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S. L. Kronberg, E. J. Scholljegerdes, E. J. Murphy, R. E. Ward, T. D. Maddock and C. S. Schauer

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Treatment of flaxseed to reduce biohydrogenation of α-linolenic acid by ruminal microbes in sheep and cattle, and increase n-3 fatty acid concentrations in red meat[1,2]

S. L. Kronberg,*[3] E. J. Scholljegerdes,† E. J. Murphy,‡ R. E. Ward,§ T. D. Maddock,# and C. S. Schauer‖

*USDA-ARS, Northern Great Plains Research Laboratory, P.O. Box 459, Mandan, ND 58554; †Department of Animal and Range Sciences, New Mexico State University, Las Cruces 88003; ‡Departments of Pharmacology, Physiology and Therapeutics, and Chemistry, University of North Dakota, Grand Forks 58202; §Department of Nutrition and Food Sciences, Utah State University, Logan 84322; #Cattle Concepts, Inc., 2005 9th Street N., Fargo, ND 58102; and ‖Hettinger Research Extension Center, North Dakota State University, Hettinger 58639

ABSTRACT: The objective of the study was to determine if flaxseed treated with a formaldehyde-free process could increase n-3 fatty acid (FA) concentrations in lamb and steer muscle. Twenty-four lambs (initial BW 43.8 ± 4.4 kg) were randomly divided into 4 groups for a 90-d trial. One treatment group (FLX) was fed 136 g/d of nontreated ground flaxseed; another treatment group (FLXT1) was fed 136 g/d of flaxseed treated to protect a-linolenic acid (ALA) from microbial hydrogenation; a third treatment group (FLXT2) was fed 136 g/d of a second treated flaxseed; and a fourth treatment group (CNTL) was fed corn and soybean meal with similar CP and DE levels as the other treatments. Intake of treated flaxseed raised plasma triacylglycerol concentrations of ALA more ($P < 0.01$) than intake of nontreated flaxseed did, but there was no difference ($P = 0.65$) in ALA increase between FLXT1 and FLXT2. Intake of treated flaxseed increased ($P \leq 0.05$) muscle phospholipid ALA and eicosapentaenoic acid concentrations more than nontreated flaxseed did. There were no differences in muscle phospholipid n-3 concentrations between FLXT1 and FLXT2. Ten yearling steers (initial BW 437 ± 18 kg) were randomly divided into 2 groups. One group was fed ground flaxseed (0.05% of steer BW/d; FLX; n = 5) and a second group was fed treated flaxseed at the same rate (FLXT; n = 5). The 175-d trial was divided into 2 periods: high roughage, low concentrate period followed by high concentrate, low roughage period. Steers were fed rations that were formulated to be isonitrogenous, isocaloric, and isolipidic. There was no difference ($P = 0.37$) in increase of ALA in blood plasma of FLX and FLXT groups by the end of the first period. However, FLXT had 16% greater ($P = 0.003$) concentration of ALA in their plasma during the second period. Muscle phospholipid n-3 FA were not greater ($P \geq 0.55$) for steers in the FLXT group. Intake of treated flaxseed raised n-3 concentrations in blood and muscle of sheep, and in blood of cattle but did not raise n-3 FA concentrations in muscle of steers. Supplementing the diets of forage-fed lambs with flaxseed treated to reduce hydrogenation of ALA by ruminal microbes can increase concentrations of n-3 FA in the muscle of lambs.

Key words: cattle, linseed, n-3 fatty acids, omega-3 fatty acids, protected, sheep

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[3]Corresponding author: scott.kronberg@ars.usda.gov

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INTRODUCTION

The n-3 fatty acids (**FA**) have beneficial effects on human health, including effects against inflammation, platelet aggregation, hypertension, and hyperlipidemia, with clinical and epidemiologic studies indicating potentially important roles against cardiovascular disease, diabetes, cancer, autoimmune diseases, and mental illnesses (Kris-Etherton et al., 2003; Riediger et al., 2009; James et al., 2010; Feskens, 2011). Additionally, greater intake of the n-6 FA linoleic acid (**LN**) and lesser intake of the n-3 FA α-linolenic acid (**ALA**) by many people over the last several decades may be an important factor for the increasing occurrence of obesity in children and adults (Ailhaud and Guesnet, 2004; Ailhaud et al., 2006; Massiera et al., 2010).

