Incremental amounts of ground flaxseed decrease milk yield but increase n-3 fatty acids and conjugated linoleic acids in dairy cows fed high-forage diets¹

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ABSTRACT

The objective of this study was to investigate the effect of incremental amounts of ground flaxseed (GFX) on milk yield and concentrations and yields of milk components, milk fatty acids (FA) profile, ruminal metabolism, and nutrient digestibility in dairy cows fed high-forage diets. Twelve multiparous Jersey cows averaging (mean $\pm$ SD) 112 ± 68 d in milk and 441 ± 21 kg of body weight and 8 primiparous Jersey cows averaging 98 ± 43 d in milk and 401 ± 43 kg of body weight were randomly assigned to treatment sequences in a replicated 4×4 Latin square design. Each period lasted 21 d with 14 d for diet adaptation and 7 d for data and sample collection. Treatments were fed as a total mixed ration (63:37 forage-to-concentrate ratio) with corn meal and soybean meal replaced by incremental levels (i.e., 0, 5, 10, or 15% diet dry matter) of GFX. The ruminal molar proportions of acetate and butyrate decreased linearly with GFX supplementation, whereas the ruminal molar proportion of propionate increased linearly resulting in decreased acetate-to-propionate ratio. Apparent total-tract digestibilities of nutrients either decreased (dry matter) or tended to decrease (organic matter, neutral detergent fiber, acid detergent fiber) linearly in cows fed GFX. Milk yield decreased linearly in cows fed increasing amounts of GFX, which is explained by the linear reduction in dry matter intake. Except for the concentrations of milk protein and urea N, which decreased linearly with GFX supplementation, no other changes in the concentration of milk components were observed. However, yields of milk protein and fat decreased linearly with GFX supplementation. The linear decrease in the yields of milk fat and protein are explained by reduced milk yield, whereas that in milk urea N is explained by decreased crude protein intake. No treatment effects were observed for plasma

urea N and nonesterified fatty acids, serum cortisol, and body weight change. Milk odd- and branched-chain FA and saturated FA decreased linearly with GFX supplementation. Milk *trans*-11 18:1, α -linolenic acid, *cis*-9, *trans*-11 18:2, and the sum of n-3 FA all increased linearly and quadratically, whereas the milk ratio of n-6 to n-3 decreased linearly in cows fed GFX. Overall, compared with the control diet (0% GFX), the diet with 15% GFX supplementation resulted in the lowest milk yield but highest milk proportions and yields (data not shown) of *cis*-9, *trans*-11 18:2 and n-3 FA.

Key words: flaxseed, lactating dairy cow, milk fatty acid, milk yield

INTRODUCTION

Flaxseed (*Linum usitatissimum*) has been extensively used as a supplemental source of fat and protein to lactating dairy cows and is one of the richest sources of the essential n-3 FA *cis*-9, *cis*-12, *cis*-15 18:3 (i.e., α -linolenic acid = ALA; Petit, 2010). It is well established that the milk concentration of functional lipids such as CLA and n-3 FA generally increases when feeding flaxseed to lactating dairy cows (Glasser et al., 2008a; Petit, 2010). Previous work has documented a variety of potential human health benefits attributed mostly to the *cis*-9, *trans*-11 isomer of CLA such as cardiovascular diseases, cancer, and modulation of immune and inflammatory responses (McCrorie et al., 2011; Dilzer and Park, 2012). Likewise, it is recognized that n-3 FA are essential for normal growth, brain development, vision, and immunity in infants and have also been linked to prevention of cardiovascular diseases (Connor, 2000; Williams, 2000).

It is noteworthy that consumers are aware of the potential human health benefits attributed to n-3 FA. For instance, a 2-yr survey conducted by Cargill revealed that approximately 98% of consumers who do grocery shopping were aware of n-3 FA and more likely to purchase products containing ALA, eicosapentaenoic acid, or docosahexaenoic acid (Anonymous, 2014).

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Participants of an Internet survey (Pirog, 2004) and web forums (Schuppli et al., 2014) in the United States and Canada associated pasture-based beef or dairy with benefits for human health. In addition to consumers' awareness about the potential health benefits of n-3 FA, a mail survey of organic dairy farmers in the northeastern United States indicated that 84% of respondents identified development of value-added dairy products and marketing as major priorities for organic dairy enterprises (Pereira et al., 2013). Even though milk processors in the United States are not financially compensating farmers for producing milk with enriched concentrations of n-3 FA and CLA, Organic Valley, one of the largest organic milk processors in the country, is currently paying premiums to farmers producing milk with no grain supplementation to the cows (<http://www.organicvalley.coop/products/milk/grassmilk/>).

It is well established that responses of milk FA profile, ruminal fermentation, and animal production to flaxseed (e.g., whole, extruded, or ground) or flaxseed oil supplementation vary based on the composition of the basal diet (Glasser et al., 2008a; Ferlay et al., 2013), level of dietary inclusion (Collomb et al., 2004b; Benchaar et al., 2012; Soder et al., 2012; Ferlay et al., 2013), method of flaxseed processing (Gonthier et al., 2004; Martin et al., 2008), and forage-to-concentrate ratio (Ueda et al., 2003; Loores et al., 2004). A wealth of scientific information is available regarding the effects of flaxseed offered as whole or extruded grain, or as oil, on milk yield, milk FA profile, and nutrient utilization, which contrasts with less research-based information about the use of ground flaxseed (**GFX**) in dairy diets, particularly diets with high proportions of forage (i.e., >60% of diet DM). To the best of our knowledge, only one experiment has been conducted to assess the effect of different levels of GFX, as the sole fat supplemental source, on milk yield and milk FA profile in dairy cows (Collomb et al., 2004a,b), thus warranting further research.

Whereas Collomb et al. (2004a,b) supplemented (DM basis) 4.4 and 6.6% GFX to hay and fodder beet-based diets offered to dairy cows, we aimed to meet and exceed these levels to further investigate the effects of increasing amounts of GFX (0, 5, 10, or 15% DM basis) on milk yield and FA profile, markers of animal health and stress (e.g., blood NEFA and cortisol), ruminal metabolism, and nutrient digestibility in dairy cows fed mixed, mostly grass baleage diets. Specifically, we aimed to replace starch from corn meal and RDP from soybean meal with fat and RDP from GFX. It is hypothesized that incremental amounts of GFX and consequent increased intake of ALA would increase n-3 FA and CLA in milk fat. We reported previously that replacing orchardgrass (*Dactylis glomerata* L.) herb-

age with incremental amounts (0, 5, 10, or 15% of diet DM) of GFX linearly increased the molar proportion of acetate and propionate and decreased that of butyrate and apparent digestibility of DM, OM, and NDF during continuous culture (Soder et al., 2012). However, it must be emphasized that the controlled conditions of in vitro systems do not entirely capture the dynamic conditions of the gastrointestinal tract of ruminants. Therefore, in vivo validation of our in vitro work is needed.

MATERIALS AND METHODS

Care and handling of the animals used in the current study were conducted as outlined in the guidelines of the University of New Hampshire Institutional Animal Care and Use Committee (IACUC Protocol no. 110605). The 84-d-long experiment was conducted at the University of New Hampshire Burley-Demeritt Organic Dairy Research Farm (43°10'N, 70°99'W) from March 13 to June 4, 2012. Twenty cows were used for all sampling procedures and statistical analyses presented herein except for ruminal sampling and analyses, in which 12 cows were used.

