.02 | 0.09 | 0.09 |
| 20:0 | 0.1 | 0.1 | 0.0 | 0.1 | 0.01 | <0.01 | <0.01 | 0.79 |
| 18:3 *cis*-6,*cis*-9,*cis*-12 | 0.1 | 0.1 | 0.1 | 0.1 | 0.01 | 0.06 | <0.01 | 0.02 |
| 18:3 *cis*-9,*trans*-12,*cis*-15 | 0.2 | 0.1 | 0.1 | 0.1 | 0.06 | 0.34 | 0.35 | 0.01 |
| 18:3 *cis*-9,*cis*-12,*cis*-15 | 0.6 | 1.0 | 0.4 | 1.0 | 0.03 | <0.01 | 0.07 | 0.02 |
| CLA[2] *cis*-9,*trans*-11 | 0.4 | 0.8 | 0.7 | 0.9 | 0.09 | <0.01 | <0.01 | 0.59 |
| 22:0 | 0.1 | 0.1 | 0.1 | 0.1 | 0.01 | 0.24 | <0.01 | 0.22 |
| 20:3n-6 | 0.1 | 0.1 | 0.1 | 0.1 | 0.01 | 0.37 | <0.01 | <0.01 |
| 20:4n-6 | 0.1 | 0.1 | 0.3 | 0.0 | 0.01 | <0.01 | <0.01 | <0.01 |
| 23:0 | 0.1 | 0.1 | 0.1 | 0.1 | 0.01 | 0.25 | 0.83 | 0.25 |
| 20:5n-3 | 0.0 | 0.0 | 0.1 | 0.1 | 0.03 | 0.83 | <0.01 | 0.99 |
| 22:5n-3 | 0.1 | 0.1 | 0.1 | 0.1 | 0.01 | 0.26 | <0.01 | 0.04 |
| Saturated FA | 65.7 | 63.1 | 63.4 | 59.2 | 1.10 | <0.01 | <0.01 | 0.11 |
| MUFA | 27.3 | 28.3 | 28.7 | 30.4 | 0.88 | <0.01 | <0.01 | 0.41 |
| PUFA | 4.2 | 5.4 | 4.7 | 6.4 | 0.22 | <0.01 | <0.01 | 0.01 |
| C18:2 isomers | 2.9 | 3.9 | 3.6 | 4.9 | 0.19 | <0.01 | <0.01 | 0.19 |

[1]1 = Extruded flaxseed, 2 = Forage:concentrate ratio, 3 = Extruded flaxseed x forage:concentrate ratio.

[2]CLA = conjugated linoleic acid.

Extruded flaxseed supplementation and high-forage diets increased ($P < 0.05$) the concentration of C18:0 milk FA (Table 6). However, the increase was more significant when dairy cows were fed EF together with high-forage than with low-forage diets, as indicated by the forage to concentrate ratio and EF interaction. The increase in the concentrations of C18 FA with EF supplementation is likely due to an increase in mammary uptake of C18 FA absorbed in the small intestine (Gonthier et al., 2004).

In the present study, feeding high-concentrate diets reduced C18:0 and milk fat concentrations. Several researchers have reported a high correlation between C18:0 and milk fat content (Chilliard et al., 2003; Loor et al., 2005). Concentration of *cis*-9 C18:1 was higher ($P < 0.05$) in the milk of cows fed the high-forage diets than cows fed the low-forage diets. However, milk *cis*-9 C18:1 level was not influenced by EF supplementation. According to Loor and Herbein (2003), the deficiency in endogenously synthesized C18:1 is responsible for re-

duced milk fat yield as frequently observed with feeding cows high concentrate diets.

Concentrations of C18:3 and conjugated linoleic acid (**CLA**) increased by 100 and 54%, respectively, as a result of EF supplementation. Feeding high-forage diets increased ($P < 0.05$) the concentration of CLA, but not that of C18:3. Forage to concentrate ratio × EF supplementation interaction ($P < 0.05$) suggested an increase in C18:3 concentration when EF was fed with high-concentrate and not low-concentrate diets. Interactions between forage to concentrate ratio and dietary fat on milk FA composition have been reported by other authors. Effects of flaxseed supplementation were higher on C18:3 when added to high-concentrate diets and were higher on C18:0 when added to high-forage diets (Chilliard and Ferlay, 2004; Dewhurst et al., 2006). Despite significant increases in the concentrations of health-promoting FA such as C18:3 and CLA as a result of extruded flaxseed supplementation, the absolute levels of these FA remained ≤1.0% of total milk FA. These results suggest extensive ruminal biohydrogenation of dietary C18:3 despite the fact that flaxseed was extruded at temperatures approaching 122°C. Gonthier et al. (2005) also reported low transfer efficiency (i.e., 2%) of dietary C18:3 to milk as a result of EF supplementation. Similar low transfer efficiency has also been reported by Chilliard et al. (2001). Gonthier et al. (2005) reported lower milk concentration of C18:3 for cows fed EF relative to those fed micronized flaxseed; this was attributed to the fact that extrusion increased ruminal biohydrogenation of C18:3 and reduced the amount of C18:3 reaching the duodenum (Gonthier et al., 2004).

### *Relationship Between Ruminal Fermentation, Milk FA, and Milk Fat Depression*

It is well documented that diets causing milk fat depression alter ruminal biohydrogenation, causing an increased production of *trans* FA isomers that inhibits de novo milk fat synthesis (Shingfield et al., 2010). *Trans* FA, such as *trans*-10 C18:1 and *trans*-10,*cis*-12 CLA, decrease de novo milk fat synthesis by inhibiting key enzymes and proteins in mammary lipid synthesis (Harvatine and Bauman, 2006; Gervais et al., 2009; Shingfield et al., 2010). However, recent studies have shown that the increase in *trans* FA isomers is insufficient to fully explain the reduction in de novo milk fat synthesis associated with diet-induced milk fat depression (Sutton et al., 2003). On the other hand, high-concentrate diets increase ruminal propionate production, which reduces milk fat concentration and yield (Rulquin et al., 2007; Maxin et al., 2011). Yet, the mechanism by which propionate decreases de novo milk fat synthesis is still unknown. A decrease in the supply of de novo milk FA precursors (i.e., acetate and butyrate) as a result of increased propionate production may be a possible explanation (Maxin et al., 2011).

## CONCLUSIONS

Results of this study showed that most of the effects observed for dairy cow performance, ruminal fermentation and total-tract digestibility were related to the forage to concentrate ratio and not due to EF supplementation. Feed intake and milk yield were not influenced by the forage to concentrate ratio. However, feeding cows high-forage diets increased milk fat and TS concentrations but reduced protein and lactose levels in milk. The reduction in milk fat concentration as a result of feeding high-concentrate diets is likely due to alteration of ruminal fermentation, which resulted in higher levels of FA isomers, such as *trans*-10 C18:1. Extruded flaxseed supplementation beneficially altered milk FA composition, as indicated by higher concentrations of health-promoting CLA and C18:3, but significantly lowered levels of C16:0 SFA. However, the lack of a major effect of EF on milk FA may be associated with the low inclusion rate and extensive ruminal biohydrogenation.

## ACKNOWLEDGMENTS

Carolane Neveu is a recipient of an Industrial Innovation Scholarship from Fonds Quebecois de la Recherche sur la Nature et les Technologies, Natural Sciences and Engineering Research Council of Canada, and Bélisle Solution Nutrition Inc. The authors thank N. Gentesse (Bélisle Solution and Nutrition Inc., Saint-Mathias sur Richelieu, Canada) for diet formulation.

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