Fish, such as salmon and tuna, are often the n-3 FA source that people are encouraged to eat. However, necessary intake of this source of n-3 FA to reduce the risk of health problems is much less than encouraged (Kronberg et al., 2006). Red meat contains small amounts of n-3 FA and this is probably important for people who eat a lot of red meat but little or no fish. In fact, for many people, red meat can be a much more significant source of n-3 FA than fatty fish simply because they eat little, if any, fish but eat considerable amounts of red meat. Additionally, concentrations of n-3 FA in red meat can be increased when ruminants consume feedstuffs that contain increased concentrations of these FA (Kronberg et al., 2006; Scollan et al., 2006).

Red meat could be an even better source of n-3 FA for people if n-3 FA in feedstuffs were protected from hydrogenation by ruminal microbes (Ashes et al., 1992; Scott and Ashes, 1993; Scollan et al., 2003; Kronberg et al., 2007; Doreau et al., 2011). Although protecting n-3 FA in feeds by encapsulating them in a matrix of formaldehyde-treated protein can increase their concentrations in muscle (Ashes et al., 1992; Scollan et al., 2003), the use of formaldehyde, a carcinogenic substance, in the treatment process may reduce consumer acceptance of meat from animals that are fed this protected n-3 FA source. An alternative technique is needed to protect n-3 FA in feedstuffs, such as flaxseed from biohydrogenation by ruminal microbes. Therefore, the objective of this study was to determine if flaxseed, which was treated with a proprietary and formaldehyde-free process to reduce microbial hydrogenation of ALA, could increase n-3 FA concentrations in blood and muscle of sheep and cattle. Flaxseed was used as the n-3 FA source because it contains a high concentration of ALA and the initial evaluation of the treated flaxseed was done with sheep because much less of the product was needed for sheep compared with cattle.

MATERIALS AND METHODS

All procedures used in the study were approved by the animal care and use committee of the Northern Great Plains Research Laboratory (Mandan, ND).

Lamb Trial

Animals and Diets. Twenty-four Rambouillet wether lambs (initial BW 43.8 ± 4.4 kg and 7 mo old) were randomly divided into 4 groups of 6 for a 90-d trial. All groups were fed a basal ration of alfalfa pellets in the morning (0800 to 0900 h) and afternoon (1600 to 1700 h). Each lamb was fed 567 g of alfalfa pellets at each feeding for the first 51 d of the trial; then, starting on d 52 of the trial, their afternoon allotment was increased to 667 g per lamb for the remainder of the trial. One group (**FLX**) was fed 136 g/(lamb·d) of nontreated ground flaxseed; another group (**FLXT1**) was fed 136 g/(lamb·d) of flaxseed treated (Double Pass LLC, Tualatin, OR) with a proprietary process to protect ALA in flaxseed from microbial biohydrogenation in the rumen (henceforth called treated flaxseed); a third group (**FLXT2**) received 136 g/(lamb·d) of a second treated flaxseed made by the same company with a slightly different proprietary process than the first; and a fourth control group (**CNTL**) received a mixture of corn and soybean meal that had similar levels of CP and DE as the flaxseed treatments (Table 1). The processes by which the 2 treated flaxseeds were made cannot be revealed, but chemical and physical processes were used that allow the products to be classified as organically produced. A small amount [27 g/(lamb·d)] of molasses syrup was added to all 4 feed mixtures to reduce sorting and dust. The lambs were penned as a group, except during morning feeding and had free-choice access to water and trace-mineral salt in their pen. They were fed alfalfa pellets as a group in the afternoon. For morning feedings of the first 10 d, all lambs were individually fed only the alfalfa pellets and corn-soybean mixture, which was later fed only to CNTL. On d 10 d, a jugular blood sample (10 mL) was collected from each lamb between 1230 and 1330 h, and kept on ice until the plasma (1,200 × *g* for 20 min at 4°C) was harvested from each sample and frozen. For morning feedings of the next 14 d, the 4 groups were fed their treatment rations and alfalfa pellets, then a second jugular blood sample (10 mL) was collected from each lamb at the same time of day as before and plasma harvested as described above. For the next 66 d, lambs were fed their treatment rations and alfalfa pellets, then were slaughtered the next day at 10 mo of age.