Animals, Experimental Design, and Treatments

Twelve multiparous Jersey cows averaging (mean $\pm$ SD) 112 $\pm$ 68 DIM and 441 $\pm$ 21 kg of BW and 8 primiparous Jersey cows averaging (mean $\pm$ SD) 98 $\pm$ 43 DIM and 401 $\pm$ 43 kg of BW at the beginning of the study were used. Cows were randomly assigned to treatment sequences in a 4 $\times$ 4 Latin square design. Distribution of animals to squares was done to balance for differences in DIM, milk production, and parity, resulting in 3 squares of multiparous cows (square 1 = 30 $\pm$ 23 DIM; square 2 = 132 $\pm$ 20 DIM; and square 3 = 174 $\pm$ 40 DIM) and 2 squares of primiparous cows (square 4 = 57 $\pm$ 13 DIM and square 5 = 139 $\pm$ 4 DIM). Each period lasted 21 d with 14 d for diet adaptation and 7 d for data and sample collection. Diets were fed as TMR (63:37 forage-to-concentrate ratio) with corn meal and soybean meal replaced by incremental levels (0, 5, 10, or 15% of diet DM) of GFX (AgMotion Specialty Grains Inc., Minneapolis, MN). The NRC (2001) ration evaluation software was used to balance the experimental diets to meet or exceed the nutritional requirements of a cow producing 22 kg of milk daily with 4.5% milk fat and 3.5% milk protein. Animals were housed in a bedded-pack barn with dried pine shavings as bedding. The bedded area (132 m²) opened to a 478-m² cement outdoor lot (total pen area = 610 m²) allowing cows to walk freely to comply with the USDA National Organic Program Pasture Rules,

which call for year-round access to the outdoors for all ruminants (USDA-AMS, 2010). A roof-covered feeding station equipped with Calan doors (American Calan Inc., Northwood, NH) was located at the end of the outdoor lot.

First-cutting baleage was harvested from 2 fields with similar botanical compositions. Fields were composed mostly of cool-season grass species [i.e., timothy grass (*Phleum pratense*), orchardgrass, Kentucky bluegrass (*Poa pratensis* L.), and tall fescue (*Festuca arundinacea* Schreb.)] with a lower proportion of legume species [i.e., red clover (*Trifolium pratense* L.), alfalfa (*Medicago sativa* L.), and white clover (*Trifolium repens* L.)]. Forage was cut using a flail-type mower centerline conditioner (model FC353RGC; Kuhn North America Inc., Brodhead, WI), tedded (model GA7302DL; Kuhn North America), and field wilted to at least 50% DM attained approximately 36 h after cutting. Forage was then harvested as baleage using a large round baler with a crop cutter (model BR740A; New Holland Agriculture, New Holland, PA), wrapped with stretch plastic using a bale wrapper (model 991BJS; McHale Engineering Ltd., Ballinrobe, Ireland), and stored in the field. The mixed, mostly grass hay used in the experiment was harvested from the same fields used for baleage.

Animal Feeding and Feed Sampling and Analyses

All baleage bales were sampled before feeding using a Hilti model TE 7-A drill (Hilti North America, Tulsa, OK) fitted with a 40-cm-long metal core sampler. Throughout the duration of the study, baleage samples (approximately 50 g each) obtained after 3 to 4 core samplings from each bale were composited to yield individual sample sizes of 150 to 200 g. Baleage samples were dried in a forced-air oven (55°C, 48 h; VWR Scientific, Bridgeport, NJ) with resulting DM used to adjust the daily proportion of baleage in the as-fed TMR. The individual concentrate ingredients as well as the mineral and vitamin premix were mixed once a week using an electric horizontal paddle mixer (Marion Mixer; Rapids Machinery Co., Marion, IA) to yield 4 concentrate mixes that were stored in 121-L trash cans (Rubbermaid Commercial Products, Saratoga Springs, NY). Bales of hay (once a week) and baleage (once a day) were chopped using a vertical mixer (Valmetal V-Mix 400; Saint-Germain-de-Grantham, QC, Canada). Baleage, hay, and concentrate were placed into separate 121-L trash cans (Rubbermaid Commercial Products), weighed on a portable digital ground scale (Rubbermaid Pelouze Digital Receiving Scale; Rubbermaid Commercial Products) with 0.1-kg readability, and finally mixed on a self-propelled vertical TMR mixer (A100 Self-Propelled; Jaylor Fabricating Inc., East Ga-

rafraxa, ON, Canada). Individual TMR were offered to the cows twice daily at approximately 9000 and 1600 h. The amount of feed offered to the cows was adjusted daily to yield refusals equal to approximately 5 to 10% of intake. Refusals were collected daily before the a.m. feeding and weighed in the same manner reported for forage and concentrate. The electronic recognition Calan doors system (American Calan Inc.) was used to individualize the dietary treatments offered to the cows. Animals had free access to water throughout the experiment. Body weights were recorded at the same time (i.e., after the a.m. milking) for 3 consecutive days at the beginning of the experiment and during the last 3 d of each period to compute BW change.

Samples of TMR, feeds, and refusals (composited by diet) were collected daily during the sampling week and composited by period. With the exception of hay and refusals, which were dried in a forced-air oven (55°C, 48 h), all remaining feeds were lyophilized before grinding. Feeds, TMR, and refusals were ground to pass through a 1-mm screen (Wiley mill; Arthur H. Thomas, Philadelphia, PA) and shipped to a commercial laboratory (Dairy One Cooperative Inc., Ithaca, NY). The following analyses were done in the feeds and TMR: DM (method 930.15; AOAC International, 2006), total N (method 990.03; AOAC International, 2006), NDF (method 6; Ankom Technology, Fairport, NY; solutions as in Van Soest et al., 1991), ADF (method 5; Ankom Technology; solutions as in method 973.18; AOAC International, 1998), crude fat (method 2003.05; AOAC International, 2006), starch (YSI 2700 Select Biochemistry Analyzer, application note no. 319; YSI Inc. Life Sciences, Yellow Springs, OH), and ash (method 942.05; AOAC International, 2006). Baleage and TMR were also analyzed for ADIN (LecoTruMac N Macro Determinator on ADF residue; Leco Corporation, St. Joseph, MI). In addition, TMR and GFX were shipped to the Pennsylvania State University (Kevin Harvatin Laboratory; University Park, PA) for individual FA analysis by GLC after direct methylation (Sukhija and Palmquist, 1988). Refusals were analyzed for DM, ash, NDF, ADF, and N according to methods and procedures used for the feed samples reported above.

Ruminal Sampling and Analyses

Samples of ruminal fluid (approximately 250 mL) were collected from 12 cows (squares 2, 3, and 5) via stomach tube (oral lavage method; Lodge-Ivey et al., 2009) for 3 consecutive days (d 17 to 19) after the p.m. milking (approximately 7 h after the a.m. feeding). During sample collection, cows were restrained in a head chute with belly straps to minimize movement. After collection, the ruminal fluid samples were squeezed

through 4 layers of cheesecloth with pH immediately measured using a bench-top pH meter (Thermo Orion SP20; Thermo Fisher Scientific, Chelmsford, MA). The initial flow of each ruminal fluid sample was discarded to minimize saliva contamination. However, saliva contamination cannot be completely disregarded, and as a result, pH data are not reported. A 10-mL aliquot of strained ruminal fluid was added to 0.2 mL of 50% (vol/vol) H_2SO_4 and stored at -20°C for later analysis of $\text{NH}_3\text{-N}$. Ammonia-N was analyzed using an Orion Ion Analyzer Model 407A (Thermo Fisher Scientific) equipped with an NH_3 gas sensing electrode no. 9512 (1 mL of ionic strength adjuster to 10 mL of ruminal fluid). A second 10-mL aliquot of strained ruminal fluid was added to 0.2 mL of 50% (vol/vol) H_2SO_4 , stored at -20°C , and retained for VFA analysis. Analysis of ruminal VFA was done at West Virginia University Rumen Fermentation Profiling Laboratory (Morgantown, WV) using a GLC instrument (Varian 3300; Varian Inc., Palo Alto, CA) equipped with flame ionization detector following the separation procedure outlined in a previous publication (Anonymous, 1975). The column was a 2-m $\times$ 2-mm glass column packed with 10% stationary phase 1200/1% H_3PO_4 on 80/100 Chromosorb WAW media (Supelco Inc., Bellefonte, PA).

Fecal Sampling and Analyses

Fecal grab samples were collected from all 20 cows once a day for 5 consecutive days (d 17 to 21) at 0000, 0800, 1200, 1600, and 2000 h by stimulating defecation or collected directly from the rectum. Samples were pooled by cow based on fresh weight (about 200 g per sampling) over the 5 d to obtain a single composite followed by storage at -20°C in plastic bags. At the end of each sampling week, composite fecal samples were thawed and placed in aluminum trays in a forced-air oven at 55°C until completely dried (approximately 72 h). Dried samples were ground to pass through a 1-mm screen (Willey mill) and analyzed for analytical DM, ash, total N, NDF, and ADF as reported previously. Both TMR and fecal samples were analyzed for indigestible ADF (ADF analysis on residue remaining after 12 d of in situ ruminal incubation; Huhtanen et al., 1994). Indigestible ADF was used as an internal marker to estimate fecal output of DM (Cochran et al., 1986).