Slaughter and Sampling Procedures. Lambs were transported to a commercial abattoir in the afternoon and harvested the next morning under USDA inspection in accordance with the Humane Slaughter Act. After slaughter,

Table 1. Chemical and fatty acid composition of feedstuffs for the lambs (DM basis)

Item	Alfalfa pellets	Soybean Meal	Corn	FLXT1[1]	FLXT2[1]	Nontreated flaxseed
CP%	21.3	48.5	7.7	20.6	20.3	22.8
TDN%[2]	62.1	83.1	86.2	54.4	60.4	74.8
Fatty acid, mg/g						
16:0	3.5	3.7	2.7	14.8	12.5	14.5
18:0	0.6	0.9	0.0	9.1	7.4	9.4
18:1n-9c	0.7	2.1	3.5	46.4	36.0	44.4
18:2n-6	2.3	13.8	13.8	45.3	37.9	46.0
18:3n-3 (ALA[3])	3.1	2.5	0.6	156.9	138.9	162.4
SFA	4.1	4.7	2.7	24.0	19.8	24.1
MUFA	0.7	2.1	3.5	46.4	36.1	44.4
PUFA	5.4	16.2	14.4	202.5	176.8	208.7
Total fatty acids	13.6	24.9	20.6	277.4	237.2	281.2

[1]FLXT1 = first treated flaxseed to reduce biohydrogenation of α-linolenic acid by ruminal microbes; FLXT2 = second treated flaxseed to reduce biohydrogenation of α-linolenic acid by ruminal microbes.

[2]TDN were calculated using the equation of Linn and Martin (1989) TDN = 0.889 – (ADF% * 0.779).

[3]ALA = alpha-linolenic acid

carcasses were hung in a cooler at 1.5°C for 22 h. Muscle tissue from the triceps brachii of the front leg was collected from each carcass into individually marked plastic freezer bags with interlocking closure, promptly frozen in liquid nitrogen, and then transported to a freezer for storage at –20°C until analysis was done.

Feedstuff Analysis. Samples of alfalfa pellets and nontreated and treated flaxseeds were dried at 45°C in a forced-air oven, and analyzed for DM (AOAC method 930.1). Nitrogen content of feedstuffs was determined using a Carlo Erba Model NA 1500 Series 2 N/C/S Analyzer (CE Elantech, Lakewood, NJ). Feedstuffs were analyzed for ADF using an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). A description of the forage quality and FA profile is presented in Table 1.

Fatty Acid Analysis of Feedstuffs, Blood Plasma, and Muscle. Samples of all feedstuffs were analyzed for FA via direct transesterification with methanolic-HCL as described by Kucuk et al. (2001). Plasma total lipid extraction and FA analysis were done as described by Kronberg et al. (2007). Muscle lipid extraction and phospholipid FA separation and analysis were done as described by Kronberg et al. (2006).

Statistical Design and Analysis. Blood plasma ALA concentrations for the lambs were evaluated with a repeated measures design using the MIXED procedure with individual animal the random effect, feed treatment (FLX, FLXT1, FLXT2) the fixed effect, and time of blood sampling the repeated measure. An unstructured covariance structure was used for the analysis because it had the smallest value for Akaike's information criterion when compared with the covariance structures compound symmetry and heterogeneous compound symmetry. Treatment means were separated with multiple runs of the MIXED procedure. Data for muscle phospholipid n-3 FA were compared with a completely randomized design using the MIXED procedure (SAS Inst. Inc., Cary, NC). Animal was the random effect and feed treatments (FLX, FLXT1, FLXT2, CNTL) were the fixed effects. Treatment means were separated with multiple runs of the MIXED procedure. Differences between treatment groups were considered significant at $P \leq 0.05$ and a trend at $P \leq 0.10$).

Steer Trial

Animals and Diets. Ten yearling Red Angus steers (initial BW 437 ± 18 kg) were randomly divided into 2 groups of 5 and penned individually in 3.7 × 3.7 m outdoor pens. One group was fed ground flaxseed (0.05% steer BW/d; **FLX**) and a second group received treated flaxseed at the same rate (**FLXT**). The FA composition of the 2 flaxseed treatments and feedstuffs fed are listed in Table 2. The treated flaxseed (Double Pass LLC) was prepared by the proprietary methods mentioned above to reduce biohydrogenation of ALA by ruminal microbes. There was a 20-d pretrial period to allow steers to adjust to pens, feed boxes, and drinking cups, followed by a 175-d trial that was divided into 2 periods: high roughage, low concentrate period

Table 2. Fatty acid composition of feedstuffs for the steer trial (DM basis)

Fatty acid, mg/g	Hay	Nontreated flaxseed	Treated flaxseed	Soybean meal	Corn
16:0	1.4	9.5	10.5	4.0	5.3
18:0	0.2	6.7	5.4	0.8	0.7
18:1n-9c	0.3	28.6	25.0	2.7	10.2
18:2n-6	0.7	30.9	29.8	13.1	21.9
18:3n-3	0.6	88.7	101.7	2.0	0.7
SFA	1.6	16.2	15.9	4.8	5.9
MUFA	0.3	28.6	25.0	2.7	10.2
PUFA	1.3	119.6	131.4	15.1	22.5
Total fatty acids	8.7	171.2	179.0	28.9	44.0

Table 3. Composition of rations and feedstuffs for the high forage, low concentrate period of the steer trial for the group fed ground flaxseed (FLX) and the group fed treated flaxseed (FLXT)

	Treatments	
Item	FLX	FLXT
Ingredient Chopped hay	45.8	45.7
Ground corn	33.4	33.3
Soybean meal	9.2	9.3
Ground flaxseed	9.8	--
Treated flaxseed	--	9.9
Trace Minerals[1]	1.1	1.1
Vitamin A,D,E[2]	0.1	0.1
Dried molasses	0.6	0.6
Composition		
DM, %	90.9	91.1
CP, % DM	14.8	14.8
TDN,[3] % DM	72.1	72.2
NE_m[4]	0.76	0.76
NE_g[4]	0.48	0.48
Ca, % DM	0.77	0.77
P, % DM	0.44	0.45
Crude fat, % DM	5.2	5.2

[1]DuMOR mineral (Brentwood, TN). guaranteed analysis: calcium [Ca; minimum (min.)] 12.00%, calcium [Ca; maximum (max.)] 14.40%, phosphorus (P; min.) 12.00%, salt (NaCl; min.) 11.75%, salt (NaCl; max.) 14.00%, sodium (Na; min.) 5.40%, sodium (Na; max.) 6.50%, magnesium (Mg; min.) 1.20%, potassium (K; min.) 0.50%, manganese (Mn; min.) 1,800 ppm, cobalt (Co; min.) 18.00 ppm, copper (Cu; min.) 500.00 ppm, copper (Cu; max.) 505.00 ppm, iodine (I; min.) 125.00 ppm, selenium (Se; min.) 20.00 ppm, selenium (Se; max.) 20.50 ppm, zinc (Zn; min.) 1,950.00 ppm, vitamin A (min.) 60,000 IU/lb [AU: lb is not an acceptable unit; please convert all "lb" to SI unit], vitamin D3 (min.) 15,000 IU/lb, vitamin E (min.) 100 IU/lb, ruminant meat and bone meal free.

[2]Super A,D,E (MS*; Hubbard Feeds, Mankato, MN). Guaranteed analysis: CP (min.) 7.2%, crude fat (min.) 2.6%, crude fiber (max.) 4.3%, ADF (max.) 2.4%, calcium (min.) 4.9%, calcium (max.) 5.9%, vitamin A (min.) 908,000 IU/kg, vitamin D (min.) 181,600 IU/kg, vitamin E (min.) 454 IU/kg.

[3]TDN were calculated using the equation of Linn and Martin (1989). TDN = 0.889 – (ADF% * 0.779).

[4]Based on published values (NRC, 2000).