Milk Sampling and Analyses

Cows were milked twice daily at approximately 0600 and 1500 h with milk yield recorded at each milking throughout the experiment. Milk samples were collected in the a.m. and p.m. milkings of d 15 and 16 of each sampling week, preserved in tubes containing

2-bromo-2-nitropropan-1,3 diol, pooled by cow according to a.m. and p.m. milk weights, and finally refrigerated at 4°C until shipped to a commercial laboratory (Dairy One Cooperative Inc.) for determination of fat, protein, lactose, and MUN using a Foss MilkoScan 4000 infrared analyzer (Foss Electric, Hillerød, Denmark).

Milk samples without preservative were collected concomitantly, pooled, and stored at -80°C until analysis for FA. Specifically, FAME were prepared by shaking a mixture of 2.5 mL of n-hexane containing 25 mg of lipids and 0.5 mL of 0.5 M sodium methoxide solution in methanol for 5 min. After completion of the methylation reaction, 1 g of sodium bisulfate was added, and vials were vortexed (20 s) and centrifuged (5 min at $2,200 \times g$ and 5°C). Next, the supernatant containing FAME was transferred to a 2-mL vial and directly used for GLC analysis (GC-2010; Shimadzu, Kyoto, Japan). The GLC was equipped with a split injector (1:100 split ratio) and a flame-ionization detector using a CP-Sil 88 WCOT fused silica column (100 m $\times$ 0.25 mm i.d. $\times$ 0.2 μm film thickness; Varian Inc., Lake Forest, CA). The total FA composition covering approximately 70 FA in the range 4:0 to 24:0, including isomers of CLA, were identified. Completeness of the reaction was regularly tested by thin-layer chromatography using n-hexane:diethyl ether:acetic acid (85:15:1) as solvent. Hydrogen was used as the carrier gas at a flow rate of 1 mL/min and for the flame-ionization detector at 40 mL/min. The other gases were purified air at 400 mL/min and N makeup gas at 30 mL/min. Injector and detector temperatures were kept at 250°C . The oven program used was as follows: initial temperature of 45°C and held for 4 min, programmed at $13^\circ\text{C}/\text{min}$ to 175°C and held for 27 min, and then programmed at $4^\circ\text{C}/\text{min}$ to 215°C held for 35 min. The injection volume was 1 μL of FAME mixtures containing about 1 to 2 $\mu\text{g}/\mu\text{L}$. Integration and quantitation was accomplished with GC solution software (version 2.30.00) and based on the flame-ionization detector response. Fatty acid methyl esters were identified by comparison of retention times with known FAME standards (Supelco 37 component FAME mix, *cis/trans* FAME mix, BAME mix, and PUFA no. 3 mix; Supelco Inc., Bellefonte, PA; CLA mixture #UC-59 M from Nu-Chek Prep, Elysian, MN). The short-chain FAME were corrected for mass discrepancy using the correction factors published by Wolff et al. (1995).

Blood Sampling and Analyses

Blood samples were taken once daily on d 15 (1100 h) and d 16 (1500 h) from the coccygeal vein into Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) and composited by cow on a volume basis. The 2

sampling time points (i.e., 1100 and 1500 h) coincided with approximately 2 and 6 h after the a.m. feeding and were chosen to be representative of the first feeding cycle (i.e., from 0900 to 1600 h). Vacutainer tubes containing EDTA were used for plasma NEFA and urea N analyses, and Vacutainer tubes without anticoagulant were used for serum cortisol analysis. After blood collection, tubes were kept on ice and immediately transported to the laboratory for processing. All blood samples were centrifuged ($3,300 \times g$ for 20 min at 5°C) using an Eppendorf Centrifuge model 5810 (Eppendorf, Hamburg, Germany) within 2 h of collection. After centrifugation, plasma and serum samples were stored at -20°C and -80°C , respectively. Concentration of plasma urea N was analyzed colorimetrically using an UV/visible spectrophotometer (Beckman Coulter Inc., Brea, CA) set at a wavelength of 540 nm. Commercial available kits were used to analyze the plasma concentration of NEFA [enzymatic colorimetric assay; HR series NEFA-HR(2); Wako Diagnostics, Richmond, VA] and the serum concentration of cortisol (competitive ELISA, immobilized antigen; catalog no. RCAN-C-270R; BioVendor LLC, Asheville, NC). A chromate microplate reader (Awareness Technology Inc., Palm City, FL) was used to read absorbance of both NEFA (550 nm wavelength) and cortisol (450 nm wavelength).

Statistical Analyses

Data were analyzed using the MIXED procedure of SAS (SAS version 9.3; SAS Institute Inc., Cary, NC) according to a 4×4 Latin square design. The 5 squares were balanced for potential first-order carryover effects in subsequent periods (Williams, 1949; Kim and Stein, 2009). Specifically, each treatment immediately preceded and followed every other exactly once in each square. A Microsoft Excel program was used to randomize treatments and balance for carryover effects (Kim and Stein, 2009). The following model was fitted for all variables:

$$Y_{ijkl} = \mu + S_i + P_j + C_{k(i)} + T_l + S \times T_{il} + E_{ijkl}$$

where Y_{ijkl} = dependent variable, μ = overall mean, S_i = fixed effect of i th square, P_j = fixed effect of j th period, $C_{k(i)}$ = random effect of k th cow within i th square, T_l = fixed effect of l th treatment, $S \times T_{il}$ = interaction between i th square and l th treatment, and E_{ijkl} = error term $\sim N(0, \sigma_e^2)$. Orthogonal polynomial contrasts were used to test responses (linear and quadratic) from incremental dietary levels of GFX. All reported values are least squares means and standard error of the mean. Significance was declared at $P \leq 0.05$ and trends

at $0.05 < P \leq 0.10$. The interaction term was removed from the final model when $P \geq 0.25$.

The ruminal concentration of $\text{NH}_3\text{-N}$ was analyzed as repeated measures over time using the same model described above with the addition of the fixed effects of day and day $\times$ treatment interaction. The subject of the repeated measures was defined as cow (square $\times$ period $\times$ treatment). The spatial covariance structure compound symmetry had the lowest Akaike information criterion and was retained in the final model. The day $\times$ treatment interaction was removed from the final model ($P > 0.25$).

RESULTS AND DISCUSSION

Nutrient Composition of the Experimental Diets

The ingredient and nutrient composition of the experimental diets and the nutrient composition of the individual feedstuffs are presented in Tables 1 and 2, respectively. The evaluation of the experimental diets using the NRC (2001) ration evaluation software is presented in Table 3. As soybean meal and corn meal were replaced by incremental levels of GFX, the concentration (DM basis) of crude fat (from 3.8 to 7.4%; Table 1) and NE_L (from 1.6 to 1.8 Mcal/kg; Table 3) increased, whereas that of starch (from 19.0 to 11.5%; Table 1) and NFC (from 25.5 to 21.4%; Table 1) decreased. These results are primarily explained by substituting starch from corn meal with crude fat from GFX (Table 2).

Ruminal Fermentation Profile and Nutrient Digestibility

The ruminal fermentation profile as well as nutrient intake and digestibility are presented in Table 4. The ruminal concentration of $\text{NH}_3\text{-N}$ and total VFA were not affected by GFX supplementation and averaged 9.01 and 67.0 mM, respectively. However, the ruminal molar proportions of acetate, butyrate, isobutyrate, valerate, and isovalerate all decreased linearly with feeding dairy cows incremental amounts of GFX. The apparent total-tract digestibilities of OM ($P = 0.06$), NDF ($P = 0.08$), and ADF ($P = 0.07$) decreased linearly, thereby suggesting that decreased ruminal molar proportions of all VFA, except propionate, were caused by reduced OM and fiber digestibility in the rumen. In fact, we demonstrated that the ruminal apparent digestibilities of both OM and NDF decreased linearly when replacing orchardgrass herbage with incremental amounts (0, 5, 10, or 15% of diet DM) of GFX (Soder et al., 2012). It is noteworthy, however, that current results partially agree with Soder et al. (2012), who re-

Table 1. Ingredient and nutrient composition of diets with incremental amounts (DM basis) of ground flaxseed offered to lactating dairy cows

Item	Dietary amounts of ground flaxseed, %			
	0	5	10	15
Ingredient composition, % of diet DM				
Mixed, mostly grass baleage	55.0	55.0	55.0	55.0
Mixed, mostly grass hay	8.00	8.00	8.00	8.00
Ground flaxseed	0.00	5.00	10.0	15.0
Soybean meal	6.00	4.80	3.50	2.00
Roasted soybean	2.00	2.00	2.00	2.00
Corn meal	27.0	23.2	19.5	16.0
Sodium bicarbonate	0.50	0.50	0.50	0.50
Minerals and vitamins premix ¹	1.50	1.50	1.50	1.50
Nutrient composition				
DM, % of fresh matter	65.5	66.2	66.6	66.8
CP, % of DM	18.3	18.4	18.4	18.3
ADIN, % of DM	0.25	0.23	0.22	0.21
NDF, % of DM	45.6	46.2	46.2	45.9
ADF, % of DM	30.0	30.2	30.8	31.1
Hemicellulose, ² % of DM	15.6	16.1	15.3	14.9
Ash, % of DM	6.84	6.78	6.94	7.02
Crude fat, % of DM	3.83	4.98	5.73	7.35
Starch, % of DM	19.0	16.4	13.9	11.5
NFC, ³ % of DM	25.5	23.7	22.8	21.4
FA, g/kg of DM				
14:0	0.10	0.11	0.11	0.11
16:0	4.36	4.85	5.06	5.42
<i>cis</i> -9 16:1	0.06	0.07	0.08	0.09
17:0	0.05	0.06	0.06	0.07
18:0	0.69	1.09	1.36	1.68
<i>cis</i> -9 18:1 (oleic acid)	2.65	4.55	5.78	7.40
<i>cis</i> -11 18:1	0.18	0.23	0.27	0.31
<i>cis</i> -9, <i>cis</i> -12 18:2 (linoleic acid)	7.09	8.59	8.96	9.80
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3 (α -linolenic acid)	5.86	10.4	14.0	19.0
20:0	0.20	0.21	0.22	0.23
<i>cis</i> -13 20:1	0.04	0.06	0.07	0.08
22:0	0.26	0.27	0.29	0.30
24:0	0.18	0.24	0.24	0.25
<i>cis</i> -15 24:1	0.12	0.11	0.10	0.10
Unidentified	3.09	3.02	3.02	3.22

¹The minerals and vitamins premix provided (as-fed basis) Ca (88.5 g), P (42 g), Mg (66.1 g), K (16.1 g), S (33.6 g), Na (16.5 g), Cl (27.4 g), Fe (1,308 mg/kg), Cu (649 mg/kg), Mn (2,099 mg/kg), Zn (3,949 mg/kg), Co (55.0 mg/kg), I (81.0 mg/kg), Mo (6.0 mg/kg), Se (24.0 mg/kg), vitamin A (138,945 IU/kg), vitamin D (37,712 IU/kg), and vitamin E (986.1 IU/kg).

²Hemicellulose = NDF – ADF.

³Calculated as $100 - (\% \text{ CP} + \% \text{ NDF} + \% \text{ crude fat} + \% \text{ ash})$.

ported linear increase or decrease for the ruminal molar proportions of acetate and butyrate, respectively.

Similar to present results, the ruminal molar proportion of propionate increased linearly in response to incremental amounts of GFX in our previous in vitro study (Soder et al., 2012). As a result of increased propionate and decreased acetate and butyrate, the acetate-to-propionate ratio decreased and the gluconic-to-lipogenic ratio increased linearly. Increased ruminal propionate may be associated with butyrate metabolism in the rumen. According to Moss et al. (2000), increased ruminal butyrate results in more H₂ available to be used as a substrate for methanogenesis. Beauchemin et al. (2009) reported that, compared with

the control diet, lactating dairy cows offered crushed flaxseed had lower ruminal molar proportion of butyrate and CH₄ production. Soder et al. (2012) observed linear decreases in ruminal butyrate and CH₄ output when orchardgrass herbage was replaced with GFX. Because ruminal butyrate decreased and propionate increased linearly in the current study, it is conceivable that H₂ was shifted from CH₄ to propionate pathway production in the rumen.

The apparent total-tract digestibility of nutrients decreased (i.e., DM) or tended (i.e., OM, NDF, and ADF) to decrease linearly with GFX supplementation (Table 4). No effect of GFX was observed for CP digestibility (mean = 68%), which agrees with our

Table 2. Nutrient composition of mixed, mostly grass baleage and hay, ground flaxseed, soybean meal, roasted soybean, and corn meal offered to lactating dairy cows

Item	Baleage	Hay	Ground flaxseed ¹	Soybean meal	Roasted soybean	Corn meal
DM, % of fresh matter	53.9	90.4	93.7	92.6	92.1	88.0
CP, % of DM	18.6	11.1	22.8	48.6	40.1	8.15
ADIN, % of DM	0.26	NA ²	NA	NA	NA	NA
NDF, % of DM	51.3	64.6	25.2	20.8	22.2	8.80
ADF, % of DM	34.3	39.5	16.3	10.3	11.8	3.90
Hemicellulose, ³ % of DM	17.1	25.1	8.93	10.6	10.4	4.90
Ash, % of DM	7.58	5.67	3.51	8.25	5.55	5.24
Crude fat, % of DM	3.75	2.00	33.6	5.95	20.4	3.65
Starch, % of DM	0.45	0.80	0.63	1.35	1.70	68.7
NFC, ⁴ % of DM	20.4	16.7	14.9	16.4	22.2	74.2
NE _L , Mcal/kg of DM	1.31	1.08	3.13	1.92	2.50	1.98

¹Ground flaxseed FA composition (g/kg of DM): 0.17 (14:0), 20.6 (16:0), 0.42 (*cis*-9 16:1), 0.24 (17:0), 13.6 (18:0), 70.4 (*cis*-9 18:1, oleic acid), 2.26 (*cis*-11 18:1), 56.6 (*cis*-9,*cis*-12 18:2, linoleic acid), 198 (*cis*-9,*cis*-12,*cis*-15 18:3, α -linolenic acid), 0.57 (20:0), 0.58 (*cis*-13 20:1), 0.04 (*cis*-8,*cis*-11,*cis*-14 20:3), 0.68 (22:0), 0.43 (24:0), 0.05 (*cis*-15 24:1), 2.84 (unidentified FA).

²NA = not analyzed.

³Hemicellulose = NDF – ADF.

⁴Calculated as 100 – (% CP + % NDF + % crude fat + % ash).

previous research (Soder et al., 2012). Similarly, apparent total-tract digestibilities of DM and OM were depressed in dairy cows fed barley silage-based diets supplemented with crushed flaxseed (Beauchemin et al., 2009). Scholljegerdes and Kronberg (2010) also observed decreased total-tract digestibility of OM, but that of NDF was not affected in beef heifers grazing summer native range herbage supplemented with a GFX–dried molasses mix (98.6% GFX and 1.6% dried molasses) or a cracked corn–soybean meal mix (65.6%

cracked corn, 32.7% soybean meal, and 1.7% dried molasses). Results from Schroeder et al. (2014) showed that GFX yielded the fastest rate of in situ DM degradation (11.3%/h), followed by flaxseed meal (7.5%/h), rolled flaxseed (4.5%/h), and whole flaxseed (0.57%/h), thus suggesting that more oil was released in the rumen when flaxseed was fed ground. Therefore, the potential increase in oil release in the rumen as corn meal and soybean meal were replaced by incremental amounts of GFX may have negatively affected ruminal function

Table 3. NRC (2001) model predictions for diets containing incremental amounts of ground flaxseed¹