Table 4. Composition of rations and feedstuffs for the high concentrate, low forage period of the steer trial for the group fed ground flaxseed (FLX) and the group fed treated flaxseed (FLXT)

	Treatments	
Item	FLX	FLXT
Ingredient Chopped hay	20	20.0
Ground corn	62.8	62.6
Soybean meal	7.3	7.4
Ground flaxseed	6.9	0
Treated flaxseed	0	7.0
Calcium Carbonate	0.9	0.9
Trace Minerals[1]	1.3	1.3
Vitamin A,D,E[2]	0.1	0.1
Dried molasses	0.7	0.7
Composition		
DM, %	90.1	90.2
CP, % DM	13.7	13.7
TDN,[3] % DM	80.04	80.08
NE_m[4]	0.88	0.88
NE_g[4]	0.58	0.58
Ca, % DM	0.81	0.81
P, % DM	0.47	0.48
Crude fat, % DM	5.2	5.2

[1]DuMOR mineral (Brentwood, TN). Guaranteed analysis: calcium [Ca; minimum (min.)] 12.00%, calcium [Ca; maximum (max.)] 14.40%, phosphorus (P; min.) 12.00%, salt (NaCl; min.) 11.75%, salt (NaCl; max.) 14.00%, sodium (Na; min.) 5.40%, sodium (Na; max.) 6.50%, magnesium (Mg; min.) 1.20%, potassium (K; min.) 0.50%, manganese (Mn; min.) 1,800 ppm, cobalt (Co; min.) 18.00 ppm, copper (Cu; min.) 500.00 ppm, copper (Cu; max.) 505.00 ppm, iodine (I; min.) 125.00 ppm, selenium (Se; min.) 20.00 ppm, selenium (Se; max.) 20.50 ppm, zinc (Zn; min.) 1950.00 ppm, vitamin A (min.) 60,000 IU/lb [AU: lb is not an acceptable unit; please convert all "lb" to SI unit], vitamin D3 (min.) 15,000 IU/lb, vitamin E (min.) 100 IU/lb, ruminant meat and bone meal free.

[2]Super A,D,E (MS*) (Hubbard Feeds, Mankato, MN). Guaranteed analysis: CP (min.) 7.2%, crude fat (min.) 2.6%, crude fiber (max.) 4.3%, ADF (max.) 2.4%, calcium (min.) 4.9%, calcium (max.) 5.9%, vitamin A (min.) 908,000 IU/kg, vitamin D (min.) 181,600 IU/kg, vitamin E (min.) 454 IU/kg.

[3]TDN were calculated using the equation of Linn and Martin (1989). TDN = (0.889 – ADF% * 0.779).

[4]Based on published values (NRC, 2000).

followed by low roughage, high concentrate period. With these periods, blood plasma ALA concentrations in respect to the 2 treatments could be determined relative to the 2 different rations. To establish baseline concentrations of n-3 FA, at the end of the pretrial period, 10 mL of whole blood was collected from each steer via coccygeal venipuncture at 1300 h into heparinized Vacutainer tubes (BD, Franklin Lakes, NJ) and kept on ice until it was centrifuged at 1,200 × *g* for 20 min at 4°C to obtain plasma. The plasma was stored at –20°C until thawed and analyzed for FA. During the pretrial period, each steer was fed ground hay ad libitum and 3.63 kg/d ground corn. After the pretrial period, all steers were randomly assigned to 1 of 2 treatments that were formulated to be isonitrogenous, isocaloric, and isolipidic (Tables 3 and 4). Steers were offered ad libitum access to this diet for 56 d. At the end of the first period, 10 mL of whole blood was collected again in the same manner described above to determine the concentrations of n-3 FA when steers consumed the high roughage, low concentrate ration. Then, the second period began. Steers were kept on the same treatments and adapted to a high concentrate diet over 2 wk with the final diet formulation presented in Table 4. During the second period of the trial, 10 mL of blood was collected from each steer 56 d after this period began—in the same manner as described above. Steers remained on their respective treatments and diets for an additional 119 d at which point they were sent to a commercial facility for slaughter.

Slaughter and Sampling Procedures. Steers were transported to a commercial slaughter facility the day before slaughter, slaughtered the next morning with USDA inspection, and in accordance with the Humane Slaughter Act. Carcasses were chilled at 2°C for 24 h, then were

ribbed between the 12th and 13th ribs, and samples of LM collected into interlocking plastic bags. The samples were then transported on ice to a freezer where they were stored at –20°C for several months until analysis. Samples of hay and nontreated and treated flaxseeds were dried and analyzed for DM, N, and ADF, as described for the lamb trial.