Item	Dietary amounts of ground flaxseed, %			
	0	5	10	15
Predicted DMI, kg/d	17.2	17.1	17.0	16.7
NE _L , Mcal/kg of DM	1.56	1.62	1.70	1.76
NE _L allowable milk, kg/d	23.1	24.5	23.6	25.5
MP allowable milk, kg/d	20.2	20.1	18.3	18.6
NE _L required, Mcal/d	24.8	24.5	24.2	23.7
NE _L supplied, Mcal/d	26.4	27.4	26.7	28.3
NE _L balance, Mcal/d	1.60	2.80	2.50	4.60
MP required, g/d	1,696	1,668	1,603	1,562
MP supplied, g/d	1,643	1,621	1,487	1,500
MP balance, g/d	–54.0	–46.0	–116	–62.0
RDP, % of diet DM	12.0	12.4	12.8	13.2
RUP, % of diet DM	5.10	4.90	4.70	4.50
RUP-MP, g/d	666	624	532	502
Bacterial-MP, g/d	897	917	881	922
Lys, % of MP	6.58	6.66	6.80	6.87
Met, % of MP	1.87	1.88	1.91	1.93
His, % of MP	2.19	2.18	2.17	2.15
Total EAA, % of MP	44.5	44.7	45.2	45.4
MP-Lys:MP-Met ratio	3.52	3.54	3.56	3.56

¹Actual feeds nutrient composition (i.e., DM, CP, NDF, ADF, and crude fat) and animal variables were used (i.e., DMI, milk yield and composition, DIM, and BW). The EAA composition used for ground flaxseed was obtained from a brown-seeded whole flaxseed (NorLin variety; Oomah and Mazza, 1993). Default NRC (2001) values for the EAA composition were used for the remaining feedstuffs.

Table 4. Least squares means for ruminal concentrations of $\text{NH}_3\text{-N}$ and total VFA, ruminal molar proportion of individual VFA, nutrient intake, and apparent total-tract digestibility of nutrients in dairy cows fed (DM basis) incremental amounts of ground flaxseed¹

Item	Dietary amounts of ground flaxseed, %				SEM	Contrast (<i>P</i> -value) ²	
	0	5	10	15		Linear	Quadratic
Ruminal $\text{NH}_3\text{-N}$, mM	8.94	9.16	8.67	9.28	0.50	0.77	0.58
Ruminal total VFA, mM	64.9	65.4	66.2	71.3	3.36	0.13	0.42
Ruminal acetate (A), mol/100 mol	72.8	72.3	72.2	71.8	0.29	<0.01	0.73
Ruminal propionate (P), mol/100 mol	14.4	14.7	15.4	16.4	0.18	<0.001	0.06
Ruminal butyrate (B), mol/100 mol	10.4	10.6	10.2	9.72	0.19	<0.001	0.08
Ruminal isobutyrate, mol/100 mol	1.03	1.02	0.92	0.87	0.02	<0.001	0.38
Ruminal valerate, mol/100 mol	0.81	0.78	0.73	0.72	0.02	<0.001	0.63
Ruminal isovalerate, mol/100 mol	0.60	0.58	0.51	0.43	0.02	<0.001	0.07
A:P ratio	5.05	4.92	4.68	4.38	0.07	<0.001	0.24
P:(A + B) ratio	0.17	0.18	0.19	0.20	0.003	<0.001	0.06
DMI, kg/d	16.9	16.9	15.7	16.1	0.25	<0.001	0.33
DM digestibility, % of DMI	67.8	68.7	67.5	64.9	1.14	0.03	0.09
OM intake, kg/d	15.7	15.7	14.6	15.0	0.24	<0.001	0.36
OM digestibility, % of OM intake	69.2	70.4	69.1	66.8	1.10	0.06	0.09
NDF intake, kg/d	7.68	7.79	7.25	7.39	0.14	<0.01	0.88
NDF digestibility, % of NDF intake	61.9	64.3	59.8	59.5	1.80	0.08	0.36
ADF intake, kg/d	5.08	5.03	4.93	4.95	0.12	0.21	0.66
ADF digestibility, % of ADF intake	56.1	58.6	54.8	53.4	2.42	0.07	0.55
CP intake, kg/d	3.08	3.09	2.88	2.94	0.06	<0.001	0.54
CP digestibility, % of CP intake	68.2	69.0	66.7	68.0	1.72	0.64	0.89

¹n = 12 cows for ruminal metabolism variables and n = 20 cows for nutrient intake and digestibility variables.

²Probability of linear and quadratic effects for incremental dietary amounts of ground flaxseed; significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

and microbial growth, particularly cellulolytic bacteria and protozoa.

Animal Production, Milk Components, and Blood Metabolites

Results of DMI are presented in Table 4, and yields of milk and milk components, feed efficiency, blood metabolites, and BW change are presented in Table 5. Intake of DM decreased linearly when soybean meal and corn meal were replaced by increasing amounts of GFX. Larsen et al. (2012) fed (DM basis) 0, 3.5, 6.8, or 10.2% of a 1:3 GFX–ground rapeseed mix during lactation wk 6 to 30 and also reported a linear decrease in DMI. As discussed earlier, apparent total-tract digestibilities of NDF and ADF were depressed in cows fed GFX (Table 4). Because NDF and ADF are primarily digested in the rumen (Huhtanen et al., 2010), it is conceivable that reduced fiber digestibility increased ruminal fill (Mertens, 1987; Dado and Allen, 1995), thereby explaining to some extent the linear decrease in DMI. Alternative mechanisms that could potentially explain the detrimental effects of supplemental fat on feed intake include depression of ruminal fermentation and gut motility, poor diet palatability, release of gut hormones, and oxidation of FA in the liver (Chilliard, 1993; Allen, 2000).

Milk yield decreased linearly with replacement of soybean meal and corn meal with increasing amounts of GFX, and this response is primarily explained by reduced feed intake (i.e., DMI and OM intake). In fact, feed intake is the most important single nutritive factor affecting production in dairy cows (Mertens, 1994; Huhtanen et al., 2011). Except for the concentrations of milk protein and MUN, which decreased linearly with GFX supplementation, no other changes in the concentration of milk components were observed (i.e., fat, lactose, SNF, and TS). Larsen et al. (2012) also reported a linear decrease in the concentration of milk protein in dairy cows offered increasing amounts of a GFX–ground rapeseed mix. Based on the NRC (2001) model, dietary energy did not limit milk protein synthesis in the current study because of a positive NE_L balance across all 4 diets (Table 3). Conversely, MP balance was negative across treatments, particularly in those with greater inclusions of GFX, suggesting that MP-predicted milk may have limited milk protein synthesis in diets with 10 and 15% GFX. However, the ratio of MP-Lys to MP-Met averaged 3.55 (Table 3), showing that all 4 diets were deficient in Met, and as a result, neither Met nor Lys status could explain the observed depression in milk protein synthesis based on the NRC (2001) model. The concentration of MP-His was quite similar among treatments, thereby indicating

Table 5. Least squares means for milk yield, concentration and yield of milk components, feed efficiency, and plasma concentration of urea N (PUN) and NEFA, and serum concentration of cortisol in lactating dairy cows fed (DM basis) incremental amounts of ground flaxseed¹

Item	Dietary amounts of ground flaxseed, %				SEM	Contrast (<i>P</i> -value) ²	
	0	5	10	15		Linear	Quadratic
Milk yield, kg/d	21.2	21.0	20.6	19.9	0.52	<0.001	0.25
Milk fat, %	4.59	4.58	4.62	4.65	0.14	0.49	0.78
Milk fat, kg/d	0.98	0.99	0.94	0.93	0.03	0.05	0.69
Milk protein, %	3.44	3.39	3.37	3.32	0.05	<0.001	0.97
Milk protein, kg/d	0.73	0.73	0.69	0.66	0.02	<0.001	0.33
Milk lactose, %	4.80	4.79	4.79	4.84	0.04	0.46	0.36
Milk lactose, kg/d	1.04	1.04	0.99	0.98	0.03	<0.01	0.65
Milk SNF, %	9.22	9.20	9.07	9.16	0.09	0.13	0.25
Milk SNF, kg/d	1.98	2.00	1.87	1.85	0.05	<0.001	0.56
Milk TS, %	13.8	13.8	13.8	13.8	0.19	0.91	0.78
Milk TS, kg/d	2.96	2.98	2.83	2.78	0.08	<0.01	0.48
4% FCM, ³ kg/d	23.4	23.6	22.5	22.0	0.63	0.01	0.51
ECM, ⁴ kg/d	25.1	25.2	24.0	23.4	0.65	<0.01	0.45
Milk yield:DMI ratio	1.24	1.28	1.26	1.19	0.05	0.29	0.09
4% FCM:DMI ratio	1.34	1.38	1.36	1.30	0.04	0.39	0.17
ECM:DMI ratio	1.44	1.48	1.45	1.38	0.05	0.28	0.15
MUN, mg/dL	12.8	12.7	12.0	10.9	0.44	<0.001	0.06
Milk N, % of N intake	23.4	23.4	23.9	22.3	0.67	0.22	0.15
PUN, mg/dL	16.8	17.1	17.6	17.0	1.07	0.83	0.66
Plasma NEFA, mEq/L	204	230	230	218	18.76	0.57	0.26
Serum cortisol, ng/mL	84.0	82.0	84.4	77.0	10.78	0.56	0.70
BW change, kg/d	-0.19	-0.20	-0.22	-0.17	0.10	0.86	0.65

¹n = 20 cows.²Probability of linear and quadratic effects for incremental dietary amounts of ground flaxseed; significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.³4% FCM = $[0.40 \times \text{milk yield (kg/d)}] + [15 \times \text{milk fat yield (kg/d)}]$ (Gaines and Davidson, 1923).⁴ECM = $[0.327 \times \text{milk yield (kg/d)}] + [12.95 \times \text{fat yield (kg/d)}] + [7.2 \times \text{protein yield (kg/d)}]$ (Orth, 1992).

that His was not a factor in the observed milk protein depression. Therefore, alternative mechanisms may have been involved. According to Casper and Schingoethe (1989), elevated blood FA caused by increased FA intake decreases the release of somatotropin (i.e., growth hormone), leading to reduced mammary uptake of AA. Cant et al. (1993) proposed that fat supplements decrease milk protein concentration by reducing blood flow through the mammary gland, ultimately reducing the extraction of blood AA for protein synthesis.

Yields of milk fat, protein, lactose, SNF, and TS all decreased linearly with increasing levels of GFX (Table 5). These linear reductions in the yields of milk components are primarily explained by decreased milk yield, which, in turn, was caused by reduced feed intake and nutrient digestibility. Responses in the yields of milk and milk components are inconsistent when comparing studies in which dairy cows were fed incremental amounts of GFX or extruded flaxseed. For instance, whereas yields of milk and milk components (e.g., fat, protein, and lactose) were not affected by feeding increasing amounts of extruded flaxseed (0, 5, 10, or 15% diet DM; Ferlay et al., 2013), these same variables decreased linearly in the current study. On the other hand, Hurtaud et al. (2010) reported linear increases in

the yields of milk and milk fat in dairy cows fed incremental amounts of extruded flaxseed (0, 2.1, or 4.3% of the diet DM). It is conceivable that oil from GFX is more rapidly released in the rumen than oil from extruded flaxseed, explaining to a certain extent the discrepancies across studies. Differences in the forage composition of the basal diet as well as amount of dietary flaxseed fed to cows further explain the inconsistencies in the production of milk and milk components reported in the literature.

Both 4% FCM and ECM decreased linearly in cows fed diets with increasing amounts of GFX (Table 5). Milk yield as well as yield of milk fat and protein also decreased linearly with incremental dietary levels of GFX, thus explaining the adverse effects on 4% FCM and ECM. Because DMI and milk yield decreased linearly, feed efficiency expressed as milk yield:DMI (average = 1.24), 4% FCM:DMI (average = 1.35), or ECM:DMI (average = 1.42) was not affected by dietary levels of GFX. Concentration of MUN decreased linearly with GFX supplementation to dairy cows, which coincided with the linear decrease in CP intake (Table 4). The negative linear relationship between CP intake and MUN is well documented in the literature (Colmenero and Broderick, 2006). Dietary levels of GFX did not

affect the concentrations of plasma urea N, plasma NEFA, and serum cortisol (mean = 17.1 mg/dL, 221 mEq/L, and 81.8 ng/mL, respectively; Table 5).

Milk FA Profile

The milk proportions of individual SFA, odd- and branched-chain FA, and total SFA are presented in Table 6. Except for the milk proportions of 4:0, 5:0, and 24:0 (this FA responded quadratically), all remaining individual SFA as well as the sum of odd- and branched-chain FA and individual SFA responded linearly in cows fed increasing amounts of GFX. Within the SFA that responded linearly, only 1 FA (i.e., 18:0) showed a positive linear response as a result of GFX supplementation. Linear responses coincided with quadratic responses for the milk proportions of 7:0, 16:0, and 18:0, most individual odd- and branched-chain FA, and the sum of odd- and branched-chain FA. Similar to current results, the milk proportions of de novo synthesized FA (i.e., 6:0 to 14:0) and 16:0 decreased linearly, whereas the milk proportion of 18:0 increased linearly in dose-response studies that fed GFX (Collomb et al., 2004b) or a GFX–ground rapeseed mix (Larsen et al., 2012). Decreased milk proportions of 6:0 to 16:0 and increased 18:0 is consistent with the inverse relationship between de novo synthesis of FA and intake of PUFA (Palmquist et al., 1993; Chilliard et al., 2000). In addition to reduced novo synthesis, Loor et al. (2004) reported that compared with the control diet, supplementation with flaxseed oil increased the ruminal biohydrogenation of ALA and the duodenal flow of 18:0 in lactating dairy cows, which may further explain the increased milk proportion of 18:0 in the present study. Linear and quadratic relationships between duodenal flow of 18:0 and 18:0 apparently absorbed in the small intestine have been reported, with the curvilinear response suggesting saturation of 18:0 intestinal absorption at high duodenal flows (Glasser et al., 2008b).

The milk proportions of individual and total unsaturated FA are presented in Table 7. Milk *cis*-9 14:1, most *cis* 16:1 FA, and *cis*-9 17:1 decreased linearly, whereas the sum of *cis*- and *trans*-18:1 MUFA increased linearly in cows fed increasing amounts of GFX. Linear responses coincided with quadratic responses for the milk proportions of several ≤ 18 -C MUFA including *cis*-9 14:1, *cis*-9 16:1, *cis*-9 18:1 (i.e., oleic acid), *cis*-12 18:1, and the sum of *cis* 18:1 MUFA and total *cis* MUFA. The Δ^9 -desaturase indices for the *cis*-9 14:1/14:0 and *cis*-9 18:1/18:0 pairs decreased linearly (Table 7), suggesting less contribution from endogenous synthesis or more reliance on dietary sources or mobilization from the adipose tissue to account for the increase in milk

oleic acid with GFX supplementation. Indeed, intake of oleic acid increased linearly when cows were fed incremental amounts of GFX (data not shown).

With the exception of *cis*-9,*cis*-12 18:2 (i.e., linoleic acid), which decreased linearly, the milk proportions of all remaining 18:2-C FA, including CLA, increased linearly with incremental amounts of GFX. Despite the linear increase in linoleic acid intake with GFX supplementation (data not shown), the proportion of this FA in milk fat and its transfer (i.e., apparent recovery) from diet to milk decreased linearly (Table 7). These results suggest that dietary linoleic acid was extensively biohydrogenated in the rumen as shown previously (Loor et al., 2004). On average, *cis*-9,*trans*-11 CLA contributed 82% of the total CLA found in milk fat. Increased *cis*-9,*trans*-11 CLA in response to incremental amounts of GFX (Collomb et al., 2004a,b) or a GFX–ground rapeseed mix (Larsen et al., 2012) is well documented in the literature. Likewise, it is well established that endogenous synthesis in the mammary gland is the major source of *cis*-9,*trans*-11 CLA secreted in milk fat through the action of Δ^9 -desaturase using *trans*-11 18:1 as substrate (Grinari et al., 2000). In fact, *trans*-11 18:1 and *cis*-9,*trans*-11 CLA increased linearly, with the largest increment observed between the 10 and 15% GFX supplementation (quadratic effects, $P < 0.01$).