Fatty Acid Analysis of Feedstuffs, Blood Plasma, and Muscle. Fatty acid analysis of feedstuffs was conducted as described for the lamb trial. Plasma FA were analyzed for FA analysis using the procedures of Lake et al. (2006). Separation of FA methyl esters was achieved by GLC (Model CP-3800, Varian Inc., Palo Alto, CA) with a 100-m capillary column (SP-2560, Supelco, Bellefonte, PA) and H_2 gas as a carrier gas at 1.5 mL min. Initial oven temperature was maintained at 120°C for 2 min, then ramped to 210°C at 6°C·min^{-1}, and then ramped to 250°C at 5°C·min^{-1}. Injector temperature was 260°C and flame ionization detector temperature was 300°C. Identification of peaks was accomplished using purified FA standards (Sigma-Aldrich, St. Louis, MO; Nu-Chek Prep, Elysian, MN; Matreya, Pleasant Gap, PA). An internal standard (1 mg of 13:0) that did not occur naturally in the samples was used to quantify FA in the samples.

Muscle fat was separated into neutral and phospholipids, and FA profiles determined as described for the lamb trial.

Statistical Design and Analysis. Blood plasma ALA concentrations from the steers were evaluated with a repeated measures design using the MIXED procedure with individual animal the random effect, feed treatment (FLX, FLXT) the fixed effect, and time of blood sampling the repeated measure. Heterogeneous compound symmetry was used as the covariance structure because it had the smallest value for Akaike's information criterion when compared with the covariance structures unstructured and compound symmetry. Treatment means were separated with multiple runs of the MIXED procedure. Muscle phospholipid n-3 FA were compared between treatments with a completely randomized design using the MIXED procedure with animal as the random effect and feed treatment as the fixed effect. Differences between treatment groups were considered significant at $P \leq 0.05$ and a trend at $P \leq 0.10$).

RESULTS AND DISCUSSION

Lamb Trial

Initial plasma triacylglycerol concentrations of ALA averaged 4.5 mol% and were similar ($P = 0.67$) for all 4 groups. Mean post-treatment plasma triacylglycerol concentrations of ALA were 4.5, 8.2, 7.6, and 4.6 mol% for the FLX, FLXT1, FLXT2, and CNTL groups, respectively. Change in these plasma triacylglycerol ALA concentrations from initial to post-treatment differed ($P = 0.001$) and were –0.5, 3.8, 3.2, and 0.24 mol% for the FLX, FLXT1, FLXT2, and CNTL groups, respectively. Changes in ALA concentrations were similar ($P = 0.17$) for CNTL and FLX groups, but ingestion of treated flaxseed by FLXT1 or FLXT2 groups resulted in greater ALA concentrations ($P = 0.005$) than ingestion of nontreated flaxseed by the FLX group. There was no difference ($P = 0.65$) in change of ALA concentrations between FLXT1 and FLXT2.

Scislowski et al. (2005) observed 123% greater concentrations (weight % of total FA methyl esters) of ALA in plasma total lipid when steers had flaxseed oil infused into their duodenum compared with steers that ingested the flaxseed oil. Therefore, the increase in ALA concentrations that we observed when the treated flaxseed was consumed indicates that the treatment process was providing some protection of the flaxseed ALA from biohydrogenation.

Muscle phospholipid concentrations of ALA were different ($P < 0.0001$) for the FLX, FLXT1, FLXT2, and CNTL groups (Table 5). Concentrations of ALA for the FLX vs. CNTL groups were different ($P < 0.0001$), and concentrations of ALA were different ($P < 0.0007$) for the FLX vs. FLXT1 and FLXT2 groups. However, there was no difference in ALA concentrations between the FLXT1 and FLXT2 groups ($P = 0.85$). Muscle phospholipid concentrations of eicosapentaenoic acid (**EPA**), docosapentaenoic acid (**DPA**), docosahexaenoic acid (**DHA**), and total n-3 (ALA + EPA + DPA + DHA) were also different ($P \leq 0.02$) among the 4 groups, and concentrations of EPA and total n-3 were greater ($P \leq 0.05$) for FLXT1 and FLXT2, than for FLX with no differences ($P \geq 0.06$) in concentrations of DPA or DHA for FLX vs. FLXT1 or FLXT2. There were no differences ($P \geq 0.17$) in ALA, EPA, DPA, or DHA concentrations between the 2 treated flaxseed groups. Alpha-linolenic acid concentrations were significantly increased in bovine muscle when flaxseed oil was infused daily into the proximal duodenum (Doreau et al., 2011). In fact, when steers were either fed extruded flaxseed for 70 d or an equivalent amount of linseed oil was infused directly into their proximal duodenum for 70 d, the percentage of ALA in the muscle of infused steers was 7-fold greater in the muscle of the infused steers (Doreau et al., 2011). Therefore, results from this trial indicate that treatments to flaxseed were probably providing some protection of ALA from biohydrogenation by ruminal microbes in lambs consuming a basal ration of finely ground and pelleted alfalfa hay. These findings have potential importance to people who consume considerable amounts of lamb and small amounts of fish high in n-3. This is because forage-fed lambs, which are common in some areas of the world, could be produced to be a source of n-3 FA, provided they were supplemented with flaxseed

Table 5. Effect of flaxseed treatment on phospholipid fatty acid concentration (mol%) of triceps brachii muscle of lambs

	Flaxseed Type					*P*-value[1]			
Fatty Acid[3]	CNTL[2]	FLX	FLXT1[2]	FLXT2[2]	SE	TRT[2]	CONT vs. FLX	FLX vs.FLXT1 or FLXT2	FLXT1 vs.FSXT2
18:3n-3 (ALA)	3.08	5.86	7.84	8.28	0.27	<0.001	<0.001	<0.001	0.85
20:5n-3 (EPA)	1.05	2.23	2.83	2.90	0.21	<0.001	<0.001	0.05	1.0
22:5n-3 (DPA)	1.99	2.89	2.65	2.85	0.14	<0.001	<0.001	0.37	0.17
22:6n-3 (DHA)	0.60	0.98	0.60	0.74	0.08	0.02	0.01	0.06	0.32
n-3 PUFA[4]	7.07	12.12	14.30	14.92	0.37	<0.001	<0.001	0.002	0.30

[1]*P*-value for *F*-test for TRT and preplanned comparisons.

[2]CNTL = control group (n = 6) of lambs that consumed a mixture of corn and soybean meal with similar CP and DE as the flaxseed treatments; FLX = treatment group (n = 6) of lambs that consumed 136 g/d of nontreated ground flaxseed; FLXT1 = treatment group (n = 6) of lambs that consumed 136 g/d of flaxseed that was treated to provide protection of ALA from microbial biohydrogenation in the rumen; FLXT2 = treatment group (n = 6) of lambs that consumed 136 g/d of flaxseed that was treated slightly differently than the other treated flaxseed to provide protection of ALA from microbial biohydrogenation in the rumen; TRT = treatment

[3]ALA = α-linolenic acid, EPA = eicosapentaenoic acid, DPA = docosapentaenoic acid, and DHA = docosahexaenoic acid.

[4]n-3 PUFA = omega-3 fatty acids 18:3n-3, 20:5n-3, 22:5n-3, and 22:6n-3.

(or other ALA-containing oilseed) that is treated to protect ALA from biohydrogenation. Lamb produced in this manner could help reduce the incidence of type 2 diabetes (Feskens, 2011) and obesity (Ailhaud et al., 2006; Massiera et al., 2010), which are serious and growing threats in many countries (Danaei et al., 2011; Finucane et al., 2011).

Steer Trial

Baseline concentrations (weight %) of ALA in blood plasma were similar ($P = 0.94$) for the 2 groups of steers before they began consuming flaxseed (2.96 and 2.94 weight % for FLX and FLXT, respectively). After consuming the high roughage, low concentrate ration plus flaxseed treatments for 56 d (first period), the plasma concentration of ALA was elevated to 8.2 and 8.9 for the FLX and FLXT groups, respectively, which did not differ ($P = 0.37$). However, during the second period with consumption of a high concentrate, low roughage diet, the FLXT group had 16% greater ($P = 0.003$) concentration of ALA in their plasma than the FLX group (9.1 vs. 7.9, respectively) and this caused a significant time × treatment interaction ($P = 0.009$) for their responses to the treatments.