The milk proportion of ALA increased linearly with the highest difference in concentration (+0.30 g/100 g of FA) observed between the 0 and the 5% GFX supplementation (quadratic effect, $P < 0.001$). Intake of ALA increased linearly as a result of GFX supplementation (data not shown), thus explaining the increase of ALA in milk fat. Scholljegerdes and Kronberg (2010) reported that compared with a cracked corn–soybean meal mix (0.32% of BW on a DM basis), GFX (0.18% of BW on a DM basis) increased the duodenal flow of ALA 11-fold when supplemented to beef heifers grazing summer native range herbage. Thus, increased duodenal supply of ALA may also explain ALA increase on milk fat. Incremental amounts of flaxseed oil fed to dairy cows led to a linear increase in ALA in milk fat, with the largest increase in concentration (+0.46 g/100 g of FA; quadratic effect) observed when flaxseed oil was increased from 0 to 2% of diet DM (Benchaar et al., 2012). Similarly, incremental amounts of a GFX–ground rapeseed mix linearly increased the milk concentration of ALA in dairy cows (Larsen et al., 2012). However, the apparent recovery of dietary ALA in milk fat decreased linearly in the current and previous studies (Benchaar et al., 2012; Larsen et al., 2012), likely as a result of extensive ruminal biohydrogenation of ALA as discussed previously. It is well known that ruminal biohydrogenation is generally recognized as a microbial strategy to mitigate PUFA toxicity (Kemp et al., 1984).

Table 6. Least squares means for milk proportion of SFA, 11-cyclohexyl 11:0, and odd- and branched-chain FA (OBCFA) in lactating dairy cows fed (DM basis) incremental amounts of ground flaxseed¹

FA, ² g/100 g of total milk FA	Dietary amounts of ground flaxseed, %				SEM	Contrast (<i>P</i> -value) ³	
	0	5	10	15		Linear	Quadratic
4:0	3.08	3.18	3.16	3.14	0.06	0.37	0.15
5:0	0.02	0.02	0.02	0.02	0.01	0.65	0.18
6:0	2.04	1.95	1.82	1.63	0.03	<0.001	0.06
7:0	0.02	0.01	0.01	0.01	0.001	<0.01	0.01
8:0	1.15	1.05	0.93	0.78	0.02	<0.001	0.16
9:0	0.02	0.02	0.01	0.01	0.001	<0.001	0.56
10:0	2.62	2.19	1.86	1.48	0.06	<0.001	0.61
11:0	0.24	0.20	0.18	0.14	0.01	<0.001	0.96
11-cyclohexyl 11:0	0.05	0.05	0.05	0.03	0.004	<0.001	0.05
12:0	2.89	2.31	1.92	1.51	0.07	<0.001	0.08
13:0	0.12	0.09	0.08	0.06	0.003	<0.001	0.11
<i>iso</i> 13:0	0.02	0.02	0.02	0.02	0.001	0.52	0.80
<i>anteiso</i> 13:0	0.06	0.05	0.04	0.03	0.002	<0.001	0.04
14:0	10.3	8.70	7.55	6.20	0.15	<0.001	0.25
<i>iso</i> 14:0	0.12	0.11	0.09	0.08	0.003	<0.001	0.50
15:0	0.76	0.65	0.59	0.53	0.01	<0.001	<0.01
<i>iso</i> 15:0	0.19	0.18	0.16	0.14	0.01	<0.001	0.25
<i>anteiso</i> 15:0	0.31	0.28	0.26	0.24	0.01	<0.001	0.29
16:0	27.4	21.6	18.6	16.0	0.41	<0.001	<0.001
<i>iso</i> 16:0	0.27	0.22	0.18	0.16	0.01	<0.001	0.03
17:0	0.48	0.40	0.35	0.32	0.01	<0.001	<0.001
<i>iso</i> 17:0	0.23	0.22	0.22	0.26	0.01	<0.01	<0.01
<i>anteiso</i> 17:0	0.22	0.20	0.18	0.17	0.01	<0.001	0.05
18:0	11.8	15.7	17.5	18.5	0.50	<0.001	<0.001
20:0	0.14	0.14	0.13	0.12	0.01	<0.01	0.38
21:0	0.03	0.03	0.02	0.02	0.001	<0.001	0.52
22:0	0.07	0.07	0.06	0.05	0.002	<0.001	0.65
24:0	0.04	0.05	0.05	0.04	0.002	0.35	0.01
Σ OBCFA ⁴	2.77	2.40	2.17	2.02	0.04	<0.001	<0.001
Σ SFA ⁵	61.9	57.2	53.8	49.7	0.49	<0.001	0.33

¹n = 20 cows.²The FA detection limit was <0.01% FAME.³Probability of linear and quadratic effects for incremental dietary amounts of ground flaxseed; significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.⁴Σ OBCFA = 13:0 + *iso* 13:0 + *anteiso* 13:0 + *iso* 14:0 + 15:0 + *iso* 15:0 + *anteiso* 15:0 + *iso* 16:0 + 17:0 + *iso* 17:0 + *anteiso* 17:0.⁵This does not include OBCFA.

With the exception of *cis*-11 20:1 (not affected by GFX) and *cis*-5,*cis*-8,*cis*-11,*cis*-14,*cis*-17 20:5 (i.e., eicosapentaenoic acid; quadratic effect, $P = 0.04$), all remaining 20-C atoms unsaturated FA decreased linearly with feeding incremental amounts of GFX (Table 7). The milk proportion of *cis*-7,*cis*-10,*cis*-13,*cis*-16,*cis*-19 22:5 also decreased linearly with GFX supplementation, and no *cis*-4, *cis*-7,*cis*-10,*cis*-13,*cis*-16,*cis*-19 22:6 (i.e., docosahexaenoic acid) was detected in milk fat. According to Shingfield and Wallace (2014), unsaturated FA with chain lengths >18-C are susceptible to extensive ruminal biohydrogenation. In addition to ruminal biohydrogenation, n-3 FA are extensively confined to plasma cholesterol ester and phospholipids (Tyburczy et al., 2008), which are poorly taken up by the mammary gland (Offer et al., 2001).

Whereas the milk proportion of n-6 FA decreased linearly, that of n-3 FA increased linearly and quadratically with GFX supplementation (Table 7). These

responses resulted in linear and quadratic decreases in the ratio of n-6 to n-3 FA, which are consistent with the linear decrease in linoleic acid as well as the linear and quadratic increases in ALA. Lowering the ratio of n-6 to n-3 FA in food has been recommended to prevent and modulate certain diseases in humans, particularly coronary heart disease (Connor, 2000; Shingfield et al., 2008). It is important to note, however, that the milk proportion of eicosapentaenoic acid was extremely low and docosahexaenoic acid was not detected in milk fat of cows fed GFX in the present study.

CONCLUSIONS

Incremental amounts of GFX linearly decreased DMI and yields of milk and milk components (i.e., fat, protein, lactose, SNF, and TS) without effect on the concentrations of milk components except milk protein and MUN, which decreased linearly. The ruminal

Table 7. Least squares means for milk proportion of unsaturated FA in lactating dairy cows fed (DM basis) incremental amounts of ground flaxseed¹