Muscle concentrations (mol%) of the n-3 phospholipid ALA, EPA, DHA, and total phospholipid n-3 were not greater in LM of steers eating the treated flaxseed ($P = 0.14$ for ALA, $P = 0.94$ for EPA, and $P = 0.55$ for DHA; Table 6). However, there was a trend for the concentration of DPA to be greater in LM of steers consuming the normal flaxseed ($P = 0.10$).

It is tempting but problematic to compare the results from the lamb and steer trial. In contrast to the treated flaxseeds used in the lamb trial, the treated flaxseed used in the steer trial had greater ALA (mg/g) than the nontreated flaxseed contained and this may have been an important factor on the greater concentration of ALA in plasma of the FLAXT steers. Also, we do not have evidence that the ALA concentration in only the triacylglycerol fraction of plasma lipids was increased in steer plasma when the treated flaxseed was consumed. This may be important because the triacylglycerol lipid fraction is probably more reflective of ALA absorbed from the diet than the plasma phospholipid fraction because the triacylglycerol fraction has much faster turnover. It is difficult for us to provide an explanation as to why the treated flaxseed seemed to be partially effective at protecting ALA in flaxseed from biohydrogenation in the rumen of forage-fed lambs and increased ALA concentration in their muscle but did not increase ALA in the muscle of steers that were fed a high concentrate, low roughage ration. Compared with high roughage rations, high concentrate rations are believed to be associated with decreased quantity of ruminal microbes that hydrogenate PUFA (Kucuk et al., 2001). Therefore, when steers were consuming the high concentrate ration, per-

Table 6. Effect of type of flaxseed fed on the phospholipid fatty acid concentration (mol%) of LM of steers

	Flaxseed Type[1]			*P*-value[2]
Fatty Acid[3]	FLX	FLXT	SE	TRT
18:3n-3 (ALA)	3.34	4.00	0.28	0.14
20:5n-3 (EPA)	1.46	1.43	0.52	0.94
22:5n-3 (DPA)	4.09	3.71	0.28	0.10
22:6n-3 (DHA)	0.26	0.27	0.02	0.55
n-3 PUFA[4]	9.36	9.62	0.45	0.71

[1]FLX = treatment group of steers (n = 5) that consumed nontreated ground flaxseed; FLXT = treatment group of steers (n = 5) that consumed flaxseed that was treated to provide protection from biohydrogenation of a-linolenic acid by ruminal microorganisms.

[2]*P*-value for *F*-test for treatment (TRT).

[3]ALA = α-linolenic acid, EPA = eicosapentaenoic acid, DPA = docosapentaenoic acid, and DHA = docosahexaenoic acid

[4]n-3 PUFA = omega-3 fatty acids 18:3n-3, 20:5n-3, and 22:5n-3.

haps more of the ALA in the nontreated flaxseed was able to bypass the rumen and consequently there may not have been enough difference in ALA concentrations in the triacylglycerol fraction of plasma in FLX and FLXT steers to result in a greater concentration of ALA in the muscle of FLXT steers. If this was in fact the situation, then greater ALA concentrations in LM of FLAXT steers would not be expected. Also, the muscle tissue analyzed from the sampled lamb muscle, triceps brachii, is considered an intermediate muscle, whereas the muscle tissue analyzed from the sampled steer muscle, LM, is a white muscle. Enser et al. (1998) observed that LM tended to have decreased PUFA concentrations, which they associated with its metabolic status as a white muscle, whereas triceps brachii had greater concentrations of several n-3 FA, as well as n-6 FA LN.

In summary, the treated flaxseed products that were fed to lambs and steers raised ALA concentrations by relatively small amounts in the blood of both lambs and steers, and in lamb muscle but not in bovine muscle. Results from the lamb trial indicate that feeding forage-fed lambs supplemental flaxseed treated to reduce biohydrogenation of ALA can increase concentration of healthful n-3 FA in their muscle. The lack of increase in ALA concentration in the muscle of steers fed the treated flaxseed may have been related to the fact that in contrast to the lambs, the steers were on a high concentrate diet and consequently there was probably more ruminal bypass of ALA in steers fed the nontreated flaxseed (due to fewer ruminal microbes that biohydrogenate n-3 FA in cattle on a high concentrate diet) and therefore less advantage to the small amount of protection for ALA that the treated flaxseed provided.

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