Item	Dietary amounts of ground flaxseed, %				SEM	Contrast (<i>P</i> -value) ³	
	0	5	10	15		Linear	Quadratic
FA, ² g/100 g of total milk FA							
<i>cis</i> -9 14:1	0.55	0.43	0.37	0.30	0.02	<0.001	0.04
<i>cis</i> -7 16:1	0.01	0.02	0.04	0.05	0.002	<0.001	0.88
<i>cis</i> -8 16:1	0.08	0.08	0.07	0.07	0.03	<0.01	0.51
<i>cis</i> -9 16:1	0.81	0.63	0.55	0.53	0.03	<0.001	<0.001
<i>cis</i> -11 16:1	0.01	0.01	0.01	0.01	0.002	<0.001	0.34
<i>cis</i> -8 17:1	0.04	0.04	0.04	0.05	0.003	0.07	0.10
<i>cis</i> -9 17:1	0.01	0.08	0.07	0.07	0.01	<0.001	0.07
<i>cis</i> -9 18:1 (oleic acid)	13.2	16.4	18.0	19.2	0.41	<0.001	<0.01
<i>cis</i> -11 18:1	0.26	0.26	0.24	0.27	0.01	0.82	0.04
<i>cis</i> -12 18:1	0.21	0.36	0.47	0.54	0.02	<0.001	<0.01
<i>cis</i> -13 18:1	0.04	0.05	0.07	0.09	0.003	<0.001	0.17
<i>cis</i> -14/ <i>trans</i> -16 18:1	0.25	0.48	0.68	0.87	0.01	<0.001	0.07
<i>cis</i> -15 18:1	0.13	0.26	0.44	0.60	0.01	<0.001	0.20
<i>cis</i> -16 18:1	0.03	0.06	0.08	0.11	0.01	<0.001	0.97
<i>trans</i> -9 14:1	0.01	0.01	0.01	0.01	0.001	0.20	0.71
<i>trans</i> -9 16:1	0.01	0.02	0.01	0.02	0.004	0.34	0.56
<i>trans</i> -4 18:1	0.01	0.02	0.03	0.04	0.002	<0.001	0.78
<i>trans</i> -5 18:1	0.01	0.02	0.02	0.02	0.002	<0.001	0.38
<i>trans</i> -6-8 18:1	0.15	0.23	0.29	0.37	0.01	<0.001	0.78
<i>trans</i> -9 18:1	0.14	0.19	0.23	0.28	0.01	<0.001	0.40
<i>trans</i> -10 18:1	0.21	0.29	0.33	0.48	0.03	<0.001	0.15
<i>trans</i> -11 18:1	1.17	1.41	1.55	2.29	0.10	<0.001	<0.01
<i>trans</i> -12 18:1	0.19	0.36	0.49	0.67	0.01	<0.001	0.87
<i>trans</i> -13/14 18:1	1.28	1.66	2.16	2.74	0.16	<0.001	0.49
<i>cis</i> -9, <i>cis</i> -12 18:2 (linoleic acid)	1.39	1.33	1.25	1.19	0.04	<0.001	0.88
<i>cis</i> -9, <i>trans</i> -13/ <i>trans</i> -8, <i>cis</i> -12 18:2	0.10	0.19	0.27	0.36	0.01	<0.001	0.99
<i>cis</i> -9, <i>trans</i> -14 18:2	0.03	0.08	0.11	0.16	0.01	<0.001	0.57
<i>cis</i> -12, <i>trans</i> -16 18:2	0.13	0.30	0.50	0.79	0.04	<0.001	0.03
<i>trans</i> -11, <i>trans</i> -15 18:2	0.02	0.05	0.08	0.15	0.01	<0.001	<0.01
<i>trans</i> -12, <i>cis</i> -15 18:2	0.01	0.04	0.10	0.12	0.004	<0.001	0.12
<i>cis</i> -9, <i>cis</i> -11 CLA	0.03	0.04	0.05	0.07	0.004	<0.001	0.95
<i>cis</i> -9, <i>trans</i> -11 CLA	0.41	0.47	0.52	0.76	0.04	<0.001	<0.01
<i>trans</i> -7, <i>trans</i> -9 CLA	0.02	0.03	0.04	0.06	0.003	<0.001	0.36
<i>trans</i> -11, <i>trans</i> -13 CLA	0.02	0.02	0.04	0.06	0.003	<0.001	0.01
<i>cis</i> -6, <i>cis</i> -9, <i>cis</i> -12 18:3	0.02	0.01	0.01	0.01	0.002	<0.001	0.49
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3 (α-linolenic acid)	0.49	0.79	1.04	1.11	0.04	<0.001	<0.001
<i>cis</i> -9 20:1	0.08	0.08	0.08	0.08	0.002	0.59	0.04
<i>cis</i> -11 20:1	0.02	0.02	0.02	0.02	0.002	0.99	0.45
<i>cis</i> -11, <i>cis</i> -14 20:2	0.03	0.02	0.02	0.02	0.002	<0.01	0.29
<i>cis</i> -5, <i>cis</i> -8, <i>cis</i> -11 20:3	0.06	0.05	0.04	0.04	0.003	<0.001	0.18
<i>cis</i> -5, <i>cis</i> -8, <i>cis</i> -11, <i>cis</i> -14 20:4	0.06	0.05	0.05	0.04	0.002	<0.001	0.13
<i>cis</i> -5, <i>cis</i> -8, <i>cis</i> -11, <i>cis</i> -14, <i>cis</i> -17 20:5 (EPA) ⁴	0.05	0.05	0.06	0.05	0.003	0.58	0.04
<i>cis</i> -7, <i>cis</i> -10, <i>cis</i> -13, <i>cis</i> -16, <i>cis</i> -19 22:5	0.10	0.10	0.09	0.08	0.003	<0.001	0.06
Δ ⁹ -desaturase index ₁₄ ⁵	5.04	4.64	4.66	4.54	0.17	<0.001	0.13
Δ ⁹ -desaturase index ₁₆	2.85	2.82	2.85	3.18	0.01	<0.01	0.02
Δ ⁹ -desaturase index ₁₈	53.0	51.0	50.9	51.1	1.02	0.02	0.06
Δ ⁹ -desaturase index _{<i>cis</i>-9, <i>trans</i>-11 CLA}	26.0	24.9	25.1	25.0	0.68	0.06	0.18
Σ <i>cis</i> 18:1 MUFA	13.9	17.4	19.3	20.8	0.42	<0.001	<0.01
Σ <i>cis</i> MUFA	15.6	18.8	20.6	22.0	0.43	<0.001	0.01
Σ <i>trans</i> 18:1 MUFA	3.42	4.66	5.78	7.76	0.22	<0.001	0.07
Σ <i>trans</i> MUFA	3.44	4.69	5.79	7.79	0.22	<0.001	0.07
Σ MUFA	19.1	23.4	26.4	29.8	0.40	<0.001	0.10
Σ CLA	0.48	0.56	0.65	0.94	0.04	<0.001	<0.01
Σ n-6 ⁶	1.55	1.47	1.37	1.30	0.05	<0.001	0.85
Σ n-3 ⁷	0.64	0.94	1.19	1.24	0.04	<0.001	<0.001
n-6/n-3	2.42	1.58	1.16	1.06	0.03	<0.001	<0.001
Σ PUFA	2.97	3.64	4.26	5.05	0.15	<0.001	0.52

Continued

Table 7 (Continued). Least squares means for milk proportion of unsaturated FA in lactating dairy cows fed (DM basis) incremental amounts of ground flaxseed¹

Item	Dietary amounts of ground flaxseed, %				SEM	Contrast (<i>P</i> -value) ³	
	0	5	10	15		Linear	Quadratic
Apparent recovery, ⁸ %							
<i>cis</i> -9, <i>cis</i> -12 18:2	11.4	9.17	8.46	7.06	0.43	<0.001	0.15
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	5.04	4.47	4.45	3.40	0.24	<0.001	0.14

¹n = 20 cows.²The FA detection limit was <0.01% FAME.³Probability of linear and quadratic effects for incremental dietary amounts of ground flaxseed; significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.⁴EPA = eicosapentaenoic acid.⁵ Δ^9 -desaturase index₁₄ = *cis*-9 14:1 $\div$ (14:0 + *cis*-9 14:1) $\times$ 100; a similar calculation was done for the remaining Δ^9 -desaturase indices shown in this table.⁶*cis*-9, *cis*-12 18:2 + *cis*-6, *cis*-9, *cis*-12 18:3 + *cis*-11, *cis*-14 20:2 + *cis*-5, *cis*-8, *cis*-11 20:3 + *cis*-5, *cis*-8, *cis*-11, *cis*-14 20:4.⁷*cis*-9, *cis*-12, *cis*-15 18:3 + *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17 20:5 + *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 22:5.⁸Apparent recovery = (milk FA yield $\div$ FA intake) $\times$ 100.

fermentation shifted toward less molar proportions of acetate and butyrate and more propionate. However, this fermentation shift in favor of glucogenic metabolism did not prevent the linear reduction in milk yield, which was driven by decreased DMI and total-tract nutrient digestibility. We conclude that the proportions and yields (data not shown) of milk FA with potential human health benefits such as oleic acid, ALA, *cis*-9,*trans*-11 CLA can be substantially improved through GFX supplementation, particularly the 15% GFX treatment, with concomitant reduction in total SFA, specifically 12:0, 14:0, and 16:0. However, GFX supplementation decreased the proportion of total odd- and branched-chain FA in milk fat, which have been associated with anticarcinogenic properties (Shingfield et al., 2008). Based on the results of the current study, the recommended amount of GFX to be fed on commercial dairy farms would depend on GFX costs and milk processors' premiums for high n-3 and CLA in milk fat. Further research is needed to address the effect of GFX supplementation on diets with different forage-to-concentrate ratios as well as different forage sources in the basal diet.